Genotype-Phenotype Correlations in Hereditary Multiple Exostoses in British Columbia

By

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In

THE FACULTY OF GRADUATE STUDIES

(Faculty of Medicine, Department of Surgery)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April 2003

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Date April 25 2003.

Abstract

Hereditary Multiple Exostosis is an autosomal dominant condition in which multiple benign cartilage-capped tumours grow in relation to the growth plates of long and flat bones. . HME has a wide spectrum of clinical presentations and results in considerable morbidity from lesions due to mass effect causing limb deformity, mal-alignment, and shortening. Mutations in EXT 1 and 2 genes result in multiple exostoses. The presumptive role of the EXT genes is either tumour suppression or growth plate regulation. The purpose of this study was to determine the relationship between the genotype and phenotype in HME. Ten families were identified with HME. Genotyping was completed by linkage analysis of all families and the EXT 1 or 2 gene was sequenced based on these results. Mutation identification and confirmation was performed. Phenotyping consisting of clinical and radiographic examinations generated 89 features for each subject. Thirty-two affected individuals from 10 families participated. Eight of 10 mutations were identified, confirmed and segregation verified. Six of the mutations were unique and 2 previously had been reported in the literature. Three mutations were in EXT 1 and 5 in EXT 2. Two were missense, 3 nonsense, 2 splice site and 1 frameshift. EXT 1 patients were found to have more exostoses, with a higher percentage of flat and pelvic bone involvement. EXT 1 patients had more mal-alignment and were shorter. Males also had a more severe phenotype and modulated the severity of EXT 1 expression. No other genotypic factors were found to influence phenotype. An established genotype phenotype correlation will aid in patient management in terms of surveillance, determining prognosis and mangement. In conclusion a genotype phenotype correlation exists where EXT 1 is linked to a more severe phenotype.

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Acknowledgements

Dr. Michael Hayden

Dr. Stephen Tredwell

Dr. Peter O'Brien

Odell Loubser – Lab support for genotyping

Mary De Vera – Study research assistant

Heather MacDonald – Study research assistant

Kirsten Roomp – Genotyping technical support

Kathryn Duff – Phenotyping

Susie Clee - Genotyping technical support

Keith Fichter - Genotyping technical support

Angie Brooks-Wilson - Genotyping technical support

Jennifer Collins - Genotyping technical support

Dr. Bonita Sawatzky - Phenotyping

BCCH Department of Orthopaedics – Patient recruitment

Chapter I: Background

1.1 Osteochondroma (Exostosis)

1.1.1 Definition (osteochondroma, exostosis)

An exostosis or osteochondroma is a benign, cartilage-capped, bone tumour. These lesions can grow adjacent to the physis of all bones (Solomon 1963). They have a propensity to grow at the ends of the long bones, in particular around the knee and shoulder, which account for 57% of lesions (Wold 1990,). They can also occur on flat bones and on vertebrae.

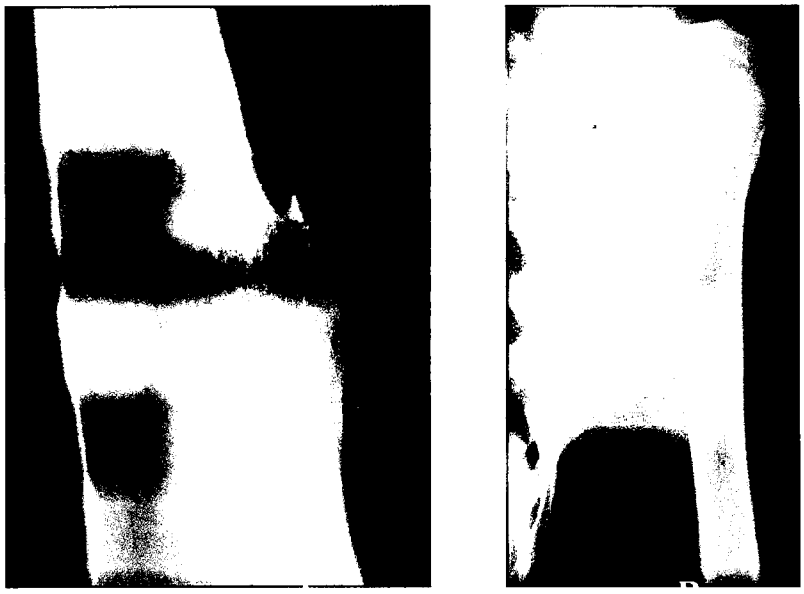


Figure 1.1 X-rays showing presence of an exostoses at the (a) distal femur (Wold 1990) and (b) proximal humerus

1.1.2 Features

1.1.1.1 Radiologic

Radiographically, these lesions appear as bony projections which are contiguous with the parent bone (Figure 1.2). The cartilage caps are radiolucent and not appreciated on plane xray when there is no mineralization in the cap. With maturity of the patient, mineralization is seen in the cartilaginous component of the tumor. The continuity of the

cortical and cancellous bone with the parent bone is reliably demonstrated using computed tomography (CT scan). Visualization of the unmineralized cartilage cap is only possible by magnetic resonance imaging (MRI) (Pierz et al. 2001).

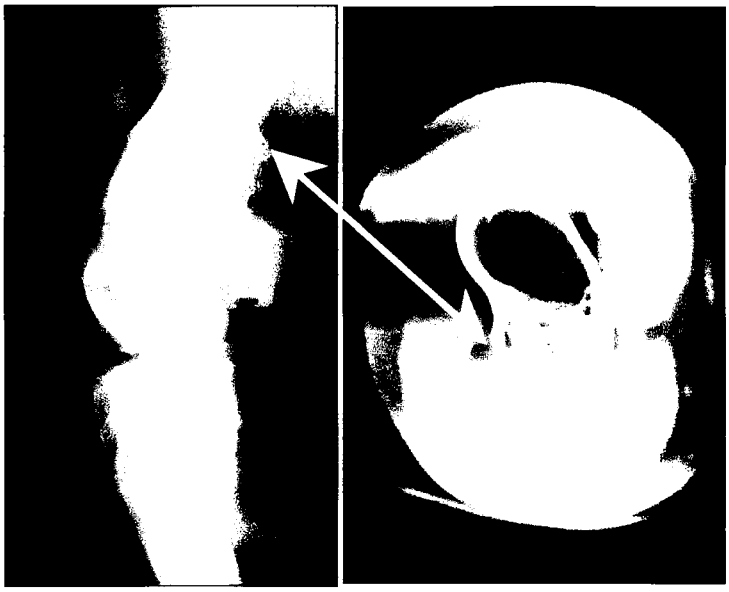


Figure 1.2 X-ray and CT scan showing location of an exostosis in relation to the parent bone

1.1.1.2 Gross Pathology

The pathology of osteochondromas was described in detail by Jaffe in 1943. The gross pathology of these lesions shows that a layer of smooth translucent, bluish cartilage is evident on the cut surface (Figure 1.3).

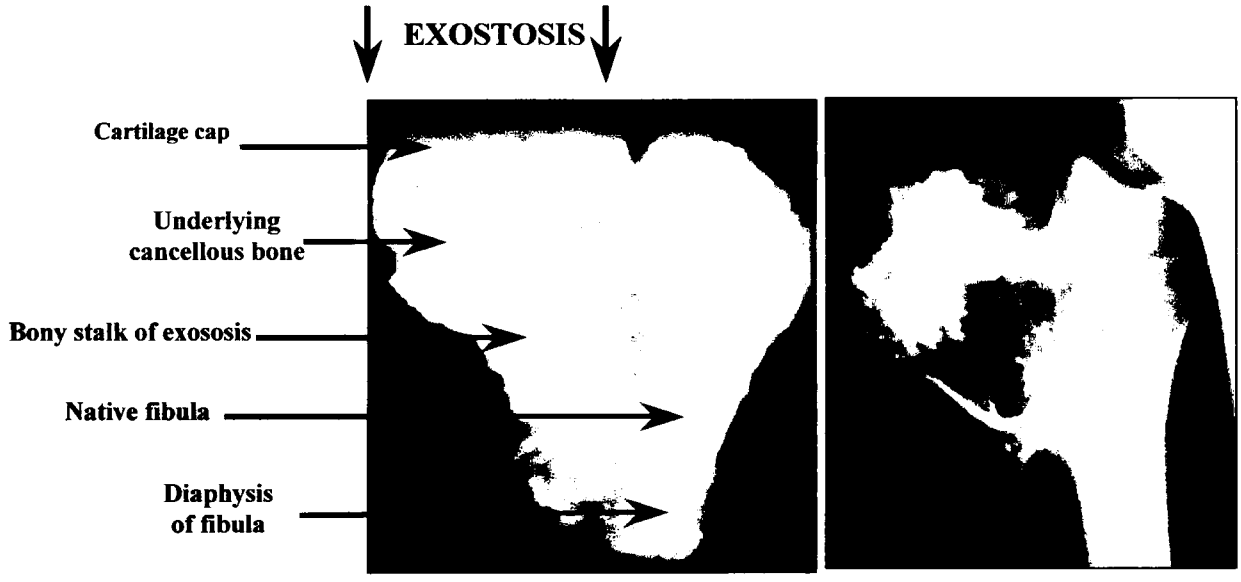


Figure 1.3 Gross pathology of an exostosis with the xray of the same lesion in situ. (Wold 1990)

The thickness of the cartilage cap varies with the activity of the lesion. Lesions typically develop and grow during childhood when the cartilage cap can be up to two centimetres thick. In contrast, adults do not develop new lesions and those that are present are quiescent with cartilage caps that are less than one centimeter in thickness. If an adult's lesion continues to grow, the cap is greater than two centimeter thick, and there is mineralization in the cap, transformation from a benign to a malignant process may have occurred (Pierz et al. 2001).

The morphology of the lesion may be sessile or pedunculated (Figure 1.4a and b). In some cases, particularly in cases of multiple exostoses, the metaphysis may be globally involved by the lesion resulting in metaphyseal flaring (Figure 1.4c).

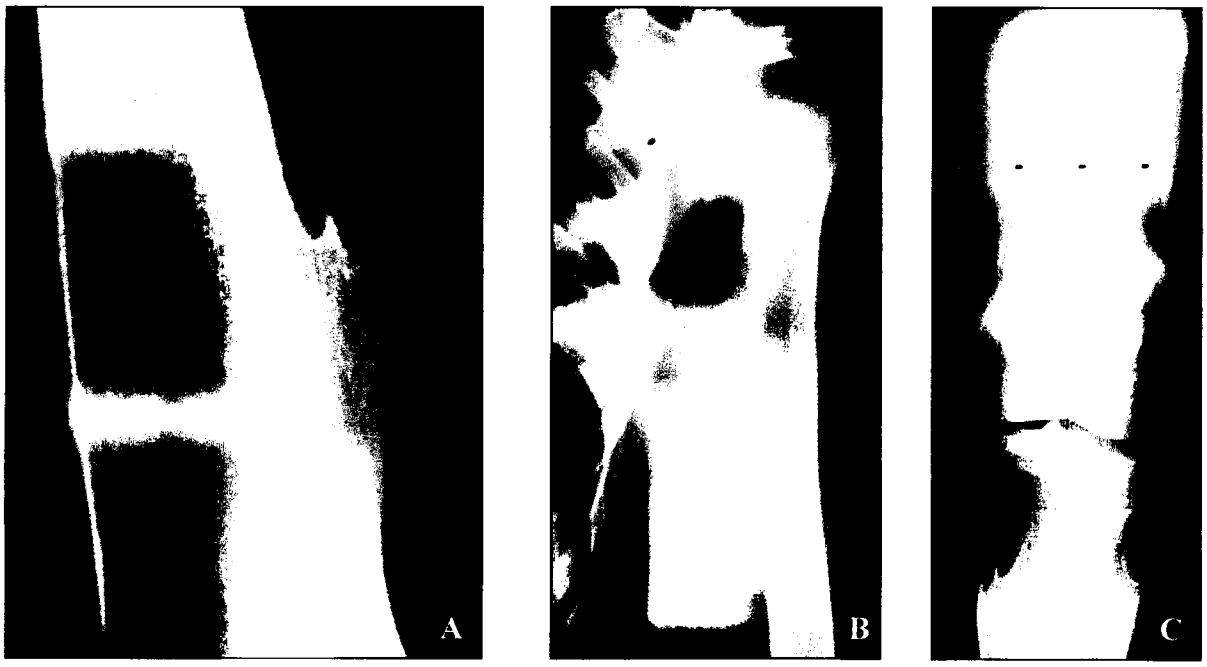


Figure 1.4 X-rays showing a (a) pedunculated exostosis (Wold 1990), (b) sessile Exostosis (solitary osteochondroma subject) and (c) a lesion causing metaphyseal flaring

1.1.1.3 Microscopic Pathology

Microscopically, the cartilaginous cap mimics the appearance of an epiphyhseal plate (physis) with the maturation architecture seen in enchondral bone formation (Figure 1.5). The chondrocytes exhibit a lack of pleomorphism, nuclear hyperchromasia, and binucleation. The underlying cancellous bone shows intertrabecular spaces filled with fatty or hematopoietic marrow (Wold 1990, 53).

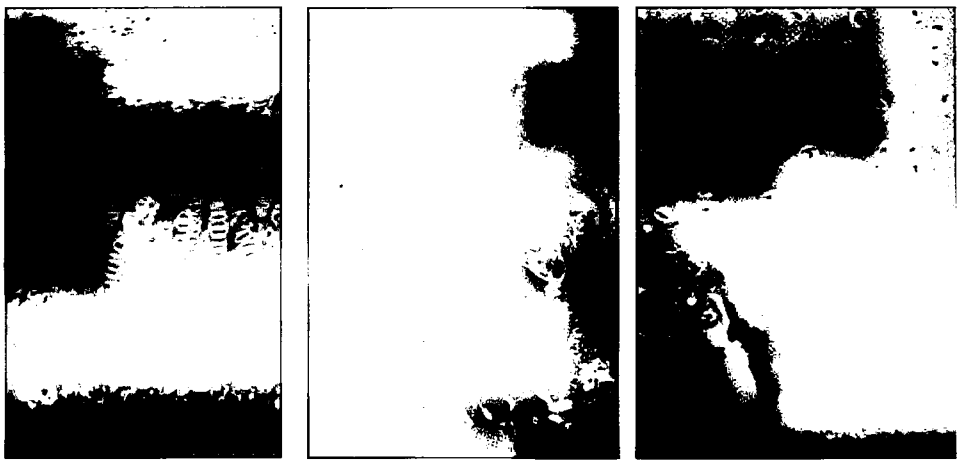


Figure 1.5 (a) Epiphyseal growth plate (Wheater 1987); (b) An osteochondroma at low magnification and (c) at high magnification (Wold 1990)

1.1.1.4 Clinical

Clinically these lesions are identified as a palpable lump; however, they cause a plethora of secondary symptoms. By mass effect alone they can compress local nerves leading to pain, paraesthesia, and paralysis. The lesion can interfere with local tendons causing locking, pain, or erosions leading to ruptures. Compression of the surrounding vasculature can also result in pain, pseudoaneurysm formation, or downstream thrombus generation.

Osteochondromas depending on size and or location may also cause unacceptable cosmetic disfigurement (Mirra 1989). Depending on their relationship with the adjacent growth plate, they may be separate and innocuous or may tether the growth plate resulting in limb malalignment (Figure 1.6), bony deformity (Figure 1.7), or growth impedance (Figure 1.8). These three effects are usually seen in patients with multiple exostoses as opposed to patients with solitary lesions. The mechanical effect of an exostosis relates to the number of lesions present in the area, how big the lesion is, and when it develops. If a lesion were to develop in isolation (especially seen in solitary osteochondromas), they tend to simply result in an innocuous bump with respect to bony deformity or joint malalignment. Often with these solitary cases, a lesion that develops early in life (before

the pubertal growth spurt) matures into a tumour that is not in contact with the growth plate and then migrates away from it as the patient grows. This is illustrated in Figure 1.4b where one sees a large solitary osteochondroma which is remote from the physis and the joint, causing no malalignment and minimal bony deformity save for the bump itself. This is in contrast to an osteochondroma that gets caught up in the growth plate and by its maturation, the stalk causes a bony bar which bridges the local physis, thereby preventing growth in that location (Figure 1.7a). This causes the growth plate and epiphysis to tilt, ultimately resulting in joint malalignment. The secondary longitudinal deformity can occur by this joint line tether or can also result from disruption of the bony architecture of the limb itself. Specifically, when looking at the forearm (Figure 1.7a), if the distal ulnar physis becomes tethered, the ulna will become shortened with respect to the radius (with which it shares an intimate relationship with respect to length), the radius will continue to grow but will become bowed because of its connections with the ulna. Another cause of deformity as well as shortening is the large lesion contained within the medullary space resulting in metaphyseal flaring (Figure 1.8a). These lesions affect the entire physis, causing severe distortion of the metaphyseal region and can cause global shortening of that limb due to the interference of the majority of that particular growth plate. Figures 1.6, 1,7, and 1.8 demonstrate all these effects.

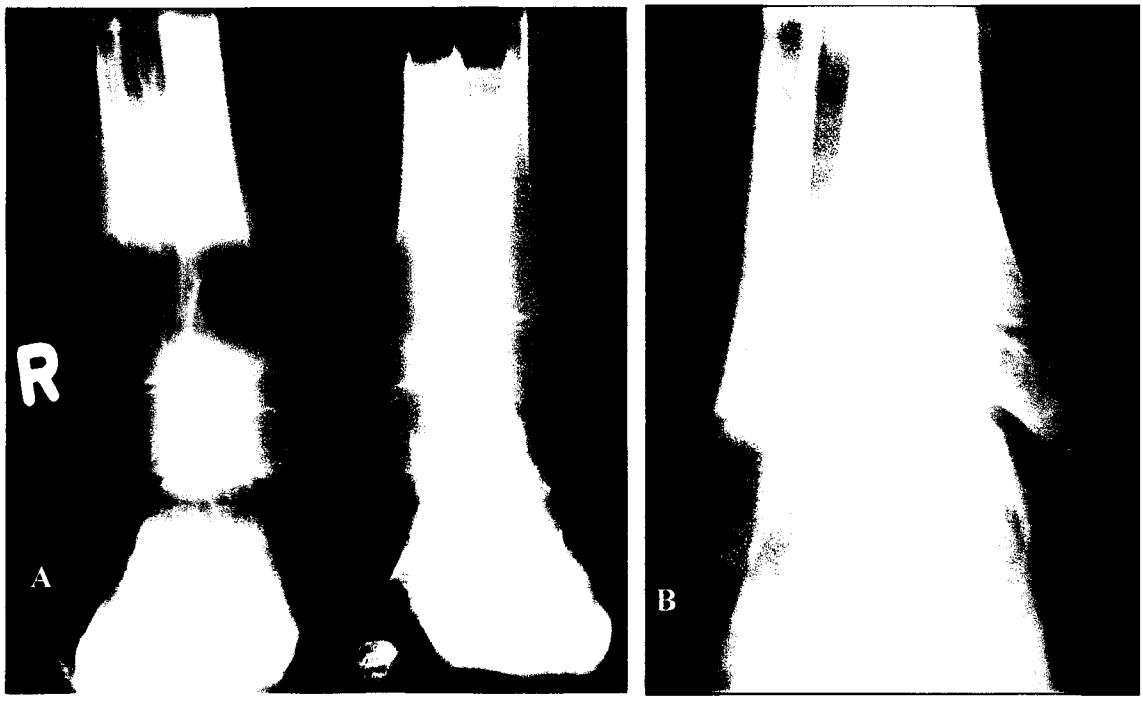


Figure 1.6 (a) X-rays showing exostosis tethering the growth plate in an affected ankle (b) A normal ankle is shown for comparison

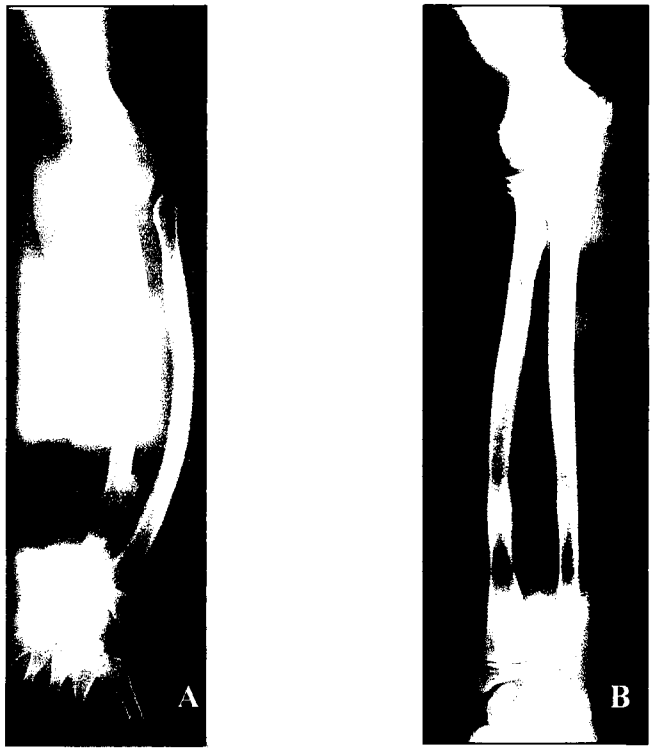


Figure 1.7 (a) X-ray showing an exostosis causing deformity in the foream; (b) a normal forearm is shown for comparison

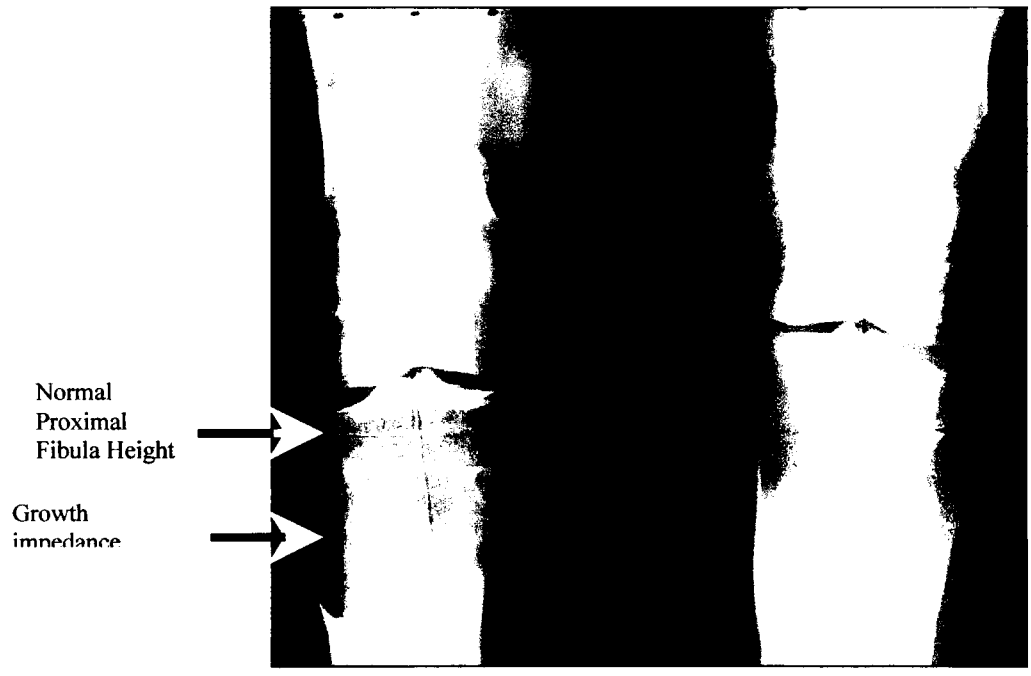


Figure 1.8 X-ray showing exostosis causing growth impedance.

1.2 Hereditary Multiple Exostoses

1.2.1 Definition

Exostoses occur either as solitary lesions or in multiples. When multiple lesions exist, they can be the result of an inherited trait called Hereditary Multiple Exostoses (HME) which accounts for two-thirds (66%) of the multiple exostoses cases (Boyer 1814) or represent sporadic cases called Spontaneous Multiple Exostoses (SME for the purposes of this thesis) which account for the remaining one-third (33%) of multiple exostoses cases. The latter case is then inherited as a dominant trait in the offspring with a 50% chance of transmitting the trait. The hereditary form of the disease, HME is the subject of this thesis.

1.2.2 Demographic features

The prevalence of HME is estimated at 1 in 50,000 (Wicklund et al. 1995; Pierz et al. 2001) with a male to female ratio of 1.5 (Schmale et al. 1994; Legeai-Mallet et al. 1997). The male to female distribution varies between 53% male and 46% female (Pierz et

al., 2002) to 49.5% male and 50.5% female (Solomon 1963). The differences between male and female distribution may be explained by a 95% penetrance rate in females.

Exostoses are typically detected as palpable lumps by the age of five in most patients (65%) and by twelve in all (100%) patients (Legeai-Mallet 1997; Solomon 1963). The proportion of individuals with HME who have clinical findings, increases from 5% at birth to 96% by age twelve (Chansky and Raskind 2002; Schmale et al. 1994; Wuyts et al. 1996). The bony distribution of exostoses found in HME patients is as follows; 50% humerus, 50% forearm, 70% knee, 25% ankle, 50% scapula (Schmale et al. 1994). The exostoses in HME cause similar symptoms to those mentioned above; however, the problem is multiplied by the number of lesions present. Their numbers also increase their potential to alter the growth of bones. Common symptoms include limb deformity (39%), limb malalignment (8%), limb length discrepancy (10%), and short stature (which is intrinsic to this disease) defined as a height two standard deviations below the mean on standard growth charts or a height less than the third percentile. (Wicklund et al. 1995).

The clinical impact on these patients is significant. Limb deformity for the purposes of this thesis and in keeping with orthopaedic opinion includes distortion of any part of a bone resulting in abnormal longitudinal or cross-sectional anatomy. Examples are abnormal bowing of the forearm or abnormal angulation of the femoral neck. Malalignment relates to the joints and longitudinal alignment of a limb. Examples are knee joint varus or increased radial inclination. Seventy-four percent of patients have removal of at least one lesion, and the average patient has three surgeries over the course of treatment (Schmale et al. 1994). The indications for surgery generally include pain, growth disturbance, angular deformity, decreased joint range of motion, degenerative arthritis, pressure on neural and vascular structures, or unacceptable appearance (Pierz et al. 2002). Furthermore, in a low percentage of HME patients (<1%), one lesion can degenerate into a

chondrosarcoma (Wicklund et al. 1995; Legeai-Mallet et al. 1997; Pierz et al. 2001) or other sarcoma (Schmale et al. 1994). The range of transformation rates in HME is reported from 0.5% to 25%. This broad range is influenced by referral bias (Pierz et al. 2001). Signs of sarcomatous degeneration of an exostosis include rapid growth and or pain in a skeletally mature individual (Lange and Rao 1984). CT and MRI imaging reveal a bulky cartilaginous cap of greater than 2 centimetres (Hudson et al. 1984) and a bone scan usually shows increased radionucleotide uptake (Bouvier et al. 1986). It is usually a low grade chodrosarcoma that develops in a pre-existing benign osteochondroma. Treatment involves wide surgical excision to reduce the local recurrence rate (Wusman 1997; Young et al. 1990).

1.2.3 Genetics and molecular biology of HME

1.2.3.1 General Information

HME is inherited as an autosomal dominant trait with a penetrance rate of 95% (Wicklund 1995; Schmale 1994) to 100% (Pierz 2002). Incomplete penetrance has been reported in female patients (Legeai-Mallet 1997). HME is a genetically heterogeneous disease as evidenced by linkage analysis (Hecht et al., 1995; 1997; Bovee et al., 1999; Phillipe et al., 1997; Wuyts et al., 1998). Two different genes, EXT 1 and EXT 2, have been associated with this disease. The exostoses genes represent a family of homologous genes consisting of six genes. EXT 1 is located on chromosome 8 (8q23-24) (Cook 1993) and EXT 2 on chromosome 11 (11p11-12) (Wuyts et al. 1996; (Wu et al. 1994). Other genes that were originally thought be associated with exostosis occurrence are EXT 3 on chromosome 19 (19p11-13) (Le Merrer et al. 1994), EXTL 1 on chromosome 1 (1p36) (Wise et al. 1997), EXTL 2 on chromosome 1 (1p11-12) (Wuyts et al. 1997), and EXTL 3 on chromosome 8 (8p12-p22) (Van Hul et al 1998). To

date, no mutations causing exostoses have been identified in these genes. EXT 3 is has recently been excluded as an EXT gene causing exostoses (Wuyts 2002).

The majority of cases (80%) of HME are accounted for by mutations in EXT 1 or EXT 2 (Cook et al. 1993; Blanton et al. 1996; Legeai-Mallet et al. 2000; Wuyts et al. 1996). Many authors, as noted in Table 1.1 have looked at the distribution of mutations between EXT 1 and EXT 2. It is most likely that there is an even distribution among EXT 1 (36%), EXT 2 (27%), and those remaining unidentified (36%) are most likely either EXT 1 or EXT 2 mutations.

Table 1.1 Summary of Family Mutations

Author	Ancestry	Number of families studied	EXT 1 Mutation		MS or non-truncating mutations		EXT 2 Mutations		# MS or non-truncating mutations		# of Unidentified Mutations
			#	(%)	#	(%)	#	(%)	#	(%)	
Philippe et al., 1997	Mixed	17	12	71	2	16.7	5	29.4	1	8.3	0
Wuyts et al., 1998	Mixed	26	10	38.5	2	7.9	10	38.5	--		6
Xu et al, 1998	Chinese	36	5	13.9	2	5.6	12	33.3	1	2.8	19
Seki et al., 2001	Japanese	43	17	39.5	--		6	13.9	1	2.3	20
Francannet et al., 2001	French	42	27	64.3	--		9	21.4	1	2.4	6
Gigante et al., 2001	Italian	9	4	44.4	--		3	33.3	--		2

Abbreviations used: MS – missense mutation

EXT 1 and EXT 2 genes have been isolated (Stickens et al. 1996; Wuyts et al. 1996). Both genes lack sequence similarity to any known gene and represent a new family of genes (Ahn et al. 1998, Stickens et al. 1996). These genes are ubiquitously expressed, with the highest expression in the liver (Stickens et al. 1996), however mutations in the EXT genes only affect growing bone (Hecht et al. 1997). EXT 1 and 2 encode homologous proteins of 746 (Ahn et al. 1998) and 718 (Stickens et al. 1996; Wuyts et al. 1996) amino

acids respectively. Thirty-one percent identity exists at the amino acid level with significant sequence similarity throughout the entire protein as can be seen in Figure 1.9 (Stickens et al. 1996). This is particularly noted in the 260 carboxy terminus tail. EXT 1 and 2 are large genes. EXT 1 has a genomic size of over 250 kilobases, with a cDNA of 3304 base pairs comprising eleven exons. The EXT 2 gene is also over 250 kilobases and has a cDNA of 3781 base pairs encoding sixteen exons. Characterization of the EXT 1 and 2 genes including the intron and exon boundaries and the translation of each gene can be found in Appendices 8.3 and 8.4.

Figure 1.9 Alignment of EXT 1 and EXT 2 genes. Identical amino acids are outlined in boxes. EXT 1 sequence from NCBI database, Accession number NM_000127 and EXT 2 sequence from NCBI database, Accession number NM_000401. Overlapping sequences detected using BLAST searching of NCBI.

1.2.3.2 EXT Physiologic Function

The function of the EXT genes remains unclear. Two theories have been brought forward for the EXT genes functioning as either a tumour suppressor gene (Hecht et al. 1995, Raskind et al. 1995, Hecht et al. 1997) or that the EXT genes act in the regulation of bone growth at the physis (Alman et al. 2002, Bornemann et al. 2002, Wuyts et al. 1998).

Evidence suggesting a tumour suppressor function played a large role in the early days of EXT gene investigations. Historically, prior to 2000, this was the main theory regarding the function of the EXT genes. This work was based on identification of the genes involved from contiguous gene syndromes and then further support by LOH studies followed by identification of two mutations in a few osteochondromas and then more consistently in chondrosarcomas. From a pathologists view point similarities were drawn between HME and other familial benign multiple tumour conditions. Since 2000 the molecular function of the gene has been further described and it puts the tumour suppressor theory into question. In general as of 2003, it is the cell-to-cell signalling and growth plate regulation roles that are receiving more attention and evidence continues to mount against the tumour suppressor role and grow towards a signalling function via heparan sulfate.

The following is a synopsis of the history to the tumour suppressor role. Exostoses were noted to develop in patients with chromosome abnormalities involving chromosome 8 such as Langer-Giedion syndrome (facial dysmorphism, mental retardation, abnormal cone-shaped phalangeal epiphyses, multiple exostoses) where 8q24.11-q24.13 is deleted (Parrish et al. 1991; Ludecke et al. 1997). In Tricho-rhino-phalangeal (TRP) syndrome (thin nails, sparse hair, short metacarpals and tarsals, unusal facies, coned shaped epiphyses of the digits) the deletion was found in the area of 8q24.12 (Buhler and Malik 1984; Fryns and Van Den Berghe 1986). TRP II has a q24.1 deletion and has mental retardation and exostosis development whereas TRP I is a deletion of 8q22.3 to 23.2 and does not develop

exostoses. Exostoses also develop in chromosomal abnormalities in chromosome 11 as in Defect 11 syndrome (multiple exostoses, enlarged parietal foramina, craniofacial dysostosis, mental retardation) where there are rearrangements at 11p11-12 (Bridge et al. 1998; Ligon et al. 1998; Bartsch, Wuyts and Van Hul 1996). These contiguous gene syndromes helped localize where the presumptive EXT genes were located.

EXT 1 and EXT 2 genes were then isolated and cloned (Stickens et al. 1996; Wuyts et al. 1996). Germline EXT mutations were then identified as being involved with the development of multiple benign bone tumours seen in HME and SME (Legeai-Mallet 1997, Hecht et al. 1995, Hecht et al. 1997, Raskind et al. 1995, Wuyts et al. 1997, Wuyts et al. 2000, Phillipe et al. 1997). It was this relationship between gene mutation and tumour formation that suggested the putative role of the EXT genes was tumour suppression and therefore the EXT genes were considered as tumour suppressor genes.

Osteochondromas were then shown to be true neoplasms by Bovee (Bovee et al. 1999), the presence of loss of heterozygosity in 6 of 14 osteochondromas and aneuploidy in 4 of 10 osteochodnromas. She concluded that this indicated a clonal origin for the cartilaginous tissue of the tumours studied. Other studies were also done looking at the genetic composition of osteochondromas. In some solitary osteochondromas both copies of the EXT gene had been mutated by somatic mutations (Porter and Stickens 1999; Mertens et al. 1994; Bovee et al. 1999, Hecht et al. 195 and 1997; Raskind et al. 1995). In addition, two mutations have been found to exist in the chondrocytes of osteochondromas in HME: one in the germline and the other in the remaining wild type or somatic allele involving EXT 1 or 2 (Bovee et al. 1999; Mertens et al. 1994). Specifically, Bovee found in two patients with HME with mutations in EXT 1, 3 of 4 osteochondromas carried two mutations, the first being the germline mutation and the second a loss of the remaining wild-type allele. The remaining osteochondroma failed to show loss of heterozygosity and

it was hypothesized by the authors it may have been a small somatic mutation that was undetected. The conclusion that Bovee proposed is that inactivation of both copies of the EXT gene is required for osteochondroma formation. However, in these mentioned papers investigating the tumours for two mutations only up to 30% (4 to 30%) of the second mutations were found in all the tumours studied (including solitary osteochondromas and those found in HME and chondrosarcomas related and unrelated to pre-existing osteochondromas). At least one mutation was always found in either EXT 1 or 2 but the second mutation was unidentified in 70 to 96% of cases, possibly due to methods used to identify mutations in EXT 1 and 2 (single strand conformaiton polymorphism (SSCP), mutation analysis, sequencing only the coding region) or possibly a different tumour suppressor system is involved, for example p53. However, it is more likely that the cells within the tumour mass are simply at a higher risk of suffering a second mutation. It is these second injuries which may be more responsible for cells that go on to become malignant cells, for example a chondrosarcoma. This then supports the two-hit hypothesis of tumourogenesis proposed by Knudson (1971), in that it takes more than just one mutated allele to result in malignant degeneration.

Inactivation of the remaining allele in HME has been seen more consistently in chondrosrcomas (Bovee et al. 1999; Mertens et al. 1994). The loss of function of the EXT genes has been shown in malignant neoplasms originating from osteochondromas, regardless if they are from a spontaneous solitary osteochondroma or found in a lesion in a subject with HME or SME. This also supported the theory that these genes serve as tumour suppressors. Loss of heterozygosity studies revealed loss of genetic markers which flank EXT 1, EXT 2, and EXT 3 loci (we now know EXT 3 has been excluded as an EXT gene) (Porter and Stickens 1999; Hecht et al. 1995; Raskind et al. 1995; Hogue et al. 1996; Hecht et al. 2002). Hogue (1996) traced mutations in an HME patient from constitutional DNA

through to osteochondroma and into chondrosarcoma. These results support Vogelstein's theory of stepwise carcinogenesis as it relates to phenotype: specifically, degeneration of a neoplasm (accumulation of mutations) undergoing malignant transformation (1992). In addition, work completed on de novo chondrosarcomas, have also shown mutations in EXT 1 and 2 (Hogue 1996).

Osteochondromas were also discussed by pathologists as having certain neoplastic pathologic behaviours reminiscent of other tumours, for example adenomas in the large bowel, which also supports the premise that EXT genes have a tumour suppressor function. Adenomas like osteochondromas are benign tumours originating in the colon versus ostechondromas which are benign tumours that originate in the proximity of the physis. They can both be solitary and benign. They can also exist in a familial multiple form: familial adenomatous polyposis (apc gene mutation) and hereditary multiple exostoses (EXT gene mutation) (Porter et al. 1999). Specific features common to neoplasms are: random location at sites of predisposition (lesions develop in HME in an asymmetric, random distribution at common juxtaphyseal sites) (Schmale et al. 1994). They demonstrate behavioural or cellular disorder, in that these lesions develop in abnormal positions for this cell type, excessive cartilage volume, and though the architecture is similar to the growth plate the zonal definition is not as succinct. Finally, lesions in HME have the potential to transform into malignancies, representing not only loss of control of cellular growth but also the ability to metastasize (Porter et al., 1999).

The underlying mechanism, or final common pathway for the tumour suppressor theory is likely due to a lack of heparan sulphate presentation on the chondrocyte cell surface. EXT genes are believed to be involved in heparan sulphate polymerization and this will be discussed in greater detail in the following paragraphs. Heparan sulphate is also part of the extracellular matrix and is known to be involved with cell mobility

adhesiveness, differentiation, and cell-to-cell signalling. Loss of these features in part describes neoplasia or tumour generation. Cell-to-cell signalling is an extracellular matrix activity and mutations involving genes acting in this system resulting in tumours, does not mean the genes are tumour suppressor genes. Tumour suppressor genes normally function as negative regulators of cell proliferation (Griffiths et al. 1996). For example p53, a known tumour suppressor gene, serves as a monitor of DNA damage. Mutations in this gene allow cell division to occur in the absence of DNA repair. There is then an accumulation of mutations, chromosomal rearrangements and aneuploidy, which increases the chances of that further uncontrolled cell proliferation occurs. EXT genes have been shown to be glycosyltransferases (see next section) involved in heparan sulfate polymerization which is not tumour suppressor activity.

The alternate and now more popular proposed physiologic function of the EXT genes is growth plate regulation. EXT gene products form a hetero-oligomeric complex involved in the regulation of cell surface heparan sulfate proteoglycan presentation (described further in the molecular function section following). Heparan sulfate is a dominant component of cartilage, which is the matrix of the growth plate. Heparan sulfate is integrally involved in the diffusion of several families of cell signalling molecules including those in the hedgehog, TGF-beta (tumour growth factor), and FGF (fibroblast growth factor) families. Specifically EXT genes are involved in the diffusion of Indian hedge hog by way of their glycosyltransferase activity. Indian hedgehog in humans, invokes osteoblast differentiation in the lower growth plate (by being in low concentration in the distal zones of the growth plate), incites chondrocyte proliferation, inhibits chondrocyte differentiation (in the proximal aspect of the growth plate where Indian hedgehog is in its highest concentration) and stimulates Parathyroid hormone related protein (PTHrP) in the perichondrium to produce chondrocytes in the zone of proliferation

of the physis and prevent movement of chondrocytes down the differentiation pathway. Mutations in the EXT genes effect the normal diffusion of Indian hedgehog (from distal to proximal) likely due to the alteration in the extracellular matrix caused by an absence of heparan sulfate. The EXT mutations may then cause a disruption in the negative-feedback loop by inhibiting Indian hedgehog diffusion, which would normally prevent chondrocyte differentiation resulting in abnormal ectopic development of chondrocytes.

It remains an abnormality of heparan sulfate polymerisation, which in turn appears to regulate growth and differentiation of the chondrocytes. The end result of EXT mutation is that Indian hedgehog does not diffuse and establish an appropriate concentration gradient in the growth plate. Proximally the concentrations are high, resulting in excessive chondrocyte proliferation without differentiation, which then becomes the nidus for tumour or osteochondroma genesis.

If this were truly the case however, one would expect the entire growth plate to be abnormal, with osteochondromas developing throughout the physis, peripherally and intramedullary, resulting predictably in juxtaphyseal/metaphyseal flaring, and multiple osteochondromas at each and every growth plate. It would be unlikely to see well-defined isolated lesions affecting only a few of the growth plates (which is a common pattern of presentation in HME/SME). On the other hand as Hecht has shown by her cross sectional studies of growth plates there are multiple niduses of presumptive osteochondroma nests in the perichondrium all along the physeal and metaphyseal zone (Hecht 2002). In her opinion there are secondary factors in the local and humoral environment affect the survival of specific nests that go on to form the clinical tumours.

The different physiologic mechanisms of action of the EXT genes should express themselves as different phenotypes at the clinical level as aluded to above. It is therefore a

useful project to determine if the phenotype varies and then how it relates to the potential physiologic role of the EXT genes.

1.2.3.3 EXT gene products and function

The proteins encoded by the EXT 1 and 2 genes are type II transmembrane glycoproteins situated in the endoplasmic reticulum (ER) (McCormick et al. 1998). The initial work done by McCormick indicated that the function of the protein expressed by EXT 1 was involved in the synthesis and presentation of heparan sulfate (HS) glycosaminoglycan (GAG) on the cell surface (McCormick et al. 1998). Biosynthesis of heparan sulfate chains involves the formation of an initial simple polysaccharide chain composed of alternating D-glucuronic acid (GlcA) and N-acetyl-D-glucuronic acid (GlcNac) units that are joined by 1-4 links. The polymer is then modified through a series of reactions involving partial N-deacetylation and N-sulfation of the GlcNac units, C-5 epimerization of GlcA to L-iduronic acid and O-sulfation at various positions (Salmivirta et al. 1996). EXT1 and 2 both possess the GlcNAc and GlcA transferase activities representative of heparan sulfate polymerase (Lind et al. 1998; Seany et al. 2000; Wei et al. 2000).

GAGs, in particular heparan sulfate, are known to function as co-factors in several signal transduction systems (as aluded to above) that affect cellular growth, differentiation, adhesion, and motility (Bernstein and Liotta 1994). GAGs may also play a role in the malignant transformation of cells, tumour adhesiveness, invasiveness, and metastasis. Given the activity of GAGs and that the EXT genes are involved with HS expression lends support that EXT genes may have either a tumor-suppression activity or growth plate regulation function.

When McCormick (McCormick et al. 1998) examined the effect of different mutations on the gene product, he found the more severe mutations such as fameshifts, nonsense, and splice sites caused truncated proteins not localized to the ER and there was no heparan sulfate presentation on the cell surface. However, in a single amino acid change, as seen in missense mutations, the protein remained located in the ER with reduced stability and yet HS cell surface display was again absent. McCormick concluded that mutation type does not differentially affect the molecular function of the EXT genes.

More recent work has shown that EXT 1 and 2 gene products though endoplasmic reticulum based proteins go on to form a hetero-oligomeric complex that leads to an accumulation of both proteins in the Golgi apparatus which in turn has the catalytic activity of heparan sulfate polymerization (Koboyashi et al. 2000; McCormick et al. 2000). McCormick demonstrates that EXT 2 does not exhibit significant glycosyltransferase activity in the absence of EXT 1 (McCormick et al. 2000). When the EXT1/2 complex exists in the Golgi apparatus, a much higher glycosyltransferase activity results compared to when EXT 1 or 2 present alone. Therefore, it is the complex of the two genes that forms the biologically relevant enzyme. This situation would explain why patients with mutations in either EXT 1 or 2 present with the formation of osteochondromas. This would also support the hypothesis that it is irrelevant which of the two genes is effected and that the phenotype would not be influenced by genotype.

Gullberg looked further into the activities of EXT 1 and 2 (Gulberg 2002). They are both catalytic enzymes as mentioned above and in both of their absence the heparan sulfate chain fails to elongate. In catalytic assays when EXT 1 alone is preserved it shows higher catalytic activity than when EXT 2 is alone. This then led to the concept that EXT 2 is a 'chaperone' or 'stabilizer' of EXT 1. Given that the two have varying impact on the

catalytic activities of heparan sulfate one may deduce that it does matter with respect to phenotype whether it is EXT 1 or EXT 2 that is mutated.

In summary, the EXT genes may have one of two physiologic functions; tumour suppressors via heparan sulfate extracellular matrix function (not tumour suppressor genes), or growth plate regulation via Indian hedgehog signalling, both contingent upon the existence of heparan sulfate presentation/presence in the physeal zone. The function of the EXT genes is to catalyze heparan sulfate polymerization. There is recent evidence that the two genes contribute differing amounts of activity whereby EXT 1 catalytic function is greater than that of EXT 2. There is also evidence showing mutation type, truncating versus nontruncating, causes different results with regards to EXT protein location but not in terms of ultimate heparan sulfate presentation. The basic science of the EXT genes suggests there may potentially be a difference in phenotype as a result of which gene is affected and by what type of mutation.

1.2.4 Mutations

Several groups have been working to identify the mutations in HME (Seki et al. 2001; Xu et al. 1998; Park et al. 2001; Raskind et al. 1998; Wells et al. 1997; Hecht et al. 1995; Wuyts et al. 1998; Philippe et al. 1997; Ahn et al. 1995). Table 1.2 and 1.3 list the known mutations in a variety of ethnic backgrounds. Figures 1.10 and 1.11 show the location of the mutations in relation to their distribution in EXT 1 and 2; more mutations have been located in EXT 1 than in EXT 2 (85 EXT 1 versus 44 EXT 2).

The most common type of mutation identified in both EXT 1 and EXT 2 is a frameshift mutation, which truncates the protein and significantly changes the portion of the protein coded for. In addition, the majority of mutations occur early in the gene. Both

genes are approximately 3300 base pairs long; in EXT 1, sixty-eight of eighty-five occur prior to base pair 1500, while in EXT 2, forty-two of forty-four occur prior to base pair 1500.

Most of the above-mentioned studies have an average of 20% percent unidentified mutations. In general, the 5' and 3' UTRs and the promoter regions were not screened and very large mutations involving one or more exons may be missed. Furthermore, EXT 3 was not studied and the missing mutations could be present in these regions. However, no mutations in EXT 3 have been found in cases of any form of exostoses and EXT 3 is now considered not to be involved with exostosis formation (Wuyts 2002). Also, not all intronic regions were investigated and these may be sites of unidentified mutations as well.

Table 1.2 Summary of Mutations Identified in the EXT 1 Gene

	cDNA change[a]	Exon	Protein Change	Type	Reference
1	42delG	1	G15	FS	Francannet et al., 2001
2	79C→A	1	Q27K	MS	D. Zaletayev, unpublished
3	118delC	1	FS H40	FS	Raskind et al., 1998
4	174-176delC	1	FS P59	FS	Philippe et al., 1997
5	204G→A	1	W68X	NS	Wuyts et al., 1998
6	242-247insC	1	FS R83	FS	Wells et al., 1997
7	248insC	1	R83	FS	Francannet et al., 2001
8	248-249delG	1	FS Q84	FS	Wells et al., 1997
9	250C→T	1	Q84X	NS	Francannet et al., 2001
10	331A→T	1	K110X	NS	Xu et al., 1999
11	352insC	1	V118	FS	Francannet et al., 2001
12	357C→A	1	Y199X	NS	Raskind, et al., 1998
13	**357C→G**	**1**		**NS**	**Alvarez et al., 2003**
14	388delAG	1	FA S130	FS	D. Zaletayev, unpublished
15	420ins4	1	FS S141	FS	Hecht et al., 1997
16	456delC	1	FS L153	FS	D. Zaletayev, unpublished
17	458delTC	1	L153	FS	Francannet et al., 2001
18	460del2T	1	F154	FS	Francannet et al., 2001
19	477delTA	1	D160	FS	Francannet et al., 2001
20	490G→C	1	D146H	MS	Bovee et al., 1999
21	515delA	1	H172	FS	Francannet et al., 2001
22	527del8	1	FS K177	FS	Hecht et al., 1997
23	549delGT	1	S180	FS	Francannet et al., 2001
24	590-591delC	1	FS S197	FS	Xu et al., 1999
25	599G→A	1	W200X	NS	Wuyts et al., 1998
26	600G→A	1	W200X	NS	Wuyts et al., 1998
27	624ins5	1	FS F209	FS	Wuyts et al., 1998
28	651-664del14	1	FS L216	FS	Seki et al., 2001
29	679delC	1	R227	FS	Francannet et al., 2001
30	679C→T	1	R227X	NS	Seki et al., 2001
31	703del15	1	PLFSKdel	5 AA del	Bovee et al., 1999
32	712delT	1	S238	FS	Francannet et al., 2001
33	713delC	1	FS S238	FS	Hecht et al., 1997 (2 families)
34	742insTT	1	FS R248	FS	D. Zaletayev, unpublished
35	820-821delGG	1	FS G274	FS	Seki et al., 2001
36	838A→G	1	R280G	MS	Wuyts et al., 1998, Raskind et al., 1998
37	840G→C	1	R280S	MS	Raskind et al., 1998
38	876-877insT	1	FS V292	FS	D. Zaletayev, unpublished
39	943delGA	1	FS D315	MS	D. Zaletayev, unpublished
40	947A→G	1	N316S	MS	Bovee et al., 1999
41	1016G→A	2	G339D	MS	Philippe et al., 1997
42	1018C→T	2	R340C	MS	Philippe et al., 1997
43	1018C→A	2	R340S	MS	Wuyts et al., 1998
44	1019G→T	2	R340L	MS	Hecht et al., 1997; Seki et al., 2001

[a]All mutations were uniformly numbered with the adenosine of the start codon nucleotide position +1.
Abbreviations used to indicate mutation types: **MS** – missense, **NS** – nonsense, **FS** - frameshift, **SS** – splice site
Blue font indicate missense or non-truncating mutations.

Table 1.2 (continued) Summary of Mutations Identified in the EXT 1 Gene

	cDNA change[a]	Exon	Protein Change	Type	Reference
45	1019G→A	2	R340H	MS	Raskind et al., 1998 (2 families); Sekit et al., 2001; **Alvarez et al., 2003**
46	1035-1056+2del24	2	FS F345	SS	Seki et al., 2001
47	1056+G→A	Intron 2		SS	Wells et al., 1997
48	1091-1093delG	3	FS E365	FS	Raskind et al., 1998
49	1122G→A	3	W374X	NS	Philippe et al., 1997
50	1157T→G	3	L386X	NS	Seki et al., 2001
51	1198-1199insA	4	FS D339	FS	Seki et al., 2001
52	1203-1204delC	4	FS L402	FS	Raskind et al., 1998
53	1213-1216del4	4	423STOP	FS	Gigante et al., 2001
53	1215del4	4	FS R405	FS	Raskind et al., 1998 (2 families)?
54	1215-1218del4	4	FS R405	FS	Seki et al., 2001
55	1370delT	4	T424	FS	Francannet et al., 2001
56	1320insT	5	441 STOP	FS	Gigante et al., 2001
57	1333-1334insG	5	FS N446	FS	Seki et al., 2001
58	1376C→G	5	S459X	NS	Wuyts et al., 1998
59	1409del10	5		SS	Park et al., 1999
60	1417+1G→A	Intron 5		SS	Philippe et al., 1997
61	1417+2del6	Intron 5		SS	Wuyts et al., 1998
62	1426-1431insC	6	FS S478	FS	Hecht et al., 1997, Raskind et al., 1998
63	1431insT	6	FS S478	FS	Wells et al., 1997
64	1457C→T	6	A486V	MS	Xu et al., 1999
65	1468-1469insC	6	FS L490	FS	Seki et al., 2001
66	1469delT	6	FS L490	FS	Wuyts et al., 1998, Ahn et al., 1995 (2 families) Wells et al., 1997, Philippe et al., 1997, Xu et al., 1999
67	1474-1475delTC	6	FS L492	FS	Seki et al., 2001
68	1487C→T	6	P496L	MS	Xu et al., 1999
69	1568delT	7	L523	FS	Francannet et al., 2001
70	1642delA	8	621 STOP	FS	Gigante et al., 2001
71	1642delA	8	S548	FS	Francannet et al., 2001
72	1679-1680insC	8	FS V561	FS	Wuyts et al., 1998
73	**1723G→C**	**8**		**SS**	**Alvarez et al., 2003**
74	1745G→A	9	W582X	NS	Francannet et al., 2001
75	1744G→A	9	W582X	NS	Francannet et al., 2001
76	1773delG	9	G591	FS	Francannet et al., 2001
77	1776C→A	9	Y592X	NS	Francannet et al., 2001
78	1784delGC	9	R595	FS	Francannet et al., 2001
79	1797G→A	9	W559X	NS	Seki et al, 2001
80	1817G→A	9	W606X	NS	Wells et al., 1997
81	1878del3	9	H627del	1 AA deletion	Raskind et al., 1998
82	1883+2T→G	9		SS	Seki et al., 2001
83	1980delG	10	664STOP	FS	Gigante et al., 2001
84	2053C→T	10	Q685X	NS	Raskind et al., 1998
85	2101C→T	11	R701X	NS	Seki et al., 2001

[a]All mutations were uniformly numbered with the adenosine of the start codon nucleotide position +1.
Abbreviations used to indicate mutation types: **MS** – missense, **NS** – nonsense, **FS** - frameshift, **SS** – splice site
Blue font indicate missense or non-truncating mutations.

Table 1.3 Summary of Mutations Identified in the EXT 2 Gene

	cDNA change[a]	Exon	Protein Change	Type	Reference
1	67C→T	2	Q23X	NS	Wuyts et al., 1998
2	77-78insT	2	FS Y26	FS	Philippe et al., 1997
3	233delC	2	FS P78	FS	Seki et al., 2001
4	239-244insG	2	FS G81	FS	Raskind et al., unpublished
5	253T→C	2	C85R	MS	Park et al., 1999
6	302del56	2	FS K101	FS	Raskind et al., unpublished
7	313A→T	2	K105X	NS	Xu et al., 1999
8	315-316insG	2	FS V106	FS	Xu et al., 1999
9	319insGT	2	FS C107	FS	Xu et al., 1999
10	374-443del70	2	FS I126	FS	Seki et al., 2001
11	449del4	2	FS A150	FS	Stickens et al, 1996
12	455T→G	2	L152R	MS	Xu et al., 1999
13	**455del4**	**2**		**FS**	**Alvarez et al., 2003**
14	495delG	2	FS L165	FS	Xu et al., 1999
15	514C→T	2	Q172X	NS	Wuyts et al., 1998; Wuyts et al., 1996; Xu et al., 1999
16	537G→C	2	R180T	MS	Francannet et al., 2001
17	537-1G→A	Intron 2		SS	Seki et al., 2001
18	580G→T	3	G193X	NS	Francannet et al., 2001
19	605C→T	3	A202V	MS	Seki et al., 2001
20	624delC	3	D208	FS	Francannet et al., 2001
21	627-2A→G	Intron 3		3' Splice Junction	Gigante et al., 2001
22	629-631insC	4	FS L211	FS	Xu et al., 1999
23	649-652delT	4	FS S218	FS	Wuyts et al., 1998
24	666C→G	4	Y222X	NS	Philippe et al., 1997
25	679G→A	4	D227N	MS	Philippet et al., 1997 (2 families); **Alvarez et al., 2003**
26	**730G→T**	**4**		NS	**Alvarez et al., 2003**
27	**751C→T**	**5**		NS	**Alvarez et al., 2003**
28	772C→T	5	Q258X	NS	Francannet et al., 2001
29	812-814delC	5	FS A271	FS	Wuyts et al., 1998
30	1079+G→T	Intron 6	FS Q313	SS	Wolf et al., 1998
31	1079+G→C	Intron 6		SS	Seki et al., 2001
32	1104insGA	7	E368	FS	Francannet et al., 2001
33	1132C→T	7	Q378X	NS	Raskind et al., unpublished
34	1139T→C	7	I380T		Gigante et al., 2001
35	1173+G→A	Intron 7	FS R360	SS	Wuyts et al., 1998 (2 families); Wuyts et al., 1996
36	1173+G→T	Intron 7	FS R360	SS	Wuyts et al., 1998
37	**1174G→A**	**Intron 7**		SS	**Alvarez et al., 2003**
38	1188G→A	8	W396X	NS	Xu et al., 1999
39	1201C→T	8	Q401X	NS	**Philippe et al., 1997**; Xu et al., 1999
40	1234C→T	8	Q412X	NS	Xu et al., (3 families)
41	1257T→A	8	Y419X	NS	Francannet et al., 2001
42	1263insAT	8	FS A422	FS	Wuyts et al., 1998
43	1669delC	11	FS R557	FS	Seki et al., 2001
44	1726G→A	11	E576K		Gigante et al., 2001

[a]All mutations were uniformly numbered with the adenosine of the start codon nucleotide position +1.Abbreviations used to indicate mutation types: **MS** – missense, **NS** – nonsense, **FS** - frameshift, **SS** – splice site; Blue font indicate missense or non-truncating mutations.

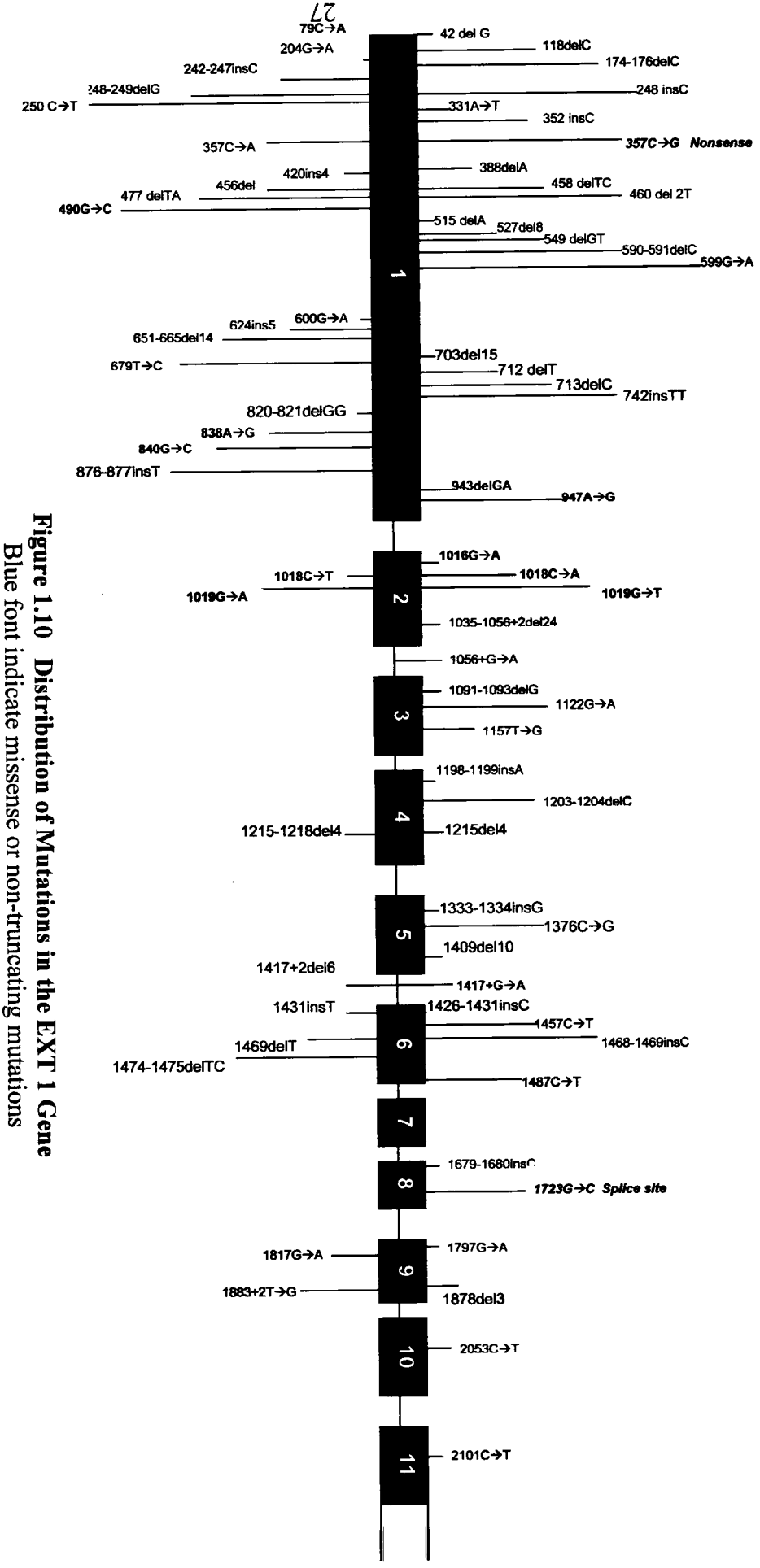


Figure 1.10 Distribution of Mutations in the EXT 1 Gene
Blue font indicate missense or non-truncating mutations

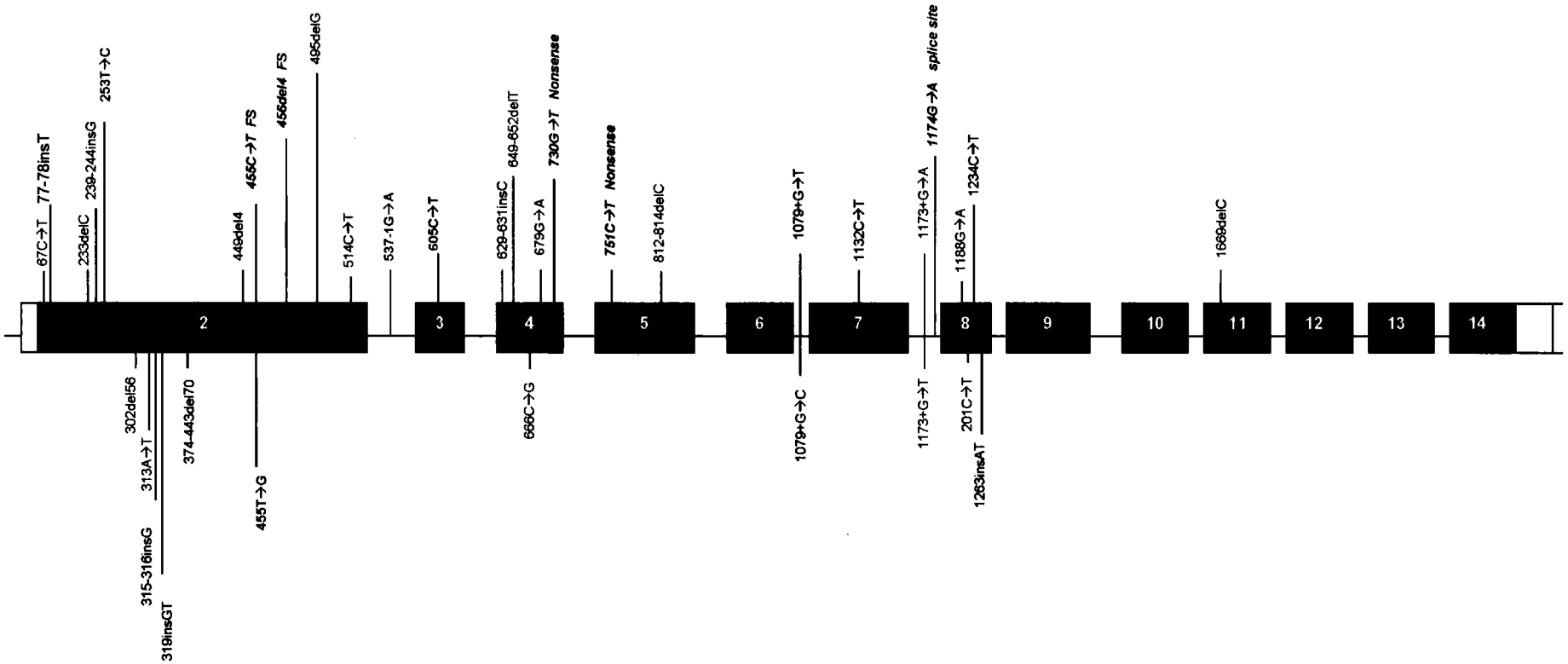


Figure 1.11 Mutation Spectrum of the EXT 2 Gene.
Blue font indicate missense or non-truncating mutations

1.2.4.1 EXT 1 Mutations Summary

Eighty-five different mutations in EXT 1 have been identified to date including the results of this study. Table 1.2 summarizes all known mutations. Some of the mutations have been found in more than one unrelated family (Table 2.1: 25, 33, 34, 53) but most are unique to each family. Of the eighty-five mutations thirteen (15%) are missense, seventeen (20%) are nonsense, forty-eight (56%) are frameshift and seven (8%) are splice site mutations. Forty of eighty-five (47%) are located in exon 1. One mutation has been identified in exon 7, and three mutations have been found in introns 2 and 5.

1.2.4.2 EXT 2 Mutations Summary

In comparison, only forty-four mutations have been identified in EXT 2. Table 1.3 summarizes all the previously published mutations plus those discovered in this study. As in EXT1, some overlap is seen in terms of unrelated families carrying the same mutation (from Table 1.2: 16, 22, 29, and 33). Of these forty-four mutations four are missense (9%), twelve (27%) are nonsense, eighteen (41%) are frameshift, and seven (16%) are splice site. Exon 1 of EXT 2 encodes the 5'UTR, and mutation analysis has not been done in this region by any of the authors. Currently, there are no identified mutations in exons 6, 9, 10, 12, 13, or 14. Exon 2 mutations (17 of 44) account for most of EXT 2 mutations.

1.2.5 Phenotyping

1.2.5.1 Schmale's Findings

Several studies and case reviews involving the phenotype of patients with HME are available. In 1994, Schmale (1994) assessed 113 individuals from forty-six families, and mapped their clinical expression. Features examined in this study included anatomical

locations, age at onset, orthopaedic operations, family pedigrees, number and location of palpable bumps, tenderness, range of motion, deformity, and limb lengths. The subject's overall functional status was evaluated using a modified version of the Musculoskeletal Tumor Society classification system (Enneking 1987, Table 2.3).

Schmale's study was a review of all patients in the state of Washington known to have HME. The prevalence in this state was estimated at 1 in 50,000; however, Schmale does admit to a variety of potential biases and therefore expects the overall frequency may be lower. The summary of their results show 49% of females at risk of having the disease were affected and 57% of males ($p>.1$), mean onset (no difference was found between genders) was 4 +/- 1 years, all cases were identified by 12 years, 4 percent of persons who carried the gene mutation did not express the disease (Schmale's coauthor Raskind had studied 34 of these families and identified the mutations and it is from this data the 96% penetrance rate was established for this population (Raskind et al. 1998)), 1% had chondrosarcoma. Figure 1.12 shows the anatomical distribution of lesions over the skeleton. With respect to the functional rating scale, 42% of males and 67% of females were rated as mild with good or excellent function; the remaining 58% of males and 33% of females were rated as severe with fair or poor function. Seventy-four percent of subjects had surgery, and on average each patient had 3 procedures.

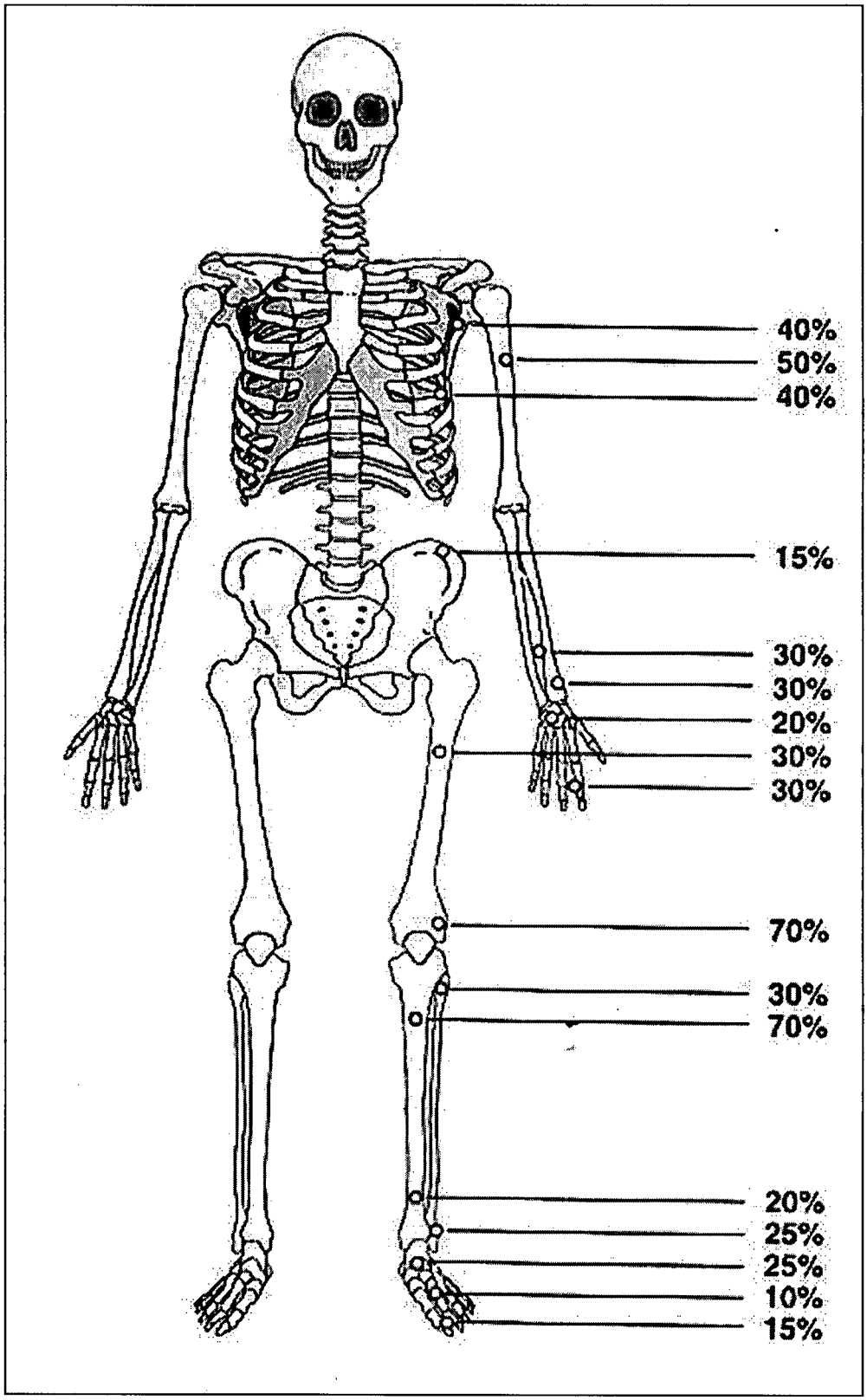


Figure 1.12 Anatomical Distribution of Lesions (Schmale 1994)

Table 1.4 Modified Functional Assessment Scale of the Musculoskeletal Tumour Society (as per Schmale 1994)

Rating	Motion *(% Total Motion of Normal Joint)*	Strength	Pain	Activity	Deformity			
					Bowing of forearm	Shortening of Forearm *(cm)*	Varus - Valgus Angulat of Knee *(°)*	Shortening of limb *(cm)*
Excel.	>90	5/5	None (no medication)	No restrict	None	None	0-5	None
Good	60 - 90	4/5	Mid (medication occasionally)	Restric. in recreational activities	Mild	<1	6-10	<1
Fair	30 - <60	3/5	Mod. (medication weekly)	Partial disability	Mod.	1-2	11-20	1-3
Poor	<30	1-2/5	Severe (narcotics or other medication daily)	Total disability	Severe	>2	>20	>3

1.2.5.2 Porter's Findings

Porter's objective was "to assess the evidence that the presence of local osteochondromas might be the major criterion affecting local bone growth" (2000). The essence behind this work was to re-define Hereditary Multiple Exostoses as a result of local bone growth interference caused by an osteochondroma rather than a dysplasia of bone (global skeletal growth disturbance). Porter based his work on sixteen of twenty-seven individuals who had forearm xrays available to examine. Comparison of palpable lesions versus radiographically present lesions revealed that on average there were twice as many radiographic lesions as there were palpable ones; therefore, radiographic data was relied upon entirely. Results showed that the greater number of lesions present the shorter the forearm. Further, the ulna was proportionately shorter than the radius in eight of ten patients, and when an osteochondroma was present near a physis, the growth of the bone as compared to normal was inhibited by as much as 80%. The forearm is a paired-bone construct, and Porter found the relative lengths of the bones correlated inversely with the

relative size of their osteochondromas. That is to say, the physical presence of the lesions results in local same-bone deformity and growth inhibition. This then leads to bony deformity, joint malalignment, and length discrepancies of a two bone system or the limb itself. If HME were a skeletal dysplasia, simple excision would not arrest the development of new, or further growth in this case, of excised lesions. Porter concluded that it is the local affect of the lesion (number of lesions, proximity to the physis and two bone systems) causing the pathology.

1.2.5.3 Genotype-Phenotype Correlations

In the past few years, despite the discoveries made in the molecular biology and genetics of exostoses, only a few papers have been published looking at the phenotype as it relates to the genotype in HME (Carroll et al. 1999; Francannet et al. 2002; Pierz et al. 2002).

1.2.5.3.1 Carroll's Findings

In 1999, Carroll assessed nine families (twenty-eight patients) with genetic mapping and evaluated the patients to determine if "genetic variations" correlated with clinical manifestations (Carroll et al. 1999). Linkage analysis was done using 6 highly polymorphic repeat (HPR) markers that flanked EXT 1. Families were assigned to either EXT 1 or not by calculating a two-point likelihood of difference using a MLINK subroutine of the computer program LINKAGE . Provisonal groupings developed from the linkage resulted in Group A representing the EXT 1 linked patients and Groups B and C representing not EXT 1 related which were clinically distinct. Clinical evaluation included range of motion of the joints, angular and limb length discrepancies, radiographs of the spine, pelvis, forearm and humeri, and hips to ankle standing films of the lower extremities. Features

evaluated were location, type and number of lesions, spine assessed for scoliosis, femur neck shaft angle, Sharp's acetabular index, Reimer migration index, radial bowing, carpal slip, radial articular angle, radial head subluxation/dislocation, ulnar shortening, femoral and tibial anatomic angle, ankle angle, and mechanical axis. Three clinical groups were identified based on the number of sessile lesions which appeared to correlate with severity of deformity and limb-length inequalities: Group A, EXT 1 linked, (87% sessile lesions) were moderately involved, Group B (95% sessile lesions) were severely involved, and Group C (72% sessile lesions) were mildly involved. Group C was ultimately deduced to be linked to EXT 2 based on the findings that chondrosarcomas were to that point only associated with chromosomes 8 and 11 and in this series of patients chondrosarcoma was identified in one patient each from Group A and C. This paper concludes that there are 3 distinct clinical groups where it was felt they represented mutations in EXT 1 (moderate phenotype, chromosome 8), EXT 2 (mild phenotype, chromosome 11) and EXT 3 (severe phenotype, chromosome 19).

The weaknesses in this paper includes first the lack of mutation identification, i.e. the true genotype, second, the inclusion of EXT 3 as one of the clinical types since EXT 3 mutations have never been shown to cause osteochondromas (though this was not known at the time of this Carroll's publication) and third, basing severity on whether lesions are sessile or pedunculated alone to categorize the patients. The strength of this paper is the extensive phenotype characterization. The main conclusion to be drawn from this work is that EXT 1 is worse that EXT 2 in this group of patients

1.2.5.3.2 Francannet's Findings

In 2001 Francannet reported on a clinical survey and mutation analysis of 42 French families. This study identified that 27 of 42 (64%) cases were accounted for by EXT 1 mutations. Of these, four were nonsense, nineteen frameshift, three missense, and one splice site. EXT 2 mutations accounted for 21% of the mutations and of these four were nonsense, 2 frameshift, two missense and one splice site. The phenotypic features assessed included a questionnaire given to the patients (the contents of the questionnaire was not included in the paper), clinical notes and xrays reviewed, the Musculoskeletal Tumor Society score for functional assessment (Enneking 1987) and development of chondrosarcoma.

Severity was described as severe or moderate and based on the following, age of onset (3 or less was severe), number of exostoses (10 or more was severe), vertebral location (presence of vertebral lesions was severe), stature (less than the 10^{th} percentile was severe) and functional rating (fair or poor was severe). The conclusion of this study was that EXT 1 caused the most severe forms of the disease and degeneration of exostosis into chondrosarcoma only occurred in EXT 1 (in clear opposition to Carroll's (1999) deductions and from the basic science literature (see section 1.2.3.2), where chondrosarcomas were found in both EXT 1 and 2).

The strength of this paper is identification of the genetic cause and its comparison with phenotypic features. An important feature that was included is the Musculoskeletal Tumour Society score (Table 1.4), which is a direct reflection of quality of life and ultimately what is the clinically relevant outcome. The phenotyping however in general is weak, not only in terms of only a few features being interpreted but also how they were applied. The 5 features were helpful in describing a portion of the phenotype, however they may not contribute to severity. For example, simply the presence of an exostosis in the

spine does not necessarily cause a problem as in pain, or deformity, in particular scoliosis. Also, as the number of lesions increase they may cause more secondary problems as in joint malalignment or bony deformity but this is also contributed to by the size, location and morphology (sessile or pedunculated) of the lesions not simply the presence or absence of lesions. Age of onset is also difficult to determine precisely and is influenced by many factors (family concerns, referral, diagnosis), which may blur the true onset date. In addition, this study identified chondrosarcoma only in patients with EXT 1 mutations whereas other authors have found these mutations in EXT 2 and 3 as well (Kivioja et al. 2000; Porter and Stickens 1999; Hecht et al. 1995; Hecht et al. 1997; Raskind et al. 1995; Hogue et al. 1996; Carroll et al. 1999). Of note once again however is that the patients with the EXT 1 mutations were phenotypically worse than the EXT 2 patients.

1.3 **Project Rationale**

Hereditary Multiple Exostosis (HME) is a relatively uncommon problem with a high clinical burden seen by Orthopaedic surgeons at British Columbia's Children's Hospital (BCCH). Most patients affected by this disease require surgical intervention an average of three times in their lifetime and usually as a child. The morbidity and complication rates of these surgeries are significant, including pain and disability, and problems implicit to surgery as a whole. Work on the genetics and molecular biology of Exostosis (EXT) genes has opened up the opportunity to further describe and examine this condition from the genotype perspective. Phenotypic features important to function and appearance are now better appreciated and readily investigated. It is the interplay between the genotype and the phenotype which has been incompletely explored.

In which gene the mutation exists, what type of mutation it is, its location, and its severity can be established. McCormick (1998; 2000) has shown that examples of both

truncating and non-truncating result in a non-functional protein and thereby in osteochondroma growth due to presumed interference in the tumour suppressor system. His original work suggested it is irrelevant where the mutation is (which gene), its type (truncating or non) or its location, the phenotype will be the same.

However, missense mutations still produce a protein that localizes to the endoplasmic reticulum. So is it true the EXT gene function is completely eradicated? Also Gullberg's (Gullberg 2002) work suggests mutations in EXT 1 and 2 have a different effect in that EXT 1 catalytic activity is greater than that of EXT 2 and this would therefore cause differing phenotypes based on which gene is mutated.

How the mutations manifest their effect on the physiologic function of the EXT genes will also then be potentially different. In terms of growth plate regulation problems if EXT 1 activity is preserved somewhat in isolation (when EXT 2 is mutated) then one would expect some preservation of the concentration gradient of Indian Hedgehog as some of the catalytic activity of heparan sulfate polymerization is preserved and thereby heparan sulfate present allowing for Indian hedgehog signalling to be partly working. This may then in turn result in less severe global growth plate changes, but should still be universal throughout the body. If the physiologic function is related to extrcellular matrix behaviour related to heparan sulfate presence then the partly preserved activity of EXT 1 in the EXT 2 mutated subject would lead to fewer chondrocyte nests developing; the fewer the nests, the fewer the lesions, the less the tumour burden and it secondary effects. Regardless of the actual physiologic function, there should be a difference in phenotype based on genotype whether it is due to which gene is affected, what type of mutation exists and possibly due to location or secondary influences such as gender remains unelucidated.

These differences based on genotype variability will then be reflected in the patient's phenotype. As we do not have the exact answer from the basic science work done on the EXT genes we may corroborate the possible mechanisms of function by looking at the phenotype. Clinically based authors suggest that the phenotype does depend on which gene is affected. For example, Carroll (Carroll et al.1999) lead us to the conclusion that if there is a mutation in EXT 1 the disease process in those individuals will be moderate versus EXT 2 which has a more mild presentation. Francennet (Francennet et al. 2001) came to the same conclusion, more specifically saying EXT 1 is worse than EXT 2. Further some authors have noted males have more severe disease and females may have incomplete penetrance, yet this is purely anecdotal (Schmale et al. 1994, Solomon et al. 1963). Neither Carroll's or Francennet's papers (Carroll et al.1999; Francennet et al. 2001) were thorough in one of the two aspects of the genotype phenotype assessment leaving their conclusions needing further exploration, but nonetheless reassuringly consistent.

There is obviously tremendous controversy about how genotype influences the phenotype. But to date researchers have worked in isolation in either the basic science or pure clinical arena except for the two above mentioned authors. This project was designed to bridge this gap by defining the genotype and the phenotype thoroughly from both aspects and then exploring the relationships. The rationale behind this study was to determine the genotype of HME: which gene is mutated, with what type of mutation, and its location, in conjunction with defining each affected individual from clinical parameters, which represent a given phenotype. The analysis of this data determines if genotype truly correlates with phenotype such that specific mutations or affected genes cause a predictable pattern of presentation, symptoms, and signs.

The results have many implications. If a correlation exists between genotype and phenotype in HME, a complete natural history for each mutation type and gene affected can be charted; this will directly influence day-to-day management of patients. For example, should particular lesions be excised early or later in its course. By knowing a patient's genotype it may be possible to determine which individuals, based on mutation type and location, are at increased risk for growth disturbance, lesion growth potential, and transformation to chondrosarcoma. It will also be possible, based on a patient's phenotype, to determine either the mutation location or type, and from that information, the individual's treatment can be managed accordingly.

1.4 Hypothesis

There is a genotype phenotype correlation in HME such that the major genotypic expressions, for example, EXT 1 versus EXT 2, will present with different phenotypic manifestations, for example, limb alignment or stature.

1.5 Objective

The objective of this study was to explore if a correlation exists between genotype and phenotype in Hereditary Multiple Exostosis in ten British Columbian families.

Chapter II: Materials and Methods

2.1 Ethical approval

The proposed study was reviewed by the Ethics Review Board of both Children's & Women's Hospital of British Columbia (C&W) and the University of British Columbia. Both boards approved of the study and its design in the fall of 1998; the projects ethical approval extended to 2004. Ethical Approval forms are found in Appendix 8.1.

2.2 Study protocol overview

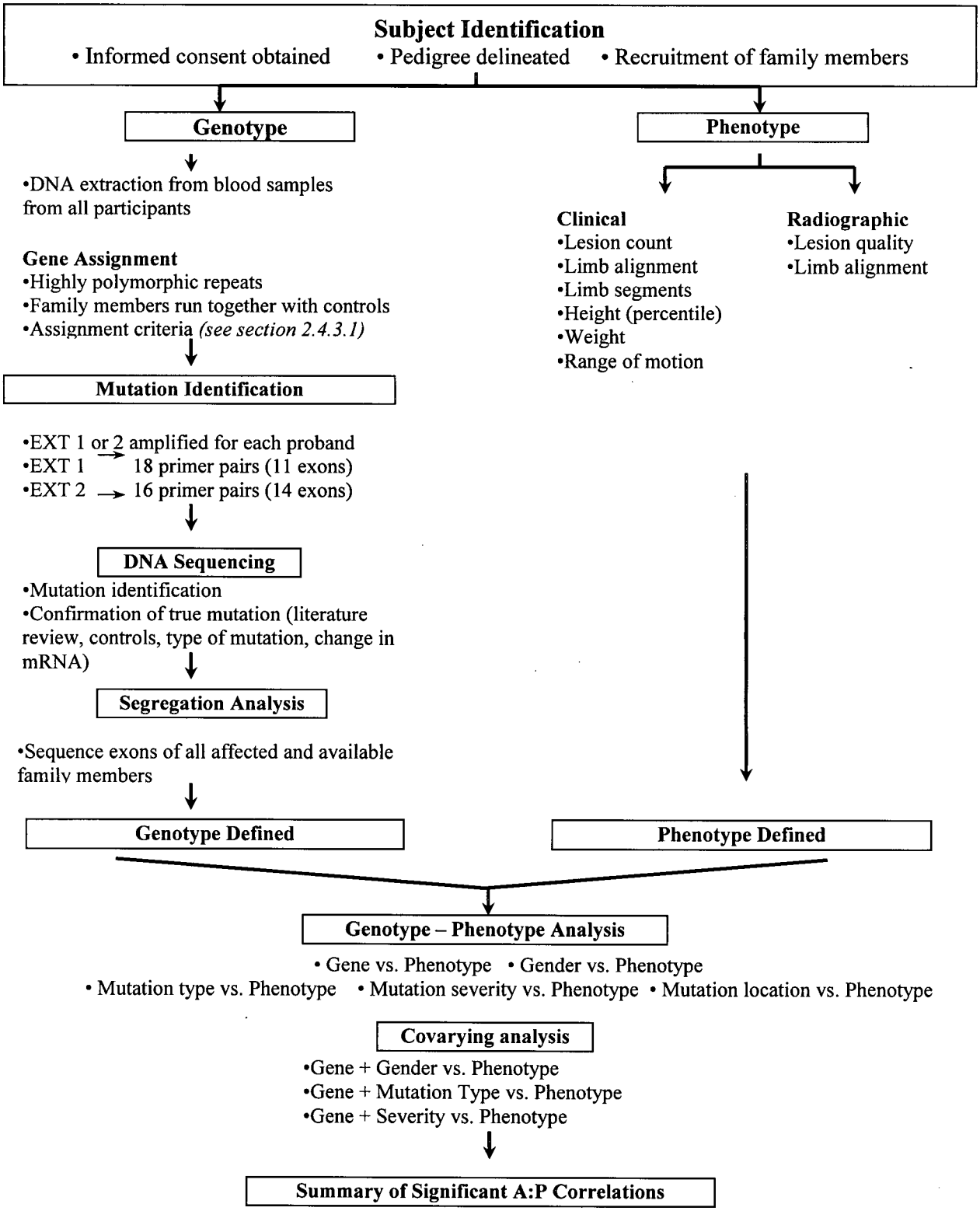


Figure 2.1 Overview of materials and methods

2.3 Subject Recruitment

2.3.1 Subject Identification

All subjects involved in this study were identified as patients of British Columbia's Children's Hospital. Patients and their families known to the paediatric orthopaedic department were approached by their respective surgeons, informed of the study, and asked if they would like to become involved. If they agreed, the principal investigator (Dr. C. Alvarez) was introduced to the family. All potential subjects were then informed of the study's rationale, purpose, and protocol. Consent was obtained from all individuals willing to participate in this study; minors consented with parental approval. The Letter of Information and Consents forms are found in Appendix 8.2. Individuals who did not wish to participate in the study continued with their regular care.

2.3.2 Pedigree Accumulation

A pedigree was designed (Cyrillic™ software) from the family history using as many corroborating family members as possible. Many extended family members did become involved in the study; however, a significant number of families had no extended members available.

2.4 Genotype

2.4.1 Sample Collection

Approximately 15 ml blood samples were collected from all participants in EDTA preserved, heparin loaded, 8 ml vacutainer tubes. Blood samples were drawn primarily by the principal investigator using universal precautions or by BCCH's laboratory accessioning personnel in the young subjects (less than 5 years of age). Blood was stored at

4°C until DNA extraction was performed. On average blood was not stored more than 1 week prior to extraction.

2.4.2 DNA Extraction

2.4.2.1 From blood

DNA extraction from patients' blood was carried out according to the NH_4Cl lysis and salt/chloroform protocol set forth by Mullenbach (1989). Red blood cell lysis solution was added: up to 45 ml per 10-15cc of sample in a 50ml falcon tube. The tube was inverted to mix and incubated at 37°C for 20 minutes with frequent mixing. The sample was then centrifuged for 5 minutes at 2000 rpm, and the supernatant was aspirated off. For the final rinse, 10-15ml of isotonic saline was added and the pellet was gently re-suspended; this solution was centrifuged for an additional 5 minutes at 2000 rpm.

The supernatant was removed down to the pellet and 10ml of saline + 500µl 20% SDS + 100µl 20mg/ml proteinase –K were added. The lysate was incubated overnight at 37°C and stored at 4°C until ready for extraction.

DNA extraction from the lysate was done using a salt/chloroform protocol (Mullenbach 1989). 3.3ml of 6M NaCl was first added to the lysate to yield a final concentration of 1.5M. The solution was mixed gently and an equal volume of chloroform was added followed by a gentle rotation for 30-60 minutes. The solution was centrifuged for 10 minutes at 2000 rpm, and the supernatant containing the DNA was transferred to a new tube. The DNA was precipitated out of the supernatant with 2x volume of 95% ETOH at room temperature. The DNA was spooled out of the liquid and re-suspended in Tris-EDTA to 2000µl. The integrity of the sample was checked on a 2% agarose gel and visualized under UV light. DNA concentration was measured using an UV/visible spectrophotometer (Ultrospec® 3000, Pharmacia Biotech).

2.4.3 Gene Assignment - Highly Polymorphic Repeats

Short Tandem Repeats (STR) were used to help trace the likelihood of the mutation being in EXT 1, 2, or 3. Initially only one marker for each of EXT 1 and 2 was used to direct which gene should be investigated primarily. Some families were too small for any meaningful segregation to occur, (families 1 and 6) and others were determined with only 2 PCRs, A01/2 and A03/4 (Families 2,3,5,16,17,18). Families 4 and 6 were assessed by all 8 markers due to lack of mutation identification when both EXT 1 and 2 were sequenced.

2.4.3.1 Marker Selection

Highly polymorphic repeat (HPR) markers were custom selected for the purposes of this project. Using the NCBI database microsatellite markers were identified for EXT 1, EXT 2, and EXT 3. Many of the markers used were the same as those used by Raskind (1995) in the project "Loss of Heterozygosity in Chondrosarcomas for Markers Linked to Hereditary Multiple Exostoses Loci on Chromosome 8 and 11" (Figure 2.2).

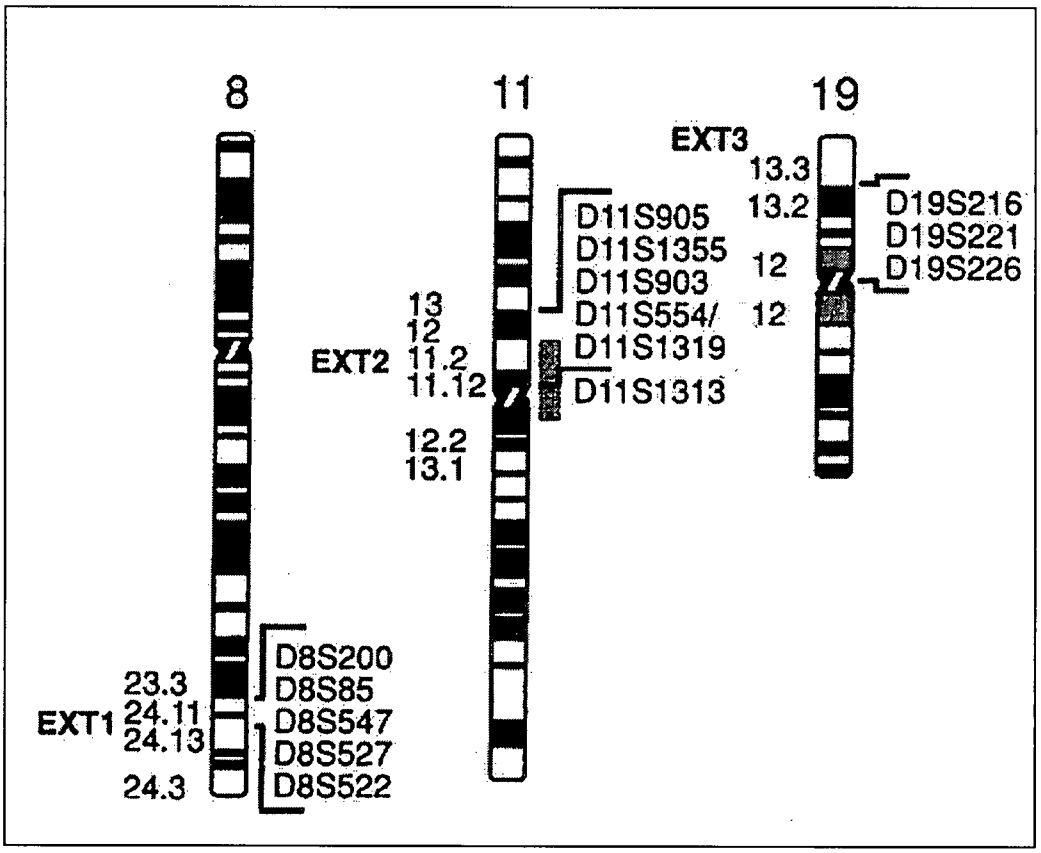


Figure 2.2 HPR marker locations in relation to EXT 1, 2, and 3. Ideograms for Chromosomes 8, 11, and 19 showing approximate locations of the EXT genes. Locations of the polymorphic microsatellite markers (CA repeats) used to determine LOH are also shown. (Raskind et al. 1995).

All markers were within a 5.4 cM span of the EXT 1 gene, for EXT 2 this was a 9 cM span and for EXT 3 it constituted a 25 cM span. Care was taken to select markers with greater than 71% heterozygosity frequency, fewer than ten alleles, and acceptable denaturation and reannealing temperatures (Table 2.1). Not all markers were required to assign the likelihood of a family carrying the mutation in one gene over another gene; however, two families did require all eight markers to help determine the likelihood of mutation location. Highly Polymorphic (HPR) markers and their features are described in appendix 8.5.1. The HPR primer pairs are named and defined in appendix 8.5.2.

2.4.3.2 PCR (with CA repeats)

PCRs were performed in a 25µl reaction volume with a final $MgCl_2$ concentration of 1.5mM, 200µM dNTP, and 0.5µM of each primer (Table 8.5.2), and 1µl Taq Polymerase (GibcoBRL) (Gene Amp-PCR system 9700, PE Applied Biosystems). Initial denaturation was done for 4 minutes at 96°C, followed by 25-30 cycles of 30 seconds at 94°C, 30 seconds at the determined temperature for each primer (see table), and 45 seconds at 72°C. Extension was performed at 72°C for 5 minutes.

2.4.3.3 PAGE (polyacrylamide gel electrophoresis)

Following the PCR, 5ul of PCR product was aliquoted into a microdish (Nunc, Intermed) well containing 5ul of denaturing loading buffer: 40% sucrose, 0.025% xylene cyanol, 0.025% bromophenol blue. Samples aliquoted in this way could be stored at -20° C for several weeks. The sample was denatured by placing the micro-dish on a heat block at 94° C for three minutes then immediately placed on ice. 4µl of the sample was loaded on a 6% denaturing polyacrylamide gel (60ml gelmix: 100ml 30% PAA, 240g urea, 50ml 10(x) TBE, 100ml dH_2O, 500µl ammonium perphospate(APS), 50µl Temed). The 0.4mm thick gel was run at 1650V with 1 x TBE running buffer for 1-2 hours on a sequencing apparatus (BRL, model S2, Life Technologies Inc.). The smaller plate used in the gel apparatus was treated with Wynn's Rain Away (Canadian Tire).

Approximately 30 minutes before the end of the running period, 500-1000ml of 0.5xTBE was prepared. A Hybond N+ membrane (positively charged nylon membrane, Amersham Life Science, UK) was trimmed to the exact size of the gel. The membrane was placed in a container with 0.5xTBE and cooled in a fridge for at 15 minutes. Five pieces of gel blotting paper (grade 238 cotton cellulose gel blot paper, Island Scientific, WA, USA) were cut slightly larger than the dimensions of the gel. When the run was completed, a

piece of blotting paper was placed over the gel, and subsequently peeled off to remove the gel from the glassplate. The wet Hybond N+ membrane was put over the gel in order to make a "gel sandwich" which was placed in a transfer apparatus (Semi-dry blotter, C.B.S. Scientific Co) with the membrane side facing down. The transfer was allowed to continue for 45 minutes at 15 volts. After the transfer was complete, the membrane was rinsed in 0.5 x TBE and dried for 1 hour at 80° C.

2.4.3.4 Hybridization and chemiluminescent detection

The following solutions were prepared for hybridization of one membrane. Stock solutions (10 x buffer, component A, component B) bought from Lifecodes Corp. (Stamford, CT, USA) were used in the "Quick-Light" hybridization protocol. Two wash solutions were prepared; Wash 1, 3ml of component A, 3.75ml of component B and 68.25ml of double distilled water, and Wash 2, 0.2ml component A, 2.5ml component B and 45.5 ml of double distilled water and 100ml 1xbuffer. The wash solutions, as well as the Quick-Light hybridization solution (15ml per membrane), were preheated at 55° C. The membrane was then soaked in 25ml of the heated Wash 1 in a hybridization tube. 4µl of a (CA)n Quick-Light research probe (Lifecodes Corp) was added to 15ml of heated hybridization solution in a 50ml Falcon tube and mixed well. The probe used was an alkaline phosphatase conjugated oligo that is vialed at 5 units per 100µl. One unit can was used in 75ml of hybridization solution when the Lifecodes Quick-Light hybridization procedure is followed. Twenty-five millilitres (25ml) of Wash I was poured out from the hybridization tube and the probe solution was added into the tube with the membrane. Hybridization was performed at 55 degrees Celsius for 30 minutes in a hybridization oven (Hybaid). The membrane was washed twice for 10minutes each with Wash 1 at 55 degrees Celsius, after which it was washed twice for 10 minutes each with Wash 2 at 55 degrees Celsius. Then the membrane was twice washed briefly at room temperature with the 1x

Quick-Light Buffer to adjust the pH of membrane to the Quick-Light chemical detection procedure.

2.4.3.5 Visualization

The membrane was then soaked in CDP-star solution (Roche Diagnostics Corporation, IN, USA) for 5min, drained and wrapped in plastic wrap. The membrane was then exposed to Kodak XAR film at room temperature for 1 hour and developed in a Kodak M35A X0Omat Processor machine. Exposure of the film to the membranes required customization for each membrane to give optimal visualization of the bands. Family members were run next to each other and each gel had control samples. Bands were labelled according to each family to aid in segregation determination (Appendix 8.5).

2.4.3.6 Exclusion Analysis

All available family members were run in adjacent lanes with two controls for each family. EXT 1 bands resulting from amplification of markers were assigned numbers (1,2,3) and EXT 2 markers were assigned letters (a,b,c). The bands were assigned numbers/letters from the top of the gel to the botton. Each member was assigned with EXT 1 numbers and EXT 2 letters. These assignments were then traced amongst the family members. Cosegregation was deemed to implicate the particular gene involved. Lack of segregation, i.e in an affected or unaffected, resulted in exclusion of that gene and proceeding to the next. From this, EXT 1 or 2 was excluded as being the source of the mutation.

2.4.4 EXT1 and EXT2 amplification

Exon 1 of EXT1 and exon 2 and 14 of EXT2 were split into overlapping fragments to obtain amplification products of less than 350 base pairs in length. Amplifications of the exons of EXT1 and EXT2 were performed in a 50µl reaction volume in 1.5mM MgCl

(except primer pair 1-9 which uses 2.5mM $MgCl_2$), 200μM dNTP, 0.5μM primer (see Table 2.2) and 1μl Taq Polymerase. 2μl of DNA (80ng/μl) was used for each sample. Samples were heated (Gene Amp PCR system 9700, PE Applied Biosystems) to 96°C for 4 minutes, and then cycled (30 times) through the following temperatures: 94°C for 30 seconds, annealing temp (Table 2) for 30 seconds, 72°C for 45 seconds, and 72°C for 5 minutes.

The PCR product (5ul) was combined with 5ul of sucrose loading dye and run on a 2% agarose gel containing ethidium bromide (1ug/ml) at constant voltage (BioRad system) for one hour in 1xTBE buffer. A 100bp DNA ladder (50ul ladder, 50ul xylene cyanol, 400ul TE) was run alongside the samples. DNA was visualized under UV light and photographed using Polaroid film.

Table 2.1 Primer pair sequences used for EXT 1

Primer Pair	Primer Name	Exon	Sequence	Length (bp)	Temp (°C)
1-1	EXT1-ex1a EXT1-ex1b	1	CAGGCGGGAAGATGGCGGACTGG*CTCCGGCTGTGGCT CCTCGATGCCC*	212	58
1-2	EXT1-ex1c EXT1-ex1d	1	TGCTCTCAGCTGGCTCTTGTCTCGGAATCCTCGT TTTCCAATTGATCCC	201	55
1-3	EXT1-ex1e EXT1-ex1f	1	CGGAGCCTCTGCGCCCCTTCGTTCC*CTAGAATGTT TTGGTAACTTTCGGCG*	232	55
1-4	EXT1-ex1g EXT1-ex1h	1	CGTATACCCACAGCAAAAAGGGGC*ATTGTTCCAC AAGTGGAGACTCTCG*	209	55
1-5	EXT1-ex1i EXT1-ex1f2	1	CCAGTTGTCACCTCAGTATGTGC*GGCTTTGGCCA GCATCGCCAGG*	168	55
1-6	EXT1-ex1k EXT1-ex1l	1	CCTGACTACACCGAGGACG*GGTGTCTGATCCTAT CCCTG*	237	55
1-7	EXT1-ex1m EXT1-ex1j	1	GGTATTCAAGGGGAAGAGGTAC*ggaccaaggccgg cagagccc*	231	55
1-8	EXT1-ex2a EXT1-ex2b	2	*ccccacattcgcaatgagtcgagaggtgataatgttaaaccc*	225	55
1-9	EXT1-ex3a EXT1-ex3b	3	cgatttggaacagcttcgtctg*gacgggggcagcaataatctgc*	224	55
1-10	EXT1-ex4a EXT1-ex4b	4	gtgcattctctttgttttacag*ctgagagaagtgtataaagg*	239	55
1-11	EXT1-ex5a EXT1-ex5b	5	cctttccaaatatcatcagg*catcttcagggtaaacaagggc*	237	55
1-12	EXT1-ex5a EXT1-ex5c	5	cctttccaaatatcatcagg*ccattttgcaatgctctgctctg*	237	55
1-13	EXT1-ex6a EXT1-ex6b	6	gctttccagcgcttcattaggc*ctggagctggagcaggcagggg*	210	55
1-14	EXT1-ex7a EXT1-ex7b	7	ggcgtacataaatacatcctaccccc*caaggctccacagtggttcc*	189	56
1-15	EXT1-ex8a EXT1-ex8b	8	caagactctgaagttacctctttccc*ggtgactgcctgaacagcccaacc*	204	58
1-16	EXT1-ex9a EXT1-ex9b	9	cattgttgattgcttgtttggcc*gtaaagtctgtaagagacatgtcc*	235	55
1-17	EXT1-ex10a EXT1-ex10b	10	cttgtcatcatgtgataatggccc*gagtgaagcaaggaagaggg*	259	55
1-18	EXT1-ex11a EXT1-ex11b	11	ccttgcacttctctcatattatcc*CCTCAAAGTCGCTCAATGTCTC GG*	230	55

NOTE: Primer names designated by "ex" followed by exon number; italics designate primers in the 3'-5'direction; lower case indicate primers located in introns; all primers used a final concentration of 1.5mM $MgCl_2$, with the exception of primer pair 1-9 which used 2.5mM $MgCl_2$; Accession Number: U67356-U67368 (Wuyts 1998)

Table 2.2 Primer pair sequences used for EXT 2

Primer Pair	Primer Name	Exon	Sequence	Length (bp)	Temp (°C)
2-1	EXT2-ex2a EXT2-ex2A8	2	Ctctcccctggtgacc*CACAGCGATAGACATCAAAACACG*	338	56
2-2	EXT2-ex2A26 EXT2-ex2A25	2	GACAGTCCCATCCCAGAGCGG*GGAGGGAACAA AACAGACAGG*	249	56
2-3	EXT2-ex2A4 EXT2-ex2b	2	ACTACACTGATGACATCAACCG*ccctttagttccctg agggcc*	176	55
2-4	EXT2-ex3a EXT2-ex3b	3	gttgacacattaattctccc*gaacaaaaatgatcttgaaccc*	184	51
2-5	EXT2-ex4a EXT2-ex4b	4	gaataaagtcctttctttctcatcg*cagtaaaggcacacctggc*	205	55
2-6	EXT2-ex5a EXT2-ex5b	5	gcaattttccaatcacctg*cctgagcctttgcgagagg*	267	51
2-7	EXT2-ex6a EXT2-ex6b	6	ctagtttgtaatctcttgcctc*tacgcagaaccactaatgtagag*	222	55
2-8	EXT2-ex7a EXT2-ex7b	7	gggatgtggggctgaaggagg*ctcctgtccctctgtatccagtc*	293	57
2-9	EXT2-ex8a EXT2-ex8b	8	gcttgctcacttaaaacagc*gcctcatgtggctagcac*	200	56
2-10	EXT2-ex8a EXT2-ex8c	8	gcttgctcacttaaaacagc*ttatgctgcccttatcaggccc*	200	56
2-11	EXT2-ex9a EXT2-ex9b	9	cagctgcttttctgacccg*gatccagctgagagaggcac*	263	55
2-12	EXT2-ex10a EXT2-ex10b	10	cctcacaaaagttaggag*aaacacactgtgtaaaacc*	240	51
2-13	EXT2-ex11a EXT2-ex11b	11	gaatggttgctgtctgaattggg*ctcagttttgtcaccttgcc*	235	55
2-14	EXT2-ex12a EXT2-ex12b	12	ccccttatttatcagctaaaggg*caagtgagtggcagagcc*	220	55
2-15	EXT2-ex13a EXT2-ex13b	13	gtccttgacactgacagccagg*tagagatcagaggctaaggcgc*	175	55
2-16	EXT2-ex14a EXT2-ex14b	14	caaacccctcctccccacctcctc*GTGGGTTAGGTGGG TGCATGCC*	318	58

NOTE: Primer names designated by "ex" followed by exon number; italics designate primers in the 3'-5'direction; lower case indicate primers located in introns; all primers used a final concentration of 1.5mM $MgCl_2$; Accession Number: U67356-U67368 (Wuyts 1998)

2.4.5 DNA Sequencing

DNA was prepared for sequencing using the polyethylene glycol 8000 precipitation protocol (Rosenthal, Coutelle and Craxton 1993). Several modifications were made including using 1.5ml Eppendorf tubes in place of 500µl tubes and allowing the sample to sit at room temperature for 20 to 30 minutes following the addition of the PEG solution to 25µl of PCR product. After re-suspending the precipitate in 11µl of H_2O, 2µl of the sample was analyzed on a 2% agarose gel (1 hour at 125V) and visualized under UV light.

Once the integrity of the product was confirmed, DNA sequencing was performed using the ABI 3100™ Sequencer (PE Biosystems, Foster City, CA, USA). This system employs capillary electrophoresis-based automated sequencing. Primer concentrations were made to 3.2 pmol.

2.4.6 Mutation Identification

PEG purified and cleaned PCR products were amplified with the ABI Prism Big Dye Terminator Cycle Sequencing Ready kit (version 2, Applied Biosystems, Foster City, CA, USA). Five ng PCR template was mixed with 3.2 pmol of sequencing primer (not nested), 2.4ul BigDye Terminator Ready reaction solution (Applied Biosystems, contains the dye terminators, dNTP's, AmpliTaq DNA polymerase FS etc.), 3ul of 5 x buffer (Applied Biosystems) to make a total volume of 20ul. (BigBye Terminator Ready reaction was diluted 1 to 4 with 5x buffer).

Amplification was done in a 96 well microamp plate at 96° C for 10 seconds, 50° C for 5 seconds, 60° C for 25 cycles in a GeneAmp PCR system 9700 thermal cycler. Precipitation of PCR products and removal of unincorporated dye terminators was done in the 96 well plate after the PCR plate was removed and spun in a table top centrifuge capable of centrifuging 96 well plates, 20µl double distilled water and 60µl of 100% isopropanol were added to each well. The plate was sealed with strips of lids or foil, inverted to mix, and left at room temperature for 15 minutes after which it was centrifuged at 1200 rpm for 5 min. Without disturbing the precipitate, the foil was removed and the supernatant discarded. A volume of 70% isopropanol was added and the plate re-centrifuged. After removing the supernatant by gently inverting the plate onto a paper towel, the samples were re-suspended in 2 µl of ultrapure formamide (Applied Biosystems). Samples were denatured by putting the plate into a thermal cycler and by

running a denaturing program at 94° C for 3 minutes and then put on ice. The plate, containing the fluorescent-labelled extension products, was loaded in the sample tray of an ABI Prism 3100 automatic sequencer (Applied Biosystems). POP-6 polymer and a 50 cm capillary array were used (both from Applied Biosystems). Data was analysed using the ABI Sequencing analysis software, version 3.2™.

Nucleotide sequences were assembled and aligned using programs in the Sequencher 3.0™ software package (Gene Codes, Ann Arbor, MI, USA). Two programs were used to analyze the DNA sequences: SEQUENCHER™ software (Gene Codes Corporation, Ann Arbor, MI, USA), and Consed (University of Washington Genome Center, Seattle, WA, USA). Sequence chromatograms for EXT1 and EXT2 were aligned into "contigs" and viewed using Phred, Phrap and Consed (version 6.0) (Ewing et al. 1998; Ewing and Green 1998; Gordon, Abajian and Green 1998). (http://www.genome.washington.edu.)

Identified mutations using these programs were confirmed using both the 5'-3' and the 3'-5' reads. Heterozygosity on both reads was required to confirm a true mutation. All probands plus the genbank sequence were compared to each other to ensure this was a true mutation versus a polymorphism. The identified mutation was compared to previously described mutations to determine whether it was novel. The translation of the gene with the new mutation was examined to determine the nature of the mutation, that is, was the mutation a missense, nonsense, frameshift (insertion, deletion), or splice site. If it was a missense, the new amino acid was interpreted in relation to whether it caused a change in the nature of the amino acid, i.e., basic vs. acidic and uncharged polar versus non-polar (hydrophilic vs. hydrophobic).

2.4.7 Segregation Analysis

Once a proband's mutation was confirmed, the available family member's DNA's were sequenced as described using the primer pair representing the location of the mutation. Contigs designed in Sequencher™ were developed using the primer pair in both 5'-3' and 3'-5' read for each family member plus the GenbBank sequence. Sequences were aligned and the identical mutation was looked for in all clinically affected family members and absent in unaffected members. Care was taken to identify subjects not affected clinically but carrying the genetic mutation.

2.5 Phenotype

All subjects identified as having at least one exostosis underwent thorough physical examinations. Xrays taken as part of the patient's care were examined. Phenotyping was divided into two categories: clinical and radiographic. Clinical features included demographics, percentile weight, percentile height, percentile limb segment lengths as well as total limb lengths, limb alignment, and range of motion. All affected patients had range of motion measured at the shoulder, elbow, wrist, ankle, knee, and hip. Method of data collection and standardization (for age and gender) is listed below. Radiographic features were obtained from available films; the data collected included lesion quality (count, size sidedness, complexity, location, and metaphyseal flaring) and angular alignments (carpal slip, radial inclination, ulnar shortening, radial head subluxation/dislocation, radial bow, elbow joint angle, femoral/tibial anatomic and mechanical angles, weight bearing axis, femoral neck-shaft angle, Sharp's Acetabular angle, fibular height, and ankle joint angle).

2.5.1 Clinical features

All physical examinations were performed by the author who is a Pediatric Orthopaedic Surgeon and a member of the Royal College of Physicians and Surgeons of Canada in Orthopaedic Surgery.

2.5.1.1 Demographics

Each affected subject's age, weight, height, ethnic background, and address were collected. The subjects' weight and height were converted to a percentile figure to standardize for age and gender to allow for comparison amongst groups. Height and weight were standardized using updated Green Anderson Charts (Hamill et al. 1979). Clinically palpable lesions were recorded, and surgically excised lesions were accounted for. All extremities and accessible flat bone were examined for exostoses.

2.5.1.2 Lesion count

All accessible aspects of the long bones, hands, fingers, feet, toes, scapulae, clavicles, ribs, sternum, spinous processes, and ilia were palpated for lesions. Any significant local deformity was also recorded (that is some lesions are so large they expand the entire local bone). All palpated lesions were recorded as present and specific location noted: for example, right distal radial radius or left proximal medial tibia. If more than one discrete lesion was palpable in a location, each was counted separately. All lesions were correlated with xray visualization; however, not all lesion areas were radiographically imaged, in particular, the hands and feet.

2.5.1.3 Limb alignment

Clinically the overall alignment of the elbow and knee were measured using a large, hand-held goniometer. The hinge of the goniometer was centred over the elbow joint which was held in full supination and extension. Each limb of the goniometer was placed along the long central axis of the upper and lower arm, and the subtended angle was measured.

Knee joint measurements were taken in a standing position. The goniometer was centred on the middle of the anterior knee joint, and each limb of the goniometer was lined up against the centre of the long axis of the femur and tibia. Again, the subtended angle was recorded. In both cases, valgus or varus alignment was denoted.

2.5.1.4 Segment and Limb Lengths

Segments and limb lengths were measured in centimetres and used surface landmarks as follows:

1. upper extremity total length – top of humeral head to ulnar styloid.
2. upper arm – top of humeral head to capitellum.
3. lower arm – tip of olecrenon to ulnar styloid.
4. lower extremity total length – anterior superior iliac spine to medial malleolus
5. upper leg – ASIS to medial condylar joint surface
6. lower leg – medial tibial joint line to tip of medial malleolus.

A conversion for femoral length was required to subtract the distance from the ASIS to the top of the femoral head. Using Caffey's method, 5% of the lower extremity length was subtracted from the total leg measurement and 10% from the upper leg length (Silverman 1985). Again using Caffey's radiologic text, each segment and total length was standardized for age and gender reduced to a percentile to allow for direct comparisons between subjects.

2.5.1.5 Range of motion

A large, hand-held goniometer was used to measure a joint's range of motion. Range of motion was measured for the shoulder (abduction, adduction, internal rotation, external rotation, and forward elevation), elbow (flexion, extension, supination, and pronation), wrist (flexion, extension, and radial and ulnar deviation), hip (flexion, extension, internal and external rotation, and abduction and adduction), knee (flexion and

extension) and ankle (flexion and extension). If no restriction in motion was identified, full ROM was indicated, if there was any reduction in the normal range, precise measurements were recorded.

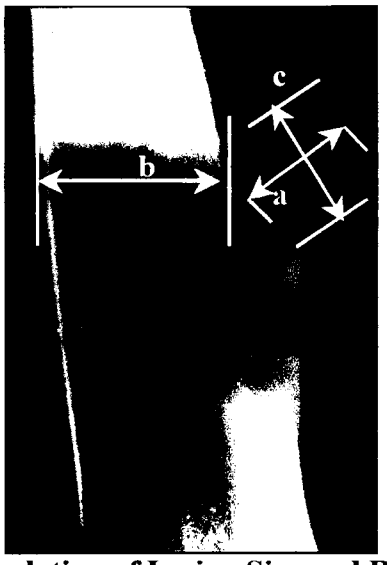
2.5.2 *Radiographic features*

Alignment and deformity measurements from the radiographs were made. Specific details covering how each measurement was calculated is outlined below. A complete radiographic record for the purposes of this project included standard images of the upper and lower extremities as well as chest and pelvis: anteroposterior (AP) proximal humerus to wrist inclusive with the elbow fully extended and forearm fully supinated, AP chest, AP standing pelvis if not incorporated into the hips to ankle film, AP standing hips to ankles inclusive. As they are not part of a patient's routine care, films of the head, hands and feet were not universally available. Lesions in areas not xrayed that were easily palpable were recorded as a clinical lesion. When orthogonal views were available from the same date, data from the two views were generally used. However, usually only one AP view was used; therefore, the size of some of the lesions may be underestimated.

2.5.2.1 Lesion quality

2.5.2.1.1 Count – all visible lesions were accounted for.

2.5.2.1.2 Size – to account for magnification and patient-size variations a standardized size calculation was obtained for every lesion. Lesion size was calculated and ranked. First the protrusion ratio (A) was obtained by dividing the protrusion distance of the lesion (bony stalk) (a) by the native bone width (b). The height ratio (B) was obtained by dividing lesion height (bony cap long axis) (c) by (b). The average of the two ratios (D) was expressed as a percentage. This average percentage was ranked as follows: $\leq$ 25% (1), 26-49% (2), 50-74% (3), $\geq$ 75% (4). The lesion ranks were also categorized as small (1), medium (2 and 3) and large (4).



Calculation of Lesion Rank

a / b = protusion = A
c / b = height = B
A / B = D%

D values	Lesion Rank	Size
< 25%	1	small
26 – 50%	2	medium
51 – 75%	3	medium
>75%	4	large

Figure 2.3 Calculation of Lesion Size and Rank

2.5.2.1.3 Side – left or right total count

2.5.2.1.4 Location – distal, proximal, metaphyseal, or flat bone (includes any of the pelvic bones, sternum, scapula, or ribs)

2.5.2.1.5 Complexity – if the lesion was multilobulated and too complex to obtain any of the three measurements it was deemed complex. In general all these lesions were also large (category 4).

2.5.2.1.6 Metaphyseal flaring – if the metaphysis of the long bone showed aneurysmal dilatation and abnormal expansion of the metaphysis globally.

2.5.2.1.7 Type – sessile versus pedunculated. If a lesions stalk is narrower than its cap, it was called pedunculated. If the stalk was equal to or larger than the cap, it was called sessile.

2.5.2.2 Limb alignment

Measurements taken are defined below and referenced accordingly. This study also introduces new measurements and these are thoroughly described in the following pages.

2.5.2.2.1 **Carpal slip** - normal value = 5 +/- 2mm (Keats 1990)

The ulnar displacement in milimetres of the ulnar edge of the lunate with respect to the ulnar border of the distal radius.

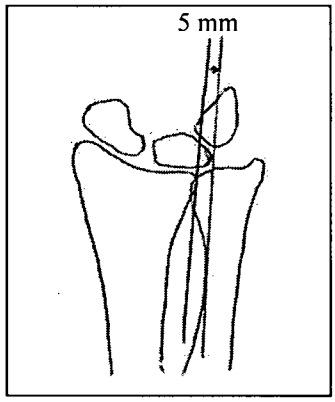


Figure 2.4 Measurement of carpal slip

2.5.2.2.2 **Radial inclination** – normal value equals 21 +/- 2° (Green 1993)

The angle between the perpendicular of the radius long axis (A – B) and a line joining the radial and ulnar distal edges of the radius (A – C).

2.5.2.2.3 **Ulnar shortening** – normal value equals 0 +/- 1mm (Green 1993)

The distance between the distal surface of the ulna and the radius.

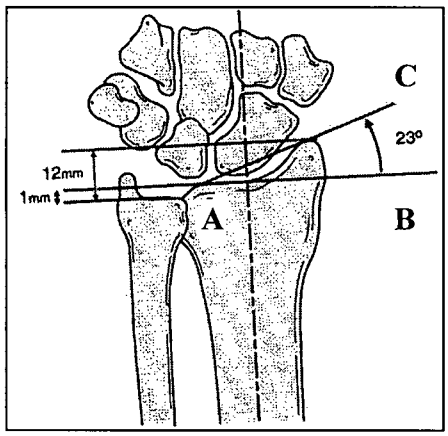


Figure 2.5 Measurement of radial inclination and ulnar shortening

2.5.2.2.4 **Radial bowing** – expected normal value equals 10 +/- 5° (Green 1993)

The angle subtended between the long mid-axis of the forearm (A – B) and the maximal radial deviated point of the radius' diaphysis (C - D).

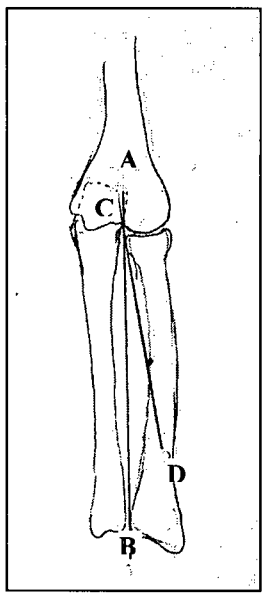


Figure 2.6 Measurement of radial bowing

2.5.2.2.5 **Radial head subluxation/dislocation** – normal value equals no subluxation or dislocation. The radial head is either subluxated/dislocated (B) or not (A).

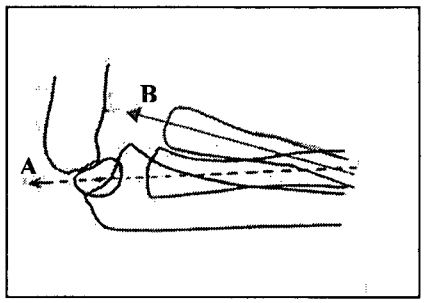


Figure 2.7 Radial head subluxation / dislocation

2.5.2.2.6 **Elbow joint angle** – normal range equals females 10+/-2° valgus, males 8+/-2° valgus (Keats 1961)

The angle subtended between a line drawn through the long axis of the humerus (A – B) and forearm (C – D).

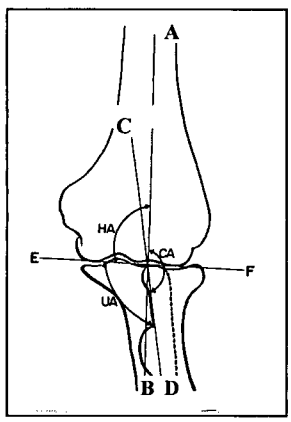


Figure 2.8 Measurement of the elbow joint angle

2.5.2.2.7 **Femoro-tibial anatomic angle** – normal value equals 7 +/- 5° valgus (Hsu et al. 1990)

The angle subtended by a line drawn between the long axis of the femur (A – B) and the tibia (C – D).

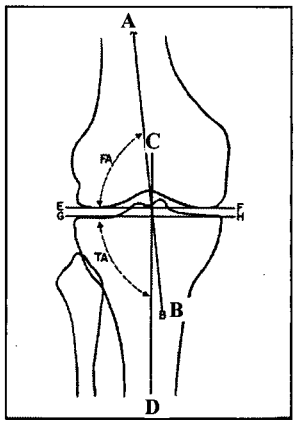


Figure 2.9 Measurement of the femoro-tibial anatomic angle.

2.5.2.2.8 **Femoro-tibial mechanical angle** – normal value equals 0 +/- 5° (Hsu et al. 1990)

The angle subtended by a line drawn from the centre of the femoral head to the centre of the knee joint (H – K) and a line from the centre of the knee to the centre of the ankle joint (K – A).

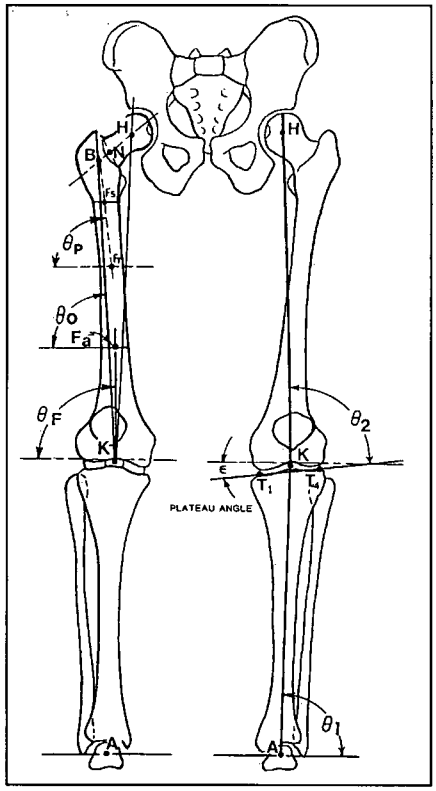


Figure 2.10 Measurement of the weight bearing axis, the femoral neck/shaft angle, and the femoral anatomic angle.

2.5.2.2.9 **Weight-bearing axis** – normal equals 50 +/- 10% (Hsu et al. 1990)

A line is drawn from the centre of the femoral head (left leg H – A) to the centre of the ankle joint. The weight-bearing axis is where this line crosses the knee joint and is expressed as a percentage of the total tibial joint surface. The distance in millimetres from the lateral tibial-joint-line border to the weight-bearing line is divided by the total joint width and expressed as a percentage. Numbers greater than 50% are in varus and those less than 50% are in valgus.

2.5.2.2.10 **Femoral neck/shaft angle** – normal equals 135 +/- 5° (Pettersson and Ringertz 1991)

The angle subtended by a line drawn between the long axis of the femoral neck (right leg B – H) and the long axis of the femoral diaphysis (right leg B – K).

2.5.2.2.11 **Sharp's Acetabular angle** equals 40 +/- 5°

(Pettersson and Ringertz 1991)

The angle subtended by a line drawn between the base of the right and left acetabular teardrops (C – E) and a line joining the tip to the lateral edge of the acetabulum (A – B).

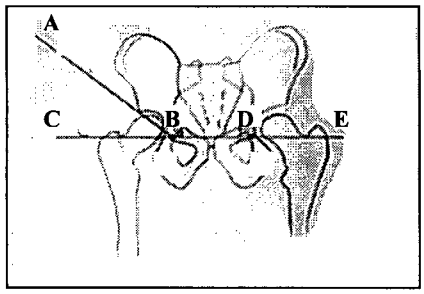


Figure 2.11 Measurement of Sharp's Acetabular angle.

2.5.2.2.12 **Fibular height** – 50 +/- 10% (described in this study)

Expressed as a percentage of the distance from the proximal tibial joint line to the proximal tip of the fibula (A) over the distance from the proximal tibial joint line to the proximal fibular physis or physeal scar (B).

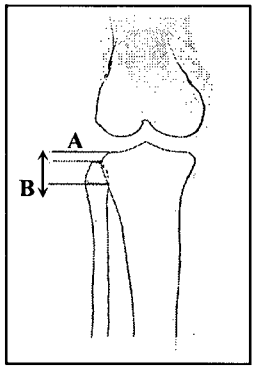


Figure 2.12 Measurement of fibular height.

2.5.2.2.13 **Ankle joint angle** – normal range equals 0+/- 5° valgus

(Hsu et al. 1990)

The angle subtended by the lines drawn between the talar dome (A – C) and the perpendicular line to the long axis of the tibia (A – B).

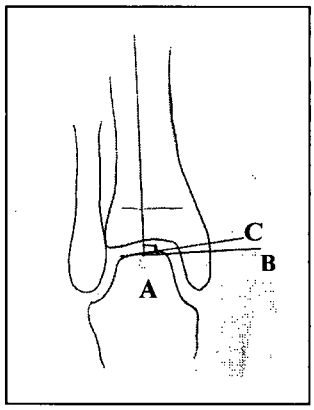


Figure 2.13 Measurement of ankle joint angle.

2.6 Data Analysis

Data was compiled as genotype and phenotype, and analysis was run on comparison groups as outlined below.

2.6.1 Genotype

Each affected individual was classified according to the following:

1. Gene affected – EXT 1 or EXT 2
2. Type of mutation – missense (MS), nonsense (NS), frameshift (FS), or splice site (SS).
3. Severity of mutation – severe or mild; severe included NS, FS, and SS, and mild included MS.
4. Location of mutation – early or late; early mutation found prior to the 1500^{th} base pair or late after the 1500^{th} basepair in either EXT 1 or 2.
5. Gender – male or female

2.6.2 Phenotype

Data were tabulated as clinical or radiographic for each affected individual. In total, 89 phenotypic parameters were collected. These were divided into three categories; lesion quality (38), limb alignment (26), limb segments (12 (x2 for left and right)) plus percentile height. Due to the large number of phenotypic features, a Pearson's correlation matrix (STATVIEW™ software) was run on the averaged data of all twenty-nine affected members to test association between any variables and to determine if any of the features were duplicated. If so, one of the variables would be eliminated as it could introduce potential statistical errors.

2.6.3 Genotype-phenotype correlation.

The genotype phenotype correlation analysis was based on comparison of the genotypic features versus the phenotypic features. For ease of presentation phenotype

features are grouped, called phenotype and represent the thirty-eight lesion quality parameters, the twenty-six limb alignment parameters, and the twenty-six limb segment parameters plus percentile height. All eighty-nine phenotypic features were evaluated versus the genotype. In the results, features showing significant differences are dissected out of the groups and discussed individually. The data analysis groupings are as follows;

1. Gene (EXT 1 vs. EXT 2) versus phenotype
2. Gene and gender versus phenotype
3. Gene and mutation type versus phenotype
4. Gene and severity versus phenotype
5. Gene and mutation location versus phenotype
6. Gender (male vs. female) versus phenotype
7. Mutation type (nonsense, missense, frameshift, splice site) versus phenotype
8. Mutation severity (severe{FS, NS, SS) vs. mild {MS}) versus phenotype
9. Mutation location (early{<1500bp} vs. late {>1500bp}) versus phenotype
10. Gender and mutation type vs. phenotype
11. Gender and severity vs. phenotype

An unpaired t-test was calculated on all 2-way analyses, and an ANOVA was calculated when the analysis was greater than 2-way. Power was calculated for every comparison because of the large variation in sample size. In many instances, sample size was too small to warrant any statistical analysis. Statistical significance was set a priori at 0.05 and power of 0.8.

As this project was designed to determine if any correlation exists between the various parameters, the data was scrutinized in terms of looking for patterns. Statistical testing was therefore done on all comparisons in an attempt to dissect out a relationship between the different categories of comparisons. This project is meant to be a descriptive

study especially since the sample sizes are small in many comparisons and therefore the power not substantial. The significant correlations gleaned from this approach will then be isolated as parameters of interest for future prospective study.

Chapter III: Results

3.1 Subject Recruitment

3.1.1 subject identification

Eleven probands and their families were provisionally diagnosed with HME. All interested members were informed of the study protocol and gave informed consent. All minors were consented for by their parents (a summary of all seventy-five study subjects follows in Table 3.1). Thirty-four individuals were found to have at least one exostosis and were deemed affected. However, proband 7-1 was later discovered to be the founder because her mother did not carry the mutation found in 7-1; and therefore, family 7 has been excluded. The final study sample includes ten families, ten probands, sixty-nine subjects, thirty-two affected individuals and 37 unaffected family members.

Table 3.1 Subject Recruitment

ID	Position	Affected	Blood	ID	Position	Affected	Blood
Family 1				**Family 5**			
1-1	P	yes	yes	5-1	F	yes	yes
1-2	M	no	yes	5-2	M	no	yes
1-3	F	yes	yes	5-3	P	yes	yes
1-4	GM	no	yes	5-4	S	no	yes
Family 2				5-5	GM	no	yes
2-1	P	yes	yes	5-6		no	yes
2-2	B	yes	yes	**Family 6**			
2-3	S	no	yes	6-1	P	yes	yes
2-4	F	yes	yes	6-2	step B	yes	yes
2-5	M	no	yes	6-3	M	yes	yes
2-6	GM	no	yes	6-4	F 6-2	no	yes
2-7	GF	no	yes	6-5	F 6-1	no	
Family 3				**Family 7**			
3-1	GM	yes	yes	7-1	P	yes	yes
3-2	P	yes	yes	7-2	M	yes	yes
3-3	S	no	yes	7-3	GF	no	yes
3-4	F	yes	yes	7-4	S	no	yes
3-5	M	no	yes	7-5	S	no	yes
3-6	B	yes	yes	7-6	GM	no	yes
3-7	step S	no	yes	**Family 8**			
3-8	M	yes	yes	8-1	P	yes	yes
3-9	F	no	yes	8-2	M	yes	yes
3-10	S	yes	yes	8-3	B	no	yes
3-11	B	no	yes	8-4	F	no	yes
3-12	B	no	yes	**Family 16**			
3-13	F	yes	yes	16-1	P	yes	yes
3-14	M	no	yes	16-2	F	yes	yes
3-15	S	yes	yes	16-3	M	no	yes
3-16	B	no	yes	16-4	S	no	yes
3-17	S	no	yes	16-5	GM	yes	yes
3-18	M	yes	yes	**Family 17**			
3-19	P	yes	yes	17-1	P	yes	yes
3-20	S	no	yes	17-2	M	yes	yes
3-21	Aunt	no	yes	17-3	B	no	yes
3-22	M	no	no	17-4	B	no	yes
3-23	P	yes	yes	17-5	GF	yes	yes
3-24	S	no	no	17-6	GM	no	yes
Family 4				**Family 18**			
4-1	M	yes	yes	18-1	P	yes	yes
4-2	F	no	yes	18-2	F	yes	yes
4-3	S	yes	yes	18-3	M	no	yes
4-4	P	yes	yes	18-4	B	no	yes

Abbreviations used: **GM**-grandmother; **GF**-grandfather; **P**-Proband; **M**-mother; **F**-father; **B**-brother; **S**-sister

3.1.2 Family pedigrees

The extended family pedigrees are located in appendix 8.6.1

3.2 Genotype Results

3.2.1 Highly Polymorphic Repeats

Eight markers were designed to assist in assigning the most likely site of mutation in either EXT 1, 2 or 3. Initially A03/04 for EXT 1 and A01/02 for EXT 2 were used on all families. Enough information was gleaned from these two markers alone to assign EXT status to families 2, 3, 5, 8, 16, 17, and 18. Additional marker information (EXT 1, 85, and 547; EXT 2, 13 and 905; EXT 3, 216 and 221) was required to further evaluate Families 4 and 6. The DNA from Families 4 and 6 were sent to Dr. Jacqueline Hecht M.D., Professor of Pediatrics at the University of Texas Medical School in Houston, Texas for more extensive linkage analysis. The results of the additional marker analysis are included.

Table 3.2 Summary of STR Markers as per family and EXT gene assignment for mutations identified in EXT 1 and EXT 2

Family	Exclusion Analysis		Mutation Found	Gene Sequenced	Location of Mutation
	EXT 1	EXT 2			
1	NI	NI	Yes	EXT 1	EXT 1 exon 2
16	NI	NI	Yes	EXT 1	EXT 1 exon 8
18	No	Yes	Yes	EXT 1	EXT 1 exon 1
6	No	No	No	EXT 1 and EXT 2	None found
2	Yes	No	Yes	EXT 2	EXT 2 exon 4
5	Yes	No	Yes	EXT 2	EXT 2 exon 4
17	Yes	No	Yes	EXT 2	EXT 2 exon 2
8	No	No	Yes	EXT 2	EXT 2 exon 7
4	Yes	No	No	EXT 1 and EXT 2	None found
3	NI	NI	Yes	EXT 1 and EXT 2	EXT 2 exon 5

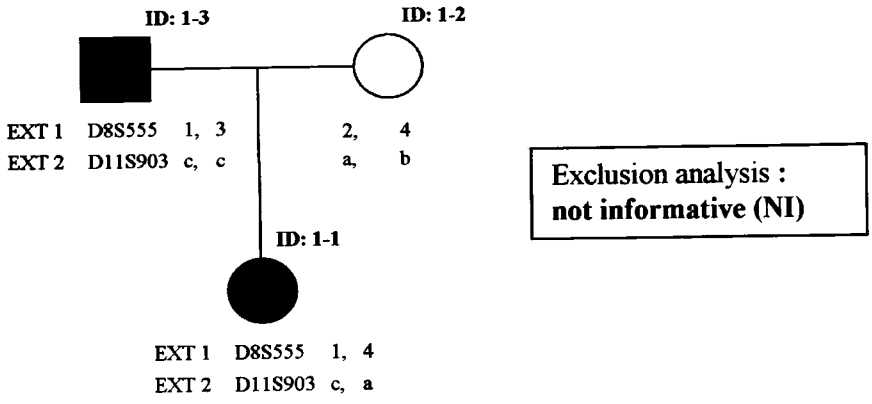


Figure 3.1a – EXT 1 and EXT 2 STR Markers. Pedigree for Family 1.

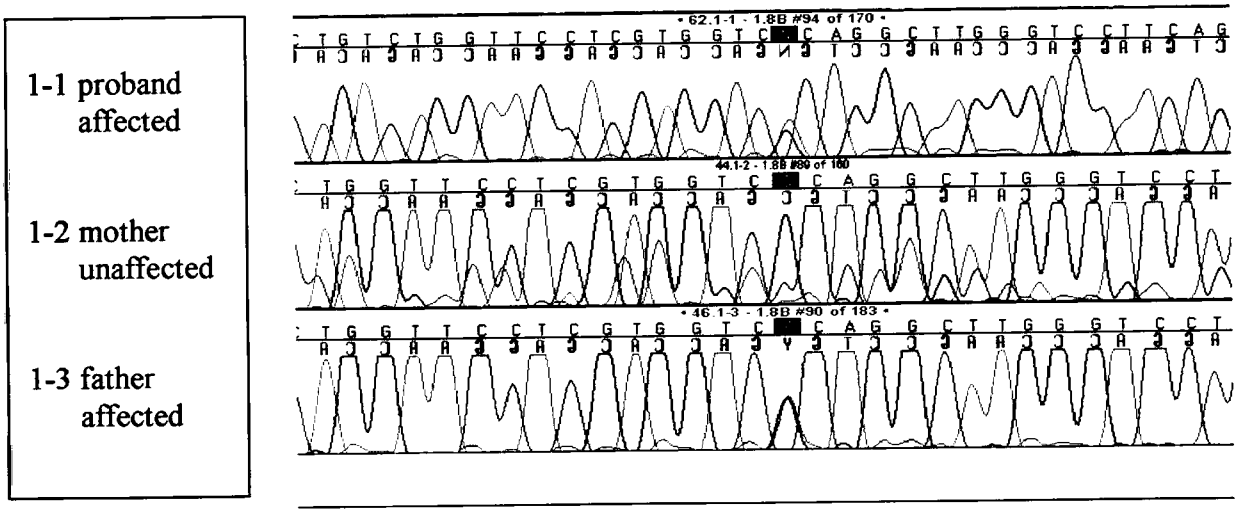


Figure 3.1b – Sequencer output for segregation analysis for Family 1. Mutation location: EXT 1 exon 2.

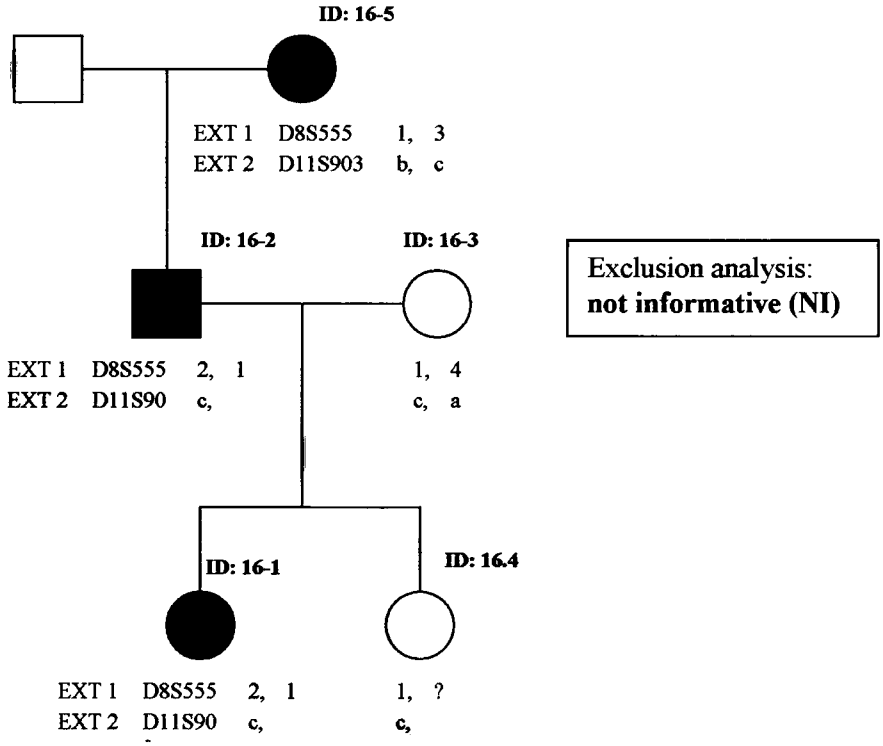


Figure 3.2a – EXT 1 and EXT 2 STR Markers. Pedigree for Family 16.

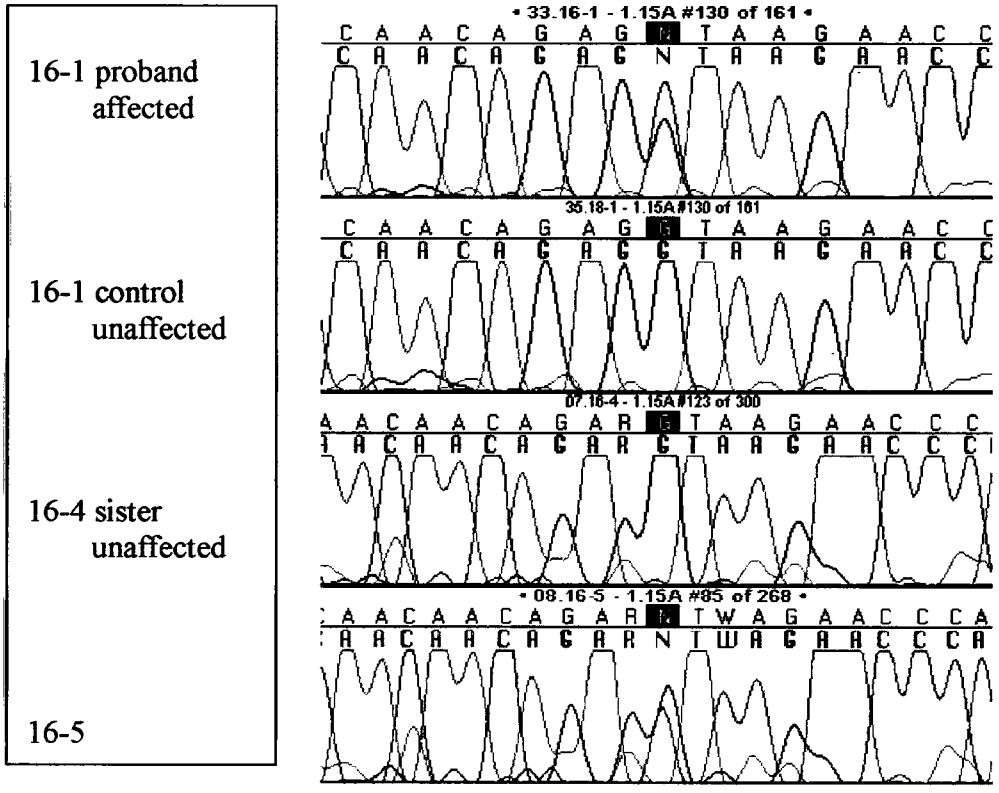
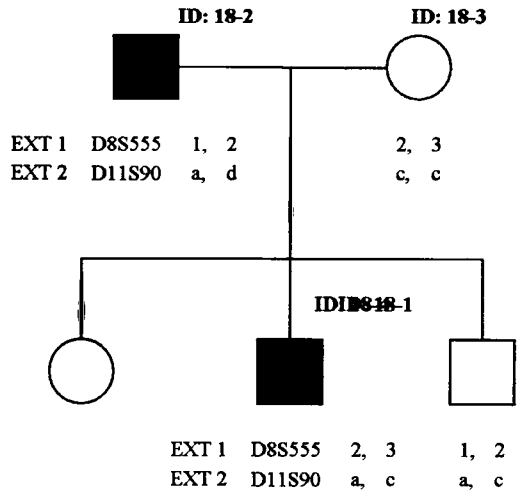


Figure 3.2b – Sequencer output for segregation analysis for Family 16. Mutation location: EXT 1 exon 8



Cannot exclude EXT 1 because affected father passed *marker 2* to the affected child and *marker 1* to the unaffected child.

Exclude EXT 2 because *marker a* was passed to an affected and an unaffected child.

Figure 3.3a – EXT 1 and EXT 2 STR Markers. Pedigree for Family 18.

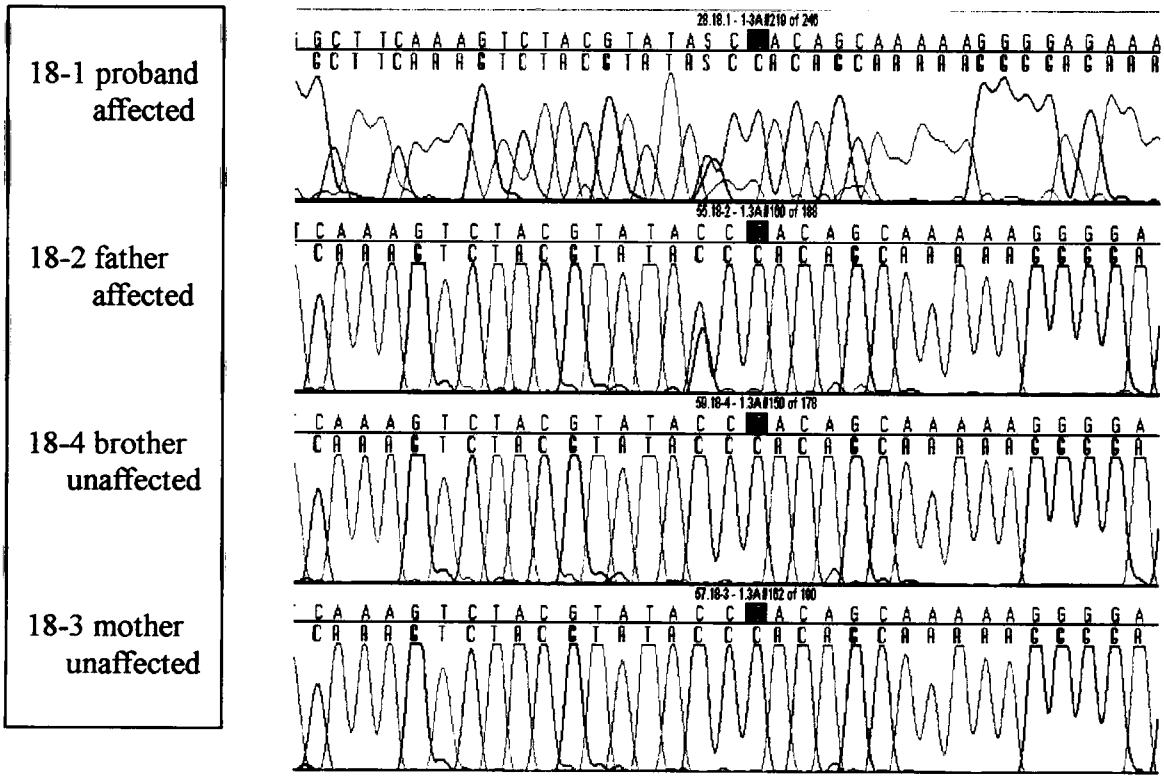


Figure 3.3b– Sequencer output for segregation analysis for Family 18. Mutation location: EXT 1 exon 1

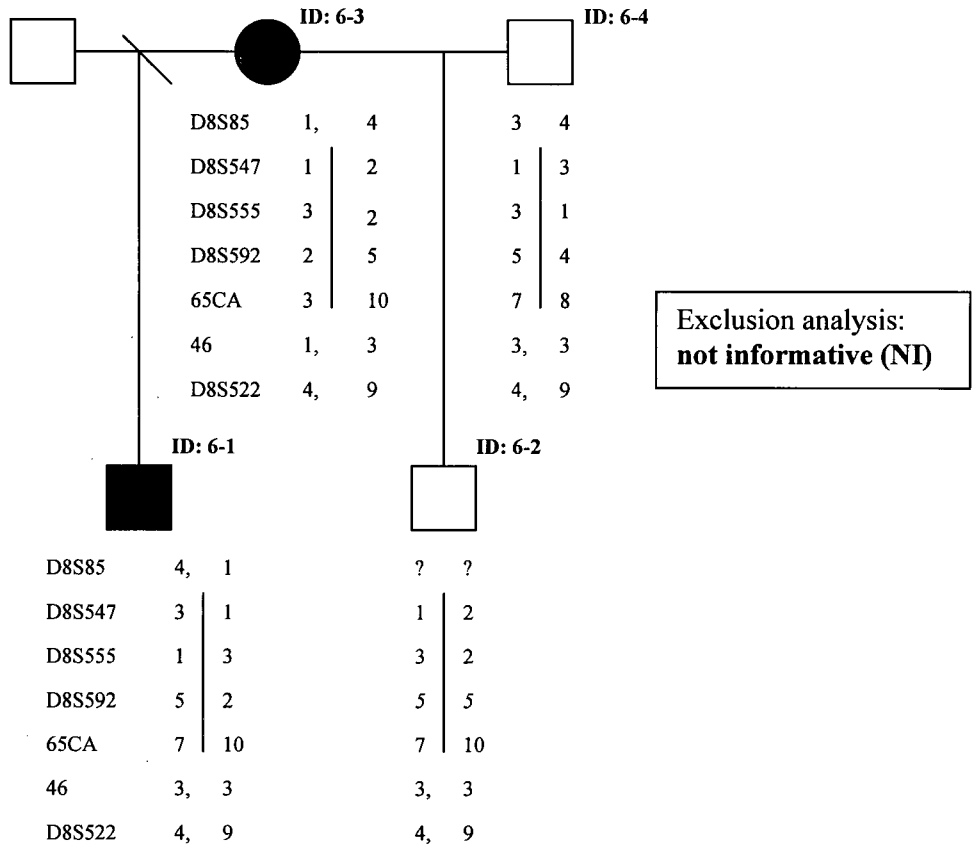


Figure 3.4a(i) EXT 1 STR Markers. Pedigree for Family 6.

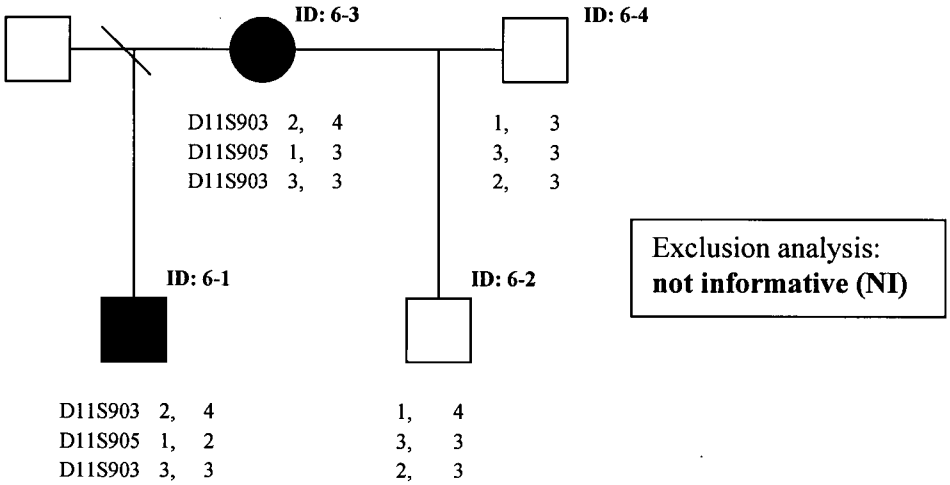
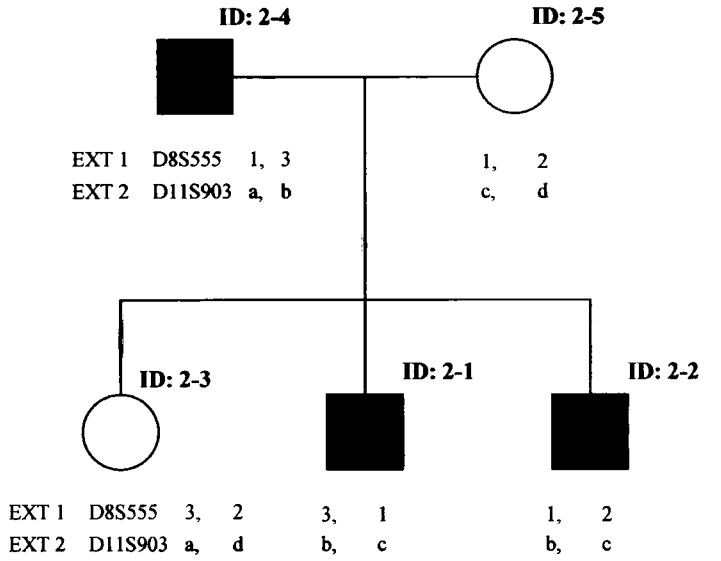


Figure 3.4 a(ii) EXT 2 STR Markers. Pedigree for Family 6.



Exclude EXT 1 because *marker 3* from the affected father was passed to an affected and an unaffected child.

Cannot exclude EXT 2 because *marker b* from the affected father was only passed onto both affected children.

Figure 3.5a – EXT 1 and EXT 2 STR Markers. Pedigree for Family 2.

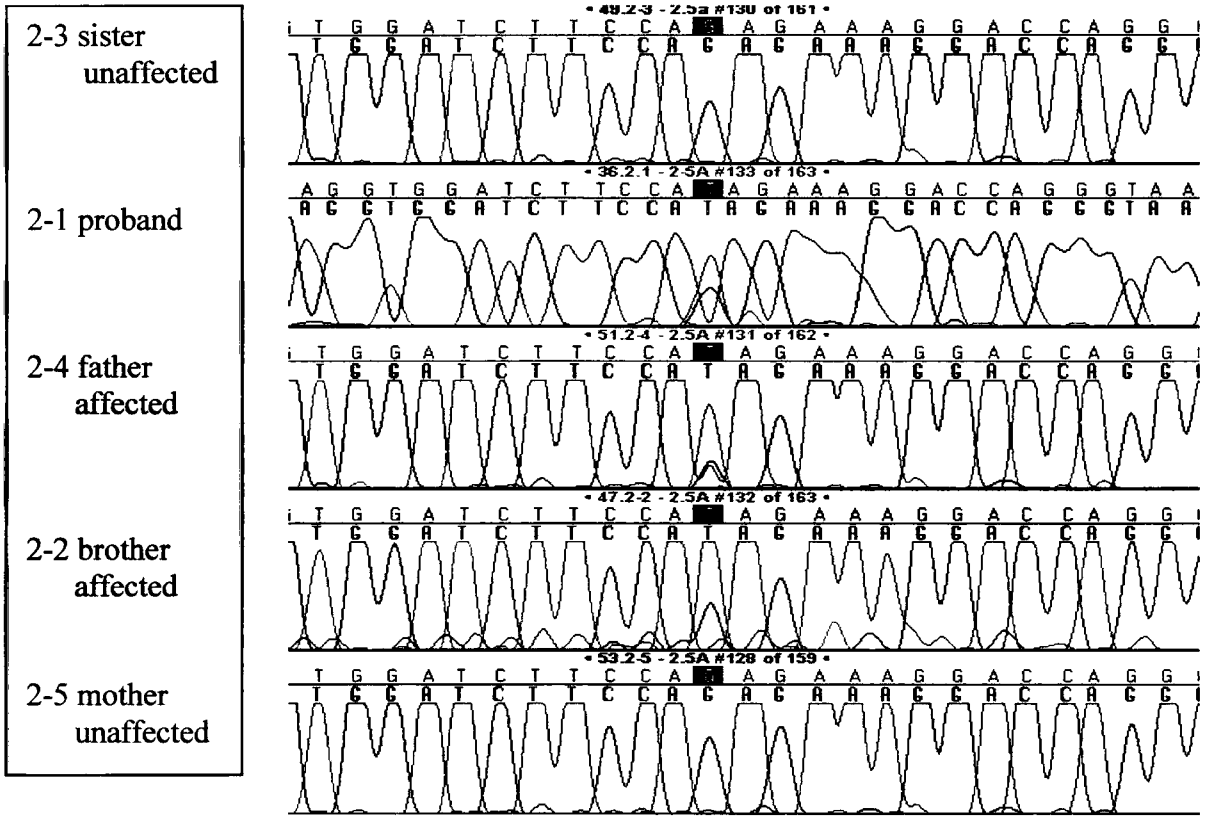
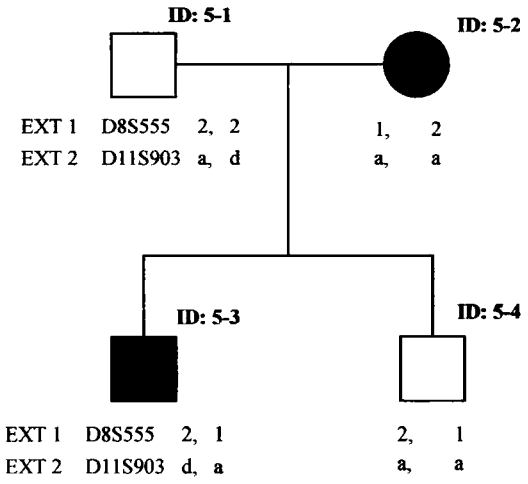


Figure 3.5b – Sequencer output for segregation analysis for Family 2. Mutation location: EXT 2 exon 4.



Can exclude EXT 1 because the affected mother passed *marker 2* to an unaffected and an affected child.

Cannot exclude EXT 2 because the affected mother passed an undistinguishable *marker a* to an unaffected and an affected child.

Figure 3.6a – EXT 1 and EXT 2 STR Marker. Pedigree for Family 5.

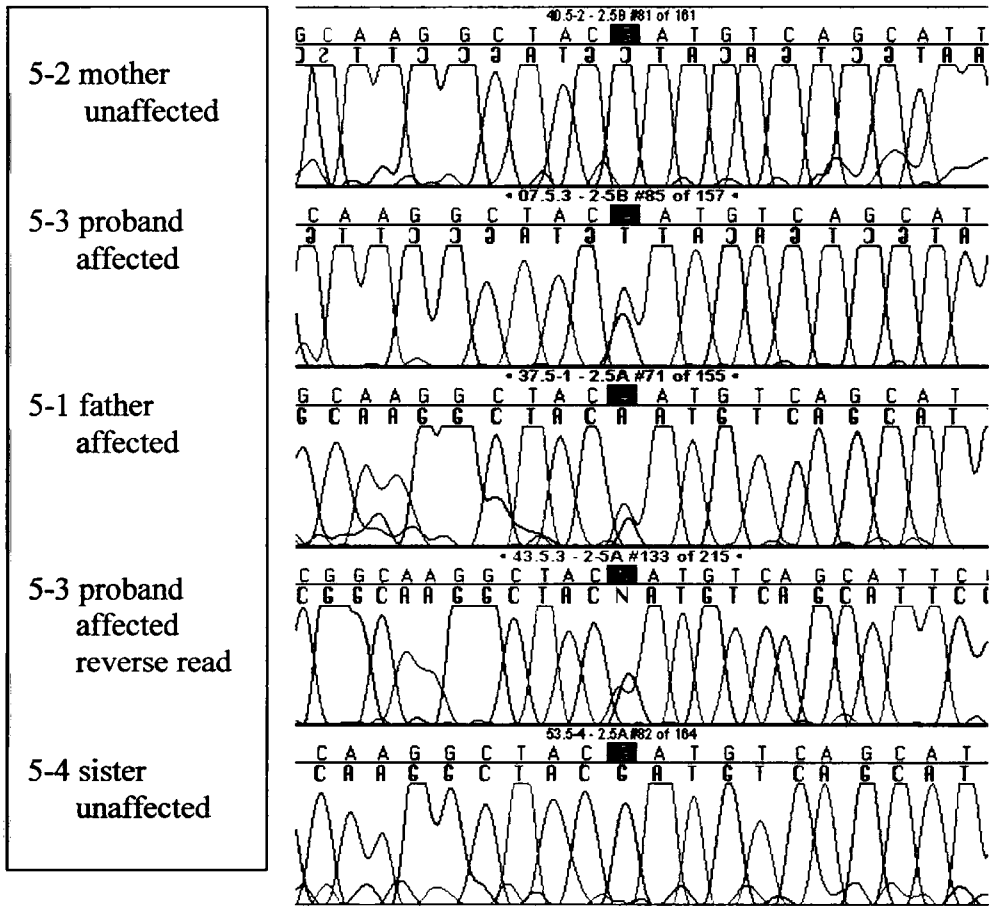
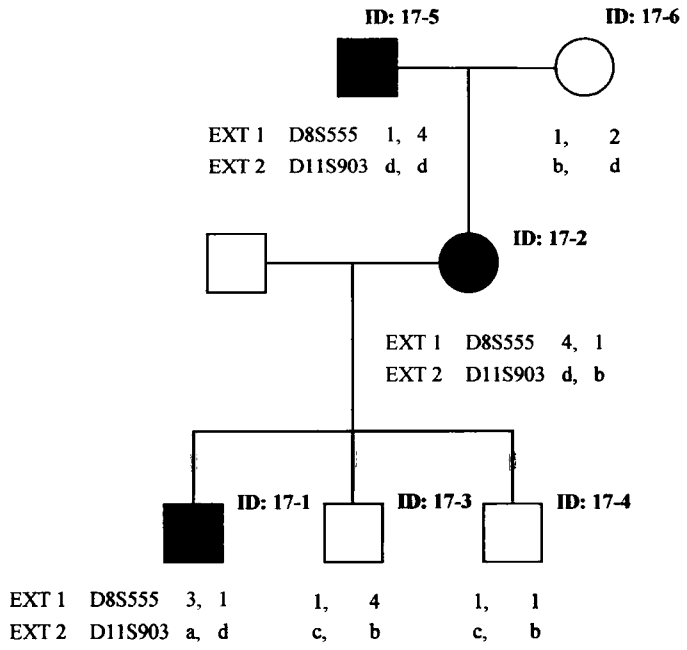


Figure 3.6b – Sequencer output for segregation analysis for Family 5. Mutation location: EXT 2 exon 4.



Can exclude EXT 1 because the affected mother passed *marker 1* to both an affected and an unaffected child and *marker 4* to an unaffected child.

Cannot exclude EXT 2 because the affected mother passed *marker d* only to an affected child and *marker b* only to unaffected children. She also received *marker d* from her affected father.

Figure 3.7a – EXT 1 and EXT 2 STR Markers. Pedigree for Family 17.

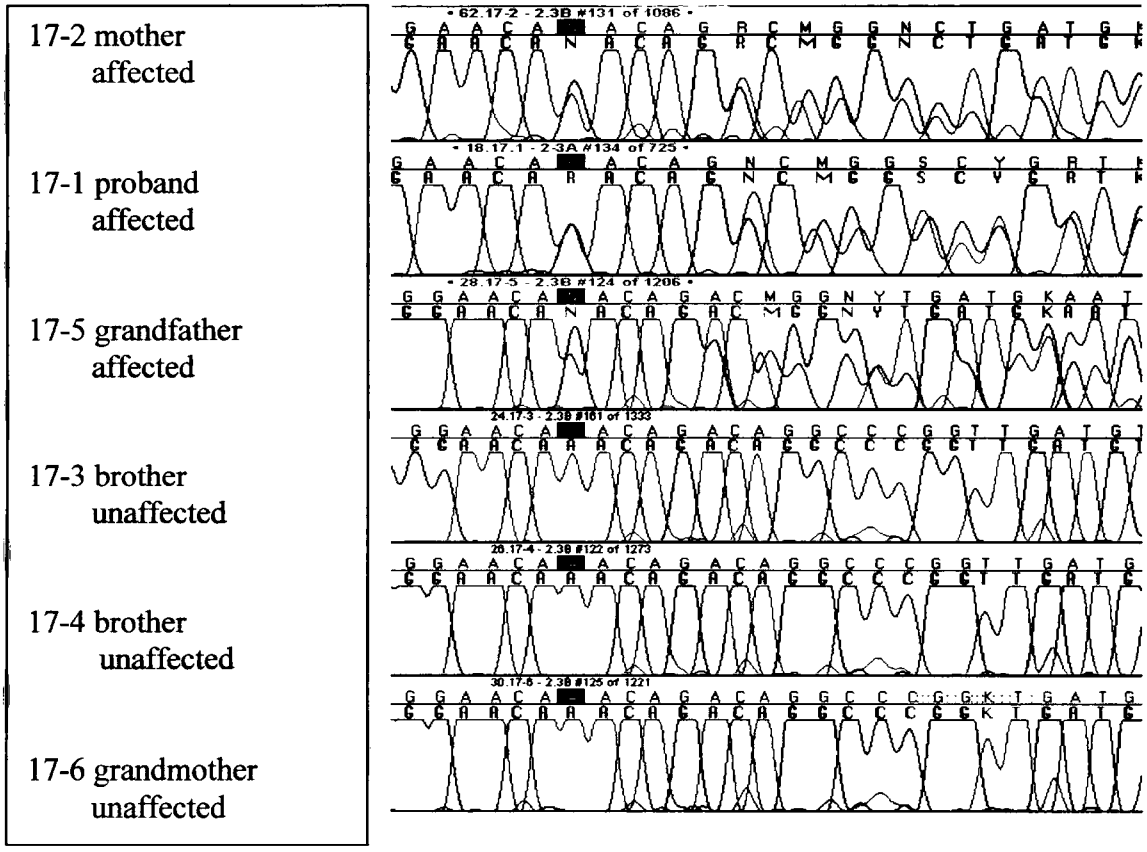


Figure 3.7b – Sequencer output for segregation analysis for Family 17. Mutation location: EXT 2 exon 2.

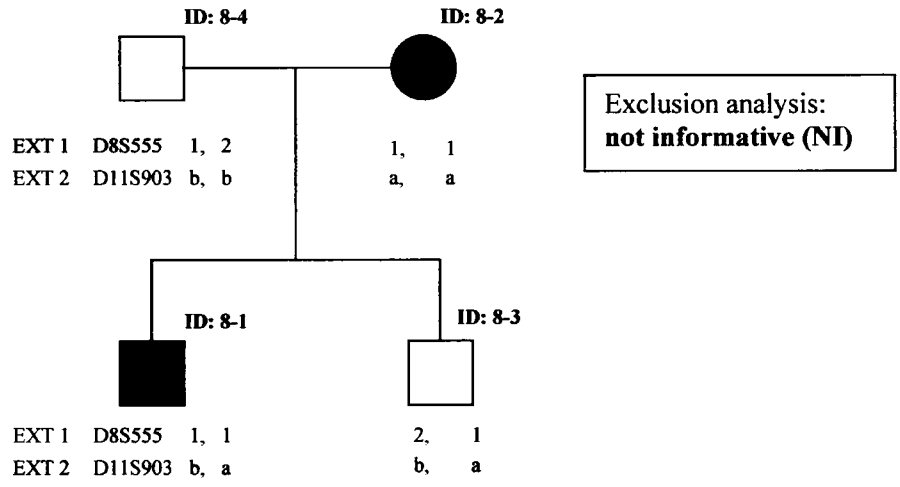


Figure 3.8a – EXT 1 and EXT 2 STR Markers. Pedigree for Family 8.

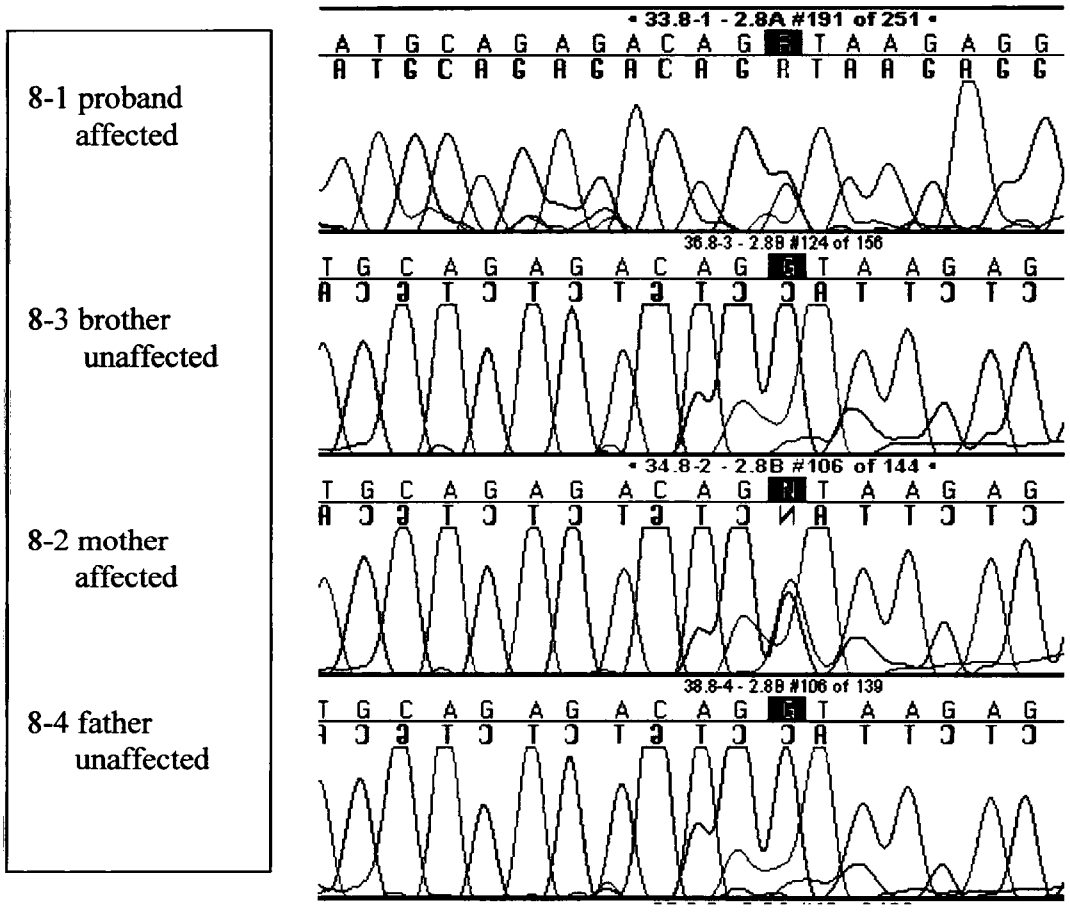
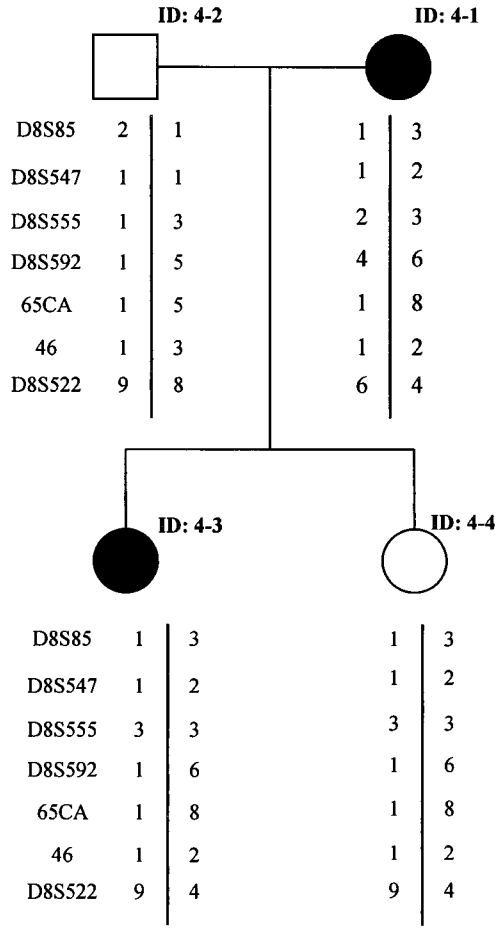


Figure 3.8b – Sequencer output for segregation analysis for Family 8. Mutation location: **EXT 2 exon 7.**



Can exclude EXT 1 because D8S85 *marker 1* from the affected mother was passed to both an affected and an unaffected child.

Cannot exclude EXT 2 because D11S903 *marker 1* from the affected mother was passed to an affected child while *marker 3* was passed to an unaffected child.

Figure 3.9a (i) EXT 1 STR Markers. Pedigree for Family 4.

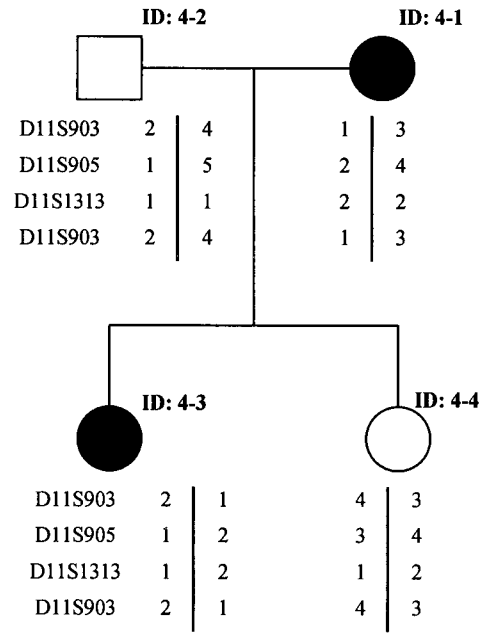


Figure 3.9a (ii) EXT 2 STR Markers. Pedigree for Family 4.

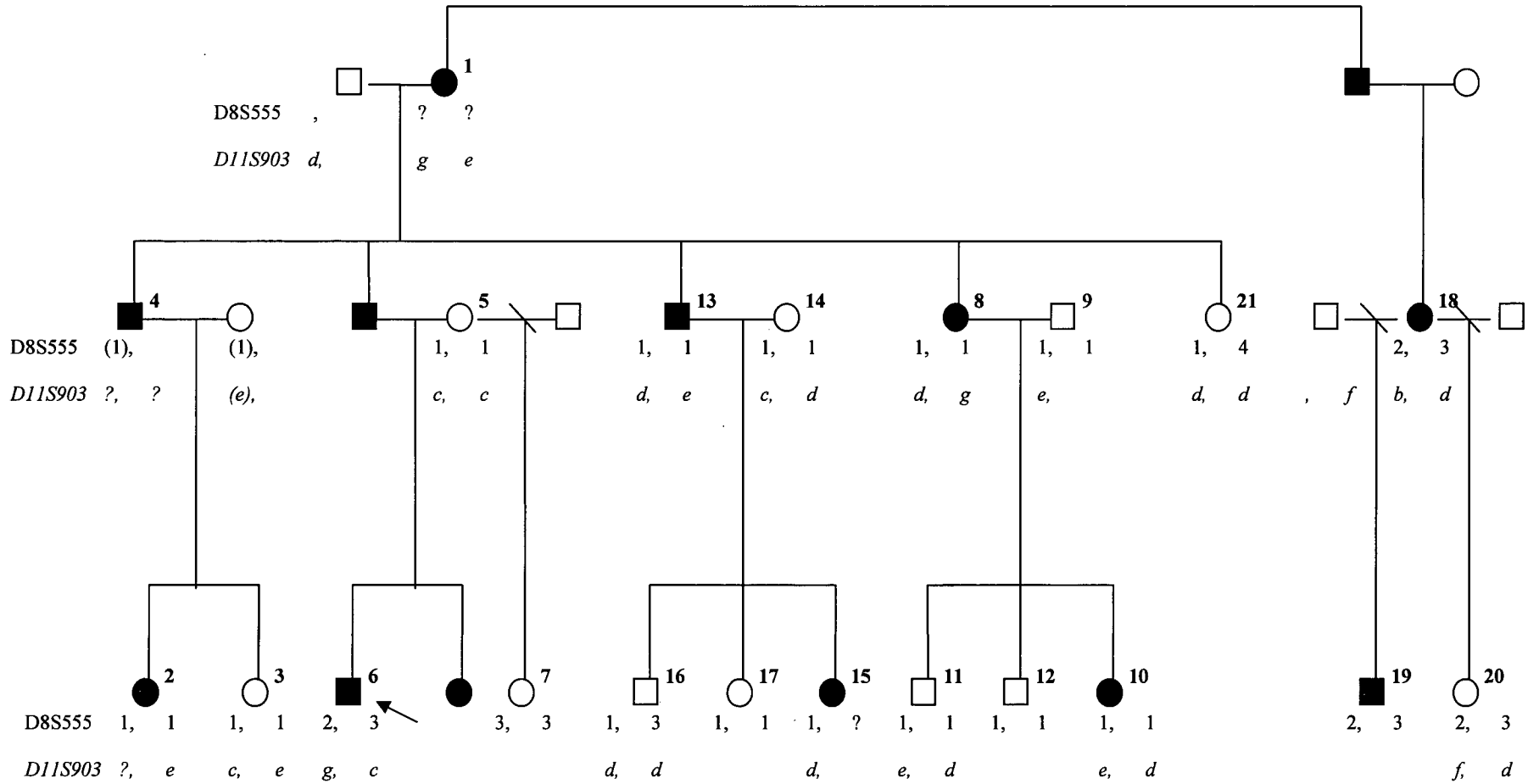


Figure 3.9a EXT 1 and EXT 2 STR Markers. Pedigree for Family 3

Exclusion analysis:
not informative (NI)

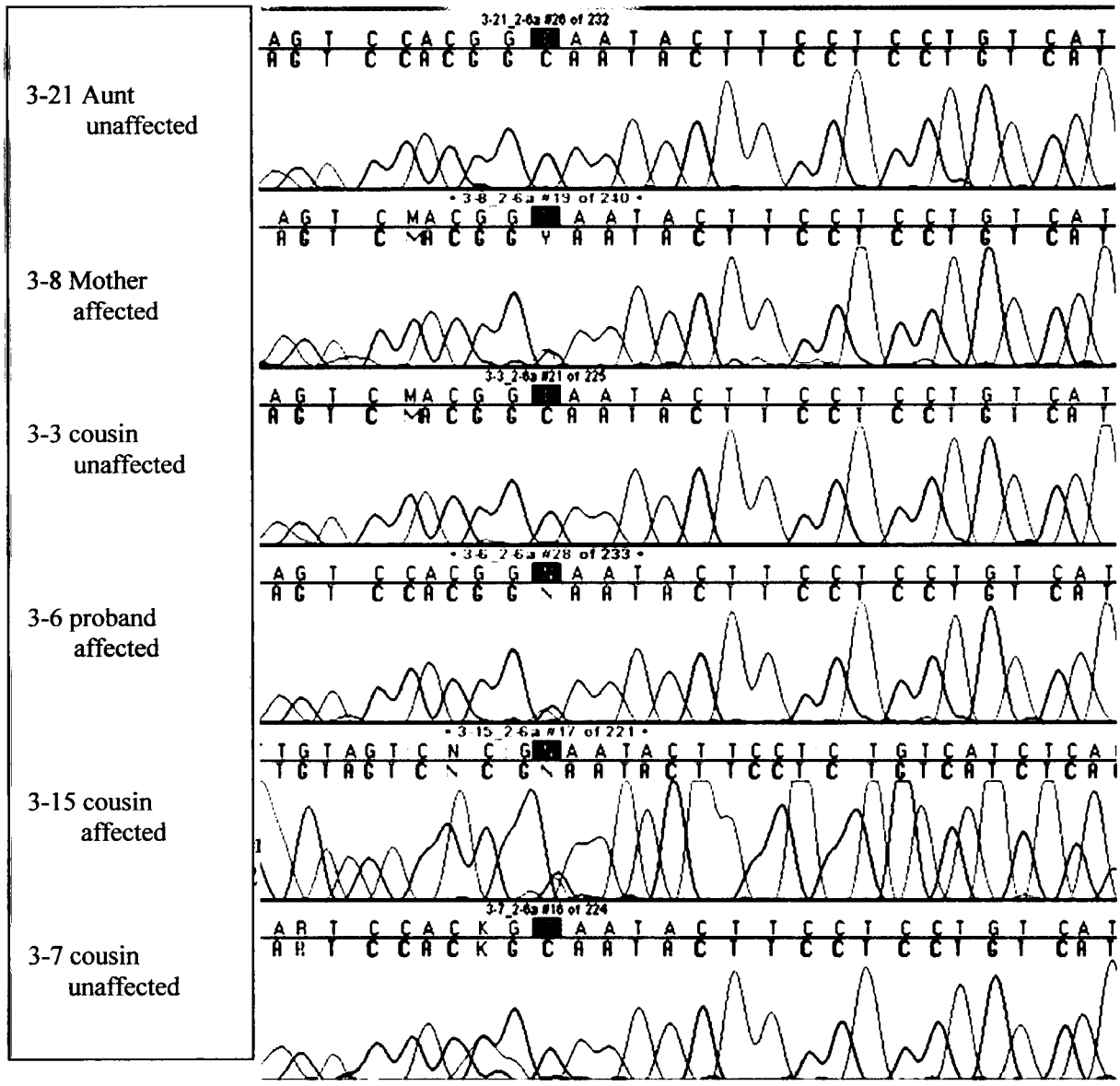


Figure 3.10b Sequencher output for segregation analysis for Family 3. Mutation location: EXT 2 exon 5.

Table 3.3 Mutations identified in each proband.

Family	EXT gene	Mutation	Exon	Amino Acid Change	Type	Unique
1	1	G1019A	2	R to H Arginine to Histidine Basic to Basic	Missense	No
2	2	G730T	4	E to X Glutamic acid to Stop	Nonsense	Yes
3	2	C751T	5	Q to X Glutamine to Stop	Nonsense	Yes
4	2	?	-	-	-	-
5	2	G679A	4	D to N Aspartic acid to Asparagines Acidic to uncharged polar	Missense	No
6	1	?	-	-	-	Yes
8	2	G1174A	7	-	Splice Site	Yes
16	1	G1723C	8	-	Splice Site	Yes
17	2	455del4	2	Premature Stop at 1293	Frameshift	Yes
18	1	C357G	1	Y to X Tyrosine to Stop	Nonsense	Yes

Once the mutations were identified in the probands confirmation of segregation was done as described in the Methods section. The Sequencher files can be reviewed in the previous section. These files confirm the appropriate identification of mutations in affected family members and the lack of mutation in the unaffected members. All family members plus controls and the GenBank sequence were tested in the same contigs.

The summary of genotyping is as follows and can be reviewed in table 4.3; All 10 families were assessed for linkage to either EXT 1 or 2 (4 EXT 1, 6 EXT 2). Eight of these 10 families had their mutation identified. Six of these eight mutations are novel and all mutations were unique to each family. Two mutations have been previously reported in the literature (Family 1 and 5). There were three nonsense, two missense, two splice site and one frameshift mutation. All mutations segregated appropriately in that those with exostoses carried the mutation and were heterozygotes at that location and those who were unaffected did not carry the mutation and had no sequence varience at that location

consistent with the Genbank sequences. Mutations in family 4 and 6 could not be identified despite sequencing both genes for two affected family members. Intronic and promoter sequences however were not sequenced. As well, very large deletions, for example an entire exon may have also been missed as the software would not pick up a heterozygozity if an entire reading frame was missing.

3.3 Phenotype Results

In the ten families represented in this study there were 32 affected individuals. Two families (4 and 6) with 6 subjects, did not have their mutation identified and therefore their data is not included in the genotype-phenotype analysis.

3.3.1 Phenotype data

Every affected individual that participated in the study including those members from families 4 and 6 completed the clinical and radiographic examinations. Save for a few data points the phenotype files were complete for every affected participant. The core data files are located in Appendix 8.6.4.1. The data includes 38 lesion quality items (8.6.4.1.1), 26 limb alignment items (8.6.4.1.2) and 25 limb segment items (8.6.4.1.3) for a total of 89 items per subject.

3.3.2 Range of Motion

Range of motion at the shoulder, elbow, forearm, wrist, hip, knee and ankle were essentially within normal limits for all subjects. In the cases of radial head dislocations in one family member of family 3 and one of family 18 there was reduction in forearm pronation and supination but the functional range was preserved (arc of 90 degrees). As

there was little effect on the clinical examination or functional range of motion, range of motion is therefore not included in this thesis, nor is this data analyzed in relation to genotype.

3.3.2 Pearson correlation matrix

All eighty-nine phenotypic parameters were placed on the x and the y-axis of the correlation matrix. Some of the limb segments correlated well but as there were so few correlations that were deemed duplicate (R> 0.8) all features were treated as separate items and therefore interpreted independently including sidedness. Appendix 8.6.4.1 contains the matrix in its entirety.

3.4 Genotype-phenotype Correlations

The data sets are based on 26 affected individuals who had both complete genotype and phenotype data. Table 3.4 outlines the breakdown of the number of subjects per category as well as the age distribution.

Table 3.4 Breakdown of Genotype Features

Genotype Feature	Number of subjects	Distribution of ages at time of study	Family 4	Family 6
EXT 1	7	9, 14, 14, 44, 47, 55, 72	-	3
EXT 2	9	7, 7, 10, 11, 14, 14, 15, 15, 16, 31, 36, 38, 39, 44, 45, 46,47, 70, 74	3	
Male	14	10, 11, 14, 14, 14, 15, 15, 39, 44, 44, 45, 47, 55, 73	-	2
Female	12	7, 7, 9, 14, 16, 31, 36, 38, 46, 47, 70, 72	3	1
MS	4	7, 9, 39, 47		
NS	14	7, 10, 14, 14, 14, 15, 15, 36, 38, 44, 46, 47, 55, 70		
FS	3	16, 45, 73		
SS	5	11, 14, 31, 44, 72		
Mild	4	7, 9, 39, 47		
Severe	22	7, 10, 11, 14, 14, 14, 14, 15, 15, 16, 31, 36, 38, 44, 44, 45, 46, 47, 55, 70, 72, 73		
Early	19	7, 7, 10, 14, 14, 14, 15, 15, 16, 36, 38, 39, 44, 45, 46, 47, 55, 70, 73		
Late	7	9, 11, 14, 31, 44, 47, 72		
EXT 1 Male	4	14, 44, 47, 55		
EXT 1 Female	3	9, 14, 72		
EXT 1 Severe	2	14, 14, 44, 55, 72		
EXT 1 Mild	5	9, 47		
EXT 1 MS	2	9, 47		
EXT 1 SS	3	14, 44, 72		
EXT 1 NS	2	14, 55		
EXT 2 Male	10	10, 11, 14, 14, 15, 15, 39, 44, 45, 73		
EXT 2 Female	9	7, 7, 16, 31, 36, 38, 46, 47, 70,		
EXT 2 Severe	17	7, 10, 11, 14, 14, 15, 15, 16, 31, 36, 38, 44, 45, 46, 47, 70, 73		
EXT 2 Mild	2	7, 39		
EXT 2 MS	2	7, 39		
EXT 2 SS	2	11, 31		
EXT 2 NS	12	7, 10, 14, 14, 14, 15, 15, 36, 38, 46, 47, 70		
Males severe	12	10, 11, 14, 14, 14, 15, 15, 44, 44, 45, 55, 73		
Males mild	2	39, 47		
Males MS	2	39, 47		
Males NS	8	10, 14, 14, 14, 15, 15, 44, 55		
Males SS	2	11, 44		
Males FS	2	45, 73		
Females severe	10	7, 14, 16, 31, 36, 38, 46, 47, 70,72		
Females mild	2	7, 9		
Females MS	2	7, 9		
Females NS	6	7, 36, 38, 46, 47, 70		
Females SS	3	14, 31, 72		
Females FS	1	16		

Phenotype parameters were grouped into lesion quality, limb alignment and limb segments. To simplify the presentation of the data they are dubbed "phenotype". From the literature review and the author's clinical experience numerous possible genotypic factors could potentially influence phenotype. Foremost was whether the EXT 1 or the EXT 2 gene mutations had a more severe clinical presentation. EXT genes were evaluated separately and then combined with other factors that were thought to potentially influence or modify the phenotype. These relationships dictated the 5 first comparisons as listed below.

Mutation type (missense, frameshift, splice site and nonsense) was looked at independently and as severity of mutation (truncating (ns, ss, fs) = severe and non-truncating (ms) = mild). Different types of mutations are often found to have different influences on the gene product and therefore the gene's function. As noted in the introduction, truncating mutations prevent localization of the EXT gene product to the endoplasmic reticulum (ER) whereas missense or nontruncating mutations result in the gene product being present in the ER. However both mutation types prevent heparan sulfate presentation on the cell surface. The question remains whether there is some preservation of EXT gene function when the product still localizes to the ER, which would then possibly result in differing phenotypes.

Gender was also analyzed as there is an anecdotal opinion that males have more severe disease (Solomon et al. 1963). This may be explained by the 100% penetrance in males and 96% in females (Schamle et al. 1998, Raskind et al. 1998), or that other growth factors are influencing tumour growth. This parameter was therefore tested as well to corroborate this unfounded opinion.

The last factor looked at was the location of the mutation. The last 780 base pairs of both EXT 1 and 2 genes is the carboxy terminus, which is highly conserved in EXT 1 and 2 and also the EXTL genes. Wuyts (Wuyts et al. 2000) believes given the conservation

of such an area, and given the ubiquitous presence of the EXT genes in human tissue, it is possible that other sources (specifically the EXTL genes (Wuyts etal. 2000)) may back up the function of the carboxy terminus thereby resulting in a milder phenotype. This suggestion is both highly speculative and paradoxical, as most highly conserved regions are crucial to function. Interestingly the fewest mutations are found in the last 780 bps (2/44 in EXT 1 and none in EXT 2). Attempts were made to look at the mutation from an early and late aspect based on most of the mutations being located prior to the 1500th base pair (bp). However given that it is only the last 780 bps that are involved in the highly conserved area and none of the mutations in this study were located so late in the gene one would expect to see no difference in these mutations. At the same time few mutations are seen beyond exon 8 (approximately at base pair 1500 for EXT 1 and 2), as can be confirmed by reviewing Figures 1.10 and 1.11 for either gene, that possibly a difference in phenotype would occur.

All the following comparisons were tabulated and are found in the indexed Appendix.

Gene versus phenotype	Appendix 8.7.1.1- 3
Gene and gender versus phenotype	Appendix 8.7.6.1 - 3
Gene and mutation type versus phenotype	Appendix 8.7.7.1 - 3
Gene and severity versus phenotype	Appendix 8.7.8.1 – 3
Gene and mutation location versus phenotype	Appendix 8.7.11.1-3
Gender versus phenotype	Appendix 8.7.2.1 - 3
Mutations type versus phenotype	Appendix 8.7.3.1 - 3
Mutation severity versus phenotype	Appendix 8.7.4.1 - 3
Mutation location versus phenotype	Appendix 8.7.5.1 - 3
Gender and severity versus phenotype	Appendix 8.7.9.1 – 3
Gender and mutation type versus phenotype	Appendix 8.7.10.1- 3

After observation of the data set lesion quality, certain features consistently showed tendencies towards differences in the various comparisons. Specifically, the average number of lesions influenced all other features and therefore percentages were looked at to standardize the data. In the lesion quality category certain items that were observed to have specific interest, or noted in the literature review were highlighted. These included: average number of lesions, size (small, medium, large), percent pedunculated lesions, percent sessile lesions, percent pelvic lesions, percent metaphyseal flaring and percent flat bone involvement. These items are highlighted below.

With regards to limb alignment, there were twenty-six items recorded for every subject. An item was categorized as abnormal if the value measured by xray analysis was greater than one standard deviation outside the published norm. The data is presented as the number of abnormal measurements (the average of each comparison group was used) out of twenty-six possible parameters.

Limb segment results were influenced by the percentile height, such that the shorter the subject was overall, the shorter the separate segment length.

Tables 3.5, 3.6 and 3.7 summarize the patterns of phenotype versus genotype. Table 3.5 summarizes the gene comparison analysis and covariant analysis data. Tables 3.6 and 3.7 summarize the mutation type, severity and location analysis and the gender covariant analysis. Only the data showing a trend or significance is included in these tables for clarity sake. Complete analysis of the data can be found in Appendices 8.7.1.1 through 8.7.11. Specific details of all comparisons is included in the text following.

Table 3.5 Summary of Results for Comparison between EXT 1 and EXT 2 Genes

EXT 1 vs. EXT 2 Comparisons	# lesions	% Pelvic	% Flatbone	% Flared	Limb Alignment	% Height
EXT1 vs. EXT2 (Appendix 8.7.1)	**1 > 2** ***p < 0.01 power .82***	**1 > 2** ***p < 0.01 power .68***	**1 > 2** ***p < 0.01 power .91***	***n/s***	**1 > 2 (17 vs 10)** ***n/s***	**1 < 2** ***p < 0.01 power .8***
Gene and Gender (Appendix 8.7.6)	1M > 1F > 2M > 2F *p < 0.01 gene and gender*	1M >1F > 2M > 2F *p < 0.01 gene*	1M > 2M > 1F > 2F ***n/s***	1M > 2M > 1F > 2F *p < 0.02 gender*	1M > 2M > 1F > 2F (16 > 13 > 10 > 8)	1F < 1M < 2M < 2F p < 0.01 gene
Gene and Mutation Type (Appendix 8.7.7)	1NS > 1SS > 1NS > 2NS > 2SS > 2MS > 2FS ***n/s***	1NS > 1MS > 1SS > 2SS > 2NS > 2MS > 2FS ***n/s***	1NS > 1MS > 1SS > 2SS > 2NS > 2MS > 2FS ***n/s***	--	1NS > 1SS > 2MS > (15) (14) (14) 2FS > 1MS > 2NS > (12) (11) (9) 2SS (9)	1NS > 1MS > 2 FS > 1SS > 2SS > 2MS > 2NS ***n/s***
Gene and Severity (Appendix 8.7.8)	*1S > 1M > 2S > 2M* ***n/s***	*1S > 1M > 2S > 2M* ***n/s***	*1S > 1M > 2S > 2M* ***n/s***	--	*1S > 2S > 1M > 2M (15 > 14 > 11 > 7)*	*1M < 1S < 2M < 2S* ***n/s***
Gene and Mutation location (Appendix 8.7.11)	1E > 2E p< 0.0021 power .95 1E > 1L	1E > 2E p < 0.001 power .99 1E > 1L 2E < 2L	1E > 2E p < 0.001 power .99 1E > 1L 2E < 2L	1E > 1L	1E = 1L > 2E > 2L (15 = 15 > 13 > 10)	1E < 1L < 2L < 2E ***n/s***

Abbreviations used: For gene comparison **1** – EXT1 and **2** – EXT2; for gender **M** – males and **F** – females; for mutation type, **MS** – missense mutation, **NS** – nonsense mutation, **FS** – frameshift mutation, and **SS** – splice site; for mutation severity, ***S*** – severe mutation and ***M*** mild mutation, E early, L late ***n/s*** – *Difference seen but not statistically significant; -- no difference seen*

3.4.1 Gene versus phenotype (Appendix 8.7.1.1-.3)

Subjects with EXT 1 mutations had more lesions than those with EXT 2 mutations, 32.7, versus 19.1 (p-value 0.0036). EXT 1 subjects have more percent pelvic lesions, 9.6 versus 2.3 (p-value 0.012) and more involvement of the flat bones, 11.8% versus 3.0% (p-value 0.0019). There were no differences noted between EXT 1 and 2 in terms of size, percent pedunculated versus percent sessile, percent complex versus percent simple or

percent metaphyseal flaring. EXT 1 subjects had more mal-alignment than subjects with EXT 2 mutations, 17 versus 10 of 26 possible parameters. EXT 1 subjects were shorter than EXT 2 subjects, 9.3 percentile versus 42.5 percentile (P-value .0081) and the overall upper extremity length (right and left) was shorter for EXT 1 patients (p-value 0.026, right and 0.027 left). Even though there were no significant differences in the remaining 10 segments measured, EXT 1 subject's measurements were always less than those of EXT 2 subjects.

3.4.2 Gene and gender versus phenotype (Appendix 8.7.6.1)

In general (not exclusively nor statistically significant in all cases) the following were noted; EXT 1 was worse than EXT 2 (See table 3.5), when further subdivided males were worse than females, nonsense mutations were worse than splice site which were worse than frameshift which were worse than missense; and severe mutations were worse than mild ones.

In detail, EXT 1 males have more lesions, 37.3, than EXT 1 females, 26.7, who had more than EXT 2 males, 24.0, who had more than EXT 2 females, 13.6. This is significant with regards to both gender (p-value 0.0032) and gene (p-value 0.0011). The same pattern exists when looking at percent pelvic lesions and percent flat bone involvement but it is only significant with regard to gene (p-value 0.015 and 0.0026) and not gender (p-value 0.51 and 0.52); %flared, EXT 1 male, 54.6, EXT 2 male, 40.6, Ext 2 female, 18.9, EXT 1 female, 17.3; % pelvic, EXT 1 males, 11.3, EXT 1 females, 7.3, EXT 2 males, 2.5, EXT 2 females, 2.1. EXT 1 and 2 males have more metaphyseal flaring than females and by gender the p-value is 0.0097. The pattern of mal-alignment also reflects males being worse than females with EXT 1 males having 16 of 26 parameters abnormal, EXT 2 males 13, EXT 1 females 10 and EXT 2 females, 8. Percentile height showed EXT 1 females to be

the shortest, 5th percentile, then EXT 1 males, 12.5th percentile, followed by EXT 2 males, 40th percentile and EXT 2 females at the 45th percentile. This was significant for gene (p-value 0.011) but not gender (p-value 0.79). If you were to cross reference to number of lesions it is as follows respectively; 26.7, 37.3, 24, 13.6.

3.4.3 Gene and mutation type versus phenotype (Appendix 8.7.7.1-.3)

EXT 1 missense had more lesions, 27.0 than EXT 2 missense, 15.0 (p-value 0.013), and EXT 1 nonsense also had more lesions, 43.5, than EXT 2 nonsense, 19.4, (p-value 0.0071) but the splice site mutation numbers between EXT 1 and 2 were similar. Further EXT 1 nonsense (43.5) mutations followed by EXT 1 splice site (29.3) had more lesions than EXT 1 missense (27). Similarly, in its series EXT 2 splice site (25.5) then nonsense (19.4) and then missense (15) followed by frameshift (11).

This relationship (EXT 1 worse than 2) also held true for percent pelvic lesions and percent flat bone involvement. That is to say EXT 1 is significantly more involved than EXT 2. But again no difference was noted in the EXT 1 and 2, splice site subjects. More specifically when looking within a group for % flat bone the data is for EXT 1; nonsense 19.3, missense, 9.2 then splice site, 8.4. For EXT 2; splice site 6.1, nonsense 3.3 and missense and splice site 0.

With regards to limb alignment EXT 1 missense mutation subjects had more abnormal values, 15, than EXT 1 splice site, 14, which had more than EXT 2 missense, 14, than EXT 2 frameshift, 12, followed by EXT 1 missense, 11, and EXT 2 nonsense and splice site at 9 each. When evaluating percentile height, EXT 1 was always shorter than EXT 2 with respect to the same mutation type. This was statistically significant only with respect to nonsense mutations (p-value 0.026). EXT 1 missense, nonsense, and splice site

were shorter than any of the EXT 2 mutation types except for the only frameshift identified in EXT 2.

3.4.4 Gene and severity versus phenotype (Appendix 8.7.8.1-.3)

EXT 1 severe, 35, and mild, 27, mutations had more lesions than their EXT 2 counterparts, 20.9 and 15 respectively (p-value 0.012 and 0.014). EXT 1 severe, 11.3, and mild, 5.4, mutations had more involvement of the pelvic bones than EXT 2, 3.2 and 0.0 (p-values 0.017 and 0.42). Similarly EXT 1 severe, 12.9, and mild, 9.2, mutations involved the flat bones more than the EXT 2 mutations, 3.9 and 0.0 respectively (p-value 0.0081 and 0.026). Alignment data showed EXT 1 severe had more abnormal values than EXT 2 severe, 15 versus 7, but EXT 2 mild had more mal-alignment than EXT 1 mild mutation subjects, 14 versus 11. However the EXT 2 mild data was only from one individual for most parameters. As for percentile height EXT 1 severe, 11.4 and mild, 4.0, were shorter than EXT 2 severe, 42.9 and mild, 39.0 (p-values 0.035 and 0.28 respectively).

3.4.5 Gene and Mutation location versus phenotype (Appendix 8.7.11.1-.3)

When comparing EXT 1 early versus EXT 2 early, EXT 1 early had more lesions, 43.5 vs. 18.3 (p-value <0.0021) more pelvic bone involvement, 18.4 vs. 1.8 (p-value < 0.001) and more flat bone involvement, 19.3 versus 2.6 (p-value <0.001). There were no differences or even trends towards differences between EXT 1 and EXT 2 late mutations. When looking at EXT 1 independently early mutations tended to have more lesions, 43.5 vs. 28.4, more pelvis involvement, 18.4 vs. 6.1, more flat bone involvement, 19.3 vs. 8.8 and more metaphyseal flaring, 61.3 vs. 29.6. This is in contrast to EXT 2 where the early mutations had fewer pelvis lesions, 1.8 vs. 6.1 and less flat bone involvement, 33.5 vs. 73.

Limb alignment data showed EXT 1 early and late mutations to have the most malalignment with 15 abnormal parameters each followed by EXT 2 early mutations and then EXT 2 late mutations.

Limb segment abnormalities were confined to percentile height where EXT 1 early subjects were the shortest at the 3rd percentile, followed by EXT 1 late mutations at the 11.8th percentile, then EXT 2 late mutations at the 25th percentile and finally EXT 2 early mutations at the 44.6th percentile.

Table 3.6 Summary of Results for remaining unvariant data

Comparisons	# lesions	% Pelvic	% Flatbone	% Flared	Limb Alignment	% Height
Males vs. Females (Appendix 8.7.2)	M > F *p < 0.01*	--	--	--	F > M (12 vs 9) ***n/s***	--
MS vs. NS vs. SS vs. FS (Appendix 8.7.3)	--	--	--	--	MS > SS = FS > NS (13 > 12 = 12 > 11) ***n/s***	FS < MS < SS < NS *p < 0.01*
Severe vs. Mild (Appendix 8.7.4)	--	--	--	--	Severe = Mild (11 vs 11) ***n/s***	Mild < Severe ***n/s***
Early vs. Late (Appendix 8.7.5)	--	--	--	--	Early = Mild (11 vs 11) ***n/s***	Late < Early ***n/s***

Abbreviations used: ***n/s*** *– Difference seen but not statistically significant; -- no difference seen; MS – missense mutation; NS – nonsense mutation; SS – splice site; FS – frameshift mutation*

3.4.6 Gender versus phenotype (Appendix 8.7.2.1-.3)

Male subjects had more lesions than females, 28.1 versus 17.2 (p-value 0.01) and males had more metaphyseal flaring than females, 45% versus 18.5% (p-value 0.01) while females had less flaring than males, 81.5% versus 55% (p-value .0079). No differences were noted in any of the other lesion quality items. Males had nine of twenty-six abnormal alignment parameters and females had 12. There was no difference with respect to percentile height or the 12 segments measured between males and females.

3.4.7 Mutation type versus phenotype (Appendix 8.7.3.1-.3)

Missense mutations had the highest percentage of small lesions, 48.5% (p-value 0.045) and splice site mutations had the highest percentage of large lesions, 48.5% but this was not statistically different than the other mutation types. Though not statistically significant, splice site mutation subjects also had the highest percentage of pelvic lesions and flat bone involvement. There were no differences between the four mutation types with respect to mal-alignment. Frameshift subjects, represented by one family, were the shortest at the 9.7th percentile and nonsense mutation subjects, represented by 3 families, were the tallest, 51.3rd percentile (p-value 0.048).

3.4.8 Mutation severity versus phenotype (Appendix 8.7.4.1-.3)

No differences were identified between severe and mild mutations. Both groups had eleven of twenty-six abnormal mal-alignment parameters and there were no significant differences in limb segment features except mild mutation subjects were consistently shorter in all characteristics.

3.4.9 Mutation location versus phenotype (Appendix 8.7.5.1-.3)

There were no differences noted in any of the thirty-eight lesion quality items when comparing early and late mutations. There were the same number of mal-alignment abnormalities between mild and severe mutations, eleven of twenty-six. Subjects with a late mutation were shorter than those with an early mutation. Limb segments and percentile height were not significantly different between the two groups.

Table 3.7 Summary of Results for Comparison between Males and Females Covariant data

Male and Female Comparisons	# lesions	% Pelvic	% Flatbone	% Flared	Limb Alignment	% Height
Gender and Severity (Appendix 8.7.9)	M s > M m > F m > Fs *p < 0.01*	--	--	M s > M m > F m > Fs *p < 0.01*	F m > M m > (12) (11) M s > F s (10) (9)	Mm smaller than the rest ***n/s***
Gender and Mutation Type (Appendix 8.7.10)	M ns > F ns > M ss > M ns > F ss > F fs > M fs > F ms > F ns	--	--	M ns > F ns > M ns > M ss > M ms > M fs > F ms > F ns > F ss > F fs **n/s**	F fs > M ss > (16) (14) F ss > M fs > (13) (12) M ms > F ms > (11) (11) M ns > F ns (9) (9)	F fs < M fs < M ms < F ss < F ms < M ss < M ns < F ns ***n/s***

Abbreviations used: For gene comparison **1** – EXT1 and **2** – EXT2; for gender **M** – males and **F** – females; for mutation type, **ms**– missense mutation, **ns** – nonsense mutation, **fs** – frameshift mutation, and **ss** – splice site; for mutation severity, **s** – severe mutation and **m** mild mutation
n/s *– Difference seen but not statistically significant;* ***--*** *no difference seen*

3.4.10 Gender and Severity versus phenotype (Appendix 8.7.9.1-.3)

Males versus females with severe mutations showed a significant difference in regards to lesion number, 29.9 versus 17.1 (p-value 0.0061), but in comparison to males and females with mild mutations there was no difference with 21 lesions each. The only remaining difference noted was again between males and females with severe mutations for percent flared metaphyses, 46.1 and 17.4 (p-value 0.0049) and the converse held true where females with severe mutations had the least flared metaphyses, 81.3 versus 53.9 (p-value 0.0075). Trends existed where males had more sessile lesions and females more pedunculated lesions. Females with a mild mutation had more limb mal-alignment, 12, followed by males with mild mutations, 11, males with severe mutations, 10 and females with severe mutations, 9, last. There was no difference in the overall percentile height for this grouping. Males with missense mutations had the greatest shortening, 10^{th} percentile.

3.4.11 Gender and mutation type versus phenotype (Appendix 8.7.10.1-.3)

Males with splice site mutations had the most number of lesions at 34.5, versus males with nonsense mutations who had 30.4. The remaining groupings had about the same number of lesions. The only significant difference noted was between male and female nonsense subjects (p-value 0.005). Males for all mutations had more sessile lesions whereas females had more pedunculated lesions, but this was not significant. Males in all categories showed more flaring than females. Mal-alignment was not different between the males and females for each mutation type. Females with frameshift mutations (1 subject) had 16 abnormal parameters, followed by males and females with splice site mutations, 14 and 13 respectively, then males with frameshift mutation, followed by missense where both genders had 11 and for the nonsense subjects, 9. As for height the one female frameshift patient was the shortest at the 8^{th} percentile followed by males with misssense and frameshift mutations at the 10^{th} percentile, the females with splice site, 12^{th} percentile, and the rest were greater than the 30^{th} percentile.

Chapter IV: Discussion

4.1 Subject Recruitment

HME is a relatively rare disorder and this is reflected in the sample size assembled (69 total participants), ten families with thirty-two affected individuals. Access to extended family members is also limited in Canada due to our multinational population and the geography. As such, family members often reside at great distances and are unavailable for recruitment. Despite this, a satisfactory sampling of all the families was obtained for a pilot project designed to determine if a trend exists between genotype and phenotype. Only one family, Family 3, was of sufficient size (36) and subjects available (24) for analysis of intra-familial correlations (the data can be found in appendix 8.6.4.1). Family 1 was the smallest with only 4 participating members.

Due to the limited sample size this thesis is designed to explore correlations between phenotype and genotype. Statistical testing, paired t-tests and ANOVA where appropriate, were used to assist the observational analysis of the data. The relations being tested between genotype and phenotype generated a large number of p-values, which were used only to focus the attention on any pattern generation as opposed to determining statistical significance. Consistent patterns were identified to generate hypotheses of association that will be tested in future larger collaborative studies.

4.2 Genotype

Previous studies (Cook et al. 1993; Blanton et al. 1996; Legeai-Mallet et al. 1997; Wuyts et al. 1995; Wuyts et al. 1998; Philippe et al. 1997; Xu et al. 1998; Seki et al. 2001) have identified from 30 to 100% of the mutations in the families studied. This study found 80% or eight out of the ten family's mutations. The remaining 2 families had multiple HPR studies done resulting in Family 4 having a high probability of a mutation in EXT 2 and Family 6 with an

EXT 1 mutation. However despite sequencing both genes twice for the proband and then once for another affected family member no mutation could be identified. As the purpose of this study was to explore correlations between phenotype and confirmed genotypes these six subjects were excluded.

The 8 families included had their mutations identified and confirmed as described above in section 3.4 and segregation analysis confirmed only affected family members carried the mutation and were heterozygous at the locus of interest. One silent polymorphism was also discovered in EXT 1 in exon 9 as G1761A. This was noted in five of eight subjects sequenced at this locus and was compared to the GenBank sequence of GAG. There was no change in the amino acid as both GAG and GAA code for glutamic acid.

Thirty percent of the ten families had mutations in EXT 1 and 50% were in EXT 2, while 20% remained unidentified. This is in contrast to the overall reported mutations where 36% are in EXT 1, 27% in EXT 2 and 36% unidentified (section 2.2.4.1 and 2.2.4.2) (Philippe et al. 1997). In this study 3 of 8 mutations (37.5%) were identified in EXT 1 and 5 of 8 (62.5%) were in EXT 2. If the two families with unidentified mutations are included based on their linkage analysis alone then 40% are found in EXT 1 and 60% in EXT 2. The ratio of EXT 1 to EXT 2 in this population is therefore 2:3 in contrast to the literature where the ratio of EXT 1 to EXT 2 mutations is 2:1. It is likely these differences relate to the small sample size available in reported studies as well as this study. It would appear the previously reported ratio is suspect and requires further study.

Many of the previous studies have looked at primarily one race. Seki looked at Japanese families where the ratio of EXT 1 to 2 was 3:1 (Seki et al. 2001), Xu looked at Chinese families where this ratio was 7:1 (Xu et al.1998), Wuyts (Wuyts et al.1998) looked at a variety of nationalities including European and Middle Eastern families and found a ratio of 1:1 and Phillipe (Phillipe et al. 1997) looked at French families and found a ratio of 2.5:1. This study

(EXT 1:2, 3:5) includes a number of ethnic groups including, East Indian (EXT 1), Welsh (EXT 2), Austrian (EXT 2), Japanese (EXT 2), German (EXT 1 and 2), and British (EXT 1 and 2). It is likely that once enough races and cultures are evaluated the ratio between EXT 1 and 2 may be 1:1.

The mutations identified were in keeping with those found in the literature in terms of the type. The literature suggests frameshift mutations are the most common and yet it was the least common in this study. However this sample size is likely a skewed sampling simply because of the small size. The most common mutation in this series is the nonsense mutation (3), followed by splice site and missense (2 each) and one frameshift.

Most mutations quoted in the literature occur in the early half of EXT 1, 80% (Table 2.1), and EXT 2, 93% (Table 2.2). Similarly in this study 67% of EXT 1 mutations occur in the first half of the gene and 100% of the EXT 2 mutations occur in the first half. In summary the mutational profile with respect to gene effected, mutation type, mutation location and mutation severity are in keeping with what is reported in the literature as of January 2003.

The mutations identified were not unique in two of eight families. Family 1 carries an EXT 1 G1019A missense mutation and has previously been described by Raskind (1998) and Seki (2001). This base change causes a change in the amino acid from arginine to histidine, which are both basic. However the amino acid change is sufficient to cause a conformational change in the EXT 1 protein thereby precluding its function and ultimately the presentation of heparan sulfate on the cell surface. This was confirmed previously by Raskind (1998). The second previously described mutation was also a missense mutation and was found in Family 5. The base change was in EXT 2 G679A causing an aspartic acid, which is acidic to be replaced by, an asparagine, which is uncharged polar. This mutation has been previously described by Phillipe (1997) and here again this work showed that the amino acid change ultimately caused alteration in the EXT 2 protein sufficient enough to result in exostosis formation.

[Phenotypically, these 2 missense mutation families were indistinguishable from the other mutation types other than a slight tendency for them to be more malaligned (13/26 versus 12/26), and slightly shorter (21st versus 27th percentile average for the others). No other features of the 89 were significant of any trend. However when looked at in the context of EXT 1 and 2 missense mutation phenotypes were always milder than nonsense and generally milder than the other truncating mutation types table 3.5 for the highlighted phenotypic features].

The remaining six mutations were unique. Three were nonsense mutations resulting in early stop codons. This, as McCormick (1998) has shown, results in a protein which is truncated and does not localize to the endoplasmic reticulum and therefore no heparan sulfate presentation on the cell surface. Two of the mutations were found in EXT 2 and one in EXT 1. One frameshift mutation was identified in EXT 2 and caused an early stop codon downstream. This would have a similar effect as a nonsense early stop.

There were two splice site mutations, one both in each EXT 1 and 2. The one located in EXT 2 (G1174A) was located in intron 7 at the 5' splice site in the first intronic position. Interestingly this is one base pair further along than the 1173 +1G→A that Wuyts described in 1998 (1998). Both however cause the first base pair in the intron to be an adenine instead of guanine resulting in splice site malfunction. The mutation found by Wuyts occurred in a Dutch family and in this study the mutation occurred in a German family. Confirmation of this splice site mutation was done by Wuyts, by amplifying the 5' splice site with custom designed primers to flank the region. The wild-type PCR fragment contains an ScrFI restriction site where the mutant allele of this splice site does not. The end result causes a skipping of exon 7 and leads to a truncated protein (Wuyts 1996). It is likely the mutation found in this study has an identical effect.

The EXT 1 splice site mutation occurred in intron eight. It too was located at the first intronic position at the 5' splice site. There have been no other described mutations in the area of

this splice site mutation. There is likely a downstream stop codon resulting in a truncated product. It was beyond the scope of the present study to fully describe the actual end result of such a mutation. Suffice is to say, multiple exostoses were still the ultimate outcome.

Mutations caused by nonsense, splice site and frameshifts result in truncated proteins. This results in the complete absence of the EXT proteins in the endoplasmic reticulum and ultimately no hetero-oligomeric complex in the Golgi apparatus. On the other hand missense mutations are altered as a result of an amino acid change but the EXT product nonetheless locates to the endoplasmic reticulum. It has not been shown how this affects the hetero-oligomeric complex in the golgi apparatus, only that there is still no identification of heparan sulfate on the cell surface in in vitro studies. These two findings were identified by McCormick (1998) and are now universally accepted. It is therefore fair to suggest that missense mutations have a mild effect on the localization of the EXT protein whereas nonsense, frameshift and splice site mutations have a severe effect, by there being no EXT protein localized to the endoplasmic reticulum. This then leads one to think there should be a difference in the phenotype caused by a mild versus severe mutation. One then concludes that, phenotype could be influenced by the type of mutation or the severity of the mutation. This appears to be the case in the 26 individuals studied here. In general, though not statistically significant or universally correct, nonsense and truncating mutations had a worse phenotype. This was far from as impressive a negative effect that EXT 1 has on phenotype. When mutation type was looked at in the context of gene mutated missense mutations tended to have the mildest presentation. It is possible then that some function of the EXT genes is preserved when missense mutations occur by the protein being present in the ER.

Gullberg (2002) has gone on to show recently that the function of the two EXT gene products in fact do vary in terms of the effect on the elongation of the heparan sulfate chains. EXT 2 is believed to chaperone or modify the activity of EXT 1 and therefore in EXT 2's

absence, chain elongation is altered but not negated as it is when EXT 1 is absent. The two genes work in synergy, but given the differential effect of EXT 1 and 2 on heparan sulfate chain elongation it is very possible that the phenotype is truly affected by which of the two genes is mutated into inactivity. Given the dominant role on the enzymatic activity that EXT 1 has one would then assume EXT 1 phenotype would be more negatively influenced. That is to say, subjects with EXT 1 mutations should have a more severe form of the disease. The results of this study support the findings of Gullberg in that subjects with EXT 1 mutations have a more severe expression of the disease.

Even though the germ line mutation exists, how does this then translate into disease expression? There are two possible mechanisms. The first is that the germ line mutation acts in a negative dominant way resulting in exostosis formation. However, this should then result in global involvement of the entire skeleton. Specifically, all growth plates, which appear to be the cell of origin source for exostosis formation, should be affected by exostosis formation. Furthermore there should be significant deformity of the entire growth plate. This sort of effect is seen in skeletal dysplasias such as achondroplasia where all growth plates involved with enchondral bone formation are affected. In achondroplasia, phenotypic features are expressed by the entire skeleton, including the skull, spine and appendicular skeleton. And yet HME rarely affects the spine, or head and has a definite propensity for the long bones including hands and feet. But not in all cases are all juxtaphyseal regions involved with exostoses. When looking at the xrays of achondroplasts the entire bone is influenced by the results of the abnormality of the growth plate function. In some cases of HME or SME, there is global effect on the bones. For example the metaphyses particularly about the knee and proximal humerus can been grossly distorted with flaring. But this is not universal in either all the bones in one subject or in all Multiple Exostoses subjects. In many cases there are simply multiple discrete lesions causing only a bump remote to the physis. This is the case in this study's population. Many subjects

showed metaphyseal flaring, but no subjects had 100% of the metaphyses distorted in this way and at the same time many subjects had some unaffected physes and metaphyses. In other subjects many discrete remote lesions existed which were completely innocuous.

The alternative mechanism is that the germ line mutation in conjunction with local influences causes exostosis formation. Hecht's (2002) work has demonstrated little nest of cells located in the perichondrium of patients with HME. These nests are possibly the result of monoclonal expression from a chondrocyte that carries the germ line mutation and its survival into a tumour is the result of local forces. This would then better explain the lack of global skeletal involvement and the lack of the entire bone being deformed.. It is then ultimately the effect on the local environment that causes the resulting deformity. As Porter (Porter et al. 2000) has shown, the more lesions on one bone and the involvement of highly integrated two bone systems the more deformity occurs. The local effect of the tumour would then be responsive to a variety of influences, including, when the tumour develops (the younger the patient the more potential for it to get bigger, the older the more likely it will migrate less from the growth plate thereby causing growth plate tethering), where in relation to the growth plate it forms (peripherally versus centrally where it can cause metaphyseal flaring), or gender given that males and females have different growing patterns and potential.

If there is a difference in which gene is affected in terms of potential for exostosis formation one would expect this to influence the phenotype. Given that EXT 1 has potentially more of a role in tumour formation secondary to a higher catalytic function than EXT 2 it would follow that EXT 1 mutations would have a greater potential for tumour formation, which is in turn influenced by the local environment. Once again the data shows EXT 1 patients have more lesions and a more severe expression of the disease.

4.3 Phenotype

In general the eighty-nine phenotypic features explored were normally distributed. All subjects, including those without mutation identification (Family 4 and 6 members), were included in the phenotype analysis. All but 18 of 2848 data points were collected from all subjects. The phenotype data were grouped into three categories reflecting significant areas of clinical concern: 1) lesion quality, 2) limb alignment and 3) limb segment lengths plus percentile height.

As the goal of this thesis was to identify a genotype phenotype correlation, phenotype alone is briefly discussed here and to greater length in the genotype phenotype section (section 4.4). In addition, the Pearson correlation matrix did not identify any two of the phenotype parameters to be correlating except with respect to limb lengths, therefore, all parameters were treated independently of each other.

4.3.1 Lesion Quality

Lesion quality was determined by radiographic evaluation of the patient. X-rays were more sensitive in detecting lesions, clinical exam underestimated the count by as much as 50% in this study. Furthermore, it was not possible to determine the morphology or the size of the lesions reliably by physical exam. Specific X-rays of hands, feet and spines were not routinely available as these sites are an uncommon source of morbidity in this population.

Using items put forward by Francennet (Francennet et al. 2002) and Carroll (Carroll et al. 1999) as a template for assessing lesion quality to reflect severity of disease expression two of the major factors used by these authors are of questionable significance in the context of the present studies results. Francannet et al. (2002) have put a large emphasis on spine lesions and if present, automatically led to the phenotype being classified as severe. Involvement of the spine in the present sample was not specifically assessed with spine x-rays. However, none of the

subjects noted any spinal lesions nor were they noted on physical examination, particularly no spinal deformity (scoliosis) was identified on physical examination. Exostoses were noted in the lower spine on some of the pelvis x-rays in this series of subjects but none were noted to be involved in spinal deformity. None of the thirty-two subjects in this study had scoliosis. Reviewing the BCCH scoliosis clinic database (containing 3137 cases) no cases were found where exostoses were the cause of spinal deformity. Similarly, Schmale (1994) reported no spinal lesions and Wold (1990) reported 3% (1% in cervical, thoracic and lumbar each). Spinal exostoses are very rare and unlikely to cause scoliosis. As a result, using the presence of spinal lesions to define severity is of questionable usefulness.

Carroll et al. (1999) in part defined the resultant phenotype of EXT 1 and 2 mutations on the basis of pedunculated versus sessile lesions. It was implied in this study that the higher percentage of sessile lesions present the worse phenotype. Presumed EXT 1 mutation subjects had 87% sessile lesions and were moderately effected, presumed EXT 2 subjects had 72% sessile lesions and were felt to have a mild phenotype and presumed EXT 3 subjects (it has since been decided that EXT 3 is not involved with exostosis formation) (Wuyts et al. 1998) were severe with 95% sessile lesions. The morphology of the lesion in isolation does not appear to be significant in terms of phenotype. Rather it is the location and influence on the growth plate, which cause deformity and mal-alignment. The present study with a larger sample size than Carroll (32 versus 29) did not observe as high a percentage of sessile lesions with the typical proportion being 60% sessile to 30% pedunculated. Therefore discussing phenotype severity on the basis of spine lesions and their morphology in isolation may not be helpful.

Involvement of flat bones, including the bones of the pelvis, was thought to be reflective of a more severe phenotype because of their increasing propensity to transform into chondrosarcoma. Fifty-six percent of chondrosarcomas occur on the flat bones with twenty-three percent originating from the pelvis (Mirra 1989). There were 14 subjects, representing both EXT

1 and 2, with flat bone involvement, and given that there is roughly a 5% transformation rate, one of the included subjects is likely to suffer from a future chondrosarcoma. Because the pelvis is capable of accommodating a large mass without obvious evidence until it is very large, transformed osteochondromas can remain hidden. In this case to ensure clear resection margins often a hemi-pelvectomy is required. In this series 11 subjects, or 35%, had a pelvic lesion identified, which is significantly higher than the 6% reported by Mirra (Mirra et al.1989) and twice that of Schmale's 15% (Schmale et al. 1994). The difference in reported pelvic lesions may reflect the routine use of pelvic x-rays in the current study that was not used in the other studies. Only Family 17 has a known case of chondrosarcoma. It occurred in a male (not a participant), which is more common (64%) (Mirra et al.1989), involved the pelvis and resulted in a partial hemi-pelvectomy to obtain clear resection margins.

The number of lesions has also been proposed to be good measure of disease severity. Porter has shown the more lesions present the greater the bony deformity (Porter et al. 2000). In this study 96% of patients had at least one lesion about the knee and 63% of subjects had knee mal-alignment. Similarly wrist, elbow and ankle alignment had a greater chance of being abnormal as the number of lesions in the involved bones increased and when it involves the two bone systems (forearm and lower leg). In this study population there was no obvious relationship between increasing number of lesions and overall mal-alignment. Besides the knee the actual number of lesions per bone was not mapped precisely but it was observed that mal-alignment and deformity occurred only in the presence of exostoses. Additionally, there were two confounding elements, gender and age. Males had more lesions, and the older an individual the more likely joint mal-alignment exists irrespective of exostoses.

The more lesions present, the higher the chance one will be on a flat bone and therefore subject to transformation. Therefore not only location of the exostosis is important but also the number, which increases the probability of their being a pelvic lesion.

The number of lesions did not correlate with the percentile height. The average number of lesions in the study was twenty-five and the percentile height thirty-three. The fewest number of lesions in one subject (EXT 2) was nine and their percentile height was at the fifty-first percentile and the subject (EXT 1) with the most lesions, fifty-three, was in the third percentile. However, five subjects were on the third percentile but their lesion counts were 34 (EXT 1), 53 (EXT 1), 11 (EXT 2), 28 (EXT 2) and 32 (EXT 1) while two subjects were above the ninetieth percentile and their lesion counts were 12 (EXT 2) and 14 (EXT 2). A relationship may truly exist here but the sample size is too small to pick this up. If however, the effect of the germline mutation is a global effect as in a skeletal dysplasia then the number of lesions should be irrelevant.

Metaphyseal flaring has also been considered to be the sign of a severe phenotype as the aneurysmal dilatation of the metaphyses was thought to cause a greater degree of mal-alignment, deformity and shortening. While a significant correlation was not found in general bony deformity, malalignment and limb length discrepancy did occur in the presence of metaphyseal flaring. The subject (EXT 1) with the highest percentage of flaring (80.5%), was on the 39th percentile for height (average 33), had 10 mal-aligned joints (average 12) including the knee and the hip on the left, but normal alignment of the right at these two joints, and a significant leg length discrepancy of 2.5 cm. The shorter leg was on the right even though both distal femurs were involved with flaring. Hence it was a combination of shortening and malalignment, which resulted in the net leg length discrepancy.

One phenotypic feature, which was overlooked in the inclusion of parameters, was a quality of life questionnaire. The Musculoskeletal tumour society functional assessment has been used in the HME setting by other authors (Schmale et al. 1994 and Francennet et al. 200). It is a validated scale reflecting the quality of life of patients with tumours. Better would be a disease specific quality of life score but no such scale exists. Pain scales have also been used in this

patient population (HME coalition in conjunction with Hecht 2002) and the results were unexpected. In particular, there is a physician/surgeon misconception that pain is not a large factor in these people's lives, but in fact the returned pain scales showed a presence of pain in 70%, with 14% greatly influencing function (personal communication Hecht 2002). Since ultimately the most important component of the clinical profile is quality of life it will be included in all future studies. Using the Musculoskeletal Tumour Society's scale as a global rating score a disease specific quality of life score will be designed concurrently.

4.3.2 Limb Alignment

Limb alignment data though done both by clinical and radiographic examinations was more accurate and complete from the radiographic examinations and therefore it is only this data that is discussed. Care was taken to standardize the data according to age and gender in the few cases where it made a difference. For example, the female carrying angle (elbow joint alignment) is in more valgus than in males.

The results are discussed in two sections using different approaches. First, when discussing phenotype alone, each subject's twenty-six alignment parameters were evaluated and classified as either within normal limits or one standard deviation outside the normal limits. The total number of abnormal results out of twenty-six was then calculated for each subject. It gives a global mal-alignment tally for each individual, which was then related to the study population. This method however did not reflect the severity of the mal-alignment. The second way the data was interpreted is more pertinent to the genotype-phenotype discussion. The group's data was collected, for example EXT 1 patients, and the values for each alignment parameter for all the subject in the group was averaged, deemed within or outside normal limits and then the abnormal alignments were tallied up for each group. By doing this only if the group as a whole had significant mal-alignment did the result register outside normal limits, thereby reflecting severity of mal-alignment. The first method looks at the data from a population perspective whereas the

second method looks specifically at severity of phenotype particularly in relation to genotypic features (discussed in section 4.4).

The femoral anatomic angle (knee joint angle) and the elbow joint angle were the most commonly abnormal alignments where 31 of 32 individuals had at least one side of knee mal-alignment and 27 of 32 had elbow joint mal-alignment. Ulnar shortening and radial inclination mal-alignment were also quite common with 27 subjects each being outside the normal limits. The least effected parameter was radial bowing where only 5 individuals were affected.

Given that the knee consists of the distal femoral, proximal tibial and fibular physes, it is not unexpected to find it the most commonly effected joint. It also involves a two-bone system where balanced bone growth is essential for alignment. The probability of having at least one of the three bones involved at the knee was reported as 94% by Schmale (1994). In this series 96% of the patients had involvement at the knee joint. On average each subject had twelve of twenty-four abnormal alignments with the range between nine and eighteen. The severity of the mal-alignment however varied considerably. As discussed above these values were obtained by averaging the alignment values for each group.

4.3.3 Limb segments and percentile height

The significance of measuring height and limb segment lengths was in order to evaluate HME as a skeletal dysplasia. Traditionally HME had been classified as a pathologic short stature (skeletal dysplasia). Short stature is defined as an individual less than the third percentile for their age and gender. The average percentile height for this series of HME subjects was the 27^{th} percentile (33^{rd} if including family 4 and 6). The range however was from the 3^{rd} (5 subjects, 3 males and 2 females) to greater than the 85^{th} (3 subjects, 1 male and 2 females) percentile. Short stature is defined as less than or equal to the 3^{rd} percentile, therefore rather than classifying HME

as a pathologic short stature or dwarfism it would better be described as having a propensity for stature below the 30th percentile, i.e. growth impedance.

The effect of the overall growth impedance correlated reliably with the lower extremity segment measurements. However when each subject's data was analyzed separately, the percentile of the upper and lower limb segments were higher than the overall percentile heights. This can be a result of a variety of factors. Firstly, the shortening experienced by these patients is not accounted for exclusively by the lower extremities and is a culmination of shortening in the pelvis, trunk and spine. Secondly, actual bony measurements are more accurately done using computed tomography versus surface landmarks. This however does not include the soft tissue envelope and therefore underestimates the total length. Thirdly, most of these subjects had a degree of mal-alignment and deformity in their limbs which effected the overall height of the patient but when broken down to the measure of each bone less shortening was identified.

Of interest was that the lower leg segment of the lower extremity was always shorter than the upper leg segment. This would be consistent with mesomelic shortening. The same pattern was noted in the upper extremity where the proximal segment was longer (based on percentiles) than the distal segment, and both were consistently below the 37th percentile. In the lower extremity they were both below the 51st percentile. In this regard, with respect to these 32 individuals, they all had mesomelic shortening in both the upper and lower extremities. Yet considering the growth plates, those with the largest growth potential would logically be the ones more significantly effected; hence it should be that the upper leg segments be shorter on a percentile basis than those of the lower leg segment (the distal femoral growth plate contributes 37% of longitudinal growth versus the proximal tibia which contributes 28%, the remaining growth plates are less still) (Morrissy and Weinstein 1996). The same can be said for the upper extremity where the proximal humeral growth plate contributes more to the overall growth of the upper extremity those of the radius and ulna. The reason behind the mesomelic shortening brings

us back to Porter's (2000) work where the two bone systems, which are mesomelic in both upper and lower extremity, are more significantly affected possibly because there is twice the chance for growth impedance. This leads us back to the hypothesis that it is partly environment that causes the effect on the phenotype, but the number of lesions controlled by genotype sets the level of severity (EXT 1 worse than 2).

Trunk measurements were not done as part of this study. On retrospect it would be worthwhile to determine if the trunk segments were also shortened. If in fact shortening does exist throughout the entire skeleton, HME may need to be reconsidered as a skeletal dysplasia. This would be supported by the observation of mesomelic shortening and that a germline mutation is present in all subjects with HME.

4.3.4 Intra-Family variability

There were three families with three generations of affected individuals with available data. Family 3 was the only family large enough and with sufficient participating subjects to look at intra-family variation. There were twenty-three participating members and nine of these were affected. There were six females and three males. However when looking at the entire Family 3 tree the ratio was closer to 1:1 between males and females for having multiple exostoses. There was one grand parent, three parents and five children studied. Intra-family variability was assessed by gender and generation, as these were the two most obvious variables.

In broad terms for the three, three generational families, the grandparents had the least number of lesions. However they were also the shortest in terms of percentile height. This is consistent with the rest of this study's data set in that number of lesions did not influence percentile height. On the other hand it is possible that since this generation is smaller, their growth potential was also less and therefore they simply did not grow as many lesions. The mal-alignment data for the three grandparents was within keeping with the entire study population.

There was no consistent pattern identified in any of the three phenotype categories between the parents and the children in Family 3 or any of the remaining families. These parameters were influenced by gender in some situations, and generation in others but no correlation could be identified.

When looking only at Family 3 lesion quality results showed an average of 17.4 lesions with a range between nine and thirty-nine. Males had twenty-six lesions on average and females had thirteen. The percent distribution of the remaining characteristics were not different between males and females except for metaphyseal flaring where all three males showed greater than thirty-five percent flaring and an average of fifty-five percent of their lesions showed metaphyseal flaring versus seventeen percent in females. All the lesion quality parameters otherwise more or less fit a normal distribution. Percentile height was 48.6 for the family with a range between eighteen and ninety (males 43rd, females 51st). Limb segment shortening was in proportion to the overall percentile height as discussed earlier in section 5.3.2 and mesomelic shortening was consistent in both the upper and lower extremities. As for alignment data, each individual had an average of 12 mal-alignments, which is also the average for the entire study population. The severity between and males and females and between generations did not show a consistent pattern.

In summary, with regards to intra-family variation, the only consistent influence was by the male gender that had more lesions and metaphyseal flaring. This was also the case in the entire study population therefore it is unlikely secondary influences within a family with a given genotype other than gender has an effect.

4.4 Genotype phenotype correlation

Phenotype as it relates to genotype was broken down into the three categories, lesion quality, limb alignment and limb segments plus percentile height. Lesion quality features that

were felt to be more representative of a worse phenotype were number of exostoses, percent flat bone involvement, percent pelvic bone involvement and percent metaphyseal flaring. The remaining parameters were not found to be indicative of severity in this population. Limb alignment severity was based on the averaged alignment data for the subjects in each group of interest and then the total number of abnormal alignment items were tallied. The more abnormal alignments present for the group being analyzed the worse the phenotype was considered. Finally the shorter the subjects which was reflected as the average for the group of interest, the worse the phenotype. The limb segment lengths were directly related to the degree of shortening of the overall stature, albeit to slightly different degrees.

The features, which appeared to represent severity of disease accounted for 38 of the 89 possible parameters explored. When the number of lesions were standardized to a percentage, the lesion quality parameters were reduced from 38 to 21. So in fact there are 72 parameters representing phenotype of which over half describe severity in this small population. However there are many trends existing amongst the other 34 parameters and all data points need to be collected to ensure differences were not missed simply due to sample bias.

Those patients with an EXT 1 mutation consistently expressed more severe phenotypic characteristics. These included a greater number of exostoses, a higher percent involvement of flat and pelvic bones, greater mal-alignment and shorter stature with corresponding limb segment shortening showing the typical mesomelic pattern. Gender in isolation appears to be a modifying feature with males tending to have more exostoses and metaphyseal flaring and perhaps a trend towards a greater degree of mal-alignment. No obvious difference in percent pelvic or flat bone involvement was seen between males and females. No evidence of phenotypic variation was observed in the other comparison groups (mutation type, mutation severity or mutation location) in isolation. One may criticize that this was not a fruitful endeavour to look at these factors independently but it does put to rest that in isolation they are not influencing factors on

phenotype. In striking contrast however, when paired with gene affected support is given to previous entertained hypotheses that in conjunction with gene affected, mutation type and severity, and mutation location, and possibly gender did have influences causing variation in the phenotype therefore these factors must differentially affect gene function.

When the relationship between EXT 1 in conjunction with gender and phenotype was explored the following was observed. In general males were worse than females within a gene; i.e. EXT 1 males were worse than EXT 1 females and EXT 2 males were worse than EXT 2 females. This was the case for number of lesions, and percent pelvic involvement. For the remaining categories showing differences males as a group were worse than females. Specifically, males were more severe than females in the following order; EXT 1 males greater than EXT 2 males greater than EXT 1 females greater than EXT 2 females in severity for degree of limb mal-alignment and percent metaphyseal flaring.

Exploration of the relationship between EXT 1 in conjunction with mutation type and phenotype revealed all EXT 1 mutation types were consistently more severe phenotypically than the EXT 2 mutations. Similarly, when EXT 1 was paired with severity EXT 1 severe and mild mutations consistently had a more severe phenotype than both EXT 2 mild and severe mutations. When dissected further for EXT 1 missense mutations were consistently milder than nonsense mutation for lesion number, percent pelvic and flat bone, limb alignment and percentile height. The same can be said for mild versus severe mutations. The EXT 2 trends were similar except for limb alignment and percentile height comparing specific mutation types. One must be reminded here that these are trends and not statistically significant. Nonetheless, this gives support to the hypothesis that mutation type affects protein localizing in the ER and therefore function and ultimately phenotype.

When gene was matched with early versus late mutation EXT 1 early was worse phenotypically than EXT 2 early for lesion number, and pelvis and flat bone involvement. This is

likely reflecting the more dominant effect the EXT 1 gene has on phenotype. However there were no differences noted in lesion quality parameters when inspecting EXT 1 versus 2 late mutations. Interestingly however when EXT 1 early was compared to EXT 1 late mutations early mutations were considerably worse with lesion number, percent pelvis, flat bone and metaphyseal involvement and percentile height (EXT 1 early mutations are the shortest, then EXT 1 late, EXT 2 late and EXT 2 early). This gives some support to Wuyts idea (Wuyts et al. 2001) idea that late mutations may be milder. This is not supported by the alignment data as EXT 1 early and late both have 15 malalignments and they are both worse than EXT 2 mutations. This again is the gene effect.

Similar exploration of gender in conjunction with mutation type and severity produced no consistent pattern on phenotype. This suggests gender may act to modify the influence that EXT 1 exerts on phenotype but in isolation has less of an impact.

Determining causation in an association found, such as EXT 1 versus EXT 2 and phenotype, can be supported by different factors. One is consistency of findings suggested above. A second is a reasonable biological rationale. The EXT 1 gene product is believed to have a higher catalytic activity in heparan sulfate chain elongation than EXT 2 "135". The extent of chain elongation may influence cell division to different degrees in EXT 1 versus EXT 2 patients.

While loss of growth regulation appears to depend partly on which EXT gene is mutated other external forces likely are modulating the extent of disease expression. Gender may variably influence clinical expression due to the difference in growing patterns in children. Females are known to have a short but rapid pre-pubertal growth spurt that comes to an end two years after menarche while boys grow more slowly over a longer period of time. In general girl's growth interval from puberty to skeletal maturity is about 4 years compared to the 6 years of males

(Lovell and Winter 1996). It is possible that gender differences in hormonal expression and duration of expressed growth factors influence this modulation.

HME has been included by some under the umbrella of skeletal dysplasia (Lovell and Wintr 1996). However, as dysplasia in general refers to an intrinsic bone disturbance and HME bony disturbance appears to be confined to those exostoses present, it may not be accurate to include HME among the skeletal dysplasias. There is a wide expression of the disease in terms of phenotype and other than each exostosis having a similar appearance at the pathologic level the variability in the skeleton from subject to subject is quite marked. Perhaps the strongest support used to place HME among the skeletal dysplasias is the associated short stature. However while most HME patients have stature less than the 50^{th} percentile, some are greater than the 85^{th} percentile and none are below the 3^{rd} percentile in these studies. On balance HME does not appear to a true skeletal dysplasia.

The disease expression is influenced by both the number of tumours present and when they occur. The number of lesions appears related in part to genotype (gene affected, mutation type, severity and location) but genotype does not seem to influence the location of tumour development. If one considers clonal expression as the mechanics of tumour development then it becomes a matter of what is causing the tumours to grow. From this work it appears that EXT mutations and their related type and location and gender predispose one to HME. There is more loss of control of tumour regulation with EXT 1 mutations, and males may have more growth potential over time to allow for more growth both with respect to actual number and to size including metaphyseal impairment. The mutation characteristics (type and location) are likely affecting the gne function at he cellular level promoting tumour development.

If the mechanism of exostosis formation was the result of a malfunctioning growth plate then all growth plates should be deformed. But this is not the case and in many cases small pedunculated exostoses are found remote from the growth plates as innocuous little bumps. This

implies that a small nest of cells developed at a point of rapid growth and that the nest did not get caught up in the growth plate thereby causing bony deformity and mal-alignment. In contrast, those joints with multiple lesions and significant mal-alignment may have had multiple monoclonal nests develop and then due to yet unknown local factors they all started to grow but the environment was such that the tumours got caught up in the growth plate and could not migrate away. Then the two bone systems would be even more sensitive to this because any disturbance in one of the bone causes significant deformity for the other.

In summary, EXT 1 mutations are associated with a more severe phenotype, which appears to be modulated both by mutation type and location and in part by gender. This may be due to the fact that EXT 1 has a more dominant effect on heparan sulfate chain elongation as a result of increased catalytic activity. Tumour suppression activity is sensitive to the heparan sulfate chain morphology, changes in which gene gives rise to varying loss of control over growth. The mechanism for expression of the disease appears to be more on of focal clonal expression dependent on the local and humoral environment rather than a skeletal dysplasia or a "sick" growth plate as there is far too much variability amongst HME patients. What causes the second hit is unknown but in the cases where both genes are mutated chondrosarcomas have been described (Hecht 1995). The phenotype is partly influenced by the location of tumour development, at what point in a child's development do they appear and in what growing milieu they develop.

An established genotype phenotype correlation has significant clinical impact. Patients with HME and in particular those with EXT 1 mutations need to be monitored to possibly avoid bony deformity and mal-alignment, which leads to surgery and the associated risks and complications of intervention. Males with HME need to be further assessed as their phenotype tends to be more severe. Phenotype profiling in relation to the gene mutated (and the mutation characteristics) will be helpful in providing families with the anticipated course of the disease for

their offspring and will aid in determining the prognosis. The relationship of chondrosarcoma to EXT 1 and 2 mutations is still unclear but surveillance for transformation of benign osteochondromas is important.

Chapter V: Summary

Ten HME families from British Columbia with thirty-two (69 total participants) affected members participated in this project. Eight mutations were identified and confirmed. Six mutations have not yet been described in the literature and two have previously been reported. The features of the mutations are in keeping with what is reported in the literature. Phenotyping was exhaustive and allowed for subjects to be described in terms of their lesion quality, limb alignment and deformity and limb segment lengths plus percentile height. A genotype phenotype correlation exists in that subjects with an EXT 1 mutation have a worse phenotype, mutation type and location also influence severity and gender appears to modulate expression of the disease. This correlation supports the hypothesis that EXT 1 has a dominant affect over EXT 2 in tumour development and that HME is unlikely to be the result of a skeletal dysplasia but rather a combination of loss of chondrocyte growth regulation and then growth parameters specific to each subject.

Chapter VI: Conclusion

There is a genotype phenotype correlation in HME where patients with EXT 1 mutations have a worse phenotype.

Chapter VII: Future work

This study was designed as a descriptive study to explore whether a genotype phenotype correlation exists in HME. This was a hypothesis-generating manoeuvre in hopes of identifying factors that represent severity of disease expression. The results of this study have provided a template from which future work can expand in a prospective fashion.

A large collaborative network has been established secondary to this pilot project. The mutations identified in the literature have been described by a variety of labs worldwide. These labs, through the assistance of the HME coalition (a non-profit support group for people living with HME and their families) and its members will provide consenting subjects whose genotypes have been identified and confirmed for the anticipated prospective project (website: http://www.geocities.com/mhecoalition/). We anticipate access to up to a minimum of 60 new families. Ethical approval has been obtained for this site and all the collaborating labs have obtained Ethical approval from their centres. Funding has been secured to carry on with this work. We anticipate approximately 200 new affected subjects from elsewhere and 30 from this centre. Our centre will genotype the BCCH new families (plus further work will be done to elucidate mutations for Family 4 and Family 6). Data will be collected as presented in this thesis. Prospective analysis will be done on the features identified as showing a trend in the pilot project. Specific hypotheses to be tested in a prospected fashion include (with regards to phenotype); EXT 1 gene mutations are worse than EXT 2, Within a gene mutated, nonsense mutations and truncating mutations are the most severe, within a gene mutated early mutations are worse than late.

Investigations also need to be done to further elucidate the local and humoral factors that are permitting specific tumours from growing from presumptive osteochondroma niduses. One potentially obvious factor is gender and it hormonal differences and growth patterns. But many other possibilities exist that influence growing bone.

Another direction this project has taken from the pilot project is to further study the hypothesis of disease expression being a result of clonal expression of perichondrial chondrocyte nests. Tumours are collected when patients undergo resection of exostoses as part of their routine care. We are working in collaboration with another group to investigate the genetic make up of the tumours themselves. Dr. Hecht's work has outlined some second mutations but her work was limited due to lack of material. Our group has access to the original 10 family's material plus the new probands presenting to the HME clinic on a regular basis. Furthermore solitary exostoses are readily available as they are routinely excised from patients. These two main patient populations will be accessed under appropriate consent and ethical approval. Funding has been obtained for this project and ethical approval is pending.

The final offshoot of the original project has been the establishment of the HME clinic at BC Children's Hospital run by the author of this thesis. This clinic provides clinical support for the families affected with HME. Disease surveillance is the main goal of the clinic. Such detailed assessments are not possible in busy orthopaedic practices and this clinic provides the consulting surgeons with the information gleaned by this detailed work. It also provides surveillance in the adults who have an increased risk of tumour transformation. In collaboration with the radiology nuclear medicine department at Children's and Women's hospital, adults will undergo bone scans every 3 years to screen for activity in known exostoses. This will be particularly beneficial to those patients with pelvic exostoses.

Bibliography

1. 1993. *The Chicago Manual of Style, 14th Ediction.* Chicago: The University of Chicago Press.

2. 1996. Multiplex-PCR-Based Single-Strand Conformation Polymorphism Protocol for Simultaneous Analysis of Up to Five Fragments of the Low-Density-Lipoprotein Receptor Gene. *BioTechniques* 20:421-429.

3. Aaltonen, Lauri A. Hereditary intestinal cancer. Cancer Biology 10, 289-298. 2001.

4. Ahn, Jung, Hermann-Josef Lüdecke, Steffi Lindow, William A. Horton, Brendan Lee, Michael J. Wagner, Bernhard Horsthemke, and Dan E. Wells. 1995. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nature Genetics.*

5. Akashi, M. and H. P. Koeffler. 1998. Li-Fraumeni syndrome and the role of the p53 tumor suppressor gene in cancer susceptibility. *Clin.Obstet.Gynecol.* 41, no. 1:172-199.

6. Arver, Brita, Quan Du, Jindong Chen, Liping Luo, and Annika Lindblom. Hereditary breast cancer: a review. Cancer Biology 10, 271-288. 2001.

7. Bartsch, O., W. Wuyts, and W. Van Hul. Delineation of a contiguous gene syndrome with multiple exostoses, enlarged parietal foramina, craniofacial dysotosis and mental retardation, caused by deletions of the short arm of chromosome 11. American Journal of Human Genetics 58, 734-742. 1996.

8. Bellaiche, Yohanns, Inge The, and Norbert Perrimon. 1998. *Tout-velu* is a *Drosophila* homologue of the putative tumour suppressor *EXT-1* and is needed for Hh diffusion. *Nature* 394:85-88.

9. Berchuck, A., M. Carney, J. M. Lancaster, J. Marks, and A. P. Futreal. 1998. Familial breast-ovarian cancer syndromes: BRCA1 and BRCA2. *Clin.Obstet.Gynecol.* 41, no. 1:157-166.

10. Bernard, Mark A., Catherine E. Hall, Deborah A. Hogue, William G. Cole, Allison Scott, Mark B. Snuggs, Gregory A. Clines, Hermann-Josef Lüdecke, Michael Lovett, W. Barry Van Winkle, and Jacqueline T. Hecht. 2001. Diminished Levels of the Putative Tumor Suppressor Proteins EXT1 and EXT2 in Exostosis Chondrocytes. *Cell Motility and the Cytoskeleton* 48:149-162.

11. Bernstein, L. R. and L. A. Liotta. Molecular mediators fo interactions with extracellular matrix components and angiogenesis. Curr.Opin.Oncol. 6, 106-113. 1994.

12. Blanton, Susan Halloran, Deborah Hogue, Michael Wagner, Dan Wells, Ian D. Young, and Jacqueline T. Hecht. 1996. Hereditary Multiple Exostoses: Confirmation of Linkage to Chromosomes 8 and 11. *American Journal of Medical Genetics* 62:150-159.

13. Bornemann, D., W. Staats, J. Duncan, S. B. Selleck, and R. Warrior. Modulation of growth factor signalling by the EXT 2 tunour suppressor gene. 2002. Tuscon, Arizona, Conference on Multiple Hereditary Exostosis.

14. Bouvier, J. F., J. L. Chassard, Brunat-Mentigny M., J. Y. Bobin, Domenach M., Roojee N., Riffat G., Mayer M., and B. E. Lahneche. Radionuclide bone imaging in diaphyseal aclasis with malignant change. Cancer 57, 2280-2284. 1986.

15. Bovee, Judith M., Anne-Marie Cleton-Jansen, Wim Wuyts, Goedele Caethoven, Antoine H. M. Taminiau, Egbert Bakker, Wim Van Hul, Cornelisse Cees J, and Pancras C. W. Hogendoom. 1999. EXT-Mutation Analysis and Loss of Heterozygosity in Sporadic and Hereditary Osteochondromas and Secondary Chondrosarcomas. *American Journal of Human Genetics* 65:689-698.

16. Bovee, Judith M., Anne-Marie Cleton-Jansen, Nel J. Kuipers-Dijkshoorn, Antoine H. M. Taminiau, Cees J. Cornelisse, and Pancras C. W. Hogendoom. 1999. Loss of Heterozygosity and DNA Ploidy Point to a Diverging Genetic Mechanism in the Origin of Peripheral and Central Chondrosarcoma. *Genes, Chromosomes & Cancer* 26:237-246.

17. Boyer. 1814. *Traite des Maladies Chirugicales*. Vol. 3 Paris: Mignuet.

18. Bridge, Julia A, SB Paramjit, JR Anderson, and James R. Neff. Biologic and clinical significance of cytogenetic and molecular cytogenetic abnormalities in benign and malignant cartilaginous lesions. Cancer Genetics and Cytogenetics 69, 79-90. 1993.

19. Bridge, Julia A, Mari Nelson, Charlotte Örndal, Paramjit Bhatia, and James R. Neff. 1998. Clonal Karyotypic Abnormalities of the Hereditary Multiple Exostoses Chromosomal Loci 8q24.1 (EXT1) and 11p11-12 (EXT2) in Patients with Sporadic and Hereditary Osteochondromas. *Cancer* 82, no. 9:1657-1663.

20. Brugieres, L., M. Gardes, C. Moutou, A. Chompret, V. Meresse, A. Martin, N. Poisson, F. Flamant, C. Bonaiti-Pellie, J. Lemerie, and J. Feunteun. Screening for Germ Line p53 Mutations in Children with Malignant Tumors and a Family History of Cancer. Cancer Research 53, 452-455. 1993.

21. Buhler, E. M. and N. J. Malik. The Trichorhinophalangeal Syndrome(s): chromosome 8 long arm deletion: is there a shorteset region of overlap between reported cases?TRP I and TRP II syndromes:are they separate entities? American Journal of Human Genetics 19[1], 113-119. 1984.

22. Carroll, Kristen L., Suzanne M. Yandow, Ken Ward, and John C. Carey. Clinical correlation to genetic variations of hereditary multiple exostosis. Journal of Paediatric Orthopaedics 19, 785-791. 1999.

23. Chansky, Howard A and Wendy Raskind. Hereditary Multiple Exostosis. Gene Clinics , 1-10. 2002. Seattle, University of Washington.

24. Cheung, P. K., C. McCormick, B. E. Crawford, J. D. Esko, F. Tufaro, and G. Duncan. Etiological Point Mutations in Hereditary Multiple Exostoses Gene EXT1: A functional analysis of Heparan Sulfate Polymerase Activity. American Journal of Medical Genetics 69, 55-66. 2001.

25. Clines, Gregory A., Jennifer A. Ashley, Sangeeta Shah, and Michael Lovett. 1997. The Structure of the Human Multiple *Exostoses 2* Gene and Characterization of Homologs in Mouse and *Caenorhabditis elegans*. *Genome Research* 7:359-367.

26. Cook, April, Wendy H. Raskind, Susan Halloran Blanton, Richard M. Pauli, Ronald G. Gregg, Claire A. Francomano, Eric Puffenberger, Ernest U. Conrad, Gregory A. Schmale, Gerard Schellenberg, Ellen Wijsman, Jacqueline T. Hecht, Dan Wells, and Michael J. Wagner. 1993. Genetic Heterogeneity in Families with Hereditary Multiple Exostoses. *American Journal of Human Genetics* 53:71-79.

27. Dianzani, I., C. Camaschella, A. Ponzone, and R. G. H. Cotton. 1993. Dilemmas and progress in mutation detection. *TIG* 9, no. 12:403-405.

28. DiCiommo, David, Brenda L. Gallie, and Rod Bremner. Retinoblastoma: the disease, gene and protein provide critical leads to understand cancer. Cancer Biology 10, 255-269. 2001.

29. Enneking W.E. Modification of the system for functional evaluation in the surgical mangement of musculoskeletal tumours. In: Limb salvage in musculoskeletal oncology. 626-639. 1987. New York, Bristol-Myers orthopaedic symposium, Churchill Livingstone.

30. Ewing, B., L. Hillier, M. C. Wendl, and P. Green. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* 8, no. 3:175-185.

31. Ewing, B. and P. Green. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* 8, no. 3:186-194.

32. Francannet, C, A Cohen-Tanugi, Martine Le Merrer, A. Munnich, Jacky Bonaventure, and Laurence Legeai-Mallet. Genotype-phenotype Correlation in hereditary multiple exostosis. J Med Genet 38[7], 430-434. 2002.

33. Frebourg, Thierry and Stephen M. Friend. 19920. Cancer Risks from Germline P53 Mutations. *Journal of Clinical Investigation* 90:1637-1641.

34. Fryns, J. P. and H. Van Den Berghe. 8q24.12 interstitial deletion in Trichorhinophalangeal syndrome type I. Human Genetics 74[2], 188-189. 1986.

35. Gigante, M. 2001. Ext-mutation analysis in Italian sporadic and hereditary osteochondromas. *International Journal of Cancer* 95, no. 6:378-383.

36. Gordon, D., C. Abajian, and P. Green. 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* 8, no. 3:195-202.

37. Green, D. P. Operative Hand Surgery, 3rd ed. 1993. New York, NY USA, Churchill Livingstone Inc.

38. Griffiths, A, J Miller, D Suzuki, R Lewontin, and W Gelbart. 1996. *An Introduction to Genetic Analysis 6th Edition.*: WH Freeman and Company, USA.

39. Gullberg, M. K. EXT 1 and 2 proteins and heparan sulfate biosynthesis. 2002. Tuscon, Arizona, Conference on Multiple Hereditary Exostoses.

40. Hamill, P. V., T. A. Drizd, C. L. Johnson, R. B. Reed, A. F. Roche, and W. M. Moore. 1979. Physical Growthe: National Centre for Health Statistics Percentiles. *American Journal of Clinical Nutrition*:607-629.

41. Hansen, Marc F. Molecular Genetic Considerations in Osteosarcoma. Clinical Orthopaedics and Related Research 270, 237-246. 1990.

42. Hecht, Jacqueline T., Deborah A. Hogue, Louise C. Strong, Marc F. Hansen, Susan H. Blanton, and Michael Wagner. 1995. Hereditary Multiple Exostosis and Chondrosarcoma: Linkage to Chromosome 11 and Loss of Heterozygosity for EXT-Linked Markers on Chromosomes 11 and 8. *American Journal of Human Genetics* 56:1125-1131.

43. Hecht, Jacqueline T., Deborah Hogue, Yang Wang, Susan H. Blanton, Michael Wagner, Louise C. Strong, Wendy Raskind, Marc F. Hansen, and Dan Wells. 1997. Hereditary Multiple Exostoses (EXT): Mutational Studies of Familial EXT1 Cases and EXT-Associated Malignancies. *American Journal of Human Genetics* 60:80-86.

44. Hecht, Jacqueline T., E. Hayes, Catherine E. Hall, H. Li, R. Haynes, William G. Cole, R. Long, M. C. Farach-Carson, and D. D Carson. EXT 1 and EXT 2 germline mutations are the most common cause of diminshed heparan sulfate in exostosis growth plates. 2002. Tuscon, Arizona, Conference on Multiple Hereditary Exostosis.

45. Hogue, Deborah, Gregory Clines, Michael Lovett, Marc F. Hansen, and Jacqueline T. Hecht. Mutational Analysis of hereditary multiple exostosis. American Journal of Human Genetics 59, A263. 1996.

46. Hollstein, M., D. Sidransky, B. Vogelstein, and C. C. Harris. 1991. p53 mutations in human cancers. *Science* 253, no. 5015:49-53.

47. Hooper, Martin L. Tumour suppressor gene mutations in humans and mice: parallels and contrasts. The EMBO Journal 17[23], 6783-6789. 2001.

48. Hori, Tada-aki, Naohiko Seki, Miki Ohira, Toshiyuki Saito, Masatake Yamauchi, Masahi Sagara, Akiko Hayashi, Satsuki Tsuji, Hiroko Ito, and Takashi Imai. 1998. A Distamycin A-Inducible Fragile Site, FRA8E, Located in the Region of the Hereditary Multiple Exostoses Gene, Is Not Involved in HPV16 DNA Integration and Amplification. *Cancer Genetics and Cytogenetics* 101:24-34.

49. Hsu, R. W., S. Himeno, M. B. Coventry, and E. Y. Chao. Normal Axial Alignment of the lower extremity and load bearing distribution. Clinical Orthopaedics and Related Research 255, 215-227. 1990.

50. Hudson, T. M., D. S. Springfield, S. S. Spanier, W. F. Ennekin, and D. J. Hamlin. Benign exostoses and exostotic chondrosarcomas: evaluation of cartilage thickness. Radiology 152, 595-599. 1984.

51. Humma, L. M., W. G. Farmerie, M. R. Wallace, and J. A. Johnson. 2000. Sequencing of beta 2-adrenoceptor gene PCR products using Taq BigDye terminator chemistry results in inaccurate base calling. *BioTechniques* 29, no. 5:962-4, 966, 968.

52. Jaffe H.L. Tumors and tumorous conditions of the Bones and Joints. 1968. Philadelphia, Lea and Febiger.

53. Jaffe, H. L. 1943. Hereditary Multiple Exostosis. *Archives of Pathology* 33:335.

54. Keats, T. E. Atlas of Roentgenographic Measurement, 6th Ed. 259.

55. Keats, T. E. Normal axial relationships of the major joints. Radiology 87, 904. 1961.

56. Kephart, Dan. 1999. Rapid Isolation of Genomic DNA from Small Quantities of Human Tissue. *Profiles in DNA* 2, no. 3.

57. Kitagawa, Hiroshi and Hiromi Shimakawa. 1999. The Tumor Suppressor EXT-like Gene EXTL2 Encodes an α1, 4-N-Acetylhexosaminytransferase That Transfers N-Acetylgalactosamine and N-Acetylglucosamine to the Common Glycosaminoglycan-Protein Linkage Region. *The Journal of Biological Chemistry* 274, no. 20:13933-13937.

58. Kivioja, A., H. Ervasti, I. Kinnunen, M. Kaitila, M. Wolf, and T. Böhling. 2000. Chondrosarcomas in a family with multiple hereditary exostoses. *The Journal of Bone and Joint Surgery* 82-B, no. 2:261-266.

59. Knudson, A. Mutation and Cancer: statistical study of retinoblastoma. Proceedings of the National Academy of Science of the USA 68, 820-823. 1971.

60. Kobayashi, S., K. Morimoto, T Shimizu, Takahashi, M., H. Kurosawa, and T. Shirasawa. Association of EXT 1 and EXT 2 hereditary multiple exostose gene products, in Golgi apparatus. Biochemical and Biophysical Research Communications 268, 860-867. 2000.

61. Lange R.H., Lange T.A., and Rao B.K. Correlative radiographic, scintigraphic, and histological evaluation of exostoses. jbjs 66, 1454-1459. 1984.

62. Lavigueur, A., V. Maltby, D. Mock, J. Rossant, T. Pawson, and A. Bernstein. 1989. High incidence of lung, bone, and lymphoid tumors in transgenic mice overexpressing mutant alleles of the p53 oncogene. *Mol.Cell Biol.* 9, no. 9:3982-3991.

63. Le Merrer, Martine, Laurence Legeai-Mallet, P. M. Jeannin, Bernhard Horsthemke, A. Schnizel, Henry Plauchu, A. Toutain, F. Achard, A. Munnich, and Pierre Maroteaux. A Gene for Hereditary Multiple Exostosis Maps to Chromosome 19p. Hum Mol Genet 3, 717-722. 1994.

64. Legeai-Mallet, Laurence, Arnold Munnich, Pierre Maroteaux, and Martine Le Merrer. 1997. Incomplete penetrance and expressivity skewing in hereditary multiple exostoses. *Clinical Genetics* 52:12-16.

65. Legeai-Mallet, Laurence, Patricia Margaritte-Jeannin, Mohamed Lemdani, Martine Le Merrer, Henry Plauchu, Pierre Maroteaux, Arnold Munnich, and Françoise Clerget-Darpoux. 1997. An extension of the admixture test for the study of genetic heterogeneity in hereditary multiple exostoses. *Human Genetics* 99:298-302.

66. Legeai-Mallet, Laurence, Antonio Rossi, Catherine Benoist-Lasselin, Rocco Piazza, Jean-François Mallet, Anne-Lise Delezoide, Arnold Munnich, Jacky Bonaventure, and Louise Zylberberg. 2000. *EXT1* Gene Mutation Induces Chondrocyte Cytoskeletal Abnormalities and Defective Collagen Expression in the Exostoses. *Journal of Bone and Mineral Research* 8:1489-1500.

67. Ligon, Azra H, Lorraine Potocki, Lisa G. Shaffer, Dominique Stickens, and Glen A. Evans. 1998. Gene for Multiple Exostoses (EXT2) Maps to 11(p11.2p12) and Is Deleted in Patients With a Contiguous Gene Sydrome. *American Journal of Medical Genetics* 75:538-540.

68. Lin, Xin, Lin Gan, William H. Klein, and Dan E. Wells. 1998. Expression and Functional Analysis of Mouse EXT1, a Homolog of the Human Multiple Exostoses Type 1 Gene. *Biochemical and Biophysical Research Communications* 248:738-743.

69. Lin, Xin, Ge Wei, Zhengzheng Shi, Laurence Dryer, Jeffrey D. Esko, Dan E. Wells, and Martin M. Matzuk. 2000. Disruption of Gastrulation and Heparan Sulfate Biosynthesis in EXT1-Deficient Mice. *Developmental Biology* 224:299-311.

70. Lind, Thomas, Frank Tufaro, Craig McCormick, Ulf Lindahl, and Kerstin Lidholt. 1998. The Putative Tumor Suppressors EXT1 and EXT2 Are Glycosyltransferases Required for the Biosysnthesis of Heparan Sulfate. *The Journal of Biological Chemistry* 273, no. 41:26265-26268.

71. Lohmann, D. R. 1999. RB1 gene mutations in retinoblastoma. *Hum.Mutat.* 14, no. 4:283-288.

72. Ludecke, H. J., M. J. Wagner, and J. Nardmann. Molecular dissection of a contiguous gene syndrome: localisation of the genes involved in the Langer-Geidieon syndrome. Hum Mol Genet 4, 31-36. 1995.

73. Lüdecke, Hermann-Josef, Jung Ahn, X. Lin, A. Hill, Michael J. Wagner, Lutz Schomburg, Bernhard Horsthemke, and Dan E. Wells. 1997. Genomic Organization and Promoter Structure of the Human EXT1 Gene. *Genomics* 40, no. 351:354.

74. Malkin, D., F. P. Li, L. C. Strong, J. F. Fraumeni, Jr., C. E. Nelson, D. H. Kim, J. Kassel, M. A. Gryka, F. Z. Bischoff, M. A. Tainsky, and . 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250, no. 4985:1233-1238.

75. Marx, Jean L. 1988. Multiplying Genes by Leaps and Bounds. *Science* 240:1408-1410.

76. Masuda, H., C. W. Miller, H. P. Koeffler, H. Battifora, and Cline.MJ. Rearrangement of the p53 gene in human osteogenic sarcomas. Proceedings of the National Academy of Science of the USA 84, 7716-7719. 1997.

77. McCormick, Craig, Yves Leduc, Diane Martindale, Kirsten Mattison, Lesley E. Esford, Angela P. Dyer, and Frank Tufaro. 1998. The putative tumour suppressor EXT1 alters the expression of cell-surface heparan sulfate. *Nature Genetics* 19:158-161.

78. McCormick, Craig, Gillian Duncan, K. Tina Goutsos, and Frank Tufaro. 2000. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumlates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proceedings of the National Academy of Science* 97, no. 2:668-673.

79. Mertens, F, A Rydholm, A Kriecbergs, H Willen, K Jonsson, S Heim, and N Mandahl. Loss of chromosome band 8q24 in sporadic osteocartilaginous exostoses. Genes, Chromosomes & Cancer 9, 8-12. 1994.

80. Miller, C. W., A. Aslo, C. Tsay, D. Slamon, K. Ishizaki, J. Toguchida, T. Yamamuro, B. Lampkin, and H. P. Koeffler. 1990. Frequency and structure of p53 rearrangements in human osteosarcoma. *Cancer Res.* 50, no. 24:7950-7954.

81. Mirra, JM. Picci P. Gold RH. Bone Tumors. 2, 1626-1645. 1989. Philidelphia and London, Lea and Febiger.

82. Morrissy, R. T. editor and S. L. editor Weinstein. 1996. *Lovell and Winter's Pediatric Orhtopaedics*. Philadelphia, Pensylvania: Lippincott-Raven.

83. Mullenbach, R., P. J. L. Lagoda, and C. Welter. 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends in Genetics* 5:391.

84. Oliner, J. D., K. W. Kinzler, P. S. Meltzer, D. L. George, and B. Vogelstein. 1992. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* 358, no. 6381:80-83.

85. Park, Kyu Joo, Ki-Hyuk Shin, Ja-Lok Ku, Tae-Joon Cho, Sang Hoon Lee, In Ho Choi, Christophe Philippe, Anthony P. Monaco, Daniel E. Porter, and Jae-Gahb Park. Germline mutations in the *EXT1* and *EXT2* genes in Korean patients with hereditary multiple exostoses. Journal of Human Genetics 44, 230-234. 2001.

86. Parrish, J. E., Michael Wagner, Jacqueline T. Hecht, C. I. Scott, and D. E. Wells. Molecular analysis of overlapping chromosomal deletions in patients with Langer-Geidion syndrome. Genomics 11, 54-61. 1991.

87. Peterson, HA. Multiple Hereditary Osteochondromata. Clinical Orthopaedics 239, 222-230. 1989.

88. Pettersson, H. and H. Ringertz. Measurements in Paediatric Radiology. 1991. London, Springer-Verlag.

89. Philippe, Christophe, Daniel E. Porter, Mark E. Emerton, Dan E. Wells, A. Hamish R. W. Simpson, and Anthony P. Monaco. 1997. Mutation Screening of the EXT1 and EXT2 Genes in Patients with Hereditary Multiple Exostoses. *American Journal of Human Genetics* 61:520-528.

90. Piao, Zhe, Hoguen Kim, Bong Kyun Jeon, Woo Jung Lee, and Chanil Park. 1997. Relationship between Loss of Heterozygosity of Tumor Suppressor Genes and Histologic Differentiation in Hepatocellular Carcinoma. *Cancer* 80, no. 5:865-872.

91. Pierz, K. A., J. R. Stieber, K. Kususmi, and J. P. Dormans. Hereditary Multiple Exostosis: One Centre's Experience and Review of Etiology. Clinical Orthopaedics and Related Research 1[401], 49-59. 2002.

92. Pierz, KA. Womer RB. Dormans JP. Paediatric Bone Tumours: Osteosarcoma, Ewing's Sarcoma, and Chondrosarcoma Associated with Multiple Hereditary Osteochondromas. Journal of Paediatric Orhtopaedics 21, 412-418. 2001.

93. Porter, D. E. and Dominique Stickens. 1999. The Neoplastic Pathogenesis of Solitary and Multiple Osteochondromas. *Journal of Pathology* 188:119-125.

94. Porter, D. E., Mark E. Emerton, F. Villaneuva-Lopez, and A. Hamish R. W. Simpson. Clinical and radiographic analysis of osteochondromas and growth disturbance in hereditary multiple exostosis. Journal of Paediatric Orthopaedics 20, 246-250. 2000.

95. Potocki, Lorraine and Lisa G. Shaffer. 1996. Interstitial Deletion of 11(p11.2p12): A Newly Described Contiguous Gene Deletion Sydrome Involving the Gene for Hereditary Multiple Exostoses (EXT2). *American Journal of Medical Genetics* 62:319-325.

96. Raskind, Wendy, Ernest U. Conrad III, H Chansky, and Mark Matsushita. Loss of Heterozygosity in chondrosarcomas for markers linked to hereditary multiple exostoses loci on chromosome 8 and 11. American Journal of Human Genetics 56, 1132-1139. 1995.

97. Raskind, Wendy H., Ernest U. Conrad III, Mark Matsushita, Ellen M. Wijsman, Dan E. Wells, Nicola Chapman, Linda J. Sandell, Michael Wagner, and John Houck. 1998. Evaluation of Locus Heterogeneity and EXT1 Mutations in 34 Families With Hereditary Multiple Exostoses. *Human Mutation* 11:231-239.

98. Rosenthal, A., O. Coutelle, and M. Craxton. 1993. Large-scale production of DNA sequencing templates by microtitre format PCR. *Nucleic Acids Res.* 21, no. 1:173-174.

99. Rosenthal, A., O. Coutelle, and M. Craxton. 1996. Multiplex-PCR-Based Single-Strand Conformation Polymorphism Protocol for Simultaneous Analysis of Up to Five Fragments of the Low-Density-Lipoprotein Receptor Gene. *BioTechniques* 20:421-429.

100. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239, no. 4839:487-491.

101. Saito, Toshiyuki, Naohiko Seki, Masatake Yamauchi, Satsuki Tsuji, Akiko Hayashi, Sumie Kozuma, and Tada-aki Hori. 1998. Structure, Chromosomal Location, and Expression Profile of EXTR1 and EXTR2, New Members of the Multiple Exostoses Gene Family. *Biochemical and Biophysical Research Communications* 243, no. 1:61-66.

102. Schmale, Gregory A., Ernest U. Conrad III, and Wendy H. Raskind. 1994. The Natural History of Hereditary Multiple Exostoses. *The Journal of Bone and Joint Surgery* 76-A, no. 7:986-992.

103. Seki, Hiroshi, Takeo Kubota, Shiro Ikegawa, Nobuhiko Haga, Fumio Fujioka, and Obzeki. 2001. Mutation Frequencies of *EXT1* and *EXT2* in 43 Japanese Families With Hereditary Multiple Exostoses. *American Journal of Medical Genetics* 99, no. 1:59-62.

104. Shi, Yi-Ru, Jer-Yuarn Wu, Fuu-Jen Tsai, Cheng-Chun Lee, and Chang-Hai Tsai. 2001. An R223P mutation in EXT2 gene causes hereditary multiple exostoses. *Human Mutation.*

105. Shi, Yi-Ru, Jer-Yuarn Wu, Fuu-Jen Tsai, Cheng-Chun Lee, and Chang-Hai Tsai. A 651-665delinsTT mutation in EXT1 causes hereditary multiple exostoses. Human Mutation 17[2], 158. 2001.

106. Silverman F.N. Caffey Paediatric Xray Diagnosis: an integrated imaging approach. [8th edition]. 1985. Yearbook Publishers Inc, Chicago.

107. Simmons, Andrew D., Maurice M. Musy, Carla S. Lopes, Larn-Yuan Hwang, Ya-Ping Yang, and Michael Lovett. 1999. A direct interaction between EXT proteins and glycosyltransferases is defective in hereditary multiple exostoses. *Human Molecular Genetics* 8, no. 12:2155-2164.

108. Smith, T. P., R. A. Godtel, and R. T. Lee. 2000. PCR-based setup for high-throughput cDNA library sequencing on the ABI 3700 automated DNA sequencer. *BioTechniques* 29, no. 4:698-700.

109. Solomon, L. Hereditary Multiple Exostosis. J Bone Joint Surg 45, 292-304. 1963.

110. Stickens, Dominique, Gregory Clines, David Burbee, Purita Ramos, Sylvia Thomas, Deborah Hogue, Jacqueline T. Hecht, Michael Lovett, and Glen A. Evans. 1996. The EXT2 multiple exostoses gene defines a family of putative tumour suppressor genes. *Nature Genetics* 14:25-32.

111. Stickens, Dominique and Glen A. Evans. 1998. A sugar fix for bone tumours? *Nature Genetics* 19:110-111.

112. Stickens, Dominique, Doris Brown, and Glen A. Evans. 2000. EXT Genes Are Differentially Expressed in Bone and Cartilage During Mouse Embryogenesis. *Developmental Dynamics* 2:452-464.

113. Taylor, E., D. Cogdell, K. Coombes, L. Hu, L. Ramdas, A. Tabor, S. Hamilton, and W. Zhang. 2001. Sequence verification as quality-control step for production of cDNA microarrays. *BioTechniques* 31, no. 1:62-65.

114. Towbin, Jeffrey A. 1995. Polymerase Chain Reaction and Its Uses as a Diagnostic Tool for Cardiovascular Disease. *Trends in Cardiovascular Medicine* 5:175-185.

115. Van Hul, Wim, Wim Wuyts, Jan Hendrickx, Frank Speleman, Jan Wauters, Kristel De Boulle, Nadine Van Roy, Paul Bossuyt, and Patrick J. Willems. 1998. Identification of a Third EXT-like Gene (EXTL3) Belonging to the EXT Gene Family. *Genomics* 47:230-237.

116. Vogelstein, B. and K. W. Kinzler. 1992. p53 function and dysfunction. *Cell* 70, no. 4:523-526.

117. Wai, Albert W. K., Ling Jing Ng, Hideto Watanabe, Yoshihiko Yamada, Patrick P. L. Tam, and Kathryn S. E. Cheah. 1998. Disrupted Expression of Matrix Genes in the Growth Plate of the Mouse cartilage matrix deficiency (cmd) Mutant. *Developmental Genetics* 22:349-358.

118. Wei, G., X. Bai, K. J. Barne, T. I. Koshy, PG Spear, and J. D. Esko. Location of the gluconyltransferase domain in heparan sulfate copolynerase EXT 1 by analysis of Chinese hamster ovary cell mutants. J Biol Chem 275, 27733-27740. 2000.

119. Wells, Dan E., April Hill, Xin Lin, Jung Ahn, Nicholas Brown, and Michael J. Wagner. 1997. Identification of novel mutations in the human EXT1 tumor suppressor gene. *Human Genetics* 99:612-615.

120. Wheater, PR., H. G. Burkitt, and V. G. Daniels. Functional Histology. 2nd edition. 1987. Churchill Livingstone.

121. White, Marga Belle, Magda Carvalho, David Derse, Stephen J. O'Brien, and Michael Dean. 1992. Detecting Single Base Substitutions as Heteroduplex Polymorphisms. *Genomics* 12:301-306.

122. White, Marga Belle, Magda Carvalho, David Derse, Stephen J. O'Brien, and Michael Dean. 1992. Detecting Single Base Substitutions as Heteroduplex Polymorphisms. *Genomics* 12:301-306.

123. Wicklund, C. Luckert, R. M. Pauli, D. Johnston, and Jacqueline T. Hecht. 1995. Natural History Study of Hereditary Multiple Exostoses. *American Journal of Medical Genetics* 55:43-46.

124. Wise, Carol A., Gregory A. Clines, Hillary Massa, Barbara J. Trask, and Michael Lovett. 1997. Identification and Localization of the Gene for EXTL, a Third Member of the Multiple Exostoses Gene Family. *Genome Research* 7:10-16.

125. Wold, LE. Mcleod RA. Sim FH. Unni KK. Atlas of Orthopaedic Pathology. 50-55. 1990. W.B. Saunders Company.

126. Wu, Y. Heutnik P. de Vries B. Sandkuijl L. A. van den Ouweland A. M. W. Niermeijer M. F. Galjaard H. Reyniers E. Wilems P. J. Halley D. J. J. Assignment of a second locus for multiple exostosis to the pericentromeric region of chromosome 11. Hum Mol Genet 3, 167-171. 1994.

127. Wuisman, PI. Jutte PC. Ozaki T. Secondary Chondrosarcoma in Osteochondromas. Acta Orthop Scand 68, 396-400. 1997.

128. Wuyts, W., W. Van Hul, Jan Hendrickx, F. Speleman, Jan Wauters, K. De Boulle, N. Van Roy, T. Van Agtmael, Paul Bossuyt, and P. J. Willems. Identification and characterization of a novel member of the EXT gene family, EXTL2. Eur J Hum Genet 5, 382-389. 1997.

129. Wuyts, W., N. Spieker, N. Van Roy, K. De Boulle, A. De Paepe, P. J. Willems, W. Van Hul, R. Versteeg, and F. Speleman. 1999. Refined physical mapping and genomic structure of the EXTL1 gene. *Cytogenetic and Cell Genetics* 86:267-270.

130. Wuyts, W. personal communication. 2002. Conference on Hereditary Multiple Exostosis, Tuscon, Arizona.

131. Wuyts, Wim, Sarvan Ramlakhan, Wim Van Hul, Jacqueline T. Hecht, Ans M. W. van den Ouweland, Wendy H. Raskind, Floris C. Hofstede, Edwin Reyniers, Dan E. Wells, Bert de Vries, Ernest U. Conrad, April Hill, Dmitry Zalatayev, Jean Weissenbach, Michael J. Wagner, Egbert Bakker, Dicky J. J. Halley, and Patrick J. Willems. 1995. Refinement of the Multiple Exostoses Locus (EXT2) to a 3-cM Interval on Chromosome 11. *American Journal of Human Genetics* 57:382-387.

132. Wuyts, Wim, Wim Van Hul, Marina Nemtsova, Edwin Reyniers, Els Van Hul, Kristel De Boulle, Bert de Vries, Jan Hendrickx, Ilde Herrygers, Paul Bossuyt, Wendy Balemans, Erik Fransen, Erik Vits, Lieve Vits, Paul Coucke, Norma J. Nowak, Thomas B. Shows, Laurence Mallet, Ans M. W. van den Ouweland, Julie McGaughran, Dicky J. J. Halley, and Patrick J. Willems. 1996. Positional cloning of a gene involved in hereditary multiple exostoses. *Human Molecular Genetics* 5, no. 10:1547-1557.

133. Wuyts, Wim, Wim Van Hul, Kristel De Boulle, Jan Hendrickx, Egbert Bakker, Filip Vanhoenacker, Florinda Mollica, Hermann-Josef Lüdecke, Bekir Sitki Sayli, Ugo E. Pazzaglia, Gert Mortier, Ben Hamel, Ernest U. Conrad, Mark Matsushita, and Wendy H. Raskind. 1998. Mutations in the EXT1 and EXT2 Genes in Hereditary Multiple Exostoses. *American Journal of Human Genetics* 62:346-354.

134. Wuyts, Wim and Wim Van Hul. 2000. Molecular Basis of Multiple Exostoses: Mutations in the EXT1 and EXT2 Genes. *Human Mutation* 15:220-227.

135. Xu, Lei, Jiahui Xia, Hujun Jiang, Jiangnan Zhou, Hejun Li, Daping Wang, Qian Pan, Zhigao Long, Chaohong Fan, and Han-Xiang Deng. 1998. Mutation analysis of hereditary multiple exostoses in the Chinese. *Human Genetics* 105:45-50.

136. Young, CL. Sim FH. Unni KK. et al. Chondrosarcoma of Bone in Children. Cancer 66, 1641-1648. 1990.

Appendix

Appendix 8.1 Ethics Approval

8.1.1 Ethics Approval Certificate from Children's and Women's Hospital of British Columbia

CHILDREN'S & WOMEN'S HEALTH CENTRE OF BRITISH COLUMBIA

January 14, 1998

Dr. Christine M. Alvarez

Dear Dr. Alvarez,

Your proposed research project, **"Establishing the Genetic Profile of Multiple Hereditary Exostoses in Families of British Columbia"** was reviewed and **APPROVED** by the In-Hospital Research Review Committee of Children's Hospital at its January 14, 1999 meeting. The In-Hospital Research Review Committee approval is valid until February 15, 2001 providing there are no changes in the research procedures.

Sincerely yours,

Nevio Cimolai, MD, FRCPC
Ad Hoc Chair, In-Hospital Research Review Committee

BRITISH COLUMBIA'S CHILDREN'S HOSPITAL
BRITISH COLUMBIA'S WOMEN'S HOSPITAL AND HEALTH CENTRE
SUNNY HILL HEALTH CENTRE FOR CHILDREN

C&W IS AN ACADEMIC HEALTH CENTRE AFFILIATED WITH THE UNIVERSITY OF BRITISH COLUMBIA AND THE B.C. RESEARCH INSTITUTE FOR CHILDREN'S & WOMEN'S HEALTH

Appendix 8.2 Patient Consent Form

British Columbia's
Children's Hospital

Department of Pediatric Orthopedic Surgery

Stephen J. Tredwell, MD, FRCSC, Department Head
Richard D. Beachamp, MD, FRCSC
H. Michael Bell, MD, FRCSC
Kenneth L. B. Brown, MD, FRCSC
Christopher Reilly, MD, FRCSC

Bonita J. Sawatzy, Ph.D., Research
Sharon A. Secord, BSc.N., Nursing Associate

Telephone: (604) 875-3187
Facsimile Line: (604) 875-2275

Letter of Information

Project: Establishing the Genetic Profile of Multiple Hereditary Exostoses in Families of British Columbia

Investigators: Dr. C. Alvarez, Dr. S. Tredwell, Dr. M. Hayden,

Osteochondromas, also known as exostoses, are benign bone tumors, which arise near growth plates at the end of long bones or on flat bones. They can occur as solitary lesions as in solitary osteochondromas (SOC) or in multiples as seen in Multiple Hereditary Exostoses (MHE). Osteochondromas do not usually cause symptoms but on occasion can cause mechanical problems due to their size and or location by causing pain, nerve compression or deformity. Less commonly, they can cause asymmetrical growth of the long bones resulting in limb malalignment or limb length discrepancy. A very rare complication of osteochondromas, particularly in MHE is the transformation of the benign lesion into a malignant one. This however is an exceptionally rare occurrence and usually occurs after skeletal maturity.

It is known that MHE is an inherited condition 95% of the time but may also occur sporadically. On the other hand, solitary osteochondromas are thought to be random occurrences. In MHE, 3 principal genes have been identified. Reported in the literature to date, is that most families with MHE have an abnormality identified in one of these 3 principal genes. No study has been done to confirm whether patients with solitary osteochondromas or patients without a family history of osteochondromas have similar genetic changes.

The purpose of this study is to establish the genetic make-up of families with MHE, patients with multiple lesions but no family history, and patients with solitary osteochondromas. This entails identifying patients with MHE and SOC. This will occur as the patient presents to a regular clinic visit. Dr. Alvarez will be introduced to interested patients and their parent(s) and a brief discussion about the project will occur. If the patient and their direct family are interested they will be entered into the study. This will involve interviewing the patients and their direct family. This interview will take about 1 hour. We are interested in

Letter of consent:

"Establishing the Genetic Profile of Multiple Hereditary Exostoses in Families of British Columbia"

I,_____ understand the above study and hereby give my assent to participate in the study.

Signature_____

Date_____

I, _____ have read and understood the letter of information regarding the above study. I hereby give my consent for_____ (participant)

My_____ (relationship)

to participate in the above titled study.

Signature _____

Date _____

Witness _____

Date _____

Appendix 8.3 EXT 1

8.3.1 EXT 1 – cDNA showing sequence and primer positions (Ref: GenBank Accession: NM_000127)

1 gcgaccgaac gcggcggtcg gcagcgttcg cgcgggggcc tgcgaagcgc tgctcggggc

61 cggcactgcc cgcggggagg acgcgccgcc gccgccaccc agcgccgccg ccgccgccgc

121 ctccagccgg gccgccgcgc gtcccggggg ccggccccgc gagcgcagga gtaaacaccg

181 ccggagtctt ggagccgctg cagaagggaa taaagagaga tgcagggatt tgtgaggtta

241 cggcgcccca gctgcaagat gcactagccg gctgaacccg ggatcggctg acttgttgga

301 accggagtgc tctgcacgga gagtggtgga tgagttgaag ttgccttccc ggggctcatt

361 ttccacgctg ccgagaggaa tccgagaggc aaggcaatca cttcgtcttg ccattgattg

421 ggtatcggga gctttttttt tctcccctct ctctttcttt tcctccgtct tgttgcatgc

481 aagaaaatta cagtccgctg ctcgcccgcc ctgggtgcga gatattcagc cccgctctct

541 cccgtgcatt gtgcaaccca aagatgaaag accgaagggg agaaagttaa agaaatcgcc

601 cacatgcgct ggatcagtcc acggcttggg gaaaggcatc cagagaaggt gggagcggag

661 agtttgaagt cttta caggc gggaagatgg cggactgg ag ctgaaagtgt tgattgggaa

ex 1 a

Exon 1 Start

721 acttgggtga ttcttgtgtt tatttacaa t cctcttgacc caggcag gac ac**atg**caggc

apr 111

781 caaaaaacgc tatttcatcc tgctctcagc tggctcttgt ctcg cccttt tgttttattt

ex 1c

841 cggaggcttg cagttta ggg catcgaggag ccacagccgg ag agaagaac acagcggtag

ex 1b

901 gaatggcttg caccacccca gtccggatca tttctggccc cgcttcc cgg agcctctgcg

ex 1e

961 ccccttcgtt cc tt gggatc aattggaaaa cgaggattc c agcgtgcaca tttccccccg

ex 1d

apr 110

1021 gcagaagcga gatgccaa ct ccagcatcta caaaggcaag aagtgccgca tggagtcctg

apr 211

1081 cttcgatttc acccttgca agaaaaacgg cttcaaagtc ta cgtatacc cacagcaaaa

ex 1g

1141 agggg agaaa atc gccgaaa gttaccaaaa cattctag cg gc catcgagg gctccaggtt

ex 1f

apr 109

1201 ctacacctcg gaccccagc c aggcgtgcct ctttgtc ctg agtctggata ctttagacag

apr 210

1261 aga ccagttg tcacctcagt atgtgc acaa tttgagatcc aaagt gcaga gtctccactt

ex 1i *ex 1i*

1321 gtggaacaat g gtaggaatc atttaatttt taa tttatat tccggcactt gg c ctgacta

apr 107 *ex 1k*

1381 caccgaggac g tggggtttg acatcggcca ggcgatgctg gccaaagcca gcatcagta c

apr 209

1441 tgaaaacttc cgacccaact ttgatgtttc tattcccctc ttttctaagg atcatcccag

1501 gacaggaggg gagagggggt ttttgaag tt caacaccatc cctcctc tca ggaagtacat

apr 108

1561 gct ggtattc aagg ggaaga ggtac ctga c aggga tagga tcagacacc a ggaatgcctt

ex 1m *ex 1l*

apr 207

1621 atatcacgtc cataacgggg aggacgttgt gctcctcacc acctgcaagc atggcaaaga

1681 ctggcaaaag cacaaggatt ctcgctgtga cagagacaac accgagtatg a gaagtatga

exon 2

1741 ttatcgggaa atgctgcaca atgccacttt ctgtctggtt cctcgtggtc gcaggcttgg

1801 gtccttcaga ttcctggagg ctttgcag gc tgcctgcgtc cctgtgatgc tcagcaatgg

exon 3

1861 atgggagttg ccattctctg aagtgattaa ttggaaccaa gctgccgtca taggcgatga

1921 gagattgtta ttacag attc cttctacaat caggtctatt catcaggata aaatcctagc

exon 4

1981 acttagacag cagacacaat tcttgtggga ggcttatttt tcttcagttg agaagattgt

2041 attaactaca ctagag atta ttcaggacag aatattcaag cacatatcac gtaacagttt

exon 5

2101 aatatggaac aaacatcctg gaggattgtt cgtactacca cagtattcat cttatctggg

2161 agattttcct tactactatg ctaatttag g tttaaagccc ccctccaaat tcactgcagt

exon 6

2221 catccatgcg gtgacccccc tggtctctca gtcccagcca gtgttgaagc ttctcgtggc

2281 tgcagccaag tcccagtact gtgcccag at catagttcta tggaattgtg acaagccccc

exon 7

2341 accagccaaa caccgctggc ctgccactgc tgtgcctgtc gtcgtcattg aaggagagag

2401 caag gttatg agcagccgtt ttctgcccta cgacaacatc atcacagacg ccgtgctcag

exon 8

2461 ccttgacgag gacacggtgc tttcaacaac agag gtggat ttcgccttca cagtgtggca

exon 9

2521 gagcttccct gagaggattg tggggtaccc cgcgcgcagc cacttctggg ataactctaa

2581 ggagcggtgg ggatacacat caaagtggac gaacgactac tccatggtgt tgacaggagc

2641 tgct|atttac cacaaatatt atcactacct atactcccat tacctgccag ccagcctgaa
exon 10

2701 gaacatggtg gaccaattgg ccaattgtga ggacattctc atgaacttcc tggtgtctgc

2761 tgtgacaaaa ttgcctccaa tcaaagtgac ccagaagaag cagtataagg agacaatgat

2821 gggacag |act tctcgggctt cccgttgggc tgaccctgac cactttgccc agcgacagag
exon 11

2881 ctgcatgaat acgtttgcca gctggtttgg ctacatgccg ctgatccact ctcagatgag

2941 gctcgacccc gtcctcttta aagaccaggt ctctattttg aggaagaaat a |ccgagacat
pr ex11b

3001 tgagcgact| t tgagg aatcc ggct |gagtgg gggaggggaa gcaagaaggg atgggggtca
apr 215

3061 agctgctctc tcttcccagt gcagatccac tcatcagcag agccagattg tgccaactat

3121 ccaaaaactt agatgagcag aatgacaaaa aaaaaaaagg ccaatgagaa ctcaactcct

3181 ggctcctggg actgcaccag actgctccaa actcacctca ctggcttctg tgtcccaaga
Stop

3241 ctaggttggt acagtttaat tatggaacat taaataatta ttttt**gaa**aa aaaaaaaaaa

3301 aaaa

8.3.2 EXT 1 Translation

```
      M  Q  A  K  K  R  Y  F  I     L  L  S  A  G  S
  1   ATG CAG GCC AAA AAA CGC TAT TTC ATC CTG CTC TCA GCT GGC TCT    15
      C  L  A  L  L  F  Y  F  G  G  L  Q  F  R  A
 16   TGT CTC GCC CTT TTG TTT TAT TTC GGA GGC TTG CAG TTT AGG GCA    30
      C  L  A  L  L  F  Y  F  G  G  L  Q  F  R  A
 31   TCG AGG AGC CAC AGC CGG AGA GAA GAA CAC AGC GGT AGG AAT GGC    45
      L  H  H  P  S  P  D  H  F  W  P  R  F  P  D
 46   TTG CAC CAC CCC AGT CCG GAT CAT TTC TGG CCC CGC TTC CCG GAG    60
      A  L  R  P  F  V  P  W  D  Q  L  E  N  E  D
 61   CCT CTG CGC CCC TTC GTT CCT TGG GAT CAA TTG GAA AAC GAG GAT    75
      S  S  V  H  I  S  P  R  Q  K  R  D  A  N  S
 76   TCC AGC GTG CAC ATT TCC CCC CGG CAG AAG CGA GAT GCC AAC TCC    90
      S  I  Y  K  G  K  K  C  R  M  E  S  C  F  D
 91   AGC ATC TAC AAA GGC AAG AAG TGC CGC ATG GAG TCC TGC TTC GAT   105
      F  T  L  C  K  K  N  G  F  K  V  Y  V  Y  P
106   TTC ACC CTT TGC AAG AAA AAC GGC TTC AAA GTC TAC GTA TAC CCA   120
      Q  Q  K  G  E  K  I  A  E  S  Y  Q  N  I  L
121   CAG CAA AAA GGG GAG AAA ATC GCC GAA AGT TAC CAA AAC ATT CTA   135
      A  A  I  E  G  S  R  F  Y  T  S  D  P  S  Q
136   GCG GCC ATC GAG GGC TCC AGG TTC TAC ACC TCG GAC CCC AGC CAG   150
      A  C  L  F  V  L  S  L  D  T  L  D  R  D  Q
151   GCG TGC CTC TTT GTC CTG AGT CTG GAT ACT TTA GAC AGA GAC CAG   165
      L  S  P  Q  Y  V  H  N  L  R  S  K  V  Q  S
166   TTG TCA CCT CAG TAT GTG CAC AAT TTG AGA TCC AAA GTG CAG AGT   180
      L  H  L  W  N  N  G  R  N  H  L  I  F  N  L
181   CTC CAC TTG TGG AAC AAT GGT AGG AAT CAT TTA ATT TTT AAT TTA   195
      Y  S  G  T  W  P  D  Y  T  E  D  V  G  F  D
```

196 TAT TCC GGC ACT TGG CCT GAC TAC ACC GAG GAC GTG GGG TTT GAC 210
I G Q A M L A K A S I S T E N
211 ATC GGC CAG GCG ATG CTG GCC AAA GCC AGC ATC AGT ACT GAA AAC 225
F R P N F D V S I P L F S K D
226 TTC CGA CCC AAC TTT GAT GTT TCT ATT CCC CTC TTT TCT AAG GAT 240
H P R T G G E R G F L K F N T
241 CAT CCC AGG ACA GGA GGG GAG AGG GGG TTT TTG AAG TTC AAC ACC 255
I P P L R K Y M L V F K G K R
256 ATC CCT CCT CTC AGG AAG TAC ATG CTG GTA TTC AAG GGG AAG AGG 270
Y L T G I G S D T R N A L Y H
271 TAC CTG ACA GGG ATA GGA TCA GAC ACC AGG AAT GCC TTA TAT CAC 285
Y L T G I G S D T R N A L Y H
286 GTC CAT AAC GGG GAG GAC GTT GTG CTC CTC ACC ACC TGC AAG CAT 300
G K D W Q K H K D S R C D R D
301 GGC AAA GAC TGG CAA AAG CAC AAG GAT TCT CGC TGT GAC AGA GAC 315
N T E Y E K Y D Y R E M L H N
316 AAC ACC GAG TAT GAG AAG TAT GAT TAT CGG GAA ATG CTG CAC AAT 330
A T F C L V P R G R R L G S F
331 GCC ACT TTC TGT CTG GTT CCT CGT GGT CGC AGG CTT GGG TCC TTC 345
R F L E A L Q A A C V P V M L
346 AGA TTC CTG GAG GCT TTG CAG GCT GCC TGC GTC CCT GTG ATG CTC 360
S N G W E L P F S E V I N W N
361 AGC AAT GGA TGG GAG TTG CCA TTC TCT GAA GTG ATT AAT TGG AAC 375
Q A A V I G D E R L L L Q I P
376 CAA GCT GCC GTC ATA GGC GAT GAG AGA TTG TTA TTA CAG ATT CCT 390
S T I R S I H Q D K I L A L R
391 TCT ACA ATC AGG TCT ATT CAT CAG GAT AAA ATC CTA GCA CTT AGA 405
Q Q T Q F L W E A Y F S S V E
406 CAG CAG ACA CAA TTC TTG TGG GAG GCT TAT TTT TCT TCA GTT GAG 420
K I V L T T L E I I Q D R I F
421 AAG ATT GTA TTA ACT ACA CTA GAG ATT ATT CAG GAC AGA ATA TTC 435
K H I S R N S L I W N K H P G
436 AAG CAC ATA TCA CGT AAC AGT TTA ATA TGG AAC AAA CAT CCT GGA 450
G L F V L P Q Y S S Y L G D F
451 GGA TTG TTC GTA CTA CCA CAG TAT TCA TCT TAT CTG GGA GAT TTT 465
P Y Y Y A N L G L K P P S K F
466 CCT TAC TAC TAT GCT AAT TTA GGT TTA AAG CCC CCC TCC AAA TTC 480
T A V I H A V T P L V S Q S Q
481 ACT GCA GTC ATC CAT GCG GTG ACC CCC CTG GTC TCT CAG TCC CAG 495
P V L K L L V A A A K S Q Y C
496 CCA GTG TTG AAG CTT CTC GTG GCT GCA GCC AAG TCC CAG TAC TGT 510
A Q I I V L W N C D K P L P A
511 GCC CAG ATC ATA GTT CTA TGG AAT TGT GAC AAG CCC CTA CCA GCC 525
K H R W P A T A V P V V V I E
526 AAA CAC CGC TGG CCT GCC ACT GCT GTG CCT GTC GTC GTC ATT GAA 540
G E S K V M S S R F L P Y D N
541 GGA GAG AGC AAG GTT ATG AGC AGC CGT TTT CTG CCC TAC GAC AAC 555
I I T D A V L S L D E D T V L
556 ATC ATC ACA GAC GCC GTG CTC AGC CTT GAC GAG GAC ACG GTG CTT 570
S T T E V D F A F T V W Q S F
571 TCA ACA ACA GAG GTG GAT TTC GCC TTC ACA GTG TGG CAG AGC TTC 585
P E R I V G Y P A R S H F W D
586 CCT GAG AGG ATT GTG GGG TAC CCC GCG CGC AGC CAC TTC TGG GAT 600
N S K E R W G Y T S K W T N D
601 AAC TCT AAG GAG CGG TGG GGA TAC ACA TCA AAG TGG ACG AAC GAC 615
Y S M V L T G A A I Y H K Y Y
616 TAC TCC ATG GTG TTG ACA GGA GCT GCT ATT TAC CAC AAA TAT TAT 630
H Y L Y S H Y L P A S L K N M
631 CAC TAC CTA TAC TCC CAT TAC CTG CCA GCC AGC CTG AAG AAC ATG 645

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      V  D  Q  L  A  N  C  E  D  I  L  M  N  F  L
646    GTG GAC CAA TTG GCC AAT TGT GAG GAC ATT CTC ATG AAC TTC CTG    660
      V  S  A  V  T  K  L  P  P  I  K  V  T  Q  K
661    GTG TCT GCT GTG ACA AAA TTG CCT CCA ATC AAA GTG ACC CAG AAG    675
        K  Q  Y  K  E  T  M  M  G  Q  T  S  R  A  S
676    AAG CAG TAT AAG GAG ACA ATG ATG GGA CAG ACT TCT CGG GCT TCC    690
      R  W  A  D  P  D  H  F  A  Q  R  Q  S  C  M
691    CGT TGG GCT GAC CCT GAC CAC TTT GCC CAG CGA CAG AGC TGC ATG    705
      N  T  F  A  S  W  F  G  Y  M  P  L  I  H  S
706    AAT ACG TTT GCC AGC TGG TTT GGC TAC ATG CCG CTG ATC CAC TCT    720
      Q  M  R  L  D  P  V  L  F  K  D  Q  V  S  I
721    CAG ATG AGG CTC GAC CCC GTC CTC TTT AAA GAC CAG GTC TCT ATT    735
      L  R  K  K  Y  R  D  I  E  R  L
736    TTG AGG AAG AAA TAC CGA GAC ATT GAG CGA CTT TGA
```

Appendix 8.4 EXT 2

8.4.1 EXT 2 – cDNA showing sequence and primer positions (Ref: GenBank Accession: NM_000401 Version NM_000401.1)

EXT 2 exon 1

1 tcgaggttgc tgcccggaag cctctgtagg tatctagtct gagaatcatc actttgaata

61 tttaagctat cagtgacaac ttccaccaga tggcgccaaa gtacatctgg gaccagaagg

121 gatttggatc ctgtagccag acccacaact ttaccaaacc aacatcgcag gcccaggggt

181 catttcatta acctctcaat aacatcgctc tgaattttaa tttaattttt tagtttccac

241 ttactgcttt atgacagcgg ttttagtgtg catggatagg gctaaatcat gtaaataata

301 gagaaagata caaaacaaaa atgcgttttt ttttttttt tttttggaga cagggtcttg

361 ctctatcacc caggctggag tgcagtggca cgaccacggc ccactgcagc cttgacctcc

421 tgggctcaag caatcttcct gcctctgcct cctaagtagt tgggactaca agcgtgtgct

481 acgatgccta gttaactttt tatttttgt agagatgggt cttgctctgc tgcccaggct

541 ggtctcaaac tcttgggctc aagcgatcct ctcgtttagg cctccccaaa tgctggaatt

601 acaggcgtga gccaccttgc ctcgccataa atgcttccat ttccgcctcg acaactactc

661 cacctgaagc tgttcatttc ttcttgcatt ccttccagaa aaaagttata cacatgcctg

721 aatataagca cctactttat atatttctcc ctcttgtttt tgcatatgca tagtttacct

781 aaaagtgact tgcccgctgt tttggactac gctttgatct taactaatat cttggagata

841 tttccttacc caaatatatt gcactatctc acattactca aatcaatcaa attccataat

901 ttatttcgat tgtgtctagc atttcgctat gattagaaag aatgctgtca tggaacttt

961 tgacaaacat tgttgagaat atccataggg caaactccgt acagagagct tgttggaatg

1021 aagggtacca gcattttccg tttgatggat agtaccaaat tgccctccag gaatgttata

1081 cgctcaccag aactgattat aataaaacgt ctacatattt gttagtttta taagcaacgc

1141 gtggtgtctc gtttgggttt aaggattctt taattatgaa tgagg ctgtc tgagcatttc

exon 1

1201 actgcggagc ctgagcgcgc ctgcctggga aaacactgca gcggtgctcg gactcctcct

1261 gtccagcagg aggcgcggcc cggcagctcc cgcatgcgca gtgcgctcgg tgtcagacgg

1321 cccggatccc ggttaccggc ccctcgctcg ctgctcgcca gcccagactc ggccctggca

1381 gtggcggctg gcgattcgga ccgatccgac ctgggcggag gtggcccgcg ccccgcggca

1441 tgagccggtg accaagctcg gggccgagcg ggaggcagcc gtggccgag|g taagcgcggc
intron 1

1501 tctccagggc agcggccggg cgggcgctga ggcgagggct ctggcctccg ggggccgctg

1561 ctgggtcggg acaagggccg agggagcgcg gccgcgcgga ggctccctgg aggcccgtgg

1621 gctgcga

EXT 2 exon 1a

1 tcctccggcg gcggccgcgc tttcagcatc ttggtaccca cctgttctcc tagccaacct

61 tcgcccccag tccgctcctt cctttcctcc tgcgacccgc cctccgccct ccgcggcgac

121 ccctcccttc ctgctgccac cttcccgcca g|ccacaggga tctgattcct cccaggggga
exon 1a

181 tgtcctgcgc ctcagggtcc ggtggtggcc tgcggcatcc cttgcggtgc cagaagccgt

241 gggacgag|gt acggaagggg ccaggggcat gtaaggccgg ggactgggtg gtcgggggcg
intron 1a

301 tgtcaggccg gggactgggt gaccgggaac tagatggccg ggggcgtgtt a

EXT 2 exon 1b

1 acattcagtc tgttgcagtg tcatatgtca tgtagcctct ggaaaatgga agtgaataaa

61 gcaaacgtca gtattaaact agtataagcc ctttgaaagg gcctgggatg cctgaagcat

121 acttcaagaa ccagtgttct aagattttgg tatgaagcat ttgctagcct cctaaactga

181 gctctgaagc gtttcctctt ttttttctga actctggaat aatttgtgga aaattggaat

241 tattatttct tgaatatttg gtagaacttg gaaaattttc tgggcctgga attttcttta

301 taggaagatt tttaaacttt tgattcagtg tctttaa|tgt tatagagcta ctcagagttg
exon 1b

361 ctgtttctcc ttgagatgct tttg|gtaagt atattttaaa ataatttttc catgttatct
intron 1b

421 gagttttcaa atgtactggc ataaattcat tgataccatc ttatctttta aatatatgca

481 gcatttagag ttatgttccc cttttcaggt atttatttgc acctttttcc ttgaattctt

541 gattaatctt accagaattt tattagtctg tttttaaaca aacaactttt agccttgttg

601 accatttcta ttttgttttt taattaattt ctgtgctttt atttattatt ttctcctgtt

661 atctttggtt ttactttgtt gtgatatgtc ttttttatag ttaatctgca actcataaag

721 atttgtgaag ctcatgtgtg aatacagttt ttgttccctt aacctcaatt ttgtcataca

781 tagaagctat ttttcaagct atggaggacc cattattggg tggtataatt gatctagtag

841 gt

EXT 2 exon 2

1 tgcactccag cctgagtgac agagtgaaac cctgtctcaa aacaaaacaa aacaaaaaaa

61 aaggttgaat agtcttttca agtgtcattt gccatcctaa atacttggtt tttcttattt

121 ctctcccctg gtgacca g ga gtgtgaggaa gaggctgtct gtgtcattat gtgtgcgtcg
ex 2a *exon 2*

181 gtcaagtata atatccgggg tcctgccctc atcccaagaa tgaagaccaa gcaccgaatc

241 tactatatca ccctcttctc cattgtcctc ctgggcctca ttgccactgg catgtttcag

301 ttttggcccc attctatcga gtcctcaaat gactggaatg tagagaagcg cagcatccgt

361 gatgtgccgg ttgttaggct gccagcc gac agtcccatcc cagagcgg gg ggatctcagt
ex 2A26

421 tgcagaatgc aca cgtgttt tgatgtctat cgctgtg gct tcaacccaaa gaacaaaatc
ex 2A8

481 aaggtgtata tctatgctct gaaaaagtac gtggatgact ttggcgtctc tgtcagcaac

541 accatctccc gggagtataa tgaactgctc atggccatct cagacagtga ct actacact

601 gatgacatca accg gg cctg tctgtttgtt ccctcc atcg atgtgcttaa ccagaacaca
ex 2A4 *ex 2A25*

661 ctgcgcatca aggagacagc acaagcgatg gcccagctct ctag gtatct cacactcata
intron 2

721 cagcccagcc cccaggagat acttgagt gg ccctcaggga actaaaggg a agggaaggat
ex 2b

781 gggaatgctt ctgctcttga gttggtttcc cgatgctgtc ttcttgcagg acggggtgtg

841 ttggagggac tgac

EXT 2 exon 3

1 tatatttcca aattatgaca taattttatg ttcttttact atataacttt aagggttgca

61 tagtattcca ttttgcagat gttctaccat atatttaacc aggcttctct aatgtatttt

121 gtgtttcttt aaccaaatgg tgaacatttg ggtagttttc aacttttcat tattagaagc

181 aggtctgtat gggacaagct tgaagtacac gtgcgttcat ttttcccctg tcatggagcc

241 agacttgtgt ctgatgtgct gttgggattt ccaggagttt gctttgcata cctgagaagc

301 ggccctattt gggcttgggg atccttgata gttgttgtct agtaactgac tcttgtcttt

361 tcata|gttga cacattaatt ctccca| catt ttaaattttt tgacag|gtgg gatcgaggta
ex 3a *exon 3*

421 cgaatcacct gttgttcaac atgttgcctg gaggtccccc agattataac acagccctgg

481 atgtccccag agacag gtag gaggcatatg tggggctgtc cttatgat gg|gttcaagatc
intron 3 *ex3b*

541 attttgttc|a tgtgaaatta tattcctaaa tctaccacat actttgtaat cagaattgtt

601 tattaaacta gaaaattgtc ataagtattt tcctcctgaa gatttagaag tgcttaaatc

661 ttttatggaa aaccagttag ggcttatgtc ctggcatacc ctctaaaact gttttcccac

721 tctggattgt gcacttctga gtgtaacaca tccagccccc aaaagtgtga caggcttgtg

781 ctacctctct ctgaattcgg gagcatttgc cacaagtaga tgcacagctt actgagagaa

841 ggt

EXT 2 exon 4

1 gtaaatgtgt ttatttataa agtatgacta gggagaggtg aatgggatct gagggaggta

61 gcagagaggc tgtccgtaag gtgtcttctg gactatgatg tgtttcaaaa actgggaagt

121 aaggaaaggg tatttaggac cccgggggaa ggctggtgat tcaaggatag aacgcagctg

181 atggccccga gatgcgtgta taaggcattg tctttataga aaactgactc tgtaaacgtt

241 agctggtttt gataataaag actcagtaat tcctgttcct ctccacagtg tgtatca|gaa

301 taaagtcctt tctttctcat cg| tttaacaa aatactttgc tttcag g |gcc ctgttggctg
ex 4a *exon 4*

361 gtggcggctt ttctacgtgg acttaccggc aaggctacga tgtcagcatt cctgtctata

421 gtccactgtc agctgaggtg gatcttccag agaaaggacc agg gtaaggt acattcatcc
intron 4

481 ca|gccaggtg tgcctttact g|aatctgtga gatgttgatg aggtttagtg tggtgggcat
ex 4b

541 caaagcaacc aatacatcag ttacagggta gggtccttga ggcactgagg cacccatctt

601 tcccacctcc atgcagtctc attcatcttg cagttttctc tgtctcctta aattcacagt

661 gctgtctacc aagttttcta agccaggaat ccatgtggta tccttaactc cgttctctcc

721 tttgtttcct atatcaaagt aagaagtcgt attgattctg catcctaaat acttcctatg

781 tctgtctgct ccccgaa

EXT 2 exon 5

1 aaaatcagtg gagtgaagac tggtaaggaa cacttactgt cgtaagttta atatcaaagt

61 ttgtcttacc tggactaaca taccagct[gc aattttccaa tcacctg] ttt ttttcccttg
ex 5a

121 tag[tccacgg caatacttcc tcctgtcatc tcaggtgggt ctccatcctg agtacagaga
exon 5

181 ggacctagaa gccctccagg tcaaacatgg agagtcagtg ttagtactcg ataaatgcac

241 caacctctca gagggtgtcc tttctgtccg taagcgctgc cacaagcacc aggtcttcga

301 ttacccacag gtgctacag] g tgagtgtcat tcatta[cctc tcgcaaaggc tcagg agagt
intron 5 *ex 5b*

361 ttgcttacat gggttaaaat tgagcccagc gaacctgagt tgtttttcag catgcaacta

421 gaattaccca gggggaagaa aacatagcat tgctctttac tggacatgta gaccttcagg

481 tacttggatg tctggtgtct tgtgttcgtg caaagctgct tggcctatga gagtctatac

541 tcctttcaga tattcattat acttcaaaaa ga

EXT 2 exon 6

1 tgaggtaagt actgtaagag atgtcagaca gtgtgccgtg gtgtgtttac atagtacata

61 gggcttaaag agacccattt gcaggaagtc acgttgttag ctgtctaagg gaagactttg

121 acattgacct tgaacatttt cagaaggcca acagtggtgg cattgaagca atactgaaga

181 gtagaaatat taatacaaaa cattgcagcc atttaaactt ttcaagtttt acaggtgtga

241 gctgttgtct tttggcattt ttgtgtcaag atgcctcagt attgcttggc gtcaaccctt

301 gtagaaactt tgtggtctgt agggatcaaa gttagtggat cagcaaaa ct agtttgtaat

ex 6a

361 ctcttgcctc tttgtgttcc tgcag gaggc tactttctgt gtggttcttc gtggagctcg

exon 6

421 gctgggccag gcagtattga gcgatgtgtt acaagctggc tgtgtcccgg ttgtcattgc

481 agactcctat attttgcctt tctctgaagt tcttgactgg aagag gtggg tagtacctcc

intron 6

541 tagtaaa ctc tacattagtg gttctgcgta tattacaaat aaaatctcct caggtcattg

ex 6b

601 taatgtatac cctgttcaag aactactaca gatagttttt ctctattttc cattaggaga

661 gttagtacac tggtctagag cagttcacaa accaaggcca gtttgcaggc tggctgtttt

721 tgtaagtcaa gttttattga aacacagccg tgtcccttcc tttacgtata gtctggctgt

781 tattgtgtca cattggtaga gttaagtaat tgcaacagaa attggatgac atacagggct

841 taaatatcac tatctggcct ttcatcacag gggtccccaa ctcccgggct gtggcctgtt

901 tggagccggg

EXT 2 exon 7

1 tatgccagat aaatgaatag atttgcatag atagctaaag gagaaaagta tttgttaact

61 tagaatggaa taaaggaaga gtgtactagg tgggtgggat ttcacatgca aaggccctct

121 ggtagggcag agcatggtgt gttcaagtga ctgaaataat accagtgtgg ctagagcaca

181 ctagtggagt ggaggcaggg tgaaagatta atggagtagg gagtgggagg taaaaaaatg

241 gagctgtaag agaactcctt tgagaagttc agccagtgaa gaagggaggg gaaagagaca

301 atacttaccg gaa gggatgt ggggctgaag gagg tttggg atgttgtttc tgcttgtgaa

ex 7a

361 atgaaacaag actgtgtgta gaaatgcttt ctgtgaaggg ctgtgtgtat gtaaactgtt

421 ttgctgttgt ctccag agca tctgtggttg taccagaaga aaagatgtca gatgtgtaca

exon 7

481 gtattttgca gagcatcccc caaagacaga ttgaagaaat gcagagacag gtaagaggcc

intron 7

541 aagtcttggg gaggtgacat gggtggtacc gaaatggtgg cctt gactgg atacagaggg

ex 7b

601 acaggag ctg aatgcctgag tggggtttac ttcctccact agatcaacta gccaaactga

661 aacgaaagga aattaatgtt aggtgagttg catcaaataa ggtttgaaat aataactctc

721 agagaactgt gcagaggtaa gcctactgca attttagggt cttaccatag cagatgcaaa

781 gctgaagctc tttggagggt ttgtagtcac agcaggtgat agtcgtagtg actaagacag

841 ccatggaagc tggaccattt cagggcaata cttctgtgta gctattgacc atgatacatt

901 gcggcacaaa ctagcccagc tt

EXT 2 exon 8

1 tttatctgga tactaattgt aagagtatgt acatatgtat aaattcattg agctgtacac

61 aagatttgtg cactttatgt tatataagac aaaatactat aaactctgcc ataacacatg

121 gatattctca tcatcacata atttatcttc tatcttaatt gaatccaatg tgcatttcac

181 ttgctaacat tttattttga ctgcatttga taaatgccaa cttctgatgg cagctggctt

241 gaacagcagg gagcatatgc cctaggcacc cccatcccta caactttggg aataaaggaa

301 ttagcctaac ctggagttga ctatgataga gtatctagtt ttcccactct gtctc|gcttg
ex 8a

361 ctcacttaaa ac| agcattat ttttttttata g gcccggtgg ttctgggaag cgtacttcca
exon 8

421 gtcaattaaa gccattgccc tggccaccct gcagattatc aatgaccgga tctatccata

481 tgctgccatc tcctatgaag aatggaatga ccctcctgct gtg gtaagtg aattcca gtg
intron 8

541 ctagccac|at gaggcatggt ccagctgtca gggtgggtgg aaggaaaaat gtactaccat
ex 8b

601 tgtaaaggtt atttaaattc tagctttcta agatgagagt gtgcttttta tacttg |gggc
ex 8c

661 ctgataaggg cagcataa|tt ttgaaacact gacaaaagta aaaaatacgg aagcagcagc

721 ttccagtgtg ttttaagtgc ttacaaagac tgtctattta ttgcagagat aagtaaggag

781 gcatgggtct tgttggaaat caaagacatc ccggtgactt ttgcaattgt aatgcttaga

841 gcttttgaaa aacttctgta agc

EXT 2 exon 9

1 gtctcttctc ccatctcttt gtccttgtag atttatattc ttttatattc atcaactgcc

61 tttttattgg gtttggggag agaatggaga taaacgcatg ctttaatctg tcatgtttaa

121 ctagaattct tttctcagct gcaaaagttc tcagctcctt ttccagtgat atcagaacca

181 aacttaatta gtccatgcaa attttgagga ggggaagact ttgagcagtt gcttagctct

241 gggatctgtc ctggtaaaag ccatcaagcc tgccatgttt gggtttgctg acgatattgg

301 gtcagccata ttgtta cagc tgcttttctg acccg tgtta atctgtcctc ttgtag aagt
ex 9a

361 ggggcagcgt gagcaatcca ctcttcctcc cgctgatccc accacagtct caagggttca
exon 9

421 ccgccatagt cctcacctac gaccgagtag agagcctctt ccgggtcatc actgaagtgt

481 ccaaggtgcc cagtctatcc aaactacttg tcgtctggaa taatcagaat aaaaaccctc

541 cagaag gtaa gaagcctta g tgcctctctc agctggatc a attttggatg gccaaattat
intron 9 *ex 9b*

601 tcacatcctt tgttttaaat aaatttcct gctttgtcaa tagcaatacc atttctgaga

661 cagcatgcct ccatttttct cagtcatctc attcttgttc tagggtggcc catctaactc

721 caagccctgg catactctgt agccacaagt g

EXT 2 exon 10

1 gaagccaatt tgttcattct agttaggaca gtattgagaa ttagtagtgt tacaaggatt

61 tagagaggat aaatatgtat gtatatagta tgtgtgtata tatgtagtat atatgtgtat

121 gtgcagtata tatatttttt attataacaa agatgcatct gtgagaatct cccctgacac

181 agttctacct atggatttga tgagagccgt ggatacaagc tgattctccc atctcatttg
241 tgatgtcatg cttttactac tttatct cct cacaaaagtt aggag aatag taaatacctt
ex 10a

301 ttctcttttt ccag attctc tctggcccaa aatccgggtt ccattaaaag ttgtgaggac
exon 10

361 tgctgaaaac aagttaagta accgtttctt cccttatgat gaaatcgaga cagaagctgt

421 tctggccatt gatgatgata tcattatgct gacctctgac gagctgcaat ttggttatga

481 g gtaagga gg ttttacacag tgtgttt ata tgtttaatat tacttcctat gactgcttgt
intron 10 *ex 10b*

541 cttttctaaa aaagagtatt atatttcctt cttaaaagtc agagttctaa aatcttccag

601 tagagtccaa aaggtgtgcg taagagtgtg ggttatgaag ctgttctttg aagcactgga

661 gaaaccctat tccaaaatgg caactgtgcc ctccactggt tttgggaact cccaagggag

721 agtcccaggg gacaatttca aaagagcatc tatagcattt aacaagcact taattgatgt

781 ctccttgaat accacttccc ttgactcaag cagct

EXT 2 exon 11

1 taatacaaat cagggcagtt gagttgcagg gttccattta tccttcattt tgtgaattca

61 ttaagaatgg aacctatttc attaatcata tgttaagaga ttgcgtacct tggcccaact

121 cagtaagcta ttacccccac attaattgaa attcccaagc atatacaaga agattgggag

181 gaagtcagaa tcagcatctg tctttgagtt ttggcagaat aactaacacc tgtttgatgg

241 aacatctcca gaatcccatt atgaccttct taggttatga tggtttgaac ctaggaagtc

301 tgttgatacc tgtttggata actcagcact gaatggttgc tgtctgaatt ggg acttgat

ex 11a

361 tgttattatg tgtctgtcct tag gtctggc gggaatttcc tgaccggttg gtgggttacc

exon 11

421 cgggtcgtct gcatctctgg gaccatgaga tgaataagtg gaagtatgag tctgagtgga

481 cgaatgaagt gtccatggtg ctcactgggg cagcttttta tcacaag gta agggggcgca

intron 11

541 gtcct ggcaa ggtgacaaaa ctgag agaat gatacacatt ttatttgacc caatttaatt

ex 11b

601 tttcatacct gccaagaggg cttagaaaag ccatattgtg tgacagtatt ttacaaataa

661 agctatcctt tttctaatta taaaagtaat gcacgctcat agtagaaaat atgaaaatag

721 aatgaagaaa agttacttgt aatcctgtca cttcgagata accattttat cattcaggtg

781 ctatttccag cttgccgttt atttatttac ttacttgtat gtatacacag acagttgtaa

841 atattctcat tagcctgctt tttcatggat tgtattgtga gccttttctc atgtcattga

901 catttcttca taaacagtta cttgttagca taattaagat accattactt aatgttttag

961 aagtcatgta taactatttt gctatcgtgg atattacttt ctaaattttg ctattt

EXT 2 exon 12

1 tttagctgta ttcatatcga ttgtttgttg ttagctcagc actactgcct catatttttc

61 aggtctctag atccagaaat gggttttttt attttgtaat aagcaaacaa aaaaacccaa

121 aaactaaggt ttacaattca tgggatttac agtagtagac tatgtatgct ttatttttttt

181 tgacccaata atttctacac tatttcatat atatggtagt tttagaattg cctcattttt

241 cttctacttt aaaaagcaca cactttggta gaaaatgacc atattgaaca tgcttggtca

301 cttgaccaaa agcattctaa tgcctccttt tacccttcct attaatacag ccttgtgatt

361 aatcttatga gagaaagctt gtccccatgc cttggctatg ctg|ccccttа tttatcagct

ex 12a

421 aaaggg|aact gctatttttg aatatttctt ctttctgtct cacttgacag [tattttaatt

exon 12

481 acctgtatac ctacaaaatg cctggggata tcaagaactg ggtagatgct catatgaac] t

541 gtgaagatat tgccatgaac ttcctggtgg ccaacgtcac gggaaaagca gttatcaagg

intron 12

601 tagga |ggctc tgccactcac ttg| ctttgtg atcttgggca aatatctatt atctgagcct

ex 12b

661 aggaagttct tgtaactata aattaaatat aggactagat aaactttaag ctctatttta

721 gtttaaggtt ctatgattga tgcggtcaca ttgggaaatt gaagctaggc tttgacaatt

781 taaacatatt ttctttttt atacagtttc tttagttgca gtttttaacc ttcagtatca

841 gaataaaggc tatgatgatc agtctataaa tcaaaaaatt atattctcaa agctg

EXT 2 exon 13

1 gggaagctgt atttcatcgc ccttatgggt acaagaacaa atggtgttta tacaaggacc

61 ttggcagtga gaaaacagtc attaaacagg aattaaggag cttgtcatca ccacttcttt

121 ccagttacag aaggcaaaag ccctccaagc ctttttatt gggcccttgt gagttctgcc

181 gttggctgag ccagacagag ttgaatggag gaatggcgag gtgtgtgtgt gtgtgtgtgc

241 acgcgcatgc aacatctcag cttacaacac aaaagaatgc agtgtggtgt cacaagcatg

301 attttatt|gt ccttgacact gacagccagg| tatgtttttg tcctcctctg gcag [gtaacc

ex 13a *exon 13*

361 ccacgaaaga aattcaagtg tcctgagtgc acagccatag atgggctttc actagaccaa

421 acacacatgg tggagag] gta agtgagcctc caaccaaaag t|gcgccttag cctctgatct

intron 13 *ex 13b*

481 cta|tttcctg ccttag gcct gtttatgggg ctttgttgga gatataagga cagcagctgg

541 tagccatagt cacctccatg tgcactgtgg gaattgggtt agttcaagcc caggtcaccc

601 aaagaattaa tttggaatgc tactcactca atttgtaatg gctggaaggg tcttaaaaat

661 atagtgggcc ttaagctcca gaagccaaat tctccatgtg gactaagcag ttaaccatct

721 acagtcattg agtggaagct agttaattcc aaggaaatac tggattattt ttc

EXT 2 exon 14

1 ccttttaag aacctgggag cagactgtgg ctactgagct ttttttgttg atgttgaaca

61 ttatgtattt tgctgttatc tctcaacctc ttgaacatac tatcttttct ccctgccccc

121 atccttctca ttctgct caa acccctcctc cccacctcct c tccaaatcc cacag gtcag

ex 14a

181 agtgcatcaa caagtttgct tcagtcttcg ggaccatgcc tctcaaggtg gtggaacacc

exon 14

241 gagctgaccc tgtcctgtac aaagatgact ttcctgagaa gctgaagagc ttccccaaca

301 ttggcagctt atgaaacgtg tcattggtgg aggtctgaat gtgaggctgg gacagaggga

361 gagaacaagg cctcccagca ctctgatgtc agagtagtag gttaagggtg gaaggttgac

421 ctacttggat ctt ggcatgc acccacctaa cccac tttct caagaacaag aacctagaat

ex 14c

481 gaatatccaa gcacctcgag ctatgcaacc tctgttcttg tatttcttat gatctctgat

541 gggttcttct cgaaaatgcc aagtggaaga ctttgtggca tgctccagat ttaaatccag

601 ctgaggctcc ctttgttttc agttccatgt aacaatctgg aaggaaactt cacggacagg

661 aagactgctg gagaagagaa gcgtgttagc ccatttgagg tctggggaat catgtaaagg

721 gtacccagac ctcactttta gttatttaca tcaatgagtt ctttcaggga accaaaccca

781 gaattcggtg caaaagccaa acatcttggt gggatttgat aaatgccttg ggacctggag

841 tgctgggctt gtgcacagga agagcaccag ccgctgagtc aggatcctgt cagttccatg

901 agctattcct ctttggtttg gctttttgat atgattaaaa ttatttttta ttccttttc

961 tactgtgtct taaacaccaa ttcctgatag tccaaggaac caccttttctc ccttgatata

1021 tttaactccg tctttggcct gacaacagtc ttctgcccat gtctgggaac acacgccagg

1081 aggaatgtct gataccctct gcatcaagcg taagaaggtc ccaaatcata accattttaa

1141 gaacagatga ctcagaaacc tccagaggaa tctgtttgct tcctgattag atccagtcaa

1201 tgttttaaag gtattgtcag agaaaaacag agggtctgta ctagccatgc aaggagtcgc

1261 tctagctggt acccgtaaaa gttgtgggaa ttgtgacccc catcccaagg ggatgccaaa

1321 atttctctca ttcttttggt ataaacttaa cattagccag ggaggttctg gctaacgtta

1381 aatgctgcta tacaactgct ttgcaacagt tgctggtata tttaaatcat taaatttcag

1441 catttactaa t actgcacat gtgtgaatta tacctcttta agcccagttg atgaacaaat
intron 14

1501 ctaccctggc aaatgttaaa tgttatggat tcgaaacaga tttatctggc tctgatatta

1561 agattagcca cagtttgggc tttagccaca acatatgtcc ccaaaacaca aaatacataa

8.4.2 EXT 2Translation

```
   M  C  A  S  V  K  Y  N  I  R  G  P  A  L  I
 1 ATG TGT GCG TCG GTC AAG TAT AAT ATC CGG GGT CCT GCC CTC ATC  15

   P  R  M  K  T  K  H  R  I  Y  Y  I  T  L  F
16 CCA AGA ATG AAG ACC AAG CAC CGA ATC TAC TAT ATC ACC CTC TTC  30

   S  I  V  L  L  G  L  I  A  T  G  M  F  Q  F
31 TCC ATT GTC CTC CTG GGC CTC ATT GCC ACT GGC ATG TTT CAG TTT  45

   W  P  H  S  I  E  S  S  N  D  W  N  V  E  K
46 TGG CCC CAT TCT ATC GAG TCC TCA AAT GAC TGG AAT GTA GAG AAG  60

   R  S  I  R  D  V  P  V  V  R  L  P  A  D  S
61 CGC AGC ATC CGT GAT GTG CCG GTT GTT AGG CTG CCA GCC GAC AGT  75

   P  I  P  E  R  G  D  L  S  C  R  M  H  T  C
76 CCC ATC CCA GAG CGG GGG GAT CTC AGT TGC AGA ATG CAC ACG TGT  90

   F  D  V  Y  R  C  G  F  N  P  K  N  K  I  K
91 TTT GAT GTC TAT CGC TGT GGC TTC AAC CCA AAG AAC AAA ATC AAG 105

   V  Y  I  Y  A  L  K  K  Y  V  D  D  F  G  V
106 GTG TAT ATC TAT GCT CTG AAA AAG TAC GTG GAT GAC TTT GGC GTC 120

   S  V  S  N  T  I  S  R  E  Y  N  E  L  L  M
121 TCT GTC AGC AAC ACC ATC TCC CGG GAG TAT AAT GAA CTG CTC ATG 135

   A  I  S  D  S  D  Y  Y  T  D  D  I  N  R  A
136 GCC ATC TCA GAC AGT GAC TAC TAC ACT GAT GAC ATC AAC CGG GCC 150

   C  L  F  V  P  S  I  D  V  L  N  Q  N  T  L
151 TGT CTG TTT GTT CCC TCC ATC GAT GTG CTT AAC CAG AAC ACA CTG 165

   R  I  K  E  T  A  Q  A  M  A  Q  L  S  R  W
166 CGC ATC AAG GAG ACA GCA CAA GCG ATG GCC CAG CTC TCT AGG TGG 180

   D  R  G  T  N  H  L  L  F  N  M  L  P  G  G
181 GAT CGA GGT ACG AAT CAC CTG TTG TTC AAC ATG TTG CCT GGA GGT 195
```

P P D Y N T A L D V P R D R A
196 CCC CCA GAT TAT AAC ACA GCC CTG GAT GTC CCC AGA GAC AGG GCC 210

L L A G G G F S T W T Y R Q G
211 CTG TTG GCT GGT GGC GGC TTT TCT ACG TGG ACT TAC CGG CAA GGC 225

Y D V S I P V Y S P L S A E V
226 TAC GAT GTC AGC ATT CCT GTC TAT AGT CCA CTG TCA GCT GAG GTG 240

D L P E K G P G P R Q Y F L L
241 GAT CTT CCA GAG AAA GGA CCA GGT CCA CGG CAA TAC TTC CTC CTG 255

S S Q V G L H P E Y R E D L E
256 TCA TCT CAG GTG GGT CTC CAT CCT GAG TAC AGA GAG GAC CTA GAA 270

A L Q V K H G E S V L V L D K
271 GCC CTC CAG GTC AAA CAT GGA GAG TCA GTG TTA GTA CTC GAT AAA 2850

C T N L S E G V L S V R K R C
286 TGC ACC AAC CTC TCA GAG GGT GTC CTT TCT GTC CGT AAG CGC TGC 300

H K H Q V F D Y P Q V L Q E A
301 CAC AAG CAC CAG GTC TTC GAT TAC CCA CAG GTG CTA CAG GAG GCT 315

T F C V V L R G A R L G Q A V
316 ACT TTC TGT GTG GTT CTT CGT GGA GCT CGG CTG GGC CAG GCA GTA 330

L S D V L Q A G C V P V V I A
331 TTG AGC GAT GTG TTA CAA GCT GGC TGT GTC CCG GTT GTC ATT GCA 345

D S Y I L P F S E V L D W K R
346 GAC TCC TAT ATT TTG CCT TTC TCT GAA GTT CTT GAC TGG AAG AGA 360

A S V V V P E E K M S D V Y S
361 GCA TCT GTG GTT GTA CCA GAA GAA AAG ATG TCA GAT GTG TAC AGT 375

I L Q S I P Q R Q I E E M Q R
376 ATT TTG CAG AGC ATC CCC CAA AGA CAG ATT GAA GAA ATG CAG AGA 390

Q A R W F W E A Y F Q S I K A
391 CAG GCC CGG TGG TTC TGG GAA GCG TAC TTC CAG TCA ATT AAA GCC 405

I A L A T L Q I I N D R I Y P
406 ATT GCC CTG GCC ACC CTG CAG ATT ATC AAT GAC CGG ATC TAT CCA 420

Y A A I S Y E E W N D P P A V
421 TAT GCT GCC ATC TCC TAT GAA GAA TGG AAT GAC CCT CCT GCT GTG 435

K W G S V S N P L F L P L I P
436 AAG TGG GGC AGC GTG AGC AAT CCA CTC TTC CTC CCG CTG ATC CCA 450

P Q S Q G F T A I V L T Y D R
451 CCA CAG TCT CAA GGG TTC ACC GCC ATA GTC CTC ACC TAC GAC CGA 465

V E S L F R V I T E V S K V P
466 GTA GAG AGC CTC TTC CGG GTC ATC ACT GAA GTG TCC AAG GTG CCC 480

S L S K L L V V W N N Q N K N
481 AGT CTA TCC AAA CTA CTT GTC GTC TGG AAT AAT CAG AAT AAA AAC 495

```
    P  P  E  D  S  L W P  K  I  R  V P  L  K
496 CCT CCA GAA GAT TCT CTC TGG CCC AAA ATC CGG GTT CCA TTA AAA 510

    V  V  R  T  A  E  N  K  L  S  N  R  F  F  P
511 GTT GTG AGG ACT GCT GAA AAC AAG TTA AGT AAC CGT TTC TTC CCT 525

    Y  D  E  I  E  T  E  A  V  L  A  I  D  D  D
526 TAT GAT GAA ATC GAG ACA GAA GCT GTT CTG GCC ATT GAT GAT GAT 540

    I  I  M  L  T  S  D  E  L  Q  F  G  Y  E  V
541 ATC ATT ATG CTG ACC TCT GAC GAG CTG CAA TTT GGT TAT GAG GTC 555

    W  R  E  F  P  D  R  L  V  G  Y  P  G  R  L
556 TGG CGG GAA TTT CCT GAC CGG TTG GTG GGT TAC CCG GGT CGT CTG 570

    H  L  W  D  H  E  M  N  K  W  K  Y  E  S  E
571 CAT CTC TGG GAC CAT GAG ATG AAT AAG TGG AAG TAT GAG TCT GAG 585

    W  T  N  E  V  S  M  V  L  T  G  A  A  F  Y
586 TGG ACG AAT GAA GTG TCC ATG GTG CTC ACT GGG GCA GCT TTT TAT 600

    H  K  Y  F  N  Y  L  Y  T  Y  K  M  P  G  D
601 CAC AAG TAT TTT AAT TAC CTG TAT ACC TAC AAA ATG CCT GGG GAT 615

    I  K  N  W  V  D  A  H  M  N  C  E  D  I  A
616 ATC AAG AAC TGG GTA GAT GCT CAT ATG AAC TGT GAA GAT ATT GCC 630

    M  N  F  L  V  A  N  V  T  G  K  A  V  I  K
631 ATG AAC TTC CTG GTG GCC AAC GTC ACG GGA AAA GCA GTT ATC AAG 645

    V  T  P  R  K  K  F  K  C  P  E  C  T  A  I
646 GTA ACC CCA CGA AAG AAA TTC AAG TGT CCT GAG TGC ACA GCC ATA 660

    D  G  L  S  L  D  Q  T  H  M  V  E  R  S  E
661 GAT GGG CTT TCA CTA GAC CAA ACA CAC ATG GTG GAG AGG TCA GAG 675

    C  I  N  K  F  A  S  V  F  G  T  M  P  L  K
676 TGC ATC AAC AAG TTT GCT TCA GTC TTC GGG ACC ATG CCT CTC AAG 690

    V  V  E  H  R  A  D  P  V  L  Y  K  D  D  F
691 GTG GTG GAA CAC CGA GCT GAC CCT GTC CTG TAC AAA GAT GAC TTT 705

    P  E  K  L  K  S  F  P  N  I  G  S  L
706   CCT GAG AAG CTG AAG AGC TTC CCC AAC ATT GGC AGC TTA TGA
```

Appendix 8.5 Genotyping

Appendix 8.5.1 Short Tandem Repeats (STR) markers

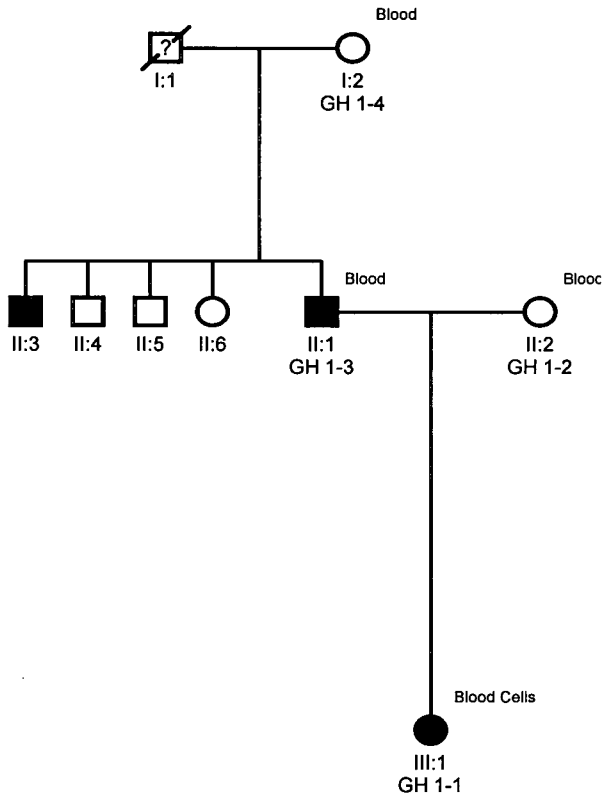
Marker name	A03/04	85	547	A01/02	905	13	216	221
Chromosome	8	8	85	11	11	11	19	19
Gene	EXT 1	EXT 1	EXT 1	EXT 2	EXT 2	EXT 2	EXT 3	EXT 3
D-number	D8S555	D8S85	D8S547	D11S903	D11S905	D11S1313	D19S216	D19S221
Gene symbol	Z24446	N/A	Z24154	Z16529	Z16575	Z23608	Z16743	Z17017
Heterozygote Frequency	75.0 %	78.9 %	71.4 %	82.1%	71.4%	89.3%	81.5%	89.29%
# of alleles		5	6	6	8	10	5	10
Allele frequencies	1 - .464 2 - .214 3 - .107 4 - .036 5 - .089 6 - .018 7 - .071	1 - .012 2 - .332 3 - .188 4 - .250 5 - .219	1 - .054 2 - .107 3 - .321 4 - .464 5 - .018 6 - .036	1 - .125 2 - .411 3 - .036 4 - .161 5 - .196 6 - .071	1 - .143 2 - .214 3 - .411 4 - .107 5 - .054 6 - .018 7 - .036 8 - .018	1 - .018 2 - .125 3 - .232 4 - .071 5 - .089 6 - .143 7 - .054 8 - .196 9 - .054 10 - .018	1 - .259 2 - .315 3 - .241 4 - .130 5 - .056	1 - .232 2 - .089 3 - .089 4 - .071 5 - .071 6 - .179 7 - .107 8 - .125 9 - .018 10 - .018
Size of fragments	1 - .177 2 - .173 3 - .167 4 - .169 5 - .165 6 - .175 7 - .171	1 - .083 2 - .081 3 - .079 4 - .075 5 - .073	1 - .193 2 - .191 3 - .189 4 - .187 5 - .195 6 - .185	1 - .101 2 - .099 3 - .105 4 - .107 5 - .103 6 - .109	1 - .222 2 - .224 3 - .210 4 - .226 5 - .208 6 - .228 7 - .212 8 - .220	1 - .202 2 - .198 3 - .196 4 - .200 5 - .192 6 - .190 7 - .204 8 - .194 9 - .188 10 - .184	1 - .191 2 - .185 3 - .179 4 - .187 5 - .189	1 - .207 2 - .209 3 - .201 4 - .195 5 - .197 6 - .199 7 - .205 8 - .203 9 - .211 10 - .191
PCR Temp	58°C	58°C	60°C	59°C	60°C	58°C	60°C	60°C

8.5.2 Short Tandem Repeats (STR) Primer Sequences

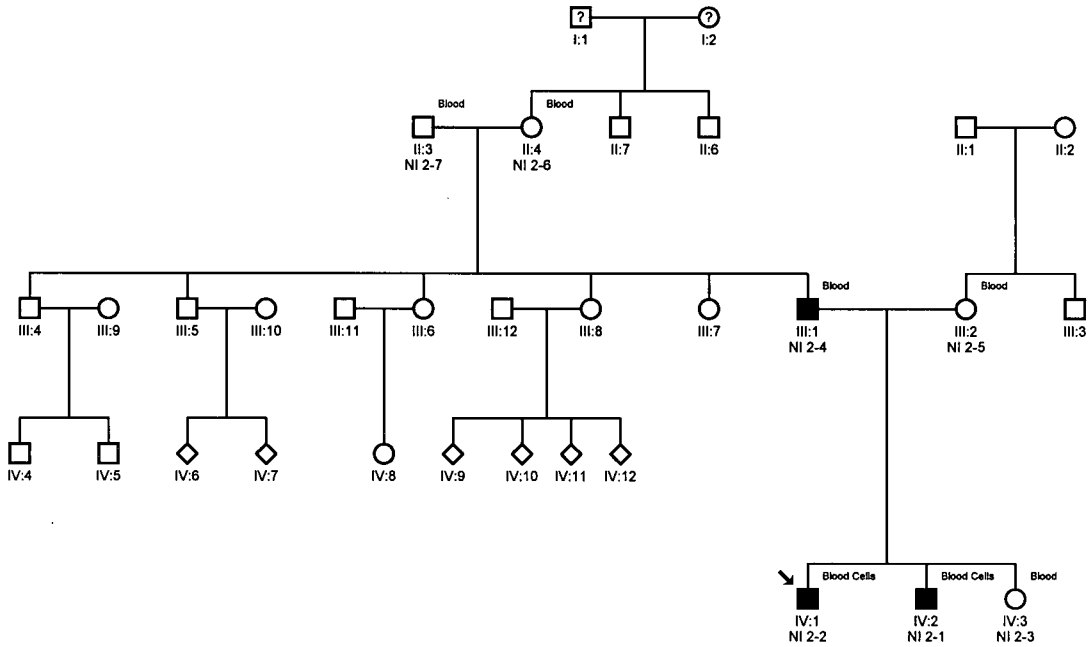
Marker Name	Sequence
A03/04	caagatggattcaaagccaaa *cattcctaaggagggttcca*
85	agctatcatcaccctataaaat *ccttgcccatcacttacac*
547	tttaaaatgcatgtggccttc *tacacacagcctcatggctc*
A01/02	caacacttcgatgttccttcc *agctgagagcgcatgtataa*
905	tctcctgtccctcacacaca *acaggggccaaataggtttc*
13	taacgatttncaacgtctaagc *gggaattttgacttcatatgca*
216	ggagacctctggctaggta *aggtacttagttactgactttg*
221	gagcaagactctgactcaac *acccagtctccagtagcag*

Appendix 8.6 Data

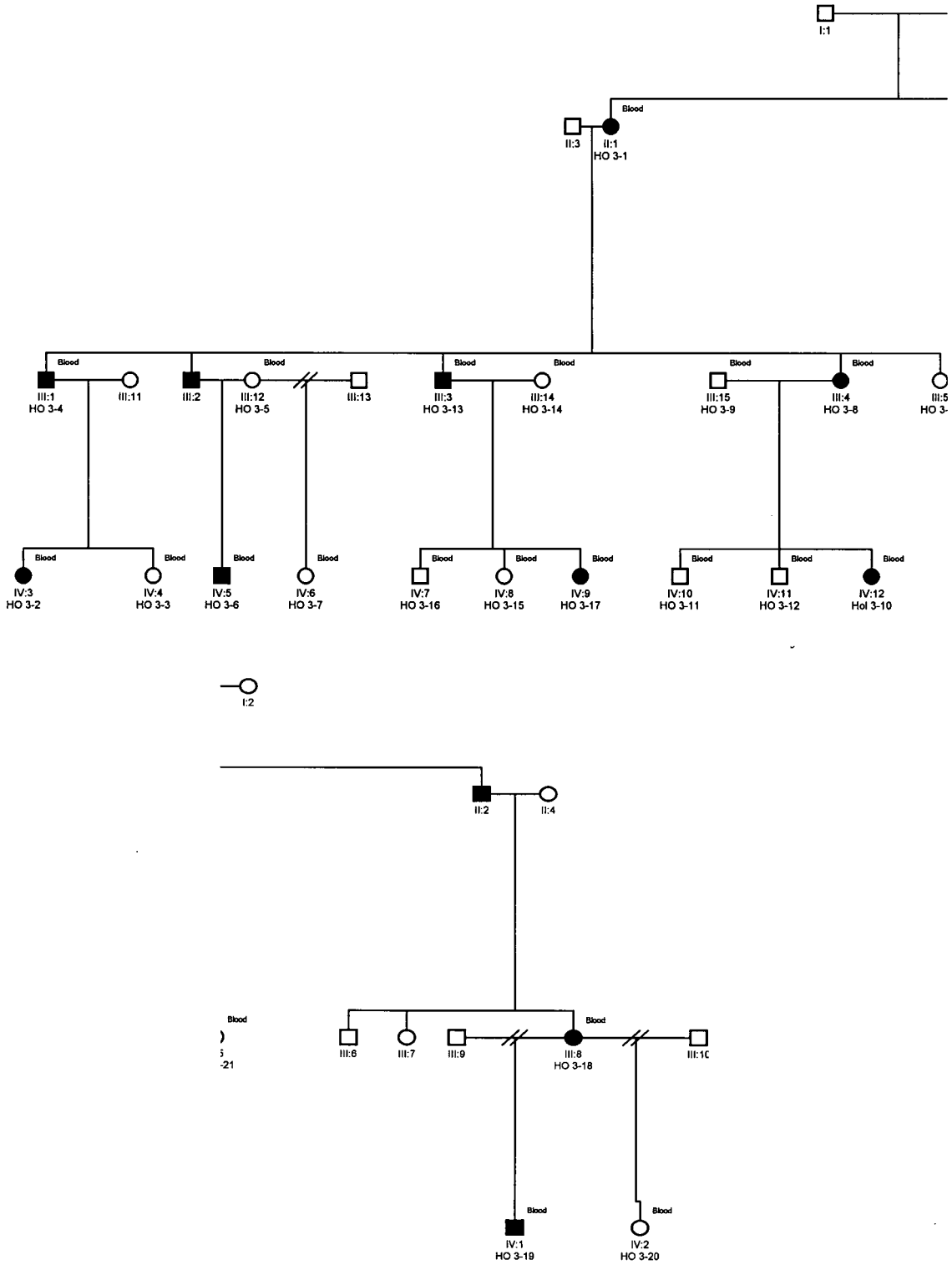
8.6.1 Cyrillic Family Pedigrees



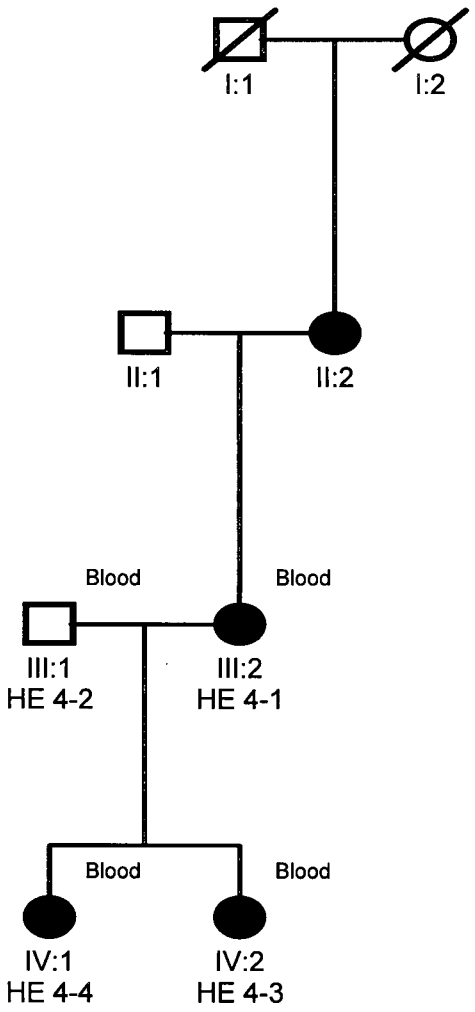
Family 1


I:1
I:2
Blood
Blood
II:3
NI 2-7
II:4
NI 2-6
II:7
II:6
II:1
II:2
Blood
Blood
III:4
III:9
III:5
III:10
III:11
III:6
III:12
III:8
III:7
III:1
NI 2-4
III:2
NI 2-5
III:3
IV:4
IV:5
IV:6
IV:7
IV:8
IV:9
IV:10
IV:11
IV:12
Blood Cells
Blood Cells
Blood
IV:1
NI 2-2
IV:2
NI 2-1
IV:3
NI 2-3

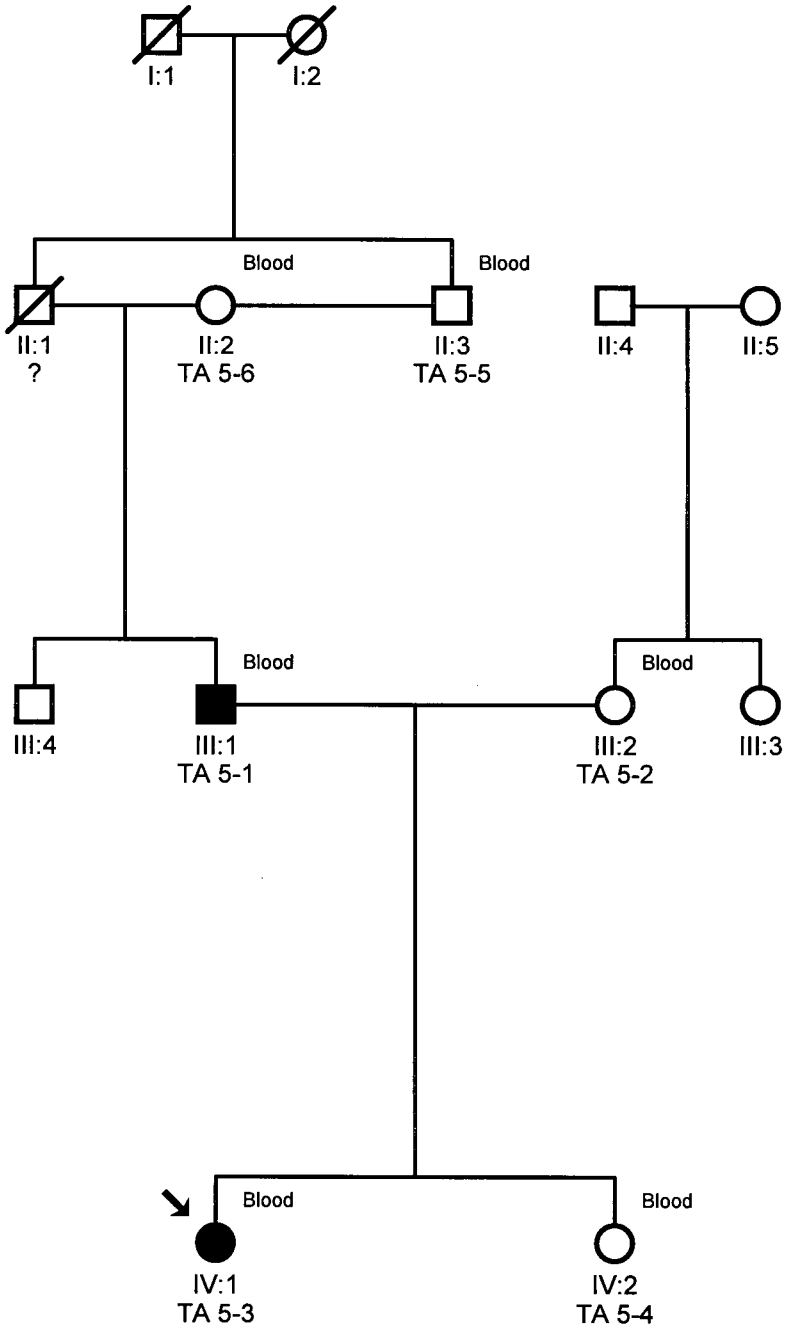
Family 2


I:1
Blood
II:3
II:1
HO 3-1
Blood
III:1
HO 3-4
III:11
III:2
Blood
III:12
HO 3-5
III:13
Blood
III:3
HO 3-13
Blood
III:14
HO 3-14
Blood
III:15
HO 3-9
Blood
III:4
HO 3-8
III:5
HO 3-
Blood
IV:3
HO 3-2
Blood
IV:4
HO 3-3
Blood
IV:5
HO 3-6
Blood
IV:6
HO 3-7
Blood
IV:7
HO 3-16
Blood
IV:8
HO 3-15
Blood
IV:9
HO 3-17
Blood
IV:10
HO 3-11
Blood
IV:11
HO 3-12
Blood
IV:12
Hol 3-10
I:2
II:2
II:4
Blood
III:6
III:7
III:9
Blood
III:8
HO 3-18
III:10
Blood
IV:1
HO 3-19
Blood
IV:2
HO 3-20

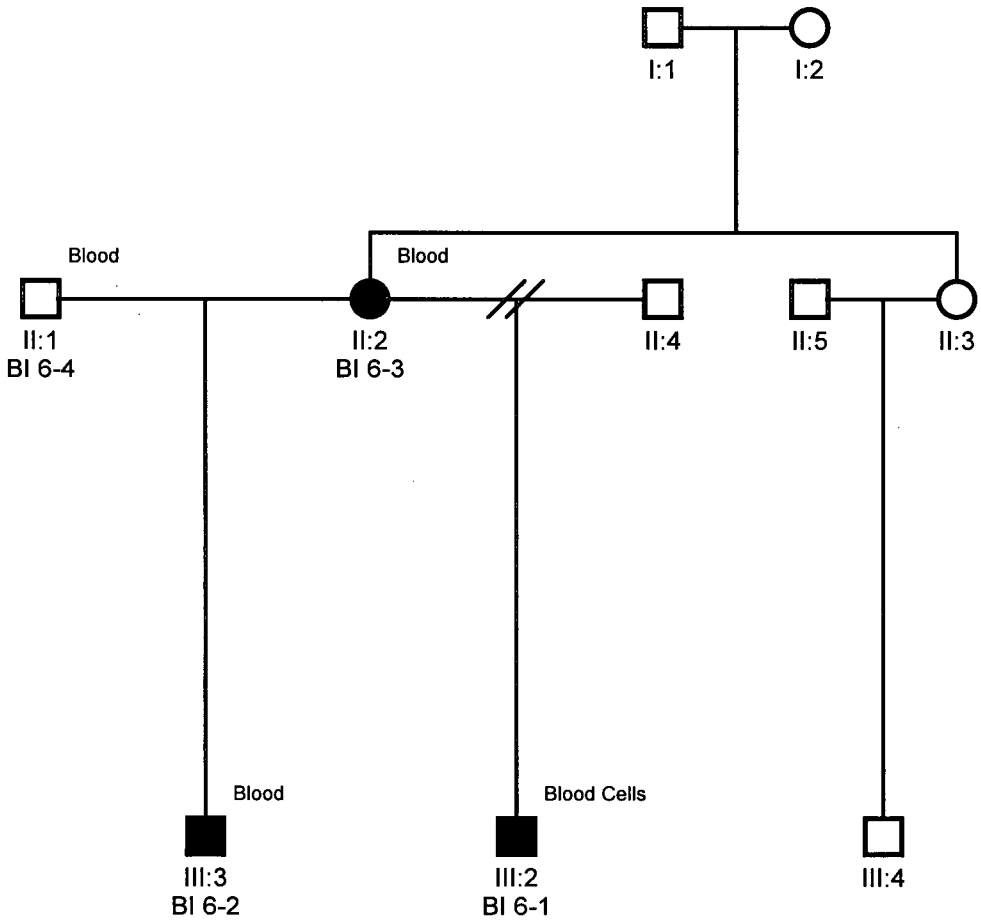
Family 3


I:1
I:2
II:1
II:2
Blood
Blood
III:1
HE 4-2
III:2
HE 4-1
Blood
Blood
IV:1
HE 4-4
IV:2
HE 4-3

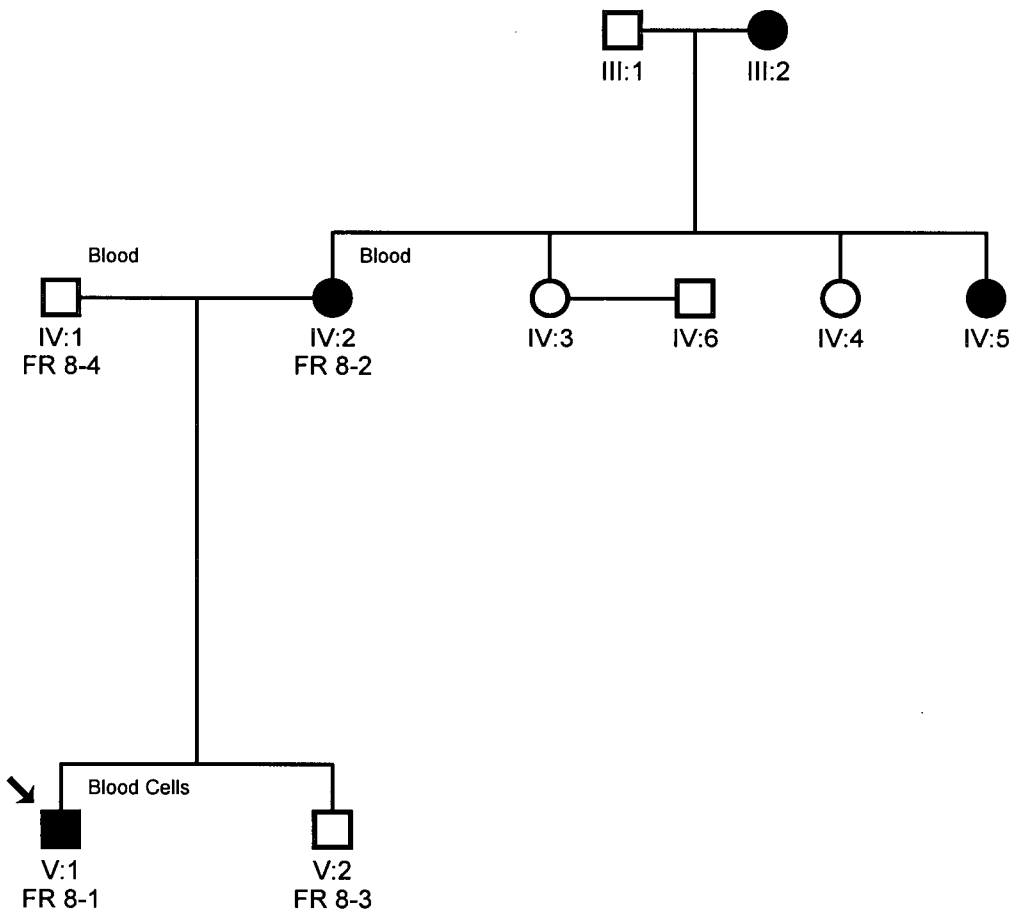
Family 4


I:1
I:2
Blood
Blood
II:1
?
II:2
TA 5-6
II:3
TA 5-5
II:4
II:5
Blood
Blood
III:4
III:1
TA 5-1
III:2
TA 5-2
III:3
Blood
Blood
IV:1
TA 5-3
IV:2
TA 5-4

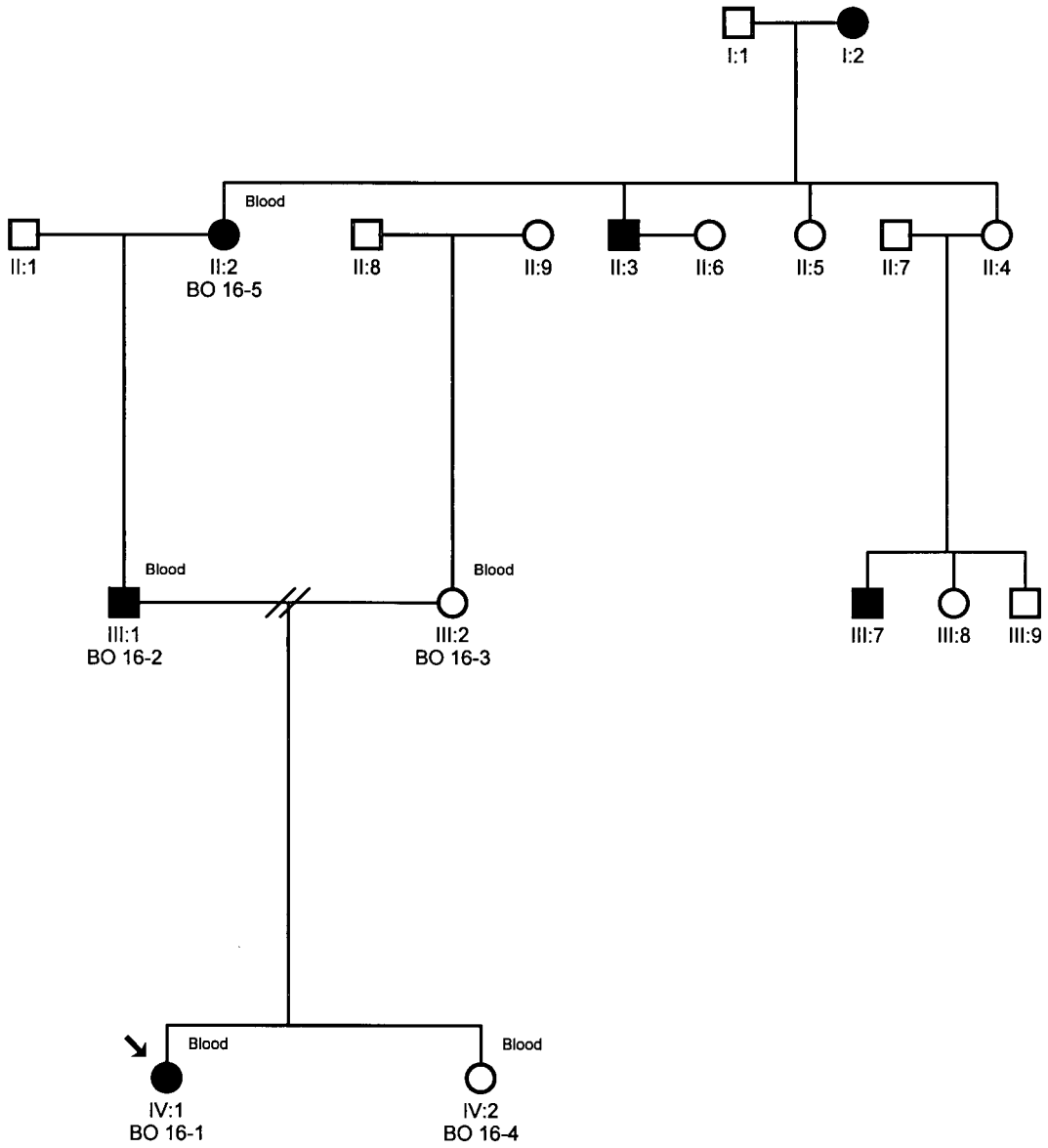
Family 5


I:1
I:2
Blood
Blood
II:1
BI 6-4
II:2
BI 6-3
II:4
II:5
II:3
Blood
Blood Cells
III:3
BI 6-2
III:2
BI 6-1
III:4

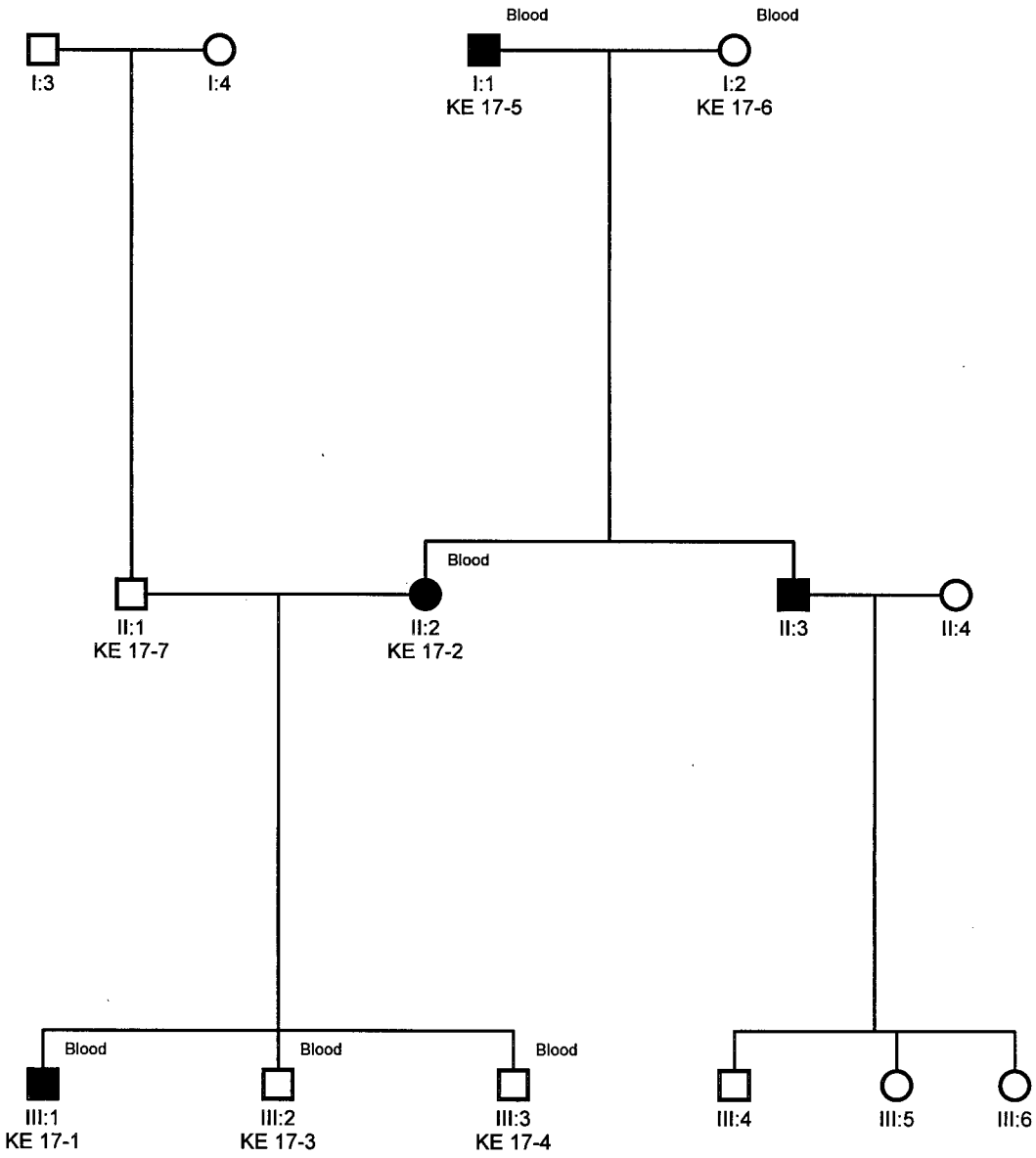
Family 6


III:1
III:2
Blood
Blood
IV:1
FR 8-4
IV:2
FR 8-2
IV:3
IV:6
IV:4
IV:5
Blood Cells
V:1
FR 8-1
V:2
FR 8-3

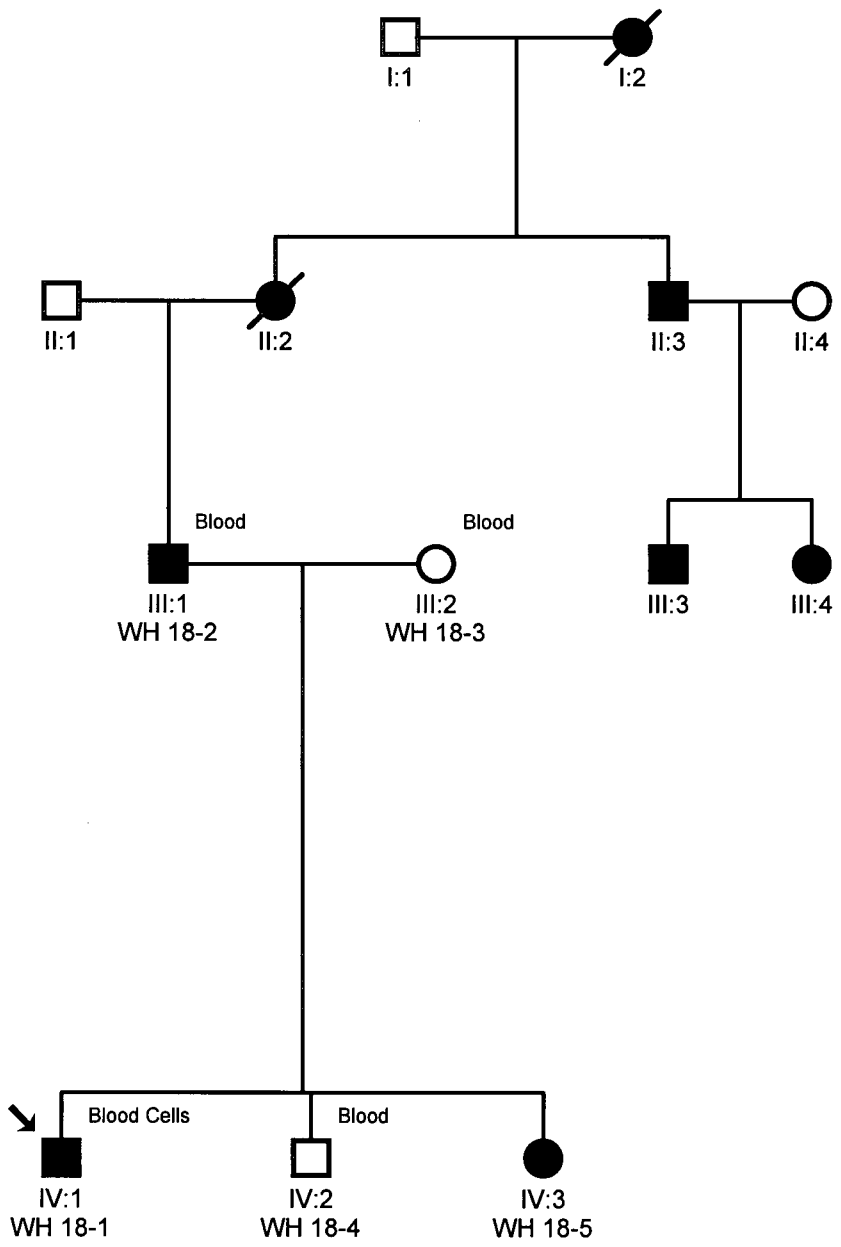
Family 8


I:1
I:2
Blood
II:1
II:2
BO 16-5
II:8
II:9
II:3
II:6
II:5
II:7
II:4
Blood
Blood
III:1
BO 16-2
III:2
BO 16-3
III:7
III:8
III:9
Blood
Blood
IV:1
BO 16-1
IV:2
BO 16-4

Family 16


Blood
Blood
I:3
I:4
I:1
KE 17-5
I:2
KE 17-6
Blood
II:1
KE 17-7
II:2
KE 17-2
II:3
II:4
Blood
Blood
Blood
III:1
KE 17-1
III:2
KE 17-3
III:3
KE 17-4
III:4
III:5
III:6

Family 17

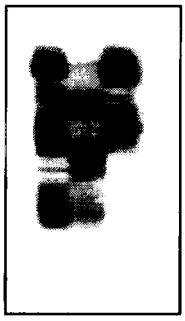

I:1
I:2
II:1
II:2
II:3
II:4
Blood
Blood
III:1
WH 18-2
III:2
WH 18-3
III:3
III:4
Blood Cells
Blood
IV:1
WH 18-1
IV:2
WH 18-4
IV:3
WH 18-5

Family 18

8.6.2 Short tandem repeat (STR) Gels

Family 1

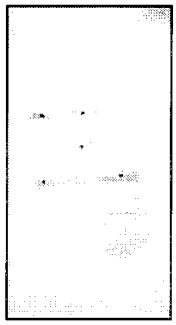
EXT 1



Family Marker Member

1_1: 1, 4
1_2: 2, 4
1_3: 1, 3

EXT 2

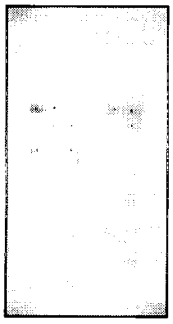


Family Marker Member

1_1: c, a
1_2: a, b
1_3: c, c

Family 2

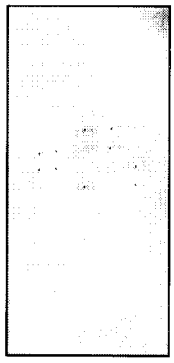
EXT 1



Family Marker Member

2_1: 3, 1
2_2: 1, 2
2_3: 3, 2
2_4:1, 3
2_5:1, 2

EXT 2



Family Marker Member

2_1: b, c
2_2: b, c
2_3: a, d
2_4:a, b
2_5:c, d

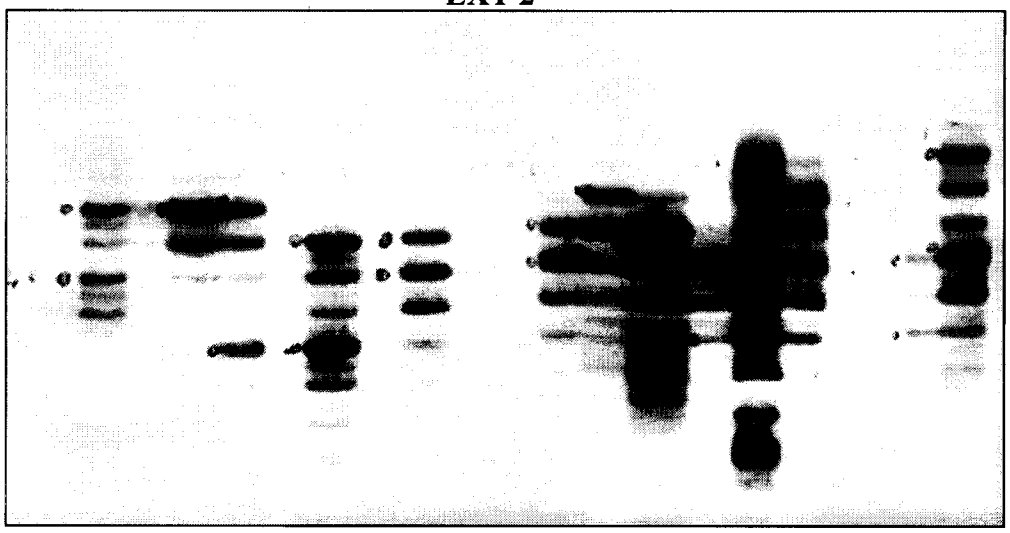
Family 3

EXT 1

Family Member	Marker
3-1	2, 3
3-2	1, 1
3-3	1, 1
3-4	(1)
3-5	1, 1
3-6	2, 3
3-7	3, 3
3-8	1, 1
3-9	1, 1
3-10	1, 1
3-11	1, 1
3-12	1, 1
3-13	1, 1
3-14	1, 1
3-15	1, 3
3-16	1, 3
3-17	1, 1
3-18	2, 3
3-19	2, 3
3-20	2, 3
3-21	1, 4

Family 3

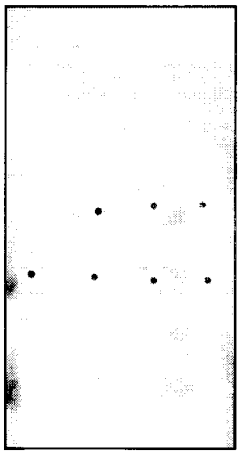
EXT 2



Family Member	Marker
3-1	g, e
3-2	a, e
3-3	c, e
3-4	c, c
3-5	c, c
3-6	g, c
3-7	
3-8	d, g
3-9	e,
3-10	e, d
3-11	e, d
3-12	
3-13	d, e
3-14	c, d
3-15	d,
3-16	d, d
3-17	
3-18	b, d
3-19	
3-20	f, d
3-21	d, d

Family 5

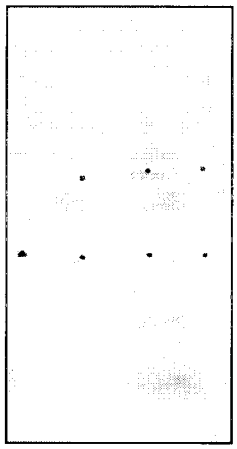
EXT 1



Family Marker Member

5_1: 2, 2
5_2: 1, 2
5_3: 2, 1
5_4: 2, 1

EXT 2

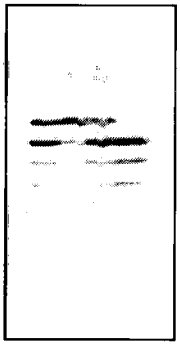


Family Marker Member

5_1: a, d
5_2: a, a
5_3: d, a
5_4: a, a

Family 8

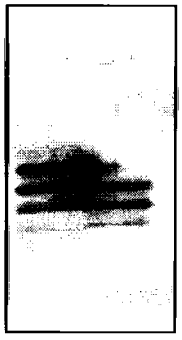
EXT 1



Family Marker Member

8_1: 1, 1
8_2: 1, 1
8_3: 2 , 1
8_4: 1 , 2

EXT 2

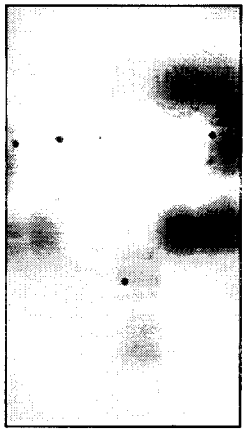


Family Marker Member

8_1:b, a
8_2:a a
8_3: b a
8_4: b b

Family 16

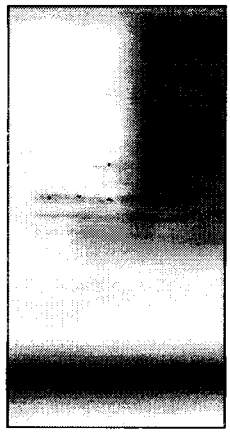
EXT 1



Family Marker Member

16_1: 2, 1
16_2: 2, 1
16_3: 1, 4
16_4:1, ?
16_5:1, 3

EXT 2

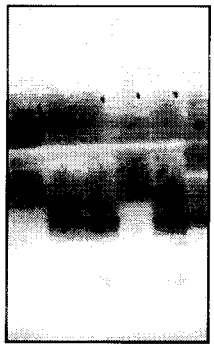


Family Marker Member

16_1: c, c
16_2: c, c
16_3: c, a
16_4:c,
16_5:b, c

Family 17

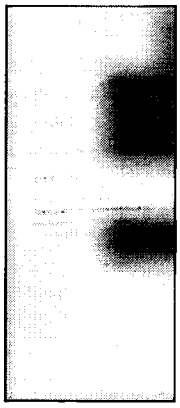
EXT 1



Family Marker Member

17_1: 3, 1
17_2: 4, 1
17_3: 1, 4
17_4:1, 1
17_5:1, 4
17_6:1, 2

EXT 2

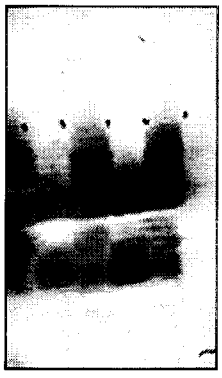


Family Marker Member

17_1: a, d
17_2: d, b
17_3: c, b
17_4:c, b
17_5:d, d
17_6:b, d

Family 18

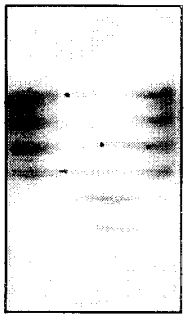
EXT 1



Family Marker Member

18_1: 2, 3
18_2: 1, 2
18_3: 2, 3
18_4:1, 2

EXT 2



Family Marker Member

18_1: a, c
18_2: a, d
18_3: c, c
18_4:a, c

8.6.3 Phenotype Data

8.6.3.1. Core Data

8.6.3.1.1 Lesion Quality Core Data

SUBJECT	GENDER	EXT	Sense	Severity	Stage of Mutation	Total # lesions	% small	% medium
Big 6-01	male					42	35.7	26.2
Big 6-02	male					27	22.2	33.3
Big 6-03	female					28	35.7	46.4
Boe 16-01	Female	1	SS	severe	Late	32	15.0	33.3
Boe 16-02	Male	1	SS	severe	Late	36	13.8	33.3
Boe 16-05	Female	1	SS	severe	Late	20	25.0	35.0
Fri 8-01	Male	2	SS	severe	Late	33	13.8	39.0
Fri 8-02	Female	2	SS	severe	Late	18	27.7	16.6
Ghu 1-01	Female	1	MS	mild	Late	28	29.6	33.3
Ghu 1-03	Male	1	MS	mild	Late	26	36.0	34.3
Heg 4-01	female					25	20.0	44.0
Heg 4-03	female					33	48.5	33.3
Heg 4-04	female					42	38.1	28.5
Hol 3-01	Female	2	NS	severe	Early	11	18.0	45.0
Hol 3-02	Female	2	NS	severe	Early	13	7.7	23.1
Hol 3-04	Male	2	NS	severe	Early	13	7.7	23.1
Hol 3-08	Female	2	NS	severe	Early	18	38.8	16.0
Hol 3-10	Female	2	NS	severe	Early	9	22.2	22.2
Hol 3-15	Female	2	NS	severe	Early	14	14.3	64.3
Hol 3-19	Male	2	NS	severe	Early	39	43.5	30.7
Hol 3-22	Female	2	NS	severe	Early	12	50.0	47.6
Hol 3-23	Male	2	NS	severe	Early	28	18.0	21.4
Ker 17-01	Male	2	FS	severe	Early	36	33.3	25.0
Ker 17-02	Female	2	FS	severe	Early	24	27.7	47.0
Ker 17-05	Male	2	FS	severe	Early	11	18.2	45.5
Nic 2-01	Male	2	NS	severe	Early	27	37.0	25.9
Nic 2-02	Male	2	NS	severe	Early	29	24.1	31.0
Nic 2-04	Male	2	NS	severe	Early	20	55.0	35.0
Tab 5-01	Male	2	MS	mild	Early	16	71.4	14.2
Tab 5-03	Female	2	MS	mild	Early	14	57.1	14.2
Whi 18-01	Male	1	NS	severe	Early	53	47.0	33.3
Whi 18-02	Male	1	NS	severe	Early	34	32.3	11.7

8.6.3.1.1 Lesion Quality Core Data (continued)

SUBJECT	% large	pelvic	%pelvic	flatbone	% flatbone	flare	%flare	Lesion Rank 1
Big 6-01	33.3	4	9.5	1	2.4	26	61.9	15
Big 6-02	44.4	2	7.4	1	3.7	13	48.1	6
Big 6-03	21.4	2	7.1	0	0	17	60.7	6
Boe 16-01	51.5	2	6.3	4	12.5	10	31.3	5
Boe 16-02	50.0	3	8.3	3	8.3	29	80.6	5
Boe 16-05	40.0	1	5.0	1	5.0	2	10.0	5
Fri 8-01	45.4	4	12.1	4	12.1	3	9.1	5
Fri 8-02	55.5	0	0.0	0	0.0	1	5.6	5
Ghu 1-01	37.0	3	10.7	3	10.7	3	10.7	7
Ghu 1-03	16.0	0	0.0	2	7.7	4	15.4	9
Heg 4-01	32.0	2	8.0	1	4.0	13	52.0	5
Heg 4-03	18.2	1	3.0	0	0.0	14	42.4	16
Heg 4-04	9.5	3	7.0	0	0	17	16.7	16
Hol 3-01	36.0	0	0.0	0	0.0	6	54.5	2
Hol 3-02	69.2	0	0.0	1	7.7	3	23.1	1
Hol 3-04	69.2	0	0.0	0	0.0	7	53.8	1
Hol 3-08	44.4	0	0.0	0	0.0	3	16.7	7
Hol 3-10	55.5	0	0.0	0	0.0	0	0.0	2
Hol 3-15	21.4	0	0.0	0	0.0	0	0.0	2
Hol 3-19	25.6	4	10.3	4	10.3	14	35.9	17
Hol 3-22	8.3	2	16.7	2	16.7	1	8.3	6
Hol 3-23	25.0	0	0.0	0	0.0	21	75.0	5
Ker 17-01	41.7	1	2.8	1	2.8	17	47.2	6
Ker 17-02	25.0	3	12.5	3	12.5	6	25.0	7
Ker 17-05	36.3	0	0.0	0	0.0	1	9.1	2
Nic 2-01	37.0	0	0.0	0	0.0	7	25.9	10
Nic 2-02	44.8	0	0.0	0	0.0	10	34.5	7
Nic 2-04	10.0	0	0.0	1	5.0	12	60.0	13
Tab 5-01	14.2	0	0.0	0	0.0	10	62.5	11
Tab 5-03	28.5	0	0.0	0	0.0	6	42.9	9
Whi 18-01	19.6	7	13.2	8	15.1	26	49.1	22
Whi 18-02	55.9	8	23.5	8	23.5	25	73.5	11

8.6.3.1.2 Limb Alignment Core Data

Subject	Gender	EXT	Sense	Severity	Stage of Mutation	# of lesions	Carpal Slip R	Carpal Slip L
Big 6-01	male					42.0	8.0	10.0
Big 6-02	male					27.0	too immature to see	to immature to see
Big 6-03	female					28.0	2.0	3.0
Boe 16-01	female	1	SS	severe	Late	32.0	3.0	4.0
Boe 16-02	male	1	SS	severe	Late	36.0	8.0	5.0
Boe 16-05	female	1	SS	severe	Late	20.0	4.0	0.0
Fri 8-01	male	2	SS	severe	Late	33.0	2.0	3.0
Fri 8-02	female	2	SS	severe	Late	18.0	2.0	6.0
Ghu 1-01	female	1	MS	mild	Late	28.0	2.0	2.0
Ghu 1-03	male	1	MS	mild	Late	26.0	9.0	8.0
Heg 4-01	female					25.0	2.0	12.0
Heg 4-03	female					33.0	1.0	0.0
Heg 4-04	female					42.0	4.0	6.0
Hol 3-01	female	2	NS	severe	Early	11.0	2.0	6.0
Hol 3-02	female	2	NS	severe	Early	13.0	4.0	5.0
Hol 3-04	male	2	NS	severe	Early	13.0	-8.0	-5.0
Hol 3-08	female	2	NS	severe	Early	18.0	2.5	3.0
Hol 3-10	female	2	NS	severe	Early	9.0	7.0	5.0
Hol 3-15	female	2	NS	severe	Early	14.0	-5.0	-5.0
Hol 3-19	male	2	NS	severe	Early	39.0	1.0	1.0
Hol 3-22	female	2	NS	severe	Early	12.0	3.0	6.0
Hol 3-23	male	2	NS	severe	Early	28.0	missing r wrist film	9.0
Ker 17-02	female	2	FS	severe	Early	24.0	3.0	6.0
Ker 17-01	male	2	FS	severe	Early	36.0	3.0	2.0
Ker 17-05	male	2	FS	severe	Early	11.0	5.0	3.0
Nic 2-01	male	2	NS	severe	Early	27.0	6.0	3.0
Nic 2-02	male	2	NS	severe	Early	29.0	2.0	3.0
Nic 2-04	male	2	NS	severe	Early	20.0	5.0	5.0
Tab 5-01	male	2	MS	mild	Early	16.0	3.0	2.0
Tab 5-03	female	2	MS	mild	Early	14.0	2.0	1.0
Whi 18-01	male	1	NS	severe	Early	53.0	5.0	6.0
Whi 18-02	male	1	NS	severe	Early	34.0	r arm and forearm not filmed	8.0

8.6.3.1.2 Limb Alignment Core Data (continued)

Subject	Rad Inclin R	Rad Inclin L	Uln Short R	Uln Short L	Rad Bow R	Rad Bow L	Rad Head Disclocation R	Rad Head Disclocation L
Big 6-01	39.0	31.0	-7.0	-10.0	9.0	12.0	N	Y
Big 6-02	22.0	25.0	0.0	6.0	5.0	7.0	N	N
Big 6-03	26.0	28.0	1.5	1.0	8.0	9.0	N	N
Boe 16-01	27.0	36.0	3.0	2.0	9.0	20.0	N	Y
Boe 16-02	28.0	32.0	0.0	8.0	7.0	11.0	Y	N
Boe 16-05	27.0	33.0	0.0	11.0	9.0	9.5	N	N
Fri 8-01	27.0	28.0	4.0	1.0	9.0	8.0	N	N
Fri 8-02	28.0	21.0	2.0	4.0	8.0	8.0	N	N
Ghu 1-01	20.0	22.0	1.0	1.0	6.0	10.5	N	N
Ghu 1-03	37.0	34.0	-3.0	-6.0	12.0	8.5	N	N
Heg 4-01	17.0	0.0	-2.0	12.0	7.0	4.0	N	N
Heg 4-03	24.0	23.0	6.0	3.0	7.0	7.0	N	N
Heg 4-04	23.0	31.0	3.0	8.0	11.0	17.0	N	Y
Hol 3-01	28.0	24.0	-4.0	3.0	8.0	5.0	N	N
Hol 3-02	34.0	30.0	-2.0	-1.0	11.0	6.0	N	N
Hol 3-04	23.0	29.0	0.0	-1.0	4.0	8.0	N	N
Hol 3-08	22.0	28.0	1.0	-9.0	5.5	7.0	N	N
Hol 3-10	26.0	22.0	-5.0	-3.0	6.0	7.0	N	N
Hol 3-15	21.0	26.0	7.0	2.0	7.0	8.0	N	N
Hol 3-19	25.0	27.0	-1.0	-3.0	6.0	12.0	N	N
Hol 3-22	21.0	23.0	0.0	1.0	4.0	4.0	N	N
Hol 3-23	missing r wrist film	30.0	missing r wrist film	11.0	9.0	9.0	missing r wrist film	N
Ker 17-02	11.0	24.0	-11.0	-7.0	9.0	11.0	N	N
Ker 17-01	30.0	34.0	-2.0	-9.0	7.0	12.0	N	N
Ker 17-05	21.0	19.0	1.0	2.0	6.0	5.0	N	N
Nic 2-01	27.0	27.0	1.5	2.0	4.0	7.0	N	N
Nic 2-02	22.0	38.0	-10.0	-5.0	8.0	6.0	N	N
Nic 2-04	21.0	20.0	-10.0	2.0	10.0	5.0	N	N
Tab 5-01	28.0	28.0	-3.0	-5.0	12.0	9.0	N	N
Tab 5-03	21.0	24.0	1.0	0.0	10.0	10.0	Y	Y
Whi 18-01	29.0	35.0	-8.0	-2.0	11.0	9.0	N	N
Whi 18-02	r arm and forearm not filmed	22.0	r arm and forearm not filmed	5.0	r arm and forearm not filmed	31.0	r arm and forearm not filmed	Y

8.6.3.1.2 Limb Alignment Core Data (continued)

Subject	Elb Jt R	Elb Jt L	Fem AA R	Fem AA L	Fem NS Ang R	Fem NS Ang L	Fem MA R	Fem MA L
Big 6-01	-7.0	12.0	-19.0	-9.0	150.0	150.0	-11.0	-4.0
Big 6-02	-17.0	-11.0	-6.0	-12.0	135.0	135.0	0.0	-3.0
Big 6-03	-4.0	-3.0	12.0	11.0	145.0	142.0	0.0	-4.0
Boe 16-01	17.0	3.0	0.0	-5.0	176.0	170.0	n/a	n/a
Boe 16-02	19.0	12.0	4.0	11.5	129.0	140.0	8.0	-10.0
Boe 16-05	-3.0	-14.0	2.5	-6.5	122.0	137.0	13.5	2.0
Fri 8-01	-12.0	-19.0	-5.0	-4.0	147.0	149.0	8.0	0.0
Fri 8-02	-16.0	-16.0	-6.0	-5.0	122.0	125.0	3.0	0.0
Ghu 1-01	-24.0	-18.0	-17.0	6.0	148.0	145.0	6.0	3.0
Ghu 1-03	-22.0	-3.0	0.0	2.0	135.0	148.0	8.0	9.0
Heg 4-01	-19.0	8.0	-5.0	-13.0	137.0	133.0	3.0	0.0
Heg 4-03	-5.0	-9.0	-12.0	14.0	139.0	135.0	-30.0	-6.5
Heg 4-04	-13.0	14.0	0.0	-7.0	139.0	149.0	5.0	0.0
Hol 3-01	-4.0	-15.0	-15.0	7.0	147.0	145.0	-9.0	6.0
Hol 3-02	13.0	9.0	10.0	10.0	133.0	134.0	2.5	3.0
Hol 3-04	29.0	15.0	10.0	9.0	139.0	137.0	8.0	5.0
Hol 3-08	-19.0	2.5	-15.0	-9.0	135.0	129.0	-5.0	6.0
Hol 3-10	-13.0	-16.0	-10.0	-11.0	139.0	132.0	-3.0	-6.0
Hol 3-15	9.0	12.0	6.0	15.0	142.0	150.0	4.0	4.0
Hol 3-19	2.0	-11.0	-12.0	-14.0	146.0	139.0	n/a	n/a
Hol 3-22	-15.0	-11.0	-11.0	-15.0	140.0	135.0	-3.0	-8.0
Hol 3-23	-12.0	-12.5	-15.0	-16.0	148.0	147.0	-10.0	-6.0
Ker 17-02	2.0	-3.0	-9.0	0.0	148.0	140.0	-4.0	6.0
Ker 17-01	-13.0	-12.0	-22.0	-11.0	127.0	130.0	-12.0	-1.0
Ker 17-05	-17.0	-7.0	5.0	-3.0	132.0	127.0	4.0	4.0
Nic 2-01	-8.0	-30.0	-10.0	-5.0	149.0	142.0	0.0	4.0
Nic 2-02	-14.0	-18.0	0.0	-11.0	150.0	135.0	6.0	-6.0
Nic 2-04	9.0	-11.0	-6.0	-3.0	133.0	130.0	4.0	8.0
Tab 5-01	-13.0	-8.0	3.0	3.0	145.0	123.0	3.0	-2.0
Tab 5-03	-14.0	-11.0	-4.0	-2.0	140.0	155.0	1.0	3.0
Whi 18-01	2.0	0.0	0.0	-7.0	151.0	142.0	5.0	-7.0
Whi 18-02	r arm and forearm not filmed	-7.0	-11.0	-12.0	141.0	143.0	-3.0	-3.0

8.6.3.1.2 Limb Alignment Core Data (continued)

Subject	Sharps R	Sharps L	Fib Ht R	Fib Ht L	Ankle Jt R	Ankle Jt L
Big 6-01	48.0	44.0	70.0	69.0	-41.0	-16.0
Big 6-02	48.0	44.0	68.0	62.5	0.0	6.0
Big 6-03	39.0	41.0	51.0	57.0	14.0	12.0
Boe 16-01	43.0	40.0	50.0	58.0	3.0	3.0
Boe 16-02	36.5	40.0	53.0	56.0	32.0	21.0
Boe 16-05	37.5	37.5	39.0	40.0	3.0	2.0
Fri 8-01	42.0	44.0	57.0	63.0	0.0	-4.0
Fri 8-02	36.0	33.0	28.0	49.0	-3.0	-5.0
Ghu 1-01	n/a	n/a	52.0	52.0	-6.0	1.0
Ghu 1-03	37.0	33.0	53.0	67.0	-7.0	2.0
Heg 4-01	41.0	39.0	75.0	62.0	19.0	3.0
Heg 4-03	37.0	42.0	63.0	66.0	-9.0	-9.0
Heg 4-04	39.5	45.0	69.0	68.0	-5.0	-10.0
Hol 3-01	32.0	35.0	32.5		-12.0	-2.0
Hol 3-02	51.0	50.0	62.0	78.0	2.0	14.5
Hol 3-04	40.0	41.0	45.0	52.0	25.0	11.0
Hol 3-08	40.0	39.0	53.0	58.0	6.0	-11.0
Hol 3-10	39.0	39.0	37.5	23.0	-7.0	-5.0
Hol 3-15	50.0	45.0	62.0	48.0	0.0	2.0
Hol 3-19	36.0	42.0	28.5	37.0	n/a	n/a
Hol 3-22	43.0	39.0	55.0	47.0	-10.0	-9.0
Hol 3-23	42.0	38.0	60.0	57.0	6.0	21.0
Ker 17-02	51.0	47.0	61.0	77.0	-20.0	-18.0
Ker 17-01	42.0	48.0	63.0	63.0	-10.0	-7.0
Ker 17-05	35.0	38.0	62.5	64.8	-9.0	-9.0
Nic 2-01	46.0	46.5	58.0	48.0	0.0	3.0
Nic 2-02	39.0	41.0	51.0	50.0	-7.0	-11.0
Nic 2-04	35.0	34.0	55.0	46.0	8.0	13.0
Tab 5-01	46.0	41.0	58.0	30.0	long films didn't include distal ankle	long films didn't include distal ankle
Tab 5-03	n/a	n/a	52.0	41.0	0.0	0.0
Whi 18-01	40.0	47.0	64.0	64.0	-31.0	-34.0
Whi 18-02	35.0	35.0	54.0	32.0	-21.0	-7.0

8.6.3.1.2 Limb Alignment Core Data (continued)

Subject	Gender	EXT	Sense	Severity	% Wt Bear R	% Wt Bear L
Big 6-01	male				15.0	40.0
Big 6-02	male				58.0	39.0
Big 6-03	female				34.0	40.0
Boe 16-01	female	1	SS	severe	68.0	60.0
Boe 16-02	male	1	SS	severe	76.0	40.0
Boe 16-05	female	1	SS	severe	70.0	74.0
Fri 8-01	male	2	SS	severe	51.0	51.0
Fri 8-02	female	2	SS	severe	58.0	50.0
Ghu 1-01	female	1	MS	mild	20.0	70.0
Ghu 1-03	male	1	MS	mild	85.0	81.0
Heg 4-01	female				56.0	46.0
Heg 4-03	female				37.0	33.0
Heg 4-04	female				50.0	50.0
Hol 3-01	female	2	NS	severe	19.0	79.0
Hol 3-02	female	2	NS	severe	61.5	56.7
Hol 3-04	male	2	NS	severe	67.0	63.0
Hol 3-08	female	2	NS	severe	19.0	59.0
Hol 3-10	female	2	NS	severe	45.0	24.0
Hol 3-15	female	2	NS	severe	58.0	38.0
Hol 3-19	male	2	NS	severe	n/a	n/a
Hol 3-22	female	2	NS	severe	32.0	15.0
Hol 3-23	male	2	NS	severe	2.0	23.5
Ker 17-02	female	2	FS	severe	30.0	75.0
Ker 17-01	male	2	FS	severe	11.0	37.0
Ker 17-05	male	2	FS	severe	68.0	68.0
Nic 2-01	male	2	NS	severe	54.0	62.0
Nic 2-02	male	2	NS	severe	77.0	29.0
Nic 2-04	male	2	NS	severe	68.0	78.0
Tab 5-01	male	2	MS	mild	56.0	52.0
Tab 5-03	female	2	MS	mild	61.0	65.0
Whi 18-01	male	1	NS	severe	69.0	57.0
Whi 18-02	male	1	NS	severe	54.0	49.0

8.6.3.1.3 Limb segments and percentile height core data

SUBJECT	GENDER	EXT	Sense	Severity	Stage of Mutation	Height (%ile)	Tot Arm Length - Left Side	Tot Arm Length - Right Side	Tot Leg Length - Left Side
Big 6-01	male					50	40	41	74
Big 6-02	male					38	31	30	46
Big 6-03	female					30	44	49.5	83
Boe 16-01	Female	1	SS	Severe	Late	3	43.0	42.0	75.0
Boe 16-02	Male	1	SS	Severe	Late	39	47.5	47.0	86.5
Boe 16-05	Female	1	SS	Severe	Late	9	50.0	51.0	86.0
Fri 8-01	Male	2	SS	Severe	Late	25	47.0	44.0	77.5
Fri 8-02	Female	2	SS	Severe	Late	25	55.5	55.5	89.0
Heg 4-01	female					8	42.5	48.0	86.0
Heg 4-03	female					38	48.0	47.0	86.0
Heg 4-04	female					60	43.0	44.0	87.0
Ghu 1-01	Female	1	MS	Mild	Late	3	38.0	37.0	62.0
Ghu 1-03	Male	1	MS	Mild	Late	5	52.5	51.0	82.5
Hol 3-01	Female	2	NS	Severe	Early	25	53.5	50.0	90.0
Hol 3-02	Female	2	NS	Severe	Early	24	49.5	46.5	77.0
Hol 3-04	Male	2	NS	Severe	Early	18	56.0	55.5	89.5
Hol 3-08	Female	2	NS	Severe	Early	25	52.0	54.5	81.0
Hol 3-10	Female	2	NS	Severe	Early	51	53.0	53.0	85.5
Hol 3-15	Female	2	NS	Severe	Early	95	40.0	40.5	67.5
Hol 3-19	Male	2	NS	Severe	Early	50	55.0	55.0	88.5
Hol 3-22	Female	2	NS	Severe	Early	90	56.0	57.0	97.0
Hol 3-23	Male	2	NS	Severe	Early	60	53.5	53.5	92.0
Ker 17-01	Male	2	FS	Severe	Early	18	50.5	52.5	86.5
Ker 17-02	Female	2	FS	Severe	Early	8	45.5	46.0	81.5
Ker 17-05	Male	2	FS	Severe	Early	3	57.0	53.5	86.5
Nic 2-01	Male	2	NS	Severe	Early	51	44.0	44.5	74.5
Nic 2-02	Male	2	NS	Severe	Early	77	52.5	50.5	94.0
Nic 2-04	Male	2	NS	Severe	Early	85	59.0	58.5	95.0
Tab 5-01	Male	2	MS	Mild	Early	15	54.0	54.5	88.0
Tab 5-03	Female	2	MS	Mild	Early	63	36.5	38.5	61.5
Whi 18-01	Male	1	NS	Severe	Early	3	43.0	43.0	79.0
Whi 18-02	Male	1	NS	Severe	Early	3	41.0	43.0	81.0

8.6.3.1.3 Limb segments and percentile height core data (continued)

SUBJECT	Tot Leg Length - Right Side	Upper Arm R	Lower Arm R	Upper Arm L	Lower Arm L	Upper Leg R	Lower Leg R	Upper Leg L	Lower Leg L
Big 6-01	75.5	25	20	24	18	38.5	28.5	35.5	31.5
Big 6-02	47	16.5	15	18	14.5	24	19	26	17.5
Big 6-03	85	28.5	23	25	20.5	47.5	31.5	44	35
Boe 16-01	75.5	26.0	21.5	26.0	18.5	39.5	30.0	38.5	30.5
Boe 16-02	89.0	27.0	21.5	28.5	19.0	43.5	37.5	42.0	39.5
Boe 16-05	85.0	30.0	24.0	30.0	23.0	42.0	35.0	43.0	37.0
Fri 8-01	77.5	28.5	21.0	30.0	21.0	37.5	32.0	38.5	31.0
Fri 8-02	89.5	35.0	26.0	35.5	25.0	47.0	37.0	47.0	37.0
Ghu 1-01	83.0	23.0	17.5	22.0	19.5	30.0	25.5	29.0	25.5
Ghu 1-03	90.0	30.0	23.0	33.0	24.5	42.5	34.5	43.0	32.5
Heg 4-01	84.0	27.5	21.0	24.5	19.0	43.5	33.5	47.0	34.0
Heg 4-03	63.5	27.0	21.0	28.0	23.0	49.0	33.5	43.0	33.0
Heg 4-04	83.5	25.0	21.0	26.0	18.0	45.0	35.0	49.0	35.0
Hol 3-01	89.0	30.0	22.0	32.5	25.0	49.5	33.0	48.5	34.0
Hol 3-02	77.5	27.5	23.5	29.5	24.5	45.0	33.5	44.5	31.0
Hol 3-04	89.0	30.5	29.5	30.5	26.5	52.0	39.5	51.0	38.5
Hol 3-08	90.0	31.0	24.0	34.5	25.0	46.0	37.0	46.0	37.0
Hol 3-10	85.5	31.0	24.0	31.0	22.5	44.0	35.0	43.5	37.0
Hol 3-15	67.5	23.0	18.0	21.5	19.5	34.0	29.5	33.0	29.0
Hol 3-19	89.0	35.5	25.5	37.0	25.0	45.0	38.0	44.5	43.0
Hol 3-22	97.0	33.0	27.5	33.5	27.5	53.0	39.0	50.5	43.5
Hol 3-23	90.5	31.5	25.0	33.5	23.5	46.0	38.5	46.0	40.0
Ker 17-01	89.5	31.5	25.0	31.0	24.0	44.5	36.5	41.5	38.0
Ker 17-02	81.0	28.0	18.5	30.0	18.0	41.5	32.5	40.0	36.0
Ker 17-05	86.5	32.0	27.0	33.5	27.0	41.0	35.0	41.5	36.0
Nic 2-01	78.0	29.0	19.5	27.5	21.0	40.5	31.0	38.0	32.0
Nic 2-02	92.0	33.0	24.0	33.0	24.5	46.5	34.5	45.5	37.0
Nic 2-04	95.5	38.0	24.0	37.5	29.5	46.5	37.0	44.5	44.0
Tab 5-01	88.5	31.0	26.0	29.5	28.0	45.5	39.5	44.5	38.0
Tab 5-03	61.5	22.5	17.5	21.0	17.5	31.0	26.5	32.5	26.0
Whi 18-01	79.0	28.0	18.0	27.0	20.0	39.0	31.0	39.0	32.0
Whi 18-02	82.0	28.0	21.0	26.0	15.0	38.0	33.0	37.5	30.0

8.6.4.2 Pearson Correlation Matrix

		1	2	3	4	5	6	7	8
	Correlation Matrix	# lesions	carpal slip r	carpal slip l	rad inclin r	rad inclin l	ulnar short r	ulnar short l	rad bow r
1	# lesions	1	0.433	0.738	0.361	0.665	-0.362	0.456	0.268
2	carpal slip r	0.433	1	0.27	0.543	0.755	-0.495	-0.194	0.022
3	carpal slip l	0.738	0.27	1	0.346	-0.18	-0.297	0.192	-0.104
4	rad inclin r	0.361	0.543	0.346	1	0.594	-0.14	0.022	0.34
5	rad inclin l	0.03	0.755	-0.18	0.594	1	-0.437	-0.323	0.089
6	ulnar short r	-0.362	-0.495	-0.297	-0.14	-0.437	1	0.475	-0.268
7	ulnar short l	0.456	-0.494	0.192	0.022	-0.323	0.475	1	0.01
8	rad bow r	0.268	0.22	-0.104	0.34	0.089	-0.268	0.01	1
9	rad bow l	0.749	0.427	0.349	0.463	0.312	-0.582	0.247	0.64
10	elb jt r	0.473	0.104	0.319	-0.55	0.159	-0.656	0.093	0.08
11	elb jt l	0.465	0.78	0.507	-0.47	-0.397	-0.133	0.324	0.128
12	fem aa r	-0.041	0.42	-0.475	0.226	0.297	0.275	0.479	0.355
13	fem aa l	-0.397	0.37	0.004	0.32	-0.161	0.301	-0.402	-0.028
14	fem ns r	0.256	0.599	0.17	0.418	0.556	-0.532	-0.03	0.271
15	fem ns l	0.364	0.614	0.144	0.709	0.444	0.079	0.352	0.312
16	fem ma r	0.217	0.143	0.379	0.213	0.24	0.388	0.593	0.355
17	fem ma l	-0.495	-0.08	-0.382	-0.285	-0.021	0.124	-0.638	-0.12
18	sharps r	-0.557	-0.073	-0.462	-0.082	0.064	0.012	-0.537	0.212
19	sharps l	-0.229	-0.116	-0.261	-0.377	-0.17	-0.278	-0.432	0.297
20	fib ht r	0.37	1	0.352	-0.355	-0.394	-0.275	-0.02	0.085
21	fib ht l	0.326	0.1	0.452	-0.159	-0.332	0.148	0.039	-0.434
22	ankle jt r	-0.505	0.137	-0.564	-0.202	-0.141	861	0.384	-0.281
23	ankle jt l	-0.552	-0.257	-0.496	-0.2	0.131	0.689	0.213	-0.398
24	% wt bear r	0.304	-0.027	-0.326	0.253	0.348	0.078	0.35	0.559
25	% wt bear l	-0.087	0.244	-0.42	0.114	0.307	-0.113	-0.405	0.376
26	% ped	-0.352	0.404	-0.58	-0.09	0.218	-0.557	-0.576	-0.08
27	% sess	0.33	0.132	0.173	0.042	-0.317	0.267	0.146	0.278
28	% distal	0.224	0.142	0.504	0.278	-0.114	0.249	0.158	-0.232
29	%prox	-0.518	0.229	-0.79	-0.407	0.083	-0.07	-0.106	0.295
30	% pelvic	0.82	-0.277	0.724	0.51	0.083	-0.413	0.279	0.265
31	%diaph	-0.244	0.288	-0.04	0.06	-0.115	0.217	-0.17	-0.219
32	%flat bones	0.002	-0.201	0.027	-0.053	0.278	-0.021	-0.229	-0.487
33	%complex	0.109	0.282	-0.05	0.435	0.346	-0.607	-0.149	0.656
34	%simple	-0.446	0.087	-0.337	-0.535	-0.389	0.514	-0.211	-0.387
35	%flared	0.164	-0.327	0.118	0.104	0.29	-0.528	0.1	0.146
36	% not flared	-0.084	0.28	-0.058	-0.045	-0.304	0.552	0.043	-0.096
37	% of 1	-0.228	-0.249	-0.155	-0.195	-0.238	-0.244	-0.286	0.529
38	% of 4	0.098	-0.233	-0.16	0.336	0.345	0.243	0.373	-0.324
39	avg #	1	0.028	0.738	0.361	0.03	-0.362	0.456	0.268
40	% left	-0.087	0.433	-0.27	0.479	0.434	-0.325	-0.215	0.787
41	% right	0.087	0.104	0.27	-0.479	-0.434	0.325	0.042	-0.787
42	% ht	-0.47	-0.104	-0.186	-0.353	-0.145	0.253	-0.075	-0.475
43	l arm upper	-0.28	-0.336	-0.28	-0.376	-0.01	0.126	-0.335	-0.432
44	l arm lower	-0.744	-0.305	-0.607	-0.519	-0.078	0.274	-0.099	-0.37
45	total arm l	-0.492	-0.42	-0.466	-0.514	-0.085	0.225	0.383	-0.439

8.6.4.2 Pearson Correlation Matrix (continued)

		1	2	3	4	5	6	7	8
		# lesions	carpal slip r	carpal slip l	rad inclin r	rad inclin l	ulnar short r	ulnar short l	rad bow r
46	ratio l arm	0.72	-0.426	0.447	0.218	0.131	-0.288	-0.073	-0.027
47	r arm upper	-0.23	0.181	-0.296	-0.393	0.016	0.015	-0.027	-0.308
48	r arm lower	-0.607	-0.321	-0.523	-0.526	-0.139	0.312	-0.048	-0.485
49	total arm r	-0.514	0.555	-0.464	-0.639	-0.194	0.209	-0.74	-0.455
50	r arm ratio	0.384	-0.527	0.167	0.017	0.196	-0.383	0.085	0.128
51	ALD	0.242	0.162	0.415	0.268	-0.423	0.265	0.522	0.302
52	l leg upper	-0.177	-0.143	-0.082	-0.655	-0.609	0.405	0.272	-0.404
53	l leg lower	-0.264	-0.662	-0.35	-0.726	-0.262	0.199	0.299	-0.49
54	total leg l	-0.056	-0.446	-0.178	-0.592	-0.233	0.072	0.347	-0.369
55	l leg ratio	0.347	-0.402	0.601	0.354	-0.525	0.299	0.376	0.273
56	r leg upper	-0.188	0.227	-0.121	-0.661	-0.414	0.279	0.262	-0.57
57	r leg lower	-0.262	-0.528	-0.423	-0.607	-0.294	0.19	0.149	-0.157
58	total leg r	-0.12	-0.574	-0.229	-0.484	-0.022	-0.03	0.158	-0.403
59	r leg ratio	0.128	-280	0.525	-0.024	-0.157	0.134	0.44	-0.679
60	LLD	0.297	0.118	0.26	-0.439	-0.485	0.308	0.047	-0.339

8.6.4.2 Pearson Correlation Matrix (continued)

		9	10	11	12	13	14	15	16	17
	Correlation Matrix	**rad bow l**	**elb jt r**	**elb jt l**	**fem aa r**	**fem aa l**	**fem ns r**	**fem ns l**	**fem ma r**	**fem ma l**
1	**# lesions**	0.749	0.473	0.465	-0.041	-0.397	0.256	0.364	0.217	-0.495
2	**carpal slip r**	0.427	0.104	0.078	0.42	-0.037	0.599	0.614	0.143	-0.008
3	**carpal slip l**	0.349	0.319	0.507	-0.475	-0.004	0.17	0.144	-0.379	-0.382
4	**rad inclin r**	0.463	-0.055	-0.047	0.058	0.132	0.418	0.709	0.213	-0.285
5	**rad inclin l**	0.312	0.159	-0.397	0.226	-0.161	0.556	0.444	0.24	-0.021
6	**ulnar short r**	-0.582	-0.656	-0.133	0.297	0.301	-0.532	0.079	0.388	0.124
7	**ulnar short l**	0.247	0.093	0.324	0.275	-0.402	-0.03	0.352	0.593	0.345
8	**rad bow r**	0.64	0.08	0.128	0.479	-0.028	0.271	0.312	0.355	0.342
9	**rad bow l**	1	0.452	0.415	0.355	-0.441	0.411	0.484	0.27	0.986
10	**elb jt r**	0.452	1	0.09	0.263	-0.704	0.421	-0.307	-0.127	0.67
11	**elb jt l**	0.415	0.09	1	0.01	0.194	0.098	0.256	-0.186	0.875
12	**fem aa r**	0.263	0.01	0.058	1	-0.158	0.281	0.487	0.741	0.423
13	**fem aa l**	-0.441	-0.704	0.194	0.158	1	-0.084	0.17	-0.311	0.34
14	**fem ns r**	0.484	0.421	0.098	0.281	-0.084	1	0.467	0.077	0.023
15	**fem ns l**	0.27	-0.307	0.256	0.487	0.17	0.467	1	0.53	0.234
16	**fem ma r**	-0.55	-0.127	-0.186	0.741	-0.311	0.077	0.53	1	0.456
17	**fem ma l**	-0.268	-0.563	-0.498	-0.489	0.44	-0.357	-0.28	-0.156	1
18	**sharps r**	0.003	-0.254	0.085	0.253	0.636	0.011	-0.121	-0.184	0.246
19	**sharps l**	0.153	0.095	0.386	0.507	0.342	-0.011	-0.321	-0.278	0.108
20	**fib ht r**	-0.114	0.269	0.72	0.615	0.208	0.063	-0.17	-0.249	-0.098
21	**fib ht l**	-0.575	-0.204	0.407	-0.023	0.235	-0.545	-0.09	-0.211	0.168
22	**ankle jt r**	-0.587	-0.626	-0.251	-0.165	0.333	-0.21	0.213	0.517	0.194
23	**ankle jt l**	0.38	-0.498	-0.238	-0.105	-0.348	0.049	0.276	0.363	0.075
24	**% wt bear r**	0.106	0.101	-0.279	-0.103	0.158	0.253	0.402	0.902	-0.062
25	**% wt bear l**	0.052	-0.423	-0.375	0.508	0.197	-0.018	0.266	0.36	0.7
26	**% ped**	-0.064	0.12	0.237	-0.145	0.088	0.31	-0.121	-0.666	0.034
27	**% sess**	-0.281	-0.028	-0.121	-0.422	0.459	-0.292	-0.136	0.3	0.534
28	**% distal**	0.044	-0.069	0.158	-0.118	-0.163	0.283	0.215	-0.055	-0.345
29	**%prox**	0.789	0.011	-0.055	0.296	-0.368	0.074	-0.122	0.178	0.543
30	**% pelvic**	-0.147	0.363	0.412	-0.431	0.136	0.068	0.316	-0.03	0.23
31	**%diaph**	-0.375	-0.556	-0.162	0.406	-0.028	-0.599	-0.016	-0.251	0.245
32	**%flat bones**	0.559	0.254	-0.35	-0.372	-0.234	-0.036	-0.311	-0.024	0.234
33	**%complex**	-0.726	0.53	0.038	0.094	0.289	0.677	0.12	-0.05	0.1
34	**%simple**	0.409	-0.58	-0.401	0.221	-0.134	-0.684	-0.416	-0.03	0.89
35	**%flared**	0.379	0.605	0.47	-0.041	0.164	0.681	0.133	-0.148	0.678
36	**% not flared**	0.015	-0.593	-0.431	0.425	0.35	-0.645	-0.08	0.202	0.345
37	**% of 1**	0.075	0.115	0.254	-0.425	-0.526	0.375	-0.165	-0.244	0.093
38	**% of 4**	0.749	0.071	-0.397	0.043	-0.397	-0.223	0.151	0.339	0.78
39	**avg #**	0.4	0.473	0.465	-0.359	0.017	0.256	0.364	0.217	0.7

8.6.4.2 Pearson Correlation Matrix (continued)

		9	10	11	12	13	14	15	16	17
		rad bow l	elb jt r	elb jt l	fem aa r	fem aa l	fem ns r	fem ns l	fem ma r	fem ma l
40	% left	-0.4	0.133	-0.203	-0.266	-0.017	0.575	0.297	0.21	0.9
41	% right	-0.656	-0.133	0.203	-0.27	0.008	-0.575	-0.297	-0.21	0.65
42	% ht	-0.389	0.188	-0.305	-0.137	-0.502	0.213	-0.401	-0.175	0.456
43	l arm upper	-0.725	-0.05	-0.735	-0.282	-0.037	-0.415	-0.521	-0.007	0.23
44	l arm lower	-0.511	-0.255	-0.759	-0.112	-0.394	-0.314	-0.547	-0.107	0.134
45	total arm l	0.567	-0.07	-0.709	-0.237	-0.765	-0.432	-0.549	-0.02	0.65
46	ratio l arm	-321	0.512	0.01	-0.308	-0.603	-0.101	0.038	0.169	0.897
47	r arm upper	-0.517	0.254	-0.76	-0.052	-0.351	-0.274	-0.593	0.029	0.568
48	r arm lower	-0.522	-0.104	-0.596	-0.113	-0.422	-0.498	-0.536	-0.075	0.123
49	total arm r	0.163	0.023	-0.508	-0.053	-0.557	-0.402	-0.632	-0.086	0.343
50	r arm ratio	-0.032	0.537	0.376	-0.165	0.574	0.181	-0.272	0.134	0.34
51	ALD	-0.334	-0.195	-0.084	-0.11	-0.465	-0.13	0.088	-0.065	0.56
52	l leg upper	-0.348	0.108	-0.309	-0.159	-0.596	-0.374	-0.448	0.033	0.67
53	l leg lower	-0.174	0.206	-0.359	-0.027	-0.742	-0.281	-0.513	0.068	0.76
54	total leg l	0.118	0.33	0.488	-0.176	0.422	-0.253	-0.498	0.079	0.435
55	l leg ratio	-0.401	-0.229	-0.219	-0.185	-0.529	-0.075	0.266	-0.1	0.346
56	r leg upper	-0.172	0.215	-0.366	-0.06	-0.619	-0.287	-0.519	-0.05	0.876
57	r leg lower	-0.171	0.102	-0.5	-0.567	-0.757	-0.352	-0.442	0.151	0.345
58	total leg r	-0.363	0.349	0.286	-0.74	0.207	-0.187	-0.48	0.044	0.234
59	r leg ratio	-0.241	0.188	0.289	-0.009	0.123	0.124	-0.097	-0.356	0.113
60	LLD	-0.214	-0.041	0.456	-0.234	0.145	-0.064	0.009	0.132	0.135

8.6.4.2 Pearson Correlation Matrix (continued)

		18	19	20	21	22	23	24	25	26
	Correlation Matrix	**sharps r**	**sharps l**	**fib ht r**	**fib ht l**	**ankle jt r**	**ankle jt l**	**% wt bear r**	**% wt bear l**	**% ped**
1	**# lesions**	-0.557	-0.229	0.37	0.326	-0.505	-0.552	0.304	-0.087	-0.352
2	**carpal slip r**	0.073	-0.116	0.1	0.137	-0.257	-0.027	0.244	0.404	0.132
3	**carpal slip l**	0.462	-0.261	0.352	0.452	-0.564	-0.496	-0.326	-0.42	-0.058
4	**rad inclin r**	0.082	-0.377	-0.355	-0.159	-0.202	-0.02	0.253	0.114	-0.09
5	**rad inclin l**	0.064	-0.17	-0.394	-0.332	-0.141	0.131	0.348	0.397	0.218
6	**ulnar short r**	0.012	-0.278	-0.275	0.148	0.861	0.689	0.078	-0.113	-0.557
7	**ulnar short l**	-0.537	-0.432	-0.02	0.039	0.384	0.213	0.35	-0.405	-0.576
8	**rad bow r**	0.212	0.297	0.085	-0.434	-0.281	-0.398	0.559	0.376	-0.08
9	**rad bow l**	0.268	0.003	0.153	-0.114	-0.575	-0.587	0.38	0.106	0.053
10	**elb jt r**	0.254	0.095	0.269	-0.204	-0.626	-0.498	0.101	-0.423	0.12
11	**elb jt l**	0.0865	0.342	0.72	0.407	-0.251	-0.238	-0.279	-0.375	0.237
12	**fem aa r**	0.253	-0.011	-0.148	-0.438	0.453	0.507	0.615	-0.023	-0.165
13	**fem aa l**	0.636	-0.321	0.208	0.235	0.235	0.333	-0.348	0.158	0.197
14	**fem ns r**	0.011	-0.278	0.063	-0.545	-0.21	0.049	0.253	-0.018	0.31
15	**fem ns l**	-0.121	0.108	-0.17	-0.09	0.213	0.276	0.402	0.266	-0.121
16	**fem ma r**	-0.184	0.156	-0.249	-0.211	0.517	0.363	0.902	0.36	-0.666
17	**fem ma l**	0.246	0.108	-0.098	0.168	0.194	0.075	-0.062	0.7	-0.034
18	**sharps r**	1	0.821	0.3	-0.102	0.079	0.254	-0.098	0.138	0.408
19	**sharps l**	0.821	1	0.685	0.062	-0.242	-0.165	-0.12	0.055	0.44
20	**fib ht r**	0.3	0.685	1	0.489	-0.343	-0.17	-0.123	-0.15	0.101
21	**fib ht l**	-0.102	0.062	0.489	1	-0.113	0.896	-0.323	-0.027	-0.214
22	**ankle jt r**	0.079	-0.242	-0.343	-0.113	1	0.895	0.226	0.082	-0.368
23	**ankle jt l**	0.254	-0.165	-0.32	-0.17	,896	1	0.109	-0.048	-0.13
24	**% wt bear r**	-0.098	-0.12	-0.123	-0.323	0.226	0.109	1	0.483	-0.618
25	**% wt bear l**	0.138	0.055	-0.15	-0.027	0.082	-0.048	0.483	1	-0.128
26	**% ped**	0.408	0.44	0.101	-0.214	-0.368	-0.13	-0.618	-0.128	1
27	**% sess**	-0.131	0.02	0.272	0.303	-0.006	-0.185	0.456	0.189	-0.797
28	**% distal**	0.012	-0.148	0.291	0.244	0.153	0.308	-0.027	-0.277	-0.33
29	**%prox**	0.436	0.479	-0.087	-0.562	0.22	0.18	0.153	0.16	0.431
30	**% pelvic**	-0.529	-0.293	0.067	0.265	-0.654	-0.68	-0.005	-0.16	-0.068
31	**%diaph**	-0.244	-0.377	-0.511	0.254	0.044	-0.111	-0.45	0.184	0.119
32	**%flat bones**	0.117	0.043	0.171	0.345	-0.013	0.185	0.093	-0.022	-0.258
33	**%complex**	0.175	0.229	-0.057	-0.76	-0.477	-0.332	0.191	0.096	0.418
34	**%simple**	0.023	-0.052	-0.066	0.392	0.456	0.224	-0.102	0.39	-0.342

8.6.4.2 Pearson Correlation Matrix (continued)

		18	19	20	21	22	23	24	25	26
	Correlation Matrix	**sharps r**	**sharps l**	**fib ht r**	**fib ht l**	**ankle jt r**	**ankle jt l**	**% wt bear r**	**% wt bear l**	**% ped**
35	**%flared**	0.315	0.45	0.389	-0.306	-0.34	-0.037	-0.058	-0.433	0.562
36	**% not flared**	-0.31	-0.447	-0.33	0.331	0.34	0.04	0.128	0.424	-0.65
37	**% of 1**	0.605	0.693	0.434	-0.455	-0.155	-0.113	-0.024	-0.038	0.379
38	**% of 4**	-0.528	-0.71	-0.694	7.95E-05	0.167	0.178	0.137	-0.168	-313
39	**avg #**	-0.557	-0.229	0.37	0.326	-0.505	-0.552	0.304	-0.087	-0.352
40	**% left**	0.331	0.175	-0.257	-0.816	-0.174	-0.085	0.428	0.252	0.201
41	**% right**	-0.331	-0.175	0.257	0.816	0.174	0.085	-0.428	-0.252	-0.201
42	**% ht**	0.088	-0.057	-0.096	-0.37	0.387	0.528	-0.182	-0.505	0.046
43	**l arm upper**	-0.496	-0.498	-0.558	-0.071	0.149	-0.016	-0.031	0.118	-0.22
44	**l arm lower**	4.69E-04	-0.161	-0.484	-0.284	0.421	0.31	-0.115	0.224	0.005
45	**total arm l**	-0.337	-0.373	-0.557	-0.165	0.306	0.135	-0.088	0.112	-0.105
46	**ratio l arm**	-0.779	-0.513	-0.132	0.246	-0.452	-0.536	0.172	-0.126	-0.326
47	**r arm upper**	-0.449	-0.401	-0.478	-0.215	0.063	-0.078	0.092	0.073	-0.238
48	**r arm lower**	-0.205	-0.276	-0.57	-0.18	0.365	0.236	-0.241	-0.094	0.055
49	**total arm r**	-0.294	-0.268	-0.449	-0.202	0.297	0.134	-0.161	-0.038	-0.022
50	**r arm ratio**	-0.283	-0.306	-0.079	-0.147	-0.364	-0.441	0.431	0.234	-0.413
51	**ALD**	-0.483	0.26	0.45	0.325	-0.082	-0.093	0.005	-0.162	-0.349
52	**l leg upper**	0.314	-0.345	-0.148	-0.031	0.355	0.107	-0.152	-0.428	-0.258
53	**l leg lower**	-0.531	-0.247	-0.221	-0.113	0.329	0.163	-0.064	-0.207	-0.087
54	**total leg l**	-0.419	-0.394	-0.277	-0.127	0.138	-0.068	0.013	-0.173	-0.194
55	**l leg ratio**	-0.619	-0.104	0.205	0.201	-0.063	-0.147	-0.151	-0.335	-0.276
56	**r leg upper**	-0.074	-0.363	-0.204	-0.053	0.308	0.152	-0.206	-0.427	-0.14
57	**r leg lower**	-0.52	-0.282	-0.391	-0.284	0.265	-0.011	0.045	-0.042	-0.118
58	**total leg r**	-0.445	-0.432	-0.406	-0.191	0.084	-0.055	0.004	-0.099	-0.097
59	**r leg ratio**	-0.044	-0.079	0.346	0.414	0.042	0.284	-0.435	-0.666	-0.003
60	**LLD**	-0.14	0.08	0.646	0.546	0.305	0.214	0.036	-0.163	-0.377

8.6.4.2 Pearson Correlation Matrix (continued)

		27	28	29	30	31	32	33	34	35
	Correlation Matrix	**% sess**	**%distal**	**%prox**	**% pelvic**	**% diaph**	**% flatbones**	**% complex**	**% simple**	**% flared**
1	**# lesions**	-0.495	-0.194	0.022	0.427	0.104	0.078	0.665	0.362	0.456
2	**carpal slip r**	-0.297	0.192	-0.104	0.349	0.319	0.507	0.755	-0.495	-0.194
3	**carpal slip l**	-0.14	0.022	0.34	0.463	-0.055	-0.047	-0.18	-0.297	0.192
4	**rad inclin r**	-0.437	-0.323	0.089	0.312	0.159	-0.397	0.594	-0.14	0.022
5	**rad inclin l**	1	0.475	-0.268	-0.582	-0.656	-0.133	-0.89	-0.437	-0.323
6	**ulnar short r**	0.475	1	0.01	0.247	0.093	0.324	-0.437	0.765	0.475
7	**ulnar short l**	-0.268	0.01	1	0.64	0.08	0.128	-0.323	0.475	0.346
8	**rad bow r**	-0.582	0.247	0.64	1	0.452	0.415	0.089	-0.268	0.01
9	**rad bow l**	-0.656	0.093	0.08	0.452	1	0.09	0.312	-0.582	0.247
10	**elb jt r**	-0.133	0.324	0.128	0.415	0.09	1	0.159	-0.656	0.093
11	**elb jt l**	0.275	0.479	0.355	0.263	0.01	0.058	-0.397	-0.133	0.324
12	**fem aa r**	0.301	-0.402	-0.028	-0.441	-0.704	0.194	0.297	0.275	0.479
13	**fem aa l**	-0.532	-0.03	0.271	0.484	0.421	0.098	-0.161	0.301	-0.402
14	**fem ns r**	0.079	0.352	0.312	0.27	-0.307	0.256	0.556	-0.532	-0.03
15	**fem ns l**	0.388	0.593	0.355	-0.55	-0.127	-0.186	0.444	0.079	0.352
16	**fem ma r**	0.124	-0.638	-0.12	-0.268	-0.563	-0.498	0.24	0.388	0.593
17	**fem ma l**	0.012	-0.537	0.212	0.003	-0.254	0.085	-0.021	0.124	-0.638
18	**sharps r**	-0.278	-0.432	0.297	0.153	0.095	0.386	0.064	0.012	-0.537
19	**sharps l**	-0.275	-0.02	0.085	-0.114	0.269	0.72	-0.17	-0.278	-0.432
20	**fib ht r**	-0.576	-0.08	-0.352	0.404	-0.58	-0.09	0.218	-0.275	-0.02
21	**fib ht l**	0.146	0.278	0.33	0.132	0.173	0.042	-0.317	0.148	0.039
22	**ankle jt r**	0.158	-0.232	0.224	0.142	0.504	0.278	-0.114	861	0.384
23	**ankle jt l**	-0.106	0.295	-0.518	0.229	-0.79	-0.407	0.083	0.689	0.213
24	**% wt bear r**	0.279	0.265	0.82	-0.277	0.724	0.51	0.083	0.078	0.35
25	**% wt bear l**	-0.17	-0.219	-0.244	0.288	-0.04	0.06	-0.115	-0.113	-0.405
26	**% ped**	-0.229	-0.487	0.002	-0.201	0.027	-0.053	0.278	-0.557	-0.576
27	**% sess**	1	0.656	0.109	0.282	-0.05	0.435	0.346	0.267	0.146
28	**% distal**	-0.211	1	-0.446	0.087	-0.337	-0.535	-0.389	0.249	0.158
29	**%prox**	0.1	0.146	1	-0.327	0.118	0.104	0.29	-0.07	-0.106
30	**% pelvic**	0.043	-0.096	-0.084	1	-0.058	-0.045	-0.304	-0.413	0.279
31	**%diaph**	-0.286	0.529	-0.228	-0.249	1	-0.195	-0.238	0.217	-0.17
32	**%flat bones**	0.373	-0.324	0.098	-0.233	-0.16	1	0.345	-0.021	-0.229
33	**%complex**	0.456	0.268	1	0.028	0.738	0.361	1	-0.607	-0.149
34	**%simple**	-0.215	0.787	-0.087	0.433	-0.27	0.479	0.434	1	-0.211
35	**%flared**	0.042	-0.787	0.087	0.104	0.27	-0.479	-0.434	-0.528	1
36	**% not flared**	-0.075	-0.475	-0.47	-0.104	-0.186	-0.353	-0.145	0.552	0.043
37	**% of 1**	-0.335	-0.432	-0.28	-0.336	-0.28	-0.376	-0.01	-0.244	-0.286
38	**% of 4**	-0.099	-0.37	-0.744	-0.305	-0.607	-0.519	-0.078	0.243	0.373

8.6.4.2 Pearson Correlation Matrix (continued)

		27	28	29	30	31	32	33	34	35
	Correlation Matrix	**% sess**	**%distal**	**%prox**	**% pelvic**	**% diaph**	**% flatbones**	**% complex**	**% simple**	**% flared**
41	**% right**	-0.027	-0.308	-0.23	0.181	-0.296	-0.393	0.016	0.325	0.042
42	**% ht**	-0.093	0.005	-0.162	-0.349	-0.113	-0.024	-0.038	0.253	-0.075
43	**l arm upper**	0.107	-0.152	-0.428	-0.258	0.178	0.137	-0.168	0.126	-0.335
44	**l arm lower**	0.163	-0.064	-0.207	-0.087	-0.552	0.304	-0.087	0.274	-0.099
45	**total arm l**	-0.068	0.013	-0.173	-0.194	-0.085	0.428	0.252	0.225	0.383
46	**ratio l arm**	-0.147	-0.151	-0.335	-0.276	0.085	-0.428	-0.252	-0.288	-0.073
47	**r arm upper**	0.152	-0.206	-0.427	-0.14	0.528	-0.182	-0.505	0.015	-0.027
48	**r arm lower**	-0.011	0.045	-0.042	-0.118	-0.016	-0.031	0.118	0.312	-0.048
49	**total arm r**	-0.055	0.004	-0.099	-0.097	0.31	-0.115	0.224	0.209	-0.74
50	**r arm ratio**	0.284	-0.435	-0.666	-0.003	0.135	-0.088	0.112	-0.383	0.085
51	**ALD**	0.214	0.036	-0.163	-0.377	-0.536	0.172	-0.126	0.265	0.522
52	**l leg upper**	-0.466	-0.514	-0.085	-0.362	0.456	0.268	0.073	0.405	0.272
53	**l leg lower**	0.447	0.218	0.131	-0.325	-0.215	0.787	-0.262	0.199	0.299
54	**total leg l**	-0.296	-0.393	0.016	0.325	0.042	-0.787	-0.233	0.072	0.347
55	**l leg ratio**	-0.113	-0.024	-0.038	0.253	-0.075	-0.475	-0.525	0.299	0.376
56	**r leg upper**	0.178	0.137	-0.168	0.126	-0.335	-0.432	-0.414	0.279	0.262
57	**r leg lower**	-0.552	0.304	-0.087	0.274	-0.099	-0.37	-0.294	0.19	0.149
58	**total leg r**	-0.085	0.428	0.252	0.225	0.383	-0.439	-0.022	-0.03	0.158
59	**r leg ratio**	0.085	-0.428	-0.252	-0.288	-0.073	-0.027	-0.157	0.134	0.44
60	**LLD**	0.528	-0.182	-0.505	0.015	-0.027	-0.308	-0.485	0.308	0.047

8.6.4.2 Pearson Correlation Matrix (continued)

		36	37	38	39	40	41	42	43	44
	Correlation Matrix	**% not flared**	**% of 1**	**% of 4**	**avg #**	**% left**	**% right**	**% ht**	**l arm upper**	**l arm lower**
1	**# lesions**	0.268	1	0.433	0.738	0.361	0.665	-0.362	0.456	0.268
2	**carpal slip r**	0.022	0.433	1	0.27	0.543	0.755	-0.495	-0.194	0.022
3	**carpal slip l**	-0.104	0.738	0.27	1	0.346	-0.18	-0.297	0.192	-0.104
4	**rad inclin r**	0.34	0.361	0.543	0.346	1	0.594	-0.14	0.022	0.34
5	**rad inclin l**	0.089	0.03	0.755	-0.18	0.594	1	-0.437	-0.323	0.089
6	**ulnar short r**	-0.268	-0.362	-0.495	-0.297	-0.14	-0.437	1	0.475	-0.268
7	**ulnar short l**	0.01	0.456	-0.494	0.192	0.022	-0.323	0.475	1	0.01
8	**rad bow r**	0.634	0.268	0.22	-0.104	0.34	0.089	-0.268	0.01	1
9	**rad bow l**	0.64	0.749	0.427	0.349	0.463	0.312	-0.582	0.247	0.64
10	**elb jt r**	0.08	0.473	0.104	0.319	-0.55	0.159	-0.656	0.093	0.08
11	**elb jt l**	0.128	0.465	0.78	0.507	-0.47	-0.397	-0.133	0.324	0.128
12	**fem aa r**	0.355	-0.041	0.42	-0.475	0.226	0.297	0.275	0.479	0.355
13	**fem aa l**	-0.028	-0.397	0.37	0.004	0.32	-0.161	0.301	-0.402	-0.028
14	**fem ns r**	0.271	0.256	0.599	0.17	0.418	0.556	-0.532	-0.03	0.271
15	**fem ns l**	0.312	0.364	0.614	0.144	0.709	0.444	0.079	0.352	0.312
16	**fem ma r**	0.355	0.217	0.143	0.379	0.213	0.24	0.388	0.593	0.355
17	**fem ma l**	-0.12	-0.495	-0.08	-0.382	-0.285	-0.021	0.124	-0.638	-0.12
18	**sharps r**	0.212	-0.557	-0.073	-0.462	-0.082	0.064	0.012	-0.537	0.212
19	**sharps l**	0.297	-0.229	-0.116	-0.261	-0.377	-0.17	-0.278	-0.432	0.297
20	**fib ht r**	0.085	0.37	1	0.352	-0.355	-0.394	-0.275	-0.02	0.085
21	**fib ht l**	-0.434	0.326	0.1	0.452	-0.159	-0.332	0.148	0.039	-0.434
22	**ankle jt r**	-0.281	-0.505	0.137	-0.564	-0.202	-0.141	861	0.384	-0.281
23	**ankle jt l**	-0.398	-0.552	-0.257	-0.496	-0.2	0.131	0.689	0.213	-0.398
24	**% wt bear r**	0.559	0.304	-0.027	-0.326	0.253	0.348	0.078	0.35	0.559
25	**% wt bear l**	0.376	-0.087	0.244	-0.42	0.114	0.307	-0.113	-0.405	0.376
26	**% ped**	-0.08	-0.352	0.404	-0.58	-0.09	0.218	-0.557	-0.576	-0.08
27	**% sess**	0.278	0.33	0.132	0.173	0.042	-0.317	0.267	0.146	0.278
28	**% distal**	-0.232	0.224	0.142	0.504	0.278	-0.114	0.249	0.158	-0.232
29	**%prox**	0.295	-0.518	0.229	-0.79	-0.407	0.083	-0.07	-0.106	0.295
30	**% pelvic**	0.265	0.82	-0.277	0.724	0.51	0.083	-0.413	0.279	0.265
31	**%diaph**	-0.219	-0.244	0.288	-0.04	0.06	-0.115	0.217	-0.17	-0.219
32	**%flat bones**	-0.487	0.002	-0.201	0.027	-0.053	0.278	-0.021	-0.229	-0.487

8.6.4.2 Pearson Correlation Matrix (continued)

		36	37	38	39	40	41	42	43	44
	Correlation Matrix	**% not flared**	**% of 1**	**% of 4**	**avg #**	**% left**	**% right**	**% ht**	**l arm upper**	**l arm lower**
33	**%complex**	0.656	0.109	0.282	-0.05	0.435	0.346	-0.607	-0.149	0.656
34	**%simple**	-0.387	-0.446	0.087	-0.337	-0.535	-0.389	0.514	-0.211	-0.387
35	**%flared**	0.146	0.164	-0.327	0.118	0.104	0.29	-0.528	0.1	0.146
36	**% not flared**	1	-0.084	0.28	-0.058	-0.045	-0.304	0.552	0.043	-0.096
37	**% of 1**	0.529	1	-0.249	-0.155	-0.195	-0.238	-0.244	-0.286	0.529
38	**% of 4**	-0.324	0.098	1	-0.16	0.336	0.345	0.243	0.373	-0.324
39	**avg #**	0.268	1	0.028	1	0.361	0.03	-0.362	0.456	0.268
40	**% left**	0.787	-0.087	0.433	-0.27	1	0.434	-0.325	-0.215	0.787
41	**% right**	-0.787	0.087	0.104	0.27	-0.479	1	0.325	0.042	-0.787
42	**% ht**	-0.475	-0.47	-0.104	-0.186	-0.353	-0.145	1	-0.075	-0.475
43	**l arm upper**	-0.432	-0.28	-0.336	-0.28	-0.376	-0.01	0.126	1	-0.432
44	**l arm lower**	-0.37	-0.744	-0.305	-0.607	-0.519	-0.078	0.274	-0.099	1
45	**total arm l**	-0.439	-0.492	-0.42	-0.466	-0.514	-0.085	0.225	0.383	-0.439
46	**ratio l arm**	-0.027	0.72	-0.426	0.447	0.218	0.131	-0.288	-0.073	-0.027
47	**r arm upper**	-0.308	-0.23	0.181	-0.296	-0.393	0.016	0.015	-0.027	-0.308
48	**r arm lower**	-0.485	-0.607	-0.321	-0.523	-0.526	-0.139	0.312	-0.048	-0.485
49	**total arm r**	-0.455	-0.514	0.555	-0.464	-0.639	-0.194	0.209	-0.74	-0.455
50	**r arm ratio**	0.128	0.384	-0.527	0.167	0.017	0.196	-0.383	0.085	0.128
51	**ALD**	0.302	0.242	0.162	0.415	0.268	-0.423	0.265	0.522	0.302
52	**l leg upper**	-0.404	-0.177	-0.143	-0.082	-0.655	-0.609	0.405	0.272	-0.404
53	**l leg lower**	-0.49	-0.264	-0.662	-0.35	-0.726	-0.262	0.199	0.299	-0.49
54	**total leg l**	-0.369	-0.056	-0.446	-0.178	-0.592	-0.233	0.072	0.347	-0.369
55	**l leg ratio**	0.273	0.347	-0.402	0.601	0.354	-0.525	0.299	0.376	0.273
56	**r leg upper**	-0.57	-0.188	0.227	-0.121	-0.661	-0.414	0.279	0.262	-0.57
57	**r leg lower**	-0.157	-0.262	-0.528	-0.423	-0.607	-0.294	0.19	0.149	-0.157
58	**total leg r**	-0.403	-0.12	-0.574	-0.229	-0.484	-0.022	-0.03	0.158	-0.403
59	**r leg ratio**	-0.679	0.128	-280	0.525	-0.024	-0.157	0.134	0.44	-0.679
60	**LLD**	-0.339	0.297	0.118	0.26	-0.439	-0.485	0.308	0.047	-0.339

8.6.4.2 Pearson Correlation Matrix (continued)

		45	46	47	48	49	50	51	52	53
	Correlation Matrix	**total arm l**	**ratio l arm**	**r arm uppder**	**r arm lower**	**total arm r**	**r arm ratio**	**ALD**	**l leg upper**	**l leg lower**
1	**# lesions**	0.749	0.473	0.465	-0.041	-0.397	0.256	0.364	0.217	0.665
2	**carpal slip r**	0.427	0.104	0.078	0.42	-0.037	0.599	0.614	0.143	0.755
3	**carpal slip l**	0.349	0.319	0.507	-0.475	-0.004	0.17	0.144	-0.379	-0.18
4	**rad inclin r**	0.463	-0.055	-0.047	0.058	0.132	0.418	0.709	0.213	0.594
5	**rad inclin l**	0.312	0.159	-0.397	0.226	-0.161	0.556	0.444	0.24	-0.89
6	**ulnar short r**	-0.582	-0.656	-0.133	0.297	0.301	-0.532	0.079	0.388	-0.437
7	**ulnar short l**	0.247	0.093	0.324	0.275	-0.402	-0.03	0.352	0.593	-0.323
8	**rad bow r**	0.64	0.08	0.128	0.479	-0.028	0.271	0.312	0.355	0.089
9	**rad bow l**	1	0.452	0.415	0.355	-0.441	0.411	0.484	0.27	0.312
10	**elb jt r**	0.452	1	0.09	0.263	-0.704	0.421	-0.307	-0.127	0.159
11	**elb jt l**	0.415	0.09	1	0.01	0.194	0.098	0.256	-0.186	-0.397
12	**fem aa r**	0.263	0.01	0.058	1	-0.158	0.281	0.487	0.741	0.297
13	**fem aa l**	-0.441	-0.704	0.194	0.158	1	-0.084	0.17	-0.311	-0.161
14	**fem ns r**	0.484	0.421	0.098	0.281	-0.084	1	0.467	0.077	0.556
15	**fem ns l**	0.27	-0.307	0.256	0.487	0.17	0.467	1	0.53	0.444
16	**fem ma r**	-0.55	-0.127	-0.186	0.741	-0.311	0.077	0.53	1	0.24
17	**fem ma l**	-0.268	-0.563	-0.498	-0.489	0.44	-0.357	-0.28	-0.156	-0.021
18	**sharps r**	0.003	-0.254	0.085	0.253	0.636	0.011	-0.121	-0.184	0.064
19	**sharps l**	0.153	0.095	0.386	0.507	0.342	-0.011	-0.321	-0.278	-0.17
20	**fib ht r**	-0.114	0.269	0.72	0.615	0.208	0.063	-0.17	-0.249	-0.394
21	**fib ht l**	-0.575	-0.204	0.407	-0.023	0.235	-0.545	-0.09	-0.211	-0.332
22	**ankle jt r**	-0.587	-0.626	-0.251	-0.165	0.333	-0.21	0.213	0.517	-0.141
23	**ankle jt l**	0.38	-0.498	-0.238	-0.105	-0.348	0.049	0.276	0.363	0.131
24	**% wt bear r**	0.106	0.101	-0.279	-0.103	0.158	0.253	0.402	0.902	0.348
25	**% wt bear l**	0.052	-0.423	-0.375	0.508	0.197	-0.018	0.266	0.36	0.307
26	**% ped**	-0.064	0.12	0.237	-0.145	0.088	0.31	-0.121	-0.666	0.218
27	**% sess**	-0.281	-0.028	-0.121	-0.422	0.459	-0.292	-0.136	0.3	-0.317
28	**% distal**	0.044	-0.069	0.158	-0.118	-0.163	0.283	0.215	-0.055	-0.114
29	**%prox**	0.789	0.011	-0.055	0.296	-0.368	0.074	-0.122	0.178	0.083
30	**% pelvic**	-0.147	0.363	0.412	-0.431	0.136	0.068	0.316	-0.03	0.083
31	**%diaph**	-0.375	-0.556	-0.162	0.406	-0.028	-0.599	-0.016	-0.251	-0.115
32	**%flat bones**	0.559	0.254	-0.35	-0.372	-0.234	-0.036	-0.311	-0.024	0.278

8.6.4.2 Pearson Correlation Matrix (continued)

		45	46	47	48	49	50	51	52	53
	Correlation Matrix	**total arm l**	**ratio l arm**	**r arm uppder**	**r arm lower**	**total arm r**	**r arm ratio**	**ALD**	**l leg upper**	**l leg lower**
35	**%flared**	0.379	0.605	0.47	-0.041	0.164	0.681	0.133	-0.148	0.29
36	**% not flared**	0.015	-0.593	-0.431	0.425	0.35	-0.645	-0.08	0.202	-0.304
37	**% of 1**	0.075	0.115	0.254	-0.425	-0.526	0.375	-0.165	-0.244	-0.238
38	**% of 4**	0.749	0.071	-0.397	0.043	-0.397	-0.223	0.151	0.339	0.345
39	**avg #**	0.4	0.473	0.465	-0.359	0.017	0.256	0.364	0.217	0.39
40	**% left**	-0.4	0.133	-0.203	-0.266	-0.017	0.575	0.297	0.21	-0.433
41	**% right**	-0.656	-0.133	0.203	-0.27	0.008	-0.575	-0.297	-0.21	0.424
42	**% ht**	-0.389	0.188	-0.305	-0.137	-0.502	0.213	-0.401	-0.175	-0.038
43	**l arm upper**	-0.725	-0.05	-0.735	-0.282	-0.037	-0.415	-0.521	-0.007	-0.168
44	**l arm lower**	-0.511	-0.255	-0.759	-0.112	-0.394	-0.314	-0.547	-0.107	-0.087
45	**total arm l**	1	-0.07	-0.709	-0.237	-0.765	-0.432	-0.549	-0.02	0.252
46	**ratio l arm**	-321	1	0.01	-0.308	-0.603	-0.101	0.038	0.169	-0.252
47	**r arm upper**	-0.517	0.254	1	-0.052	-0.351	-0.274	-0.593	0.029	-0.505
48	**r arm lower**	-0.522	-0.104	-0.596	1	-0.422	-0.498	-0.536	-0.075	0.118
49	**total arm r**	0.163	0.023	-0.508	-0.053	1	-0.402	-0.632	-0.086	0.224
50	**r arm ratio**	-0.032	0.537	0.376	-0.165	0.574	1	-0.272	0.134	0.112
51	**ALD**	-0.334	-0.195	-0.084	-0.11	-0.465	-0.13	1	-0.065	-0.126
52	**l leg upper**	-0.348	0.108	-0.309	-0.159	-0.596	-0.374	-0.448	1	0.073
53	**l leg lower**	-0.174	0.206	-0.359	-0.027	-0.742	-0.281	-0.513	0.068	1
54	**total leg l**	0.118	0.33	0.488	-0.176	0.422	-0.253	-0.498	0.079	-0.233
55	**l leg ratio**	-0.401	-0.229	-0.219	-0.185	-0.529	-0.075	0.266	-0.1	-0.525
56	**r leg upper**	-0.172	0.215	-0.366	-0.06	-0.619	-0.287	-0.519	-0.05	-0.414
57	**r leg lower**	-0.171	0.102	-0.5	-0.567	-0.757	-0.352	-0.442	0.151	-0.294
58	**total leg r**	-0.363	0.349	0.286	-0.74	0.207	-0.187	-0.48	0.044	-0.022
59	**r leg ratio**	-0.241	0.188	0.289	-0.009	0.123	0.124	-0.097	-0.356	-0.157
60	**LLD**	-0.214	-0.041	0.456	-0.234	0.145	-0.064	0.009	0.132	-0.485

8.6.4.2 Pearson Correlation Matrix (continued)

		54	55	56	57	58	59	60
	Correlation Matrix	**total leg l**	**l leg ratio**	**r leg uppder**	**r leg lower**	**total leg r**	**r leg ratio**	**LLD**
1	**# lesions**	0.362	0.456	0.34	-0.754	0.445	0.125	0.297
2	**carpal slip r**	-0.495	-0.194	0.647	-0.576	0.233	0.324	0.044
3	**carpal slip l**	-0.297	0.192	0.99	-0.75	0.34	0.859	0.26
4	**rad inclin r**	-0.14	0.022	0.322	-0.756	0.78	-0.94	-0.439
5	**rad inclin l**	-0.437	-0.323	0.538	0.34	0.98	0.23	-0.485
6	**ulnar short r**	0.765	0.475	0.283	0.76	0.5	0.35	0.308
7	**ulnar short l**	0.475	0.346	0.73	0.23	0.55	-0.433	0.44
8	**rad bow r**	-0.268	0.01	0.93	0.123	0.456	-0.354	-0.339
9	**rad bow l**	-0.582	0.247	0.833	0.345	0.76	-0.433	-214
10	**elb jt r**	-0.656	0.093	0.763	0.34	0.002	-0.43	-0.041
11	**elb jt l**	-0.133	0.324	0.3	0.56	0.213	-0.45	0.456
12	**fem aa r**	0.275	0.479	883	0.83	0.04	0.94	-0.06
13	**fem aa l**	0.301	-0.402	0.393	0.34	-0.5	0.49	0.123
14	**fem ns r**	-0.532	-0.03	0.482	0.09	-0.3	0.43	-0.064
15	**fem ns l**	0.079	0.352	0.119	0.67	-0.3	0.87	0.009
16	**fem ma r**	0.388	0.593	0.299	0.69	-0.44	0.003	-0.14
17	**fem ma l**	0.124	-0.638	0.33	0.005	-0.564	0.9	0.08
18	**sharps r**	0.012	-0.537	0.21	-0.56	0.04	0.54	0.636
19	**sharps l**	-0.278	-0.432	0.222	-0.564	0.868	0.94	0.546
20	**fib ht r**	-0.275	-0.02	0.33	0.234	0.345	0.113	0.305
21	**fib ht l**	0.148	0.039	-0.38	-0.44	0.965	0.124	0.214
22	**ankle jt r**	861	0.384	-0.734	-0.2	0.674	0.13	0.036
23	**ankle jt l**	0.689	0.213	0.823	0.609	747	0.89	-0.163
24	**% wt bear r**	0.078	0.35	0.932	0.443	0.82	0.006	-0.377
25	**% wt bear l**	-0.113	0.876	0.229	0.553	0.679	0.042	0.335
26	**% ped**	-0.557	-0.576	0.922	0.26	0.23	0.456	0.537
27	**% sess**	0.267	0.146	0.199	0.765	0.45	0.756	-0.289
28	**% distal**	0.249	0.158	0.029	0.334	0.97	0.345	-0.17
29	**%prox**	-0.07	-0.106	0.392	0.67	0.22	0.923	-0.385
30	**% pelvic**	-0.413	0.279	0.675	0.87	0.229	0.454	0.268
31	**%diaph**	0.217	-0.17	0.445	0.98	0.674	0.293	-0.562

8.6.4.2 Pearson Correlation Matrix (continued)

		54	55	56	57	58	59	60
	Correlation Matrix	**total leg l**	**l leg ratio**	**r leg uppder**	**r leg lower**	**total leg r**	**r leg ratio**	**LLD**
34	**%simple**	0.514	-0.211	0.142	0.43	0.843	0.32	0.095
35	**%flared**	-0.528	0.1	0.234	0.567	0.273	0.234	-0.019
36	**% not flared**	0.552	0.043	0.566	0.54	0.009	0.345	-331
37	**% of 1**	-0.244	-0.286	0.122	0.454	0.987	0.322	0.933
38	**% of 4**	0.243	0.373	0.677	0.465	0.09	0.233	0.84
39	**avg #**	-0.362	0.456	0.564	0.476	0.65	0.944	0.483
40	**% left**	-0.325	-0.215	0.678	0.2	0.43	0.758	0.93
41	**% right**	0.325	0.042	0.435	0.65	0.23	0.483	-0.333
42	**% ht**	0.253	-0.075	0.789	0.67	0.19	0.493	-0.843
43	**l arm upper**	0.126	-0.335	0.345	0.58	0.87	0.842	0.934
44	**l arm lower**	0.274	-0.099	0.876	0.45	0.908	0.745	0.23
45	**total arm l**	0.225	0.383	567	0.678	0.654	0.39	0.383
46	**ratio l arm**	-0.288	-0.073	0.998	0.345	0.876	0.398	0.203
47	**r arm upper**	0.015	-0.027	0.887	0.45	0.213	0.834	0.23
48	**r arm lower**	0.312	-0.048	-0.987	0.48	0.8	-0.842	0.432
49	**total arm r**	0.209	-0.74	-0.76	0.578	0.56	0.321	0.233
50	**r arm ratio**	-0.383	0.085	-0.787	0.567	0.49	0.123	0.11
51	**ALD**	0.265	0.522	-0.765	0.45	0.65	0.432	0.002
52	**l leg upper**	0.405	0.272	0.789	0.576	0.7	0.35	0.922
53	**l leg lower**	0.199	0.299	0.098	0.333	0.567	0.23	0.74
54	**total leg l**	1	0.347	0.087	0.006	0.678	0.655	0.34
55	**l leg ratio**	0.299	1	0.554	0.433	0.098	0.544	0.299
56	**r leg upper**	0.279	0.262	1	0.44	0.456	0.005	0.008
57	**r leg lower**	0.19	0.149	0.667	1	0.87	0.35	0.009
58	**total leg r**	-0.03	0.158	0.453	0.333	1	0.234	0.343
59	**r leg ratio**	0.134	0.44	0.698	0.54	0.99	1	0.493
60	**LLD**	0.308	0.047	0.184	0.254	0.666	0.54	1

Appendix 8.7 Genotype – Phenotype Correlation Tables

8.7.1 Gene

Table 8.7.1.1 Lesion Quality by Gene

Variable	EXT 1 (n=7)	EXT 2 (n=19)	P-value	Power
Lesion Rank 1	9.1 ± 6.1	6.2 ± 4.6 (n = 17)	0.21	0.25
% Rank 1	27.0 ± 10.1	31.6 ± 20.3 (n = 17)	0.58	0.073
Lesion Rank 2	6.4 ± 2.9	3.9 ± 2.8 (n = 17)	0.059	0.32
% Rank 2	19.4 ± 7.5	22.6 ± 12.7 (n = 17)	0.55	0.11
Lesion Rank 3	5.0 ± 1.9	1.9 ± 1.8 (n = 17)	< 0.01 (0.0013)	0.94
% Rank 3	16.0 ± 6.0	9.5 ± 6.9 (n = 17)	0.042	0.57
Lesion Rank 4	12.1 ± 4.1	7.1 ± 4.6 (n = 17)	0.019	0.50
% Rank 4	37.7 ± 10.1	36.1 ± 18.9 (n = 17)	0.83	0.053
Small (%)	28.4 ± 11.7	30.8 ± 17.9 (n = 19)	0.74	0.061
Medium (%)	30.6 ± 8.4	30.9 ± 13.9 (n = 19)	0.96	0.050
Large (%)	38.6 ± 15.7	36.5 ± 17.9 (n – 19)	0.79	0.058
Average Number of Lesions	32.7 ± 10.4	19.1 ± 8.8 (n = 17)	< 0.01 (0.0036)	0.82
No. Pedunculated	8.7 ± 2.9	6.1 ± 4.3 (n = 19)	0.15	0.28
% Pedunculated	26.9 ± 5.3	31.2 ± 13.8 (n = 17)	0.43	0.12
No. Sessile	21.1 ± 8.9	13.4 ± 6.9 (n = 19)	0.028	0.61
% Sessile	64.3 ± 11.1	65.1 ± 14.8 (n = 17)	0.89	0.054
No. Distal	13.1 ± 5.0	8.1 ± 4.4 (n = 19)	0.020	0.67
% Distal	40.2 ± 8.4	39.9 ± 14.5 (n = 17)	0.97	0.051
No. Proximal	14.4 ± 5.2	9.4 ± 4.6 (n = 19)	0.026	0.62
% Proximal	43.9 ± 8.9	46.4 ± 16.9 (n = 17)	0.72	0.069
No. Pelvic	3.4 ± 2.9	0.74 ± 1.4 (n = 19)	< 0.01 (0.0043)	0.87
% Pelvic	9.6 ± 7.5	2.3 ± 5.2 (n = 17)	0.012	0.68
No Diaphyseal	1.9 ± 1.7	1.3 ± 1.3 (n = 19)	0.39	0.13
% Diaphyseal	6.5 ± 6.9	8.7 ± 12.0 (n = 17)	0.66	0.063
No. Flat Bone	4.1 ± 2.8	0.84 ± 1.4 (n = 19)	< 0.01 (0.0005)	0.98
% Flat Bone	11.8 ± 6.1	3.0 ± 15.3 (n = 17)	< 0.01(0.0019)	0.91
No. Complex	4.9 ± 5.9	2.7 ± 2.1 (n = 19)	0.17	0.26
% Complex	12.4 ± 10.3	14.3 ± 9.3 (n = 17)	0.67	0.061
No. Simple	25.3 ± 5.4	17.3 ± 8.6 (n = 19)	0.32	0.58
% Simple	79.5 ± 10.6	84.1 ± 9.5 (n = 17)	0.31	0.20
No. Flared	14.1 ± 12.0	6.8 ± 5.9 (n = 19)	0.047	0.52
% Flared	38.6 ± 29.7	30.4 ± 24.2 (n = 17)	0.48	0.099
No. Not Flared	18.6 ± 7.8	12.9 ± 7.2 (n = 17)	0.10	0.34
% Not Flared	61.4 ± 29.7	69.6 ± 24.2 (n = 17)	0.48	0.091
No. Left	18.6 ± 6.7	10.1 ± 5.2 (n = 19)	< 0.01 (0.0022)	0.92
% Left	56.6 ± 7.2	49.4 ± 10.09 (n = 17)	0.13	0.39
No. Right	14.3 ± 4.9	10.2 ± 4.9 (n = 19)	0.076	0.42
% Right	43.8 ± 7.8	50.6 ± 10.9 (n = 17)	0.15	0.34

Table 8.7.1.2. Limb Alignment by Gene

Variable	Normal Values	EXT 1 (n = 7)	EXT 2 (n = 19)	P-value	Power
1. Carpal Slip Right	5 ± 2mm	5.2 ± 2.8	2.2 ± 3.6	0.08	0.41
2. Carpal Slip Left		4.7 ± 3.0	3.1 ± 3.5	0.29	0.17
3. Radial Inclination Right	21° ± 2°	28.0 ± 5.4	24.2 ± 5.0	0.13	0.31
4. Radial Inclination Left		30.6 ± 6.0	26.4 ± 4.8	0.08	0.41
5. Ulnar Shortening Right	0 ± 1 mm	-1.2 ± 3.9	-1.7 ± 4.9	0.81	0.056
6. Ulnar Shortening Left		2.7 ± 5.8	-0.8 ± 4.9	0.14	0.30
7. Radial Bow Right	10° ± 5°	9.0 ± 2.3	7.6 ± 2.4	0.20	0.23
8. Radial Bow Left		14.2 ± 8.4	7.7 ± 2.4	< 0.01	0.87
9. Radial Head Dislocation R		1 dislocation	1 dislocation		
10. Radial Head Dislocation L		2 dislocations	1 dislocation		
11. Elbow Joint Right	9° ± 3°	-1.8 ± 18.5	-5.6 ± 12.9	0.58	0.082
12. Elbow Joint Left		-3.9 ± 10.2	-8.5 ± 11.3	0.35	0.14
13. Femoral A.A. Right	7° ± 2° valgus	-3.1 ± 7.8	-5.6 ± 9.1	0.53	0.093
14. Femoral A.A. Left		-1.6 ± 8.3	-3.4 ± 9.0	0.64	0.073
15. Femoral N.S. Angle Right	135° ± 5°	143.1 ± 17.7	140.1 ± 8.0	0.55	0.088
16. Femoral N.S. Angle Left		146.4 ± 11.0	137.1 ± 9.1	0.04	0.56
17. Femoral M.A. Right	0° ± 5° varus	6.3 ± 5.4	-0.1 ± 6.0	0.03	0.59
18. Femoral M.A. Left		-1.0 ± 7.0	1.1 ± 5.0	0.42	0.12
19. Sharp's Right	35° ± 4°	38.5 ± 3.1	41.4 ± 5.7	0.29	0.23
20. Sharp's Left		38.5 ± 5.4	41.4 ± 4.9	0.31	0.16
21. Fibular Height Right	50 ± 10	52.0 ± 8.0	51.6 ± 11.7	0.94	0.051
22. Fibular Height Left		52.2 ± 13.8	51.8 ± 14.4	0.95	0.052
23. Ankle Joint Angle Right	0° ± 5°	-9.8 ± 13.6	-1.8 ± 10.1	0.14	0.062
24. Ankle Joint Angle Left		-5.5 ± 14.4	-1.0 ± 10.6	0.42	0.052
25. % Weightbear Right	50 ± 10	61.0 ± 22.4	46.5 ± 22.2	0.18	0.36
26. % Weightbear Left		65.2 ± 11.9	51.4 ± 19.7	0.12	0.21
Number of parameters that fall beyond the normal range		**15 / 24**	**4 / 24**		

Table 8.7.1.3. Segment Lengths and Percentile Height by Gene

Variable	EXT 1 (n = 7)	EXT 2 (n = 19)	P-value	Power
Total Leg Length-Right	79.6 ± 8.3	84.9 ± 9.1	0.19	0.24
Upper Leg – Right	39.2 ± 4.5	44.0 ± 5.5	0.052	0.49
Lower Leg – Right	32.4 ± 3.9	34.9 ± 3.6	0.12	0.32
Total Leg Length – Left	78.9 ± 8.4	84.3 ± 9.3	0.19	0.24
Upper Leg – Left	38.9 ± 4.9	43.2 ± 5.1	0.063	0.45
Lower Leg – Left	32.4 ± 4.6	36.2 ± 4.8	0.087	0.39
Total Arm Length – Right	44. 9 ± 5.1	50.7 ± 5.7	0.026	0.62
Upper Arm – Right	27.4 ± 2.4	30.6 ± 3.8	0.052	0.49
Lower Arm – Right	20.9 ± 2.4	23.6 ± 3.4	0.071	0.43
Total Arm Length – Left	45.0 ± 5.2	51.1 ± 5.9	0.027	0.62
Upper Arm – Left	27.5 ± 3.5	31.2 ± 4.4	0.059	0.47
Lower Arm – Left	19.9 ± 3.1	23.9 ± 3.3	0.011	0.77
Percentile Height	9.33 ± 13.3	42.5 ± 29.0	< 0.01 (0.0081)	0.80

8.7.2 Gender

Table 8.7.2.1. Lesion Quality by Gender

Variable	Males (n = 14)	Females (n = 12)	P-value
Lesion Rank 1	9.1 ± 5.9	4.6 ± 2.6	0.03
% Rank 1	27.8 ± 16.8	32.3 ± 19.1	0.55
Lesion Rank 2	5.3 ± 3.4	3.8 ± 2.4	0.24
% Rank 2	18.8 ± 9.6	25.0 ± 12.8	0.19
Lesion Rank 3	3.7 ± 2.5	1.8 ± 1.7	0.04
% Rank 3	12.4 ± 7.0	10.3 ± 7.6	0.49
Lesion Rank 4	10.0 ± 5.0	6.9 ± 4.5	0.13
% Rank 4	36.5 ± 16.3	36.7 ± 17.9	0.97
Small (%)	32.2 ± 17.9	27.8 ± 14.7	0.50
Medium (%)	28.8 ± 9.3	33.1 ± 15.6	0.39
Large (%)	35.1 ± 17.4	39.4 ± 17.1	0.53
Average Number of Lesions	28.1 ± 11.5	17.2 ± 7.2	0.01
No. Pedunculated	7.9 ± 5.0	5.6 ± 2.3	0.16
% Pedunculated	27.4 ± 12.8	36.0 ± 10.4	0.12
No. Sessile	19.1 ± 8.5	11.3 ± 5.4	0.01
% Sessile	68.0 ± 14.6	58.2 ± 6.7	0.08
No. Distal	11.2 ± 5.4	7.4 ± 3.9	0.05
% Distal	40.5 ± 14.5	39.5 ± 11.1	0.86
No. Proximal	13.4 ± 5.1	7.7 ± 3.6	< 0.01 (0.0035)
% Proximal	49.3 ± 12.1	41.4 ± 19.5	0.30
No. Pelvic	1.9 ± 2.8	0.9 ± 1.2	0.26
% Pelvic	5.2 ± 7.6	3.5 ± 5.7	0.55
No Diaphyseal	1.1 ± 1.1	1.9 ± 1.6	0.13
% Diaphyseal	5.4 ± 8.3	12.9 ± 13.7	0.16
No. Flat Bone	2.2 ± 2.9	1.2 ± 1.5	0.26
% Flat Bone	6.3 ± 7.5	4.8 ± 6.2	0.59
No. Complex	4.4 ± 4.5	1.9 ± 1.4	0.07
% Complex	14.5 ± 7.1	12.2 ± 8.0	0.51
No. Simple	23.1 ± 8.7	15.2 ± 6.3	0.01
% Simple	83.9 ± 8.7	83.3 ± 8.0	0.88
No. Flared	12.6 ± 9.1	4.3 ± 4.9	0.01
% Flared	45.0 ± 25.2	18.5 ± 17.8	0.01
No. Not Flared	15.1 ± 8.9	14.0 ± 6.1	0.74
% Not Flared	55.0 ± 25.2	81.5 ± 17.8	< 0.01 (0.0079)
No. Left	15.2 ± 7.2	9.0 ± 4.3	0.02
% Left	52.1 ± 10.6	51.0 ± 10.0	0.83
No. Right	13.4 ± 5.6	8.8 ± 3.5	0.02
% Right	47.9 ± 10.6	49.3 ± 10.1	0.77

Table 8.7.2.2. Limb Alignment by Gender

Variable	Normal Values	Males (n = 14)	Females (n = 12)	P-value
1. Carpal Slip Right	5 ± 2mm	3.4 ± 4.3 (n = 12)	2.5 ± 2.7	0.52
2. Carpal Slip Left		3.8 ± 3.6	3.3 ± 3.3	0.70
3. Radial Inclination Right	21° ± 2°	26.5 ± 4.6 (n = 12)	23.8 ± 5.8	0.22
4. Radial Inclination Left		28.8 ± 5.7	26.1 ± 4.7	0.20
5. Ulnar Shortening Right	0 ± 1 mm	-2.5 ± 4.6 (n = 12)	-0.58 ± 4.5	0.30
6. Ulnar Shortening Left		0.0 ± 5.6	0.33 ± 5.1	0.88
7. Radial Bow Right	10° ± 5°	8.1 ± 2.7 (n = 13)	7.7 ± 2.1	0.71
8. Radial Bow Left		10.0 ± 6.4	8.8 ± 4.1	0.58
9. Radial Head Dislocation R		1 dislocation	1 dislocation	
10. Radial Head Dislocation L		1 dislocation	2 dislocations	
11. Elbow Joint Right	9° ± 3°	-3.8 ± 15.2 (n = 13)	-5.6 ± 13.4	0.77
12. Elbow Joint Left		-7.9 ± 11.7	-6.5 ± 10.7	0.74
13. Femoral A.A. Right	7° ± 2° valgus	3.4 ± 4.3 (n = 12)	2.5 ± 2.7	0.52
14. Femoral A.A. Left		3.8 ± 3.6	3.3 ± 3.3	0.70
15. Femoral N.S. Angle Right	135° ± 5°	140.9 ± 8.3	141.0 ± 14.1	0.97
16. Femoral N.S. Angle Left		138.0 ± 8.1	141.4 ± 12.5	0.41
17. Femoral M.A. Right	0° ± 5° varus	2.2 ± 6.7 (n = 13)	0.55 ± 6.2 (n = 11)	0.53
18. Femoral M.A. Left		-0.39 ± 6.0 (n = 13)	1.7 ± 4.7 (n = 11)	0.36
19. Sharp's Right	35° ± 4°	39.7 ± 3.9 (n = 13)	42.3 ± 6.6 (n = 10)	0.25
20. Sharp's Left		40.7 ± 4.9 (n = 13)	40.5 ± 5.3 (n = 10)	0.93
21. Fibular Height Right	50 ± 10	54.5 ± 9.4 (n = 13)	48.7 ± 11.7	0.18
22. Fibular Height Left		51.8 ± 12.8 (n = 13)	51.9 ± 15.9 (n = 11)	0.99
23. Ankle Joint Angle Right	0° ± 5°	-4.2 ± 14.9 (n = 11)	-3.7 ± 7.6	0.92
24. Ankle Joint Angle Left		-2.0 ± 14.7 (n = 11)	-2.3 ± 8.2	0.95
25. % Weightbear Right	50 ± 10	55.2 ± 24.9 (n = 12)	45.1 ± 19.9	0.29
26. % Weightbear Left		54.2 ± 17.9 (n = 12)	55.5 ± 20.4 (n = 12)	0.87
Number of parameters that fall beyond the normal range		**9**	**12**	

Table 8.7.2.3. Segment Lengths and Percentile Height by Gender

Variable	Males (n = 14)	Females (n = 12)	P-value
Total Leg Length-Right	86.4 ± 5.5	80.2 ± 11.3	0.08
Upper Leg – Right	43.4 ± 3.9	41.9 ± 7.2	0.49
Lower Leg – Right	35.5 ± 2.9	32.8 ± 4.2	0.065
Total Leg Length – Left	85.8 ± 6.2	79.4 ± 11.2	0.081
Upper Leg – Left	42.6 ± 3.7	41.3 ± 6.9	0.54
Lower Leg – Left	36.5 ± 4.5	33.6 ± 5.3	0.14
Total Arm Length – Right	50.4 ± 5.2	47.6 ± 6.9	0.25
Upper Arm – Right	31.0 ± 3.0	28.3 ± 4.1	0.072
Lower Arm – Right	23.6 ± 3.1	22.0 ± 3.4	0.23
Total Arm Length – Left	50.8 ± 5.6	47.7 ± 6.9	0.20
Upper Arm – Left	31.3 ± 3.5	28.9 ± 5.1	0.18
Lower Arm – Left	23.5 ± 3.9	22.1 ± 3.4	0.36
Percentile Height	32.3 ± 28.2	35.1 ± 32.4	0.82

8.7.3 Mutation Type

Table 8.7.3.1. Lesion Quality by Mutation Type

Variable	Missense (n=4)	Nonsense (n=14)	Splice Site (n=5)	Frameshift (n=3)	p-value	Power
Lesion Rank 1	9.0 ± 1.6	7.6 ± 6.4	5.0 ± 0.0	5.0 ± 2.6	0.57	0.17
% Rank 1	48.3 ± 21.6	30.0 ± 16.9	19.0 ± 5.5	21.3 ± 6.9	0.054	0.62
Lesion Rank 2	4.0 ± 3.8	4.4 ± 2.6	6.0 ± 4.1	6.7 ± 3.1	0.53	0.18
% Rank 2	16.0 ± 12.2	21.5 ± 11.8	23.8 ± 9.6	31.5 ± 13.1	0.38	0.24
Lesion Rank 3	2.5 ± 1.9	2.6 ± 2.3	4.2 ± 2.6	2.3 ± 2.3	0.55	0.17
% Rank 3	10.8 ± 5.9	10.1 ± 7.6	16.0 ± 7.1	9.0 ± 4.9	0.42	0.22
Lesion Rank 4	5.5 ± 3.0	8.4 ± 5.1	12.6 ± 3.6	9.7 ± 8.1	0.22	0.36
% Rank 4	24.8 ± 5.9	38.3 ± 20.2	41.2 ± 7.6	37.9 ± 14.0	0.47	0.20
Small (%)	48.5 ± 19.3	29.7 ± 15.8	19.1 ± 6.7	26.4 ± 7.6	0.045	0.65
Medium (%)	24.0 ± 11.3	30.7 ± 13.9	31.4 ± 8.6	39.2 ± 12.3	0.49	0.19
Large (%)	23.9 ± 10.8	37.3 ± 20.1	48.5 ± 5.9	34.3 ± 8.5	0.19	0.37
Average Number of Lesions	21.0 ± 7.0	22.9 ± 12.8	27.8 ± 8.2	23.7 ± 12.5	0.81	0.10
No. Pedunculated	6.3 ± 2.1	6.7 ± 4.6	6.6 ± 2.7	8.3 ± 6.7	0.93	0.074
% Pedunculated	30.8 ± 8.9	30.4 ± 13.5	24.9 ± 9.4	35.5 ± 16.3	0.69	0.13
No. Sessile	14.8 ± 5.7	14.6 ± 9.3	18.8 ± 7.2	15.3 ± 8.1	0.81	0.10
% Sessile	69.2 ± 8.9	63.6 ± 15.7	66.9 ± 11.9	64.5 ± 16.3	0.90	0.080
No. Distal	9.0 ± 4.7	9.6 ± 5.9	10.8 ± 3.0	7.3 ± 5.0	0.84	0.095
% Distal	42.3 ± 17.5	41.1 ± 12.6	39.8 ± 8.3	18.2 ± 0.0	0.39	0.23
No. Proximal	9.5 ± 3.0	10.1 ± 5.7	12.8 ± 6.1	11.7 ± 5.5	0.76	0.11
% Proximal	48.1 ± 19.5	44.9 ± 15.9	44.1 ± 10.9	50.6 ± 3.7	0.92	0.075
No. Pelvic	0.75 ± 1.5	1.5 ± 2.8	2.0 ± 1.6	1.3 ± 1.5	0.89	0.083
% Pelvic	2.7 ± 5.4	4.5 ± 7.9	6.3 ± 4.5	0.0 ± 0.0	0.79	0.10
No Diaphyseal	1.5 ± 2.4	1.1 ± 1.3	2.0 ± 1.0	2.0 ± 1.0	0.61	0.15
% Diaphyseal	6.0 ± 8.4	7.2 ± 12.1	8.2 ± 5.3	11.4 ± 13.8	0.92	0.075
No. Flat Bone	1.3 ± 1.5	1.7 ± 2.9	2.4 ± 1.8	1.3 ± 1.5	0.89	0.081
% Flat Bone	4.6 ± 5.5	5.6 ± 7.9	7.6 ± 5.2	0.0 ± 0.0	0.78	0.11
No. Complex	2.8 ± 0.96	3.7 ± 4.8	3.0 ± 1.6	2.3 ± 1.5	0.92	0.075
% Complex	14.9 ± 9.4	14.3 ± 11.2	10.3 ± 3.5	12.6 ± 8.5	0.86	0.089
No. Simple	18.3 ± 7.5	18.2 ± 9.0	22.8 ± 6.5	21.3 ± 13.1	0.76	0.12
% Simple	85.1 ± 9.4	82.2 ± 11.3	82.6 ± 7.9	87.4 ± 8.5	0.85	0.091
No. Flared	5.8 ± 3.1	9.6 ± 8.9	9.0 ± 11.7	8.0 ± 8.2	0.89	0.081
% Flared	32.9 ± 24.3	36.5 ± 25.3	27.3 ± 31.4	27.1 ± 19.2	0.88	0.084
No. Not Flared	15.3 ± 9.6	13.2 ± 7.1	18.8 ± 8.3	14.7 ± 4.5	0.57	0.18
% Not Flared	67.1 ± 24.3	63.5 ± 25.3	72.7 ± 31.4	68.7 ± 19.8	0.92	0.075
No. Left	12.5 ± 2.6	12.1 ± 8.7	14.4 ± 2.9	9.7 ± 4.9	0.83	0.096
% Left	61.7 ± 10.2	49.1 ± 9.8	53.3 ± 6.9	40.8 ± 7.9	0.039	0.67
No. Right	8.5 ± 4.7	10.7 ± 4.5	13.6 ± 5.6	14.0 ± 8.2	0.39	0.24
% Right	38.3 ± 10.2	50.9 ± 9.8	47.3 ± 7.4	59.2 ± 7.9	0.044	0.65

Table 8.7.3.2. Limb Alignment by Mutation Type

Variable	Normal Values	Missense (n=4)	Nonsense (n=14)	Splice Site (n=5)	Frameshift (n=3)	p-value	Power
1. Carpal Slip Right	5 ± 2mm	4.0 ± 3.4	2.0 ± 4.4	3.8 ± 2.5	3.7 ± 1.2	0.71	0.13
2. Carpal Slip Left		3.3 ± 3.2	3.6 ± 4.2	3.6 ± 2.3	3.7 ± 2.1	0.99	0.052
3. Radial Inclination Right	21° ± 2°	26.5 ± 7.9	24.9 ± 4.1	27.4 ± 0.55	20.7 ± 9.5	0.36	0.25
4. Radial Inclination Left		27.0 ± 5.3	27.2 ± 5.1	30.0 ± 5.8	25.7 ± 7.6	0.70	0.13
5. Ulnar Shortening Right	0 ± 1 mm	-1.0 ± 2.3	-2.5 ± 5.1	1.8 ± 1.8	-4.0 ± 6.2	0.25	0.32
6. Ulnar Shortening Left		-4.7 ± 5.9	0.14 ± 4.8	5.2 ± 4.2	-4.7 ± 5.9	0.033	0.69
7. Radial Bow Right	10° ± 5°	10.0 ± 2.8	7.2 ± 2.5	8.4 ± 0.89	7.3 ± 1.5	0.20	0.37
8. Radial Bow Left		9.5 ± 0.91	8.9 ± 6.7	11.3 ± 5.0	9.3 ± 3.8	0.88	0.086
9. Radial Head Dislocation R		1 dislocation	0	1 dislocation	0		
10.Radial Head Dislocation L		1 dislocation	1 dislocation	1 dislocation	0		
11. Elbow Joint Right	9° ± 3°	-18.3 ± 5.6	-1.6 ± 13.9	1.0 ± 16.2	-9.3 ± 10.0	0.13	0.45
12. Elbow Joint Left		-10.0 ± 6.3	-6.6 ± 12.7	-6.8 ± 13.6	-7.3 ± 4.5	0.97	0.063
13. Femoral A.A. Right	7° ± 2° valgus	-4.5 ± 8.8	-5.6 ± 9.1	-0.9 ± 4.5	-8.7 ± 13.5	0.66	0.14
14. Femoral A.A. Left		2.3 ± 3.3	-4.4 ± 10.4	-1.8 ± 7.5	-4.7 ± 5.7	0.59	0.16
15. Femoral N.S. Angle Right	135° ± 5°	142.0 ± 5.7	142.4 ± 6.2	139.2 ± 22.9	135.7 ± 11.0	0.81	0.10
16. Femoral N.S. Angle Left		142.8 ± 13.8	138.6 ± 6.5	144.2 ± 16.8	132.3 ± 6.8	0.41	0.23
17. Femoral M.A. Right	0° ± 5° varus	4.5 ± 3.1	-0.27 ± 5.7	8.1 ± 4.3	-4.0 ± 8.0	0.027	0.73
18. Femoral M.A. Left		3.3 ± 4.5	0.0 ± 6.0	-2.0 ± 5.4	3.0 ± 3.6	0.48	0.19
19. Sharp's Right	35° ± 4°	41.5 ± 6.4	40.6 ± 5.5	39.6 ± 3.4	42.7 ± 8.0	0.90	0.078
20. Sharp's Left		37.0 ± 5.7	40.8 ± 4.9	38.6 ± 4.6	44.3 ± 5.5	0.35	0.25
21. Fibular Height Right	50 ± 10	53.8 ± 2.9	51.3 ± 11.3	43.5 ± 12.7	62.2 ± 1.0	0.15	0.43
22. Fibular Height Left		47.5 ± 15.8	49.2 ± 13.9	52.5 ± 10.1	68.3 ± 7.6	0.17	0.39
23. Ankle Joint Angle Right	0° ± 5°	-4.3 ± 3.8	-3.2 ± 14.1	0.75 ± 2.9	-13.0 ± 6.1	0.48	0.19
24. Ankle Joint Angle Left		1.0 ± 1.0	-1.1 ± 14.4	-1.0 ± 4.1	-11.3 ± 5.9	0.55	0.17
25. % Weightbear Right	50 ± 10	55.5 ± 26.8	48.1 ± 23.2	61.8 ± 8.9	36.3 ± 29.0	0.50	0.19
26. % Weightbear Left		67.0 ± 12.0	48.7 ± 20.9	58.8 ± 11.1	60.0 ± 20.2	0.34	0.26
Number of parameters that fall beyond the normal range		**13**	**11**	**12**	**12**		

Table 8.7.3.3. Segment Lengths and Percentile Height by Mutation Type

Variable	Missense (n=4)	Nonsense (n=14)	Splice Site (n=5)	Frameshift (n=3)	p-value	Power
Total Leg Length-Right	74.3 13.8	86.3 ± 8.4	83.3 ± 6.5	85.7 ± 4.3	0.16	0.42
Upper Leg – Right	37.3 ± 7.9	44.0 ± 5.3	41.9 ± 3.7	42.3 ± 1.9	0.21	0.36
Lower Leg – Right	31.5 ± 6.7	34.7 ± 3.2	34.3 ± 3.2	34.7 ± 2.0	0.55	0.17
Total Leg Length – Left	73.5 ± 13.8	85.4 ± 9.0	82.8 ± 6.2	84.8 ± 2.9	0.18	0.39
Upper Leg – Left	37.2 ± 7.7	43.0 ± 5.1	41.8 ± 3.5	41.0 ± 0.87	0.29	0.29
Lower Leg – Left	30.5 ± 5.9	36.5 ± 5.3	35.0 ± 4.0	36.7 ± 1.2	0.22	0.35
Total Arm Length – Right	45.3 ± 5.4	50.3 ± 6.1	47.9 ± 5.4	50.7 ± 4.1	0.53	0.18
Upper Arm – Right	26.6 ± 4.5	30.9 ± 3.9	29.3 ± 3.5	30.5 ± 2.2	0.28	0.29
Lower Arm – Right	21.0 ± 4.2	22.7 ± 3.0	22.8 ± 2.1	23.5 ± 4.4	0.75	0.12
Total Arm Length – Left	45.3 ± 9.3	50.2 ± 6.4	48.6 ± 4.6	51.0 ± 5.8	0.58	0.16
Upper Arm – Left	26.4 ± 5.8	31.2 ± 4.8	30.0 ± 3.5	31.5 ± 1.8	0.32	0.27
Lower Arm – Left	22.4 ± 4.8	23.2 ± 3.9	21.3 ± 2.7	23.0 ± 4.6		
Percentile Height	21.5 ± 28.2	51.3 ± 32.1	20.2 ± 14.3	9.7 ± 7.6	0.048	0.64

8.7.4 Mutation Severity

Table 8.7.4.1. Lesion Quality by Mutation Severity

Variable	Severe (n=22)	Mild (n=4)	P-value	Power
Lesion Rank 1	6.7 ± 5.5 (n=20)	9.0 ± 1.6	0.42	0.12
% Rank 1	26.7 ± 15.2	48.3 ± 21.6	0.024	0.64
Lesion Rank 2	4.8 ± 2.9	4.0 ± 3.8	0.66	0.070
% Rank 2	22.8 ± 11.2	16.0 ± 12.2	0.29	0.17
Lesion Rank 3	2.9 ± 2.4	2.5 ± 1.9	0.76	0.060
% Rank 3	11.5 ± 7.6	10.8 ± 5.9	0.85	0.054
Lesion Rank 4	9.2 ± 5.1	5.5 ± 3.0	0.18	0.25
% Rank 4	38.9 ± 17.2	24.8 ± 5.9	0.12	0.32
Small (%)	26.8 ± 13.7	48.5 ± 19.3	0.011	0.76
Medium (%)	32.0 ± 12.5	24.0 ± 11.3	0.24	0.19
Large (%)	39.4 ± 17.0	23.9 ± 10.8	0.095	0.37
Average Number of Lesions	23.5 ± 11.8	21.0 ± 7.0	0.69	0.067
No. Pedunculated	6.9 ± 4.4	6.3 ± 2.1	0.77	0.059
% Pedunculated	29.8 ± 12.7	30.8 ± 8.9	0.89	0.052
No. Sessile	15.6 ± 8.6	14.8 ± 5.7	0.85	0.054
% Sessile	63.9 ± 14.3	69.2 ± 8.9	0.49	0.10
No. Distal	9.5 ± 5.2	9.0 ± 4.7	0.85	0.054
% Distal	39.6 ± 12.2	42.3 ± 17.5	0.71	0.064
No. Proximal	10.9 ± 5.6	9.5 ± 3.0	0.62	0.076
% Proximal	45.2 ± 14.3	48.1 ± 19.5	0.73	0.063
No. Pelvic	1.6 ± 2.4	0.75 ± 1.5	0.50	0.098
% Pelvic	4.8 ± 7.0	2.7 ± 5.4	0.58	0.082
No Diaphyseal	1.5 ± 1.2	1.5 ± 2.4	0.95	0.050
% Diaphyseal	8.4 ± 11.2	6.0 ± 8.4	0.69	0.067
No. Flat Bone	1.8 ± 2.5	1.3 ± 1.5	0.66	0.070
% Flat Bone	5.8 ± 7.1	4.6 ± 5.5	0.75	0.060
No. Complex	3.4 ± 3.9	2.8 ± 0.96	0.76	0.060
% Complex	13.5 ± 9.6	14.9 ± 9.4	0.79	0.058
No. Simple	19.7 ± 8.9	18.3 ± 7.5	0.77	0.060
% Simple	82.3 ± 10.1	85.1 ± 9.4	0.62	0.076
No. Flared	9.3 ± 9.0	5.8 ± 3.1	0.45	0.11
% Flared	32.8 ± 26.4	32.9 ± 24.3	0.99	0.050
No. Not Flared	14.5 ± 7.5	15.3 ± 9.6	0.85	0.054
% Not Flared	67.2 ± 26.4	67.1 ± 24.3	0.99	0.050
No. Left	12.3 ± 7.3	12.5 ± 2.6	0.96	0.050
% Left	49.5 ± 9.4	61.7 ± 10.2	0.029	0.60
No. Right	11.8 ± 5.2	8.5 ± 4.7	0.25	0.20
% Right	50.6 ± 9.5	38.3 ± 10.2	0.028	0.61

Table 8.7.4.2. Limb Alignment by Mutation Severity

Variable	Normal Values	Severe (n=22)	Mild (n=4)	P-value
1. Carpal Slip Right	5 ± 2mm	2.7 ± 3.7	4.0 ± 3.4	0.53
2. Carpal Slip Left		3.6 ± 3.5	3.3 ± 3.2	0.86
3. Radial Inclination Right	21° ± 2°	24.9 ± 4.9	26.5 ± 7.9	0.59
4. Radial Inclination Left		27.6 ± 5.5	27.0 ± 5.3	0.83
5. Ulnar Shortening Right	0 ± 1 mm	-1.7 ± 4.9	-1.0 ± 2.3	0.79
6. Ulnar Shortening Left		0.6 ± 5.5	-2.5 ± 3.5	0.28
7. Radial Bow Right	10° ± 5°	7.5 ± 2.1	10.0 ± 2.8	0.05
8. Radial Bow Left		9.5 ± 5.9	9.5 ± 0.9	0.99
9. Radial Head Dislocation R		1 dislocation	1 dislocation	
10.Radial Head Dislocation L		2 dislocations	1 dislocation	
11. Elbow Joint Right	9° ± 3°	-2.1 ± 13.8	-18.3 ± 5.6	0.03
12. Elbow Joint Left		-6.8 ± 11.7	-10.0 ± 6.3	0.60
13. Femoral A.A. Right	7° ± 2°	-5.0 ± 8.9	-4.5 ± 8.8	0.92
14. Femoral A.A. Left	valgus	-3.9 ± 9.1	2.3 ± 3.3	0.20
15. Femoral N.S. Angle Right	135° ± 5°	140.7 ± 11.9	142.0 ± 5.7	0.84
16. Femoral N.S. Angle Left		139.0 ± 9.8	142.8 ± 13.8	0.51
17. Femoral M.A. Right	0° ± 5° varus	0.9 ± 6.8	4.5 ± 3.1	0.31
18. Femoral M.A. Left		0.1 ± 5.6	3.3 ± 4.5	0.29
19. Sharp's Right	35° ± 4°	40.7 ± 5.4	41.5 ± 6.4	0.84
20. Sharp's Left		40.9 ± 4.9	37.0 ± 5.7	0.30
21. Fibular Height Right	50 ± 10	51.3 ± 11.7	53.8 ± 2.9	0.69
22. Fibular Height Left		52.7 ± 13.9	47.5 ± 15.8	0.51
23. Ankle Joint Angle Right	0° ± 5°	-3.9 ± 12.2	-4.3 ± 3.8	0.95
24. Ankle Joint Angle Left		-2.6 ± 12.3	1.0 ± 1.0	0.62
25. % Weightbear Right	50 ± 10	49.1 ± 22.4	55.5 ± 26.8	0.62
26. % Weightbear Left		52.5 ± 19.2	67.0 ± 12.0	0.16
Number of parameters that fall beyond the normal range		**7/24**	**5/24**	**8/24**

Table 8.7.4.3. Segment Lengths and Percentile Height by Mutation Severity

Variable	Mild (n=4)	Severe (n=22)	P-value	Power
Total Leg Length-Right	74.3 ± 13.7	85.2 ± 7.2	0.022	0.65
Upper Leg – Right	37.3 ± 7.9	43.7 ± 4.7	0.031	0.59
Lower Leg – Right	31.5 ± 6.7	34.8 ± 3.0	0.11	0.34
Total Leg Length – Left	73.5 ± 13.8	84.5 ± 7.5	0.025	0.63
Upper Leg – Left	37.3 ± 7.7	42.9 ± 4.5	0.048	0.51
Lower Leg – Left	30.5 ± 5.9	36.0 ± 4.4	0.038	0.55
Total Arm Length – Right	45.3 ± 8.8	49.8 ± 5.4	0.17	0.26
Upper Arm – Right	26.6 ± 4.5	30.3 ± 3.4	0.067	0.44
Lower Arm – Right	21.0 ± 4.2	23.2 ± 3.1	0.23	0.21
Total Arm Length – Left	45.3 ± 9.3	50.2 ± 5.6	0.15	0.28
Upper Arm – Left	26.4 ± 5.8	30.9 ± 3.9	0.059	0.46
Lower Arm – Left	22.4 ± 4.8	22.9 ± 3.6	0.79	0.058
Percentile Height	21.5 ± 28.2	35.8 ± 29.9	0.39	0.13

8.7.5 Mutation Location

Table 8.7.5.1. Lesion Quality by Mutation Location

Variable	Early (n=19)	Late (n=7)	P-Value	Power
Lesion Rank 1	7.5 ± 6.0	5.9 ± 1.6	0.48	0.10
% Rank 1	33.6 ± 19.9	22.1 ± 7.6	0.16	0.28
Lesion Rank 2	3.9 ± 2.6	6.3 ± 3.5	0.085	0.39
% Rank 2	20.6 ± 12.4	24.3 ± 8.7	0.48	0.10
Lesion Rank 3	2.3 ± 2.2	4.1 ± 2.2	0.072	0.43
% Rank 3	9.6 ± 6.9	15.7 ± 6.2	0.058	0.47
Lesion Rank 4	7.5 ± 5.0	11.3 ± 3.7	0.084	0.39
% Rank 4	36.0 ± 19.2 (n=17)	37.9 ± 8.6	0.81	0.056
Small (%)	32.8 ± 17.8	22.9 ± 8.9	0.18	0.25
Medium (%)	30.3 ± 14.1	32.1 ± 7.1	0.75	0.061
Large (%)	35.1 ± 18.2	42.2 ± 13.2	0.36	0.14
Average Number of Lesions	21.2 ± 12.1	27.6 ± 6.7	0.21	0.22
No. Pedunculated	6.8 ± 4.6	6.9 ± 2.4	0.97	0.050
% Pedunculated	31.8 ± 13.1	25.6 ± 8.2	0.27	0.18
No. Sessile	14.2 ± 8.5	19.0 ± 5.9	0.19	0.24
% Sessile	63.3 ± 14.7	68.5 ± 10.4	0.41	0.12
No. Distal	8.8 ± 5.6	11.3 ± 2.8	0.27	0.18
% Distal	39.4 ± 14.3	41.7 ± 8.6	0.69	0.067
No. Proximal	10.3 ± 5.3	12.0 ± 5.3	0.47	0.11
% Proximal	47.2 ± 16.3	42.2 ± 10.8	0.46	0.11
No. Pelvic	1.3 ± 2.5	1.9 ± 1.6	0.59	0.080
% Pelvic	3.7 ± 7.4	6.1 ± 4.8	0.46	0.11
No Diaphyseal	1.2 ± 1.2	2.1 ± 1.7	0.13	0.31
% Diaphyseal	7.9 ± 12.1	8.4 ± 6.7	0.91	0.051
No. Flat Bone	1.5 ± 2.6	2.4 ± 1.5	0.37	0.14
% Flat Bone	4.6 ± 7.5	8.1 ± 4.4	0.27	0.18
No. Complex	3.4 ± 4.1	2.9 ± 1.3	0.73	0.063
% Complex	15.3 ± 10.7	10.0 ± 3.1	0.22	0.21
No. Simple	18.1 ± 9.2	23.3 ± 5.4	0.17	0.26
% Simple	81.9 ± 10.6	84.9 ± 7.7	0.50	0.097
No. Flared	9.3 ± 8.1	7.4 ± 9.9	0.63	0.074
% Flared	36.8 ± 24.8	23.2 ± 26.6	0.25	0.19
No. Not Flared	12.3 ± 6.7	20.1 ± 7.2	0.019	0.68
% Not Flared	63.2 ± 24.8	76.8 ± 26.6	0.25	0.19
No. Left	11.6 ± 7.7	14.4 ± 2.4	0.35	0.14
% Left	50.8 ± 11.9	53.4 ± 5.6	0.58	0.082
No. Right	10.6 ± 5.3	13.3 ± 4.6	0.25	0.19
% Right	49.2 ± 11.9	47.0 ± 6.0	0.65	0.072

Table 8.7.5.2. Limb Alignment by Mutation Location

Variable	Normal Values	Early (n=19)	Late (n=7)	P-Value
1. Carpal Slip Right	5 ± 2mm	2.4 ± 3.7	4.3 ± 3.0	0.25
2. Carpal Slip Left		3.4 ± 3.7	4.0 ± 2.6	0.68
3. Radial Inclination Right	21° ± 2°	24.1 ± 5.2	27.7 ± 5.0	0.13
4. Radial Inclination Left		26.8 ± 5.1	29.4 ± 5.9	0.28
5. Ulnar Shortening Right	0 ± 1 mm	-2.6 ± 4.9	1.0 ± 2.3	0.08
6. Ulnar Shortening Left		-0.9 ± 5.0	3.0 ± 5.5	0.10
7. Radial Bow Right	10° ± 5°	7.6 ± 2.5	8.6 ± 1.9	0.39
8. Radial Bow Left		9.0 ± 5.8	10.8 ± 4.2	0.47
9. Radial Head Dislocation R		1 dislocation	1 dislocation	
10. Radial Head Dislocation L		1 dislocation	1 dislocation	
11. Elbow Joint Right	9° ± 3°	-4.2 ± 13.0	-5.9 ± 17.7	0.80
12. Elbow Joint Left		-7.1 ± 11.0	-7.9 ± 12.0	0.87
13. Femoral A.A. Right	7° ± 2° valgus	-5.6 ± 9.3	-3.1 ± 7.1	0.53
14. Femoral A.A. Left		-3.9 ± 9.2	-0.1 ± 6.8	0.33
15. Femoral N.S. Angle Right	135° ± 5°	141.3 ± 7.0	139.9 ± 19.2	0.77
16. Femoral N.S. Angle Left		137.6 ± 8.3	144.9 ± 13.8	0.11
17. Femoral M.A. Right	0° ± 5° varus	-0.6 ± 5.8	7.8 ± 3.4	<0.01
18. Femoral M.A. Left		0.6 ± 5.4	0.7 ± 6.2	0.97
19. Sharp's Right	35° ± 4°	41.2 ± 5.7	39.1 ± 3.2	0.44
20. Sharp's Left		41.4 ± 4.8	37.5 ± 4.7	0.12
21. Fibular Height Right	50 ± 10	53.4 ± 10.5	46.5 ± 10.9	0.18
22. Fibular Height Left		50.9 ± 15.2	54.8 ± 9.9	0.56
23. Ankle Joint Angle Right	0° ± 5°	-4.7 ± 13.0	-1.7 ± 4.4	0.59
24. Ankle Joint Angle Left		-2.9 ± 13.3	-0.2 ± 3.4	0.63
25. % Weightbear Right	50 ± 10	47.3 ± 22.7	58.7 ± 22.2	0.30
26. % Weightbear Left		51.7 ± 19.7	64.3 ± 12.7	0.15
Number of parameters that fall beyond the normal range		**11**	**11**	

Table 8.7.5.3. Segment Lengths and Percentile Height by Mutation Location

Variable	Early (n=19)	Late (n=7)	P-Value	Power
Total Leg Length-Right	84.7 ± 9.0	80.5 ± 9.2	0.31	0.16
Upper Leg – Right	43.6 ± 5.6	40.3 ± 5.4	0.19	0.24
Lower Leg – Right	34.7 ± 3.6	33.1 ± 4.3	0.34	0.15
Total Leg Length – Left	83.9 ± 9.2	79.8 ± 9.3	0.32	0.16
Upper Leg – Left	42.7 ± 5.2	40.1 ± 5.2	0.28	0.18
Lower Leg – Left	35.9 ± 4.9	33.3 ± 4.8	0.25	0.19
Total Arm Length – Right	50.0 ± 5.9	46.8 ± 6.3	0.24	0.20
Upper Arm – Right	30.2 ± 3.7	28.5 ± 3.8	0.31	0.16
Lower Arm – Right	23.1 ± 3.5	22.1 ± 2.7	0.48	0.10
Total Arm Length – Left	50.0 ± 6.5	47.6 ± 5.9	0.39	0.13
Upper Arm – Left	30.5 ± 4.5	29.3 ± 4.4	0.54	0.089
Lower Arm – Left	23.3 ± 3.9	21.5 ± 3.9	0.26	0.19
Percentile Height	40.2 ± 31.3	15.6 ± 14.1	0.058	0.47

8.7.6 Gene and Gender

Table 8.7.6.1 Lesion Quality by Gene and Gender

Variable	EXT 1 Males (n=4)	EXT 1 Females (n=3)	EXT 2 Males (n=10)	EXT 2 Females (n=9)	P-value EXT	P-value Gender
Lesion Rank 1	11.8 ± 7.3	5.7 ± 1.2	7.9 ± 5.3	4.3 ± 2.9	0.18	0.032
% Rank 1	30.8 ± 11.9	22.0 ± 5.2	33.1 ± 22.2	29.9 ± 19.3	0.59	0.56
Lesion Rank 2	7.0 ± 2.9	5.7 ± 3.5	4.6 ± 3.5	3.1 ± 1.7	0.064	0.22
% Rank 2	18.8 ± 5.3	20.3 ± 11.1	18.8 ± 11.3	26.8 ± 13.6	0.55	0.20
Lesion Rank 3	5.8 ± 2.2	4.0 ± 1.0	2.8 ± 2.0	1.0 ± 0.93	0.0006	0.013
% Rank 3	15.8 ± 5.9	16.3 ± 7.6	10.9 ± 7.2	8.0 ± 6.7	0.049	0.47
Lesion Rank 4	12.8 ± 3.9	11.3 ± 4.9	8.8 ± 5.0	5.3 ± 3.3	0.018	0.097
% Rank 4	34.8 ± 10.0	41.7 ± 10.6	37.2 ± 18.9	34.8 ± 20.3	0.84	0.97
Small (%)	32.3 ± 13.8	23.2 ± 7.5	32.2 ± 20.0	29.3 ± 16.5	0.75	0.51
Medium (%)	28.2 ± 10.9	33.9 ±0.98	29.1 ± 9.1	32.9 ± 18.2	0.96	0.41
Large (%)	35.4 ± 20.5	42.8 ± 7.7	34.9 ± 17.2	38.2 ± 19.5	0.79	0.55
Avg # of lesions	37.3 ± 11.4	26.7 ± 6.1	24.0 ± 9.5	13.6 ± 3.2	0.0011	0.0032
No. Pedunculated	9.0 ± 3.2	8.3 ± 3.1	8.1 ± 5.6	4.9 ± 0.9	0.27	0.15
% Pedunculated	24.1 ± 4.1	30.5 ± 4.9	28.9 ± 13.9	38.8 ± 11.6	0.24	0.097
No. Sessile	24.3 ± 10.9	17.0 ± 3.5	17.6 ± 7.1	9.4 ± 5.2	0.037	0.015
% Sessile	64.4 ± 15.3	64.1 ± 4.3	67.7 ± 16.0	55.2 ± 5.7	0.75	0.15
No. Distal	14.8 ± 5.7	11.0 ± 4.0	9.8 ± 4.8	6.2 ± 3.2	0.016	0.038
% Distal	40.0 ± 10.8	40.4 ± 6.0	40.7 ± 16.5	39.2 ± 12.8	0.97	0.86
No. Proximal	17.3 ± 5.0	10.7 ± 3.8	11.8 ± 4.4	6.7 ± 3.1	0.0095	0.0016
% Proximal	46.8 ± 8.0	40.1 ±10.1	48.5 ± 13.8	42.1 ± 23.7	0.81	0.37
No. Pelvic	4.5 ± 3.7	1.0 ± 1.0	0.90 ± 1.7	0.56 ± 1.1	0.0038	0.19
% Pelvic	11.3 ± 9.8	7.3 ± 3.0	2.5 ± 4.9	2.1 ± 5.9	0.015	0.51
No Diaphyseal	2.0 ± 0.82	3.0 ± 2.0	1.2 ± 1.2	1.7 ± 1.5	0.50	0.11
% Diaphyseal	2.4 ± 1.6	11.9 ± 7.8	6.4 ± 9.1	13.4 ±16.5	0.57	0.12
No. Flat Bone	5.3 ± 3.2	2.7 ± 1.5	1.0 ± 1.6	0.67 ± 1.1	0.0004	0.15
% Flat Bone	13.7 ± 7.4	9.4 ± 3.9	2.0 ± 4.9	3.0 ± 6.1	0.0026	0.52

Table 8.7.6.1 Lesion Quality by Gene and Gender (continued)

Variable	EXT 1 Males (n=4)	EXT 1 Females (n=3)	EXT 2 Males (n=10)	EXT 2 Females (n=9)	P-value EXT	P-value Gender
No. Complex	6.8 ± 7.7	2.3 ± 1.5	3.4 ± 2.5	2.1 ± 1.3	0.24	0.15
% Complex	15.6 ± 13.1	8.2 ± 3.9	14. 9 ± 7.9	14.1 ± 9.1	0.61	0.47
No. Simple	26. ± 5.6	23.3 ± 5.5	23.1 ± 8.9	12.3 ± 4.2	0.039	0.0076
% Simple	73.6 ± 10.1	87.4 ± 4.7	85. 1 ± 7.9	81.2 ± 8.9	0.33	0.59
No. Flared	21.0 ± 11.5	5.0 ± 4.4	9.2 ± 5.7	4.1 ± 5.3	0.019	0.0043
% Flared	54.6 ± 29.4	17.3 ± 12.1	40.6 ± 23.7	18.9 ± 20.3	0.43	0.0097
No. Not Flared	16.3 ± 9.8	21.7 ± 3.5	14.6 ± 9.1	11.1 ± 3.9	0.10	0.73
% Not Flared	45.4 ± 29.4	82.7 ± 12.1	59.3 ± 23.7	81.1 ± 20.3	0.43	0.0097
No. Left	21.8 ± 7.4	14.3 ± 2.1	13.3 ± 5.4	7.7 ± 3.4	0.0030	0.0079
% Left	58.2 ± 8.9	54.5 ± 5.1	52.1 ± 11.9	49.3 ± 11.8	0.27	0.52
No. Right	15.5 ± 5.5	12.7 ± 4.5	13.2 ± 5.7	7.4 ± 2.4	0.11	0.022
% Right	41.8 ± 8.9	46.5 ± 6.6	47.9 ± 11.9	50.7 ± 11.8	0.31	0.48

Table 8.7.6.2. Limb Alignment by Gene and Gender

Variable	Normal Values	EXT 1 Males (n=4)	EXT 1 Females (n=3)	EXT 2 Males (n=10)	EXT 2 Females (n=9)	P-value EXT	P-value Gender
1. Carpal Slip Right	5 ± 2mm	7.3 ± 2.1	2.1 ± 4.1	3.0 ± 1.0	2.3 ± 3.1	0.076	0.4941
2. Carpal Slip Left		6.8 ± 1.5	2.6 ± 3.5	2.0 ± 2.0	3.7 ± 3.7	0.27	0.68
3. Radial Inclination Right	21° ± 2°	31.3 ± 4.9	24.9 ± 3.3	24.7 ± 4.0	23.6 ± 6.5	0.13	0.21
4. Radial Inclination Left		30.8 ± 5.9	28.0 ± 5.7	30.3 ± 7.4	24.7 ± 2.9	0.079	0.19
5. Ulnar Shortening Right	0 ± 1 mm	-3.7 ± 4.0	-2.2 ± 4.9	1.3 ± 1.5	-1.2 ± 5.1	0.81	0.32
6. Ulnar Shortening Left		1.3 ± 6.4	-0.50 ± 5.5	4.7 ± 5.5	-1.1 ± 4.5	0.15	0.87
7. Radial Bow Right	10° ± 5°	10.0 ± 2.6	7.5 ± 2.6	8.0 ± 1.7	7.6 ± 2.2	0.21	0.71
8. Radial Bow Left		14.9 ± 10.8	8.1 ± 2.5	13.3 ± 5.8	7.3 ± 2.2	0.0061	0.53
9. Radial Head Dislocation R		1 dislocation	0	0	1 dislocation		
10.Radial Head Dislocation L		1 dislocation	1 dislocation	0	1 dislocation		
11. Elbow Joint Right	9° ± 3°	-0.33 ± 20.6	-4.9 ± 14.4	-3.3 ± 20.5	-6.3 ± 11.8	0.59	0.77
12. Elbow Joint Left		0.50 ± 8.2	-11.4 ± 11.4	-9.7 ± 11.2	-5.4 ± 10.9	0.34	0.73
13. Femoral A.A. Right	7° ± 2° valgus	-1.8 ± 6.4	-5.2 ± 9.9	-4.8 ± 10.6	-6.0 ± 8.8	0.54	0.68
14. Femoral A.A. Left		-1.4 ± 10.4	-5.5 ± 7.8	-1.8 ± 6.8	-1.1 ± 10.1	0.65	0.40
15. Femoral N.S. Angle R	135° ± 5°	139 ± 9.4	141.6 ± 8.2	148.7 ± 27.0	138.4 ± 7.9	0.55	0.97
16. Femoral N.S. Angle L		143.3 ± 3.4	135.9 ± 8.6	150.7 ± 17.2	138.3 ± 9.9	0.040	0.38
17. Femoral M.A. Right	0° ± 5° varus	4.5 ± 5.2	1.2 ± 7.4	9.8 ± 5.3	-1.5 ± 4.4	0.033	0.49
18. Femoral M.A. Left		-2.8 ± 8.3	0.68 ± 4.9	2.5 ± 0.71	1.6 ± 5.2	0.43	0.37
19. Sharp's Right	35° ± 4°	37.3 ± 2.5	40.3 ± 4.1	40.3 ± 3.9	42.8 ±7.3	0.30	0.26
20. Sharp's Left		38.3 ± 7.6	41.4 ± 4.2	38.8 ± 1.8	40.9 ± 5.9	0.33	0.93
21. Fibular Height Right	50 ± 10	57.0 ± 6.1	53.8 ± 10.4	47.0 ± 7.0	49.2 ± 13.2	0.94	0.19
22. Fibular Height Left		54.3 ± 19.4	51.1 ± 11.5	50.0 ± 9.2	52.6 ± 18.3	0.96	0.99
23. Ankle Joint Angle Right	0° ± 5°	-19.7 ± 12.1	1.6 ± 11.6	0.0± 5.2	-4.9 ± 8.1	0.099	0.90
24. Ankle Joint Angle Left		-13.0 ± 18.7	2.1 ± 11.0	2.0 ± 1.0	-3.7 ± 9.1	0.40	0.95
25. % Weightbear Right	50 ± 10	69.3 ± 15.5	50.4 ± 26.3	52.7 ± 28.3	42.6 ± 17.9	0.19	0.29
26. % Weightbear Left		62.3 ± 16.7	51.5 ± 8.4	68.0 ± 7.2	51.3 ± 21.9	0.14	0.87
Parameters outside of normal range		**16**	**13**	**10**	**8**		

Table 8.7.6.3. Segment Lengths and Percentile Height by Gene and Gender

Variable	EXT 1 Males (n=4)	EXT 1 Females (n=3)	EXT 2 Males (n=10)	EXT 2 Females (n=9)	P-value EXT	P-value Gender
Total Leg Length-Right	83.4 ± 4.2	74.7 ± 10.8	87.6 ± 5.7	82.1 ± 11.5	0.082	0.18
Upper Leg – Right	40.8 ± 2.7	37.2 ± 6.3	44.5 ± 4.0	43.4 ± 7.1	0.47	0.058
Lower Leg – Right	34.0 ± 2.7	30.2 ± 4.8	36.2 ± 2.9	33.7 ± 3.9	0.059	0.11
Total Leg Length – Left	82.3 ± 3.2	74.3 ± 12.0	87.2 ± 6.6	81.1 ± 11.2	0.080	0.18
Upper Leg – Left	40.4 ± 2.6	36.8 ± 7.1	43.6 ± 3.8	42.8 ± 6.5	0.53	0.069
Lower Leg – Left	33.5 ± 4.1	31.0 ± 5.8	37.8 ± 4.1	34.5 ± 5.2	0.13	0.084
Total Arm Length – Right	46.0 ± 3.8	43.3 ± 7.1	52.2 ± 4.7	49.1 ± 6.6	0.22	0.028
Upper Arm – Right	28.3 ± 1.3	26.3 ± 3.5	32.1 ± 2.9	29.0 ± 4.2	0.058	0.043
Lower Arm – Right	20.9 ± 2.1	21.0 ± 3.3	24.7 ± 2.8	22.3 ± 3.6	0.21	0.069
Total Arm Length – Left	46.0 ± 5.1	43.7 ± 6.0	52.9 ± 4.6	49.1 ± 6.9	0.17	0.026
Upper Arm – Left	28.6 ± 3.1	26.0 ± 4.0	32.3 ± 3.2	29.9 ± 5.3	0.17	0.058
Lower Arm – Left	19.6 ± 3.9	20.3 ± 2.4	25.0 ± 2.8	22.7 ± 3.6	0.30	0.011
Percentile Height	12.5 ± 17.7	5.0 ± 3.5	40.2 ± 28.3	45.1 ± 31.4	0.79	0.011

8.7.7 Gene and Mutation Type

Table 8.7.7.1 Lesion Quality by Gene and Mutation Type

Variable	EXT 1 Missense (n=2)	EXT 2 Missense (n=2)	P-value	Power	EXT 1 Nonsense (n=2)	EXT 2 Nonsense (n=12)	P-value	Power
Lesion Rank 1	8.0 ± 1.4	10.0 ± 1.4	0.29	0.14	16.5 ± 7.8	6.1 ± 5.1	0.026	0.64
% Rank 1	30.0 ± 7.1	66.5 ± 3.5	0.022	0.88	37.0 ± 7.1	28.9 ± 18.0	0.55	0.086
Lesion Rank 2	7.0 ± 2.8	1.0 ± 0.0	0.096	0.39	7.0 ± 4.2	3.9 ± 2.2	0.13	0.31
% Rank 2	25.5 ± 9.2	6.5 ± 0.71	0.10	0.37	15.5 ± 4.9	22.5 ± 12.4	0.46	0.11
Lesion Rank 3	4.0 ± 1.4	1.0 ± 0.0	0.096	0.39	5.0 ± 2.8	2.2 ± 2.1	0.11	0.34
% Rank 3	15.0 ± 5.7	6.5 ± 0.71	0.17	0.24	11.0 ± 2.8	9.9 ± 8.2	0.87	0.053
Lesion Rank 4	8.0 ± 1.4	3.0 ± 0.0	0.038	0.72	15.0 ± 1.4	7.3 ± 4.6	0.041	0.55
% Rank 4	29.5 ± 3.5	20.0 ± 1.4	0.072	0.48	36.5 ± 14.8	38.6 ± 21.5	0.89	0.052
Small (%)	32.8 ± 4.5	64.3 ± 10.1	0.057	0.57	39.7 ± 10.4	28.0 ± 16.3	0.36	0.14
Medium (%)	33.8 ± 0.71	14.2 ± 0.0	0.0007	1.0	22.5 ± 15.3	32.1 ± 13.9	0.39	0.13
Large (%)	26.5 ± 14.8	21.4 ± 10.1	0.72	0.058	37.8 ± 25.7	37.2 ± 20.5	0.97	0.050
Average Number of Lesions	27.0 ± 1.4	15.0 ± 1.4	0.013	0.97	43.5 ± 13.4	19.4 ± 9.3	0.007 1	0.86
No. Pedunculated	7.5 ± 2.1	5.0 ± 1.4	0.29	0.14	11.5 ± 2.1	5.9 ± 4.5	0.12	0.33
% Pedunculated	27.6 ± 6.4	33.9 ± 12.6	0.59	0.068	26.9 ±3.5	31.0 ± 14.5	0.71	0.064
No. Sessile	19.5 ± 0.71	10.0 ± 2.8	0.044	0.66	27.5 ± 17.7	12.4 ± 6.2	0.027	0.64
% Sessile	72.3 ± 6.4	66.1 ± 12.6	0.59	0.068	59.8 ± 22.2	64.2 ± 15.6	0.73	0.062
No. Distal	12.5 ± 2.1	5.5 ± 3.5	0.14	0.28	17.0 ± 8.5	8.3 ± 4.8	0.051	0.51
% Distal	46.6 ± 10.3	37.9 ± 27.1	0.72	0.058	37.9 ± 7.8	41.6 ± 13.4	0.72	0.063
No. Proximal	10.0 ± 2.8	9.0 ± 4.2	0.81	0.054	18.0 ± 5.7	8.8 ± 4.7	0.027	0.64
% Proximal	37.4 ± 12.4	58.9 ± 22.7	0.36	0.11	41.3 ± 0.24	45.6 ± 17.3	0.74	0.061
No. Pelvic	1.5 ±2.1	0.0 ± 0.0	0.42	0.095	7.5 ± 0.71	0.50 ± 1.2	<0.00 01	1.0
% Pelvic	5.4 ± 7.6	0.0 ± 0.0	0.42	0.095	18.4 ± 7.3	2.2 ± 5.4	0.002 7	0.95
No Diaphyseal	2.5 ± 3.5	0.50 ± 0.71	0.51	0.078	1.5 ± 0.71	1.1 ± 1.4	0.69	0.066
% Diaphyseal	8.9 ± 12.6	3.1 ± 4.4	0.60	0.067	3.4 ± 0.59	7.8 ± 13.0	0.65	0.071
No. Flat Bone	2.5 ± 0.71	0.0 ± 0.0	0.038	0.72	8.0 ±0.0	0.67 ± 1.2	<0.00 01	1.0
% Flat Bone	9.2 ± 2.1	0.0 ± 0.0	0.026	0.85	19.3 ± 5.9	3.3 ± 5.5	0.002 7	0.95
No. Complex	2.5 ± 0.71	3.0 ± 1.4	0.69	0.059	9.5 ± 12.0	2.8 ± 2.6	0.060	0.47
% Complex	9.3 ± 3.1	20.5 ± 11.4	0.31	0.13	18.5 ± 21.9	13.6 ± 10.1	0.59	0.079
No. Simple	24.5 ± 2.1	12.0 ± 2.8	0.038	0.72	29.5 ± 7.8	16.3 ± 7.9	0.051	0.50

Table 8.7.7.1 Lesion Quality by Gene and Mutation Type (continued)

Variable	EXT 1 Missense (n=2)	EXT 2 Missense (n=2)	P-value	Power	EXT 1 Nonsense (n=2)	EXT 2 Nonsense (n=12)	P-value	Power
% Simple	90.7 ± 3.1	79.5 ± 11.4	0.31	0.13	68.3 ± 3.2	84.5 ± 10.5	0.057	0.48
No. Flared	3.5 ± 0.71	8.0 ± 2.8	0.16	0.25	25.5 ± 0.71	7.0 ± 6.4	0.0019	0.96
% Flared	13.0 ± 3.3	52.7 ± 13.9	0.059	0.55	61.3 ± 17.3	32.3 ± 24.5	0.14	0.29
No. Not Flared	23.5 ± 2.1	7.0 ± 1.4	0.011	0.98	18.0 ± 12.7	12.4 ± 6.3	0.32	0.15
% Not Flared	86.9 ± 3.3	47.3 ± 13.9	0.059	0.55	38.7 ± 17.3	67.7 ± 24.5	0.14	0.29
No. Left	14.5 ± 0.71	10.5 ± 2.1	0.13	0.31	27.5 ± 4.9	9.6 ± 6.1	0.0021	0.96
% Left	53.7 ± 0.19	69.6 ± 7.6	0.097	0.38	64.5 ± 8.6	46.5 ± 7.5	0.0093	0.82
No. Right	12.5 ± 0.71	4.5 ± 0.71	0.0077	0.99	16.0 ± 8.5	9.8 ± 3.4	0.072	0.43
% Right	46.3 ± 0.19	30.4 ± 7.6	0.097	0.38	35.5 ± 8.6	53.5 ± 7.5	0.0093	0.82

Table 8.7.7.1 Lesion Quality by Gene and Mutation Type (continued)

Variable	EXT 1 Splice Site (n=3)	EXT 2 Splice Site (n=2)	P-value	Power	EXT 2 FS (n=3)
Lesion Rank 1	5.0 ± 0.0	5.0 ± 0.0			2.0 ± 0.0
% Rank 1	18.3 ± 5.9	20.0 ± 7.1	0.79	0.055	18.0 ± 0.0
Lesion Rank 2	5.7 ± 3.5	6.5 ± 6.4	0.86	0.052	4.0 ± 0.0
% Rank 2	18.0 ± 7.6	32.5 ± 0.71	0.082	0.43	36.0 ± 0.0
Lesion Rank 3	5.7 ± 2.1	2.0 ± 1.4	0.12	0.32	1.0 ± 0.0
% Rank 3	20.0 ± 6.2	10.0 ± 1.4	0.12	0.32	9.0 ± 0.0
Lesion Rank 4	13.0 ± 4.6	12.0 ± 2.8	0.81	0.054	4.0 ± 0.0
% Rank 4	44.0 ± 7.8	37.0 ± 7.1	0.39	0.11	36.0 ± 0.0
Small (%)	17.9 ± 6.1	20.8 ± 9.8	0.71	0.060	26.4 ± 7.6
Medium (%)	33.9 ± 0.98	27.8 ± 15.8	0.52	0.082	39.2 ± 12.3
Large (%)	47.2 ± 6.3	50.5 ± 7.1	0.62	0.068	34.3 ± 8.5
Average Number of Lesions	29.3 ± 8.3	25.5 ± 10.6	0.68	0.063	11.0 ± 0.0
No. Pedunculated	7.7 ± 3.1	5.0 ± 1.4	0.35	0.12	8.3 ± 6.7
% Pedunculated	26.3 ± 7.5	22.7 ± 14.9	0.74	0.058	45.5 ± 0.0
No. Sessile	18.0 ± 4.6	20.0 ± 12.7	0.81	0.054	15.3 ± 8.1
% Sessile	61.8 ± 2.9	74.5 ± 18.9	0.30	0.14	54.5 ± 0.0
No. Distal	11.0 ± 4.0	10.5 ± 2.1	0.89	0.052	7.3 ± 5.0
% Distal	37.5 ± 8.4	43.2 ± 9.6	0.53	0.080	18.2 ± 0.0
No. Proximal	15.0 ± 6.0	9.5 ± 6.4	0.39	0.11	11.7 ± 5.5
% Proximal	50.1 ± 7.2	35.1 ± 10.4	0.15	0.28	54.5 ± 0.0
No. Pelvic	2.0 ± 1.0	2.0 ± 2.8		0.050	1.3 ± 1.5
% Pelvic	6.5 ± 1.7	6.1 ± 8.6	0.93	0.051	0.0 ± 0.0
No Diaphyseal	1.7 ± 1.2	2.5 ± 0.71	0.44	0.097	2.0 ± 1.0
% Diaphyseal	6.9 ± 6.9	10.1 ± 1.4	0.59	0.072	27.3 ± 0.0
No. Flat Bone	2.7 ± 1.5	2.0 ± 2.8	0.75	0.058	1.3 ± 1.5
% Flat Bone	8.6 ± 3.8	6.1 ± 8.6	0.66	0.064	0.0 ± 0.0
No. Complex	3.3 ± 2.1	2.5 ± 0.71	0.64	0.066	2.3 ± 1.5
% Complex	10.5 ± 4.8	10.1 ± 1.4	0.93	0.051	18.2 ± 0.0

Table 8.7.7.1 Lesion Quality by Gene and Mutation Type (continued)

Variable	EXT 1 Splice Site (n=3)	EXT 2 Splice Site (n=2)	P-value	Power	EXT 2 FS (n=3)
No. Simple	23.0 ± 5.3	22.5 ± 10.6	0.95	0.050	21.3 ± 13.1
% Simple	79.6 ± 8.8	87.1 ± 5.4	0.37	0.12	81.8 ± 0.0
No. Flared	13.7 ± 13.9	2.0 ± 1.4	0.34	0.13	8.0 ± 8.0
% Flared	40.6 ± 36.2	7.3 ± 2.5	0.31	0.14	9.1 ± 0.0
No. Not Flared	15.7 ± 7.8	23.5 ± 9.2	0.38	0.12	10.0 ± 0.0
% Not Flared	59.4 ± 36.2	92.7 ± 2.5	0.31	0.14	90.9 ± 0.0
No. Left	15.3 ± 3.1	13.0 ± 2.8	0.45	0.094	9.7 ± 4.9
% Left	53.3 ± 5.8	53.3 ± 11.1	0.99	0.050	36.4 ± 0.0
No. Right	14.3 ± 5.5	12.5 ± 7.8	0.77	0.056	14.0 ± 8.2
% Right	47.7 ± 6.9	46.7 ± 11.1	0.91	0.051	63.6 ± 0.0

Table 8.7.7.2. Limb Alignment by Gene and Mutation Type

Variable	Normal Values	EXT 1 MS (n=2)	EXT 2 MS (n=2)	P-value	Power	EXT 1 NS (n=2)	EXT 2 NS (n=12)	P-value	Power
1. Carpal Slip Right	5 ± 2mm	5.5 ± 4.9	2.5 ± 0.71	0.49	0.083	5.0	1.8 ± 4.5	0.51	0.093
2. Carpal Slip Left		5.0 ± 4.2	1.5 ± 0.71	0.37	0.11	7.0 ± 1.4	3.0 ± 4.2	0.22	0.21
3. Radial Inclination Right	21° ± 2°	28.5 ± 12.0	24.5 ± 4.9	0.71	0.059	29.0	24.5 ± 4.0	0.32	0.15
4. Radial Inclination Left		28.0 ± 8.5	26.0 ± 2.8	0.78	0.055	28.5 ± 9.2	27.0 ± 4.7	0.71	0.064
5. Ulnar Shortening Right	0 ± 1 mm	-1.0 ± 2.8	-1.0 ± 2.8		0.050	-8.0	-2.0 ± 5.0	0.28	0.17
6. Ulnar Shortening Left		-2.5 ± 4.9	-2.5 ± 3.5		0	1.5 ± 4.9	-0.83 ± 4.9	0.68	0.067
7. Radial Bow Right	10° ± 5°	9.0 ± 4.2	11.0 ± 1.4	0.59	0.068	11.0	6.9 ± 2.4	0.12	0.32
8. Radial Bow Left		9.5 ± 1.4	9.5 ± 0.71		0.050	20.0 ± 15.6	7.0 ± 2.1	0.0048	0.89
9. Radial Head Dislocation R		0	1 dislocation			0	0		
10. Radial Head Dislocation L		0	1 dislocation			1 dislocation	0		
11 Elbow Joint Right	9° ± 3°	-23.0 ± 1.4	-13.5 ± 0.71	0.014	0.97	2.0	-1.9 ± 14.5	0.80	0.056
12. Elbow Joint Left		-10.5 ± 10.6	-9.5 ± 2.1	0.91	0.051	-3.5 ± 4.9	-7.2 ± 13.7	0.72	0.063
13. Femoral A.A. Right	7° ± 2° valgus	-8.5 ± 12.0	-0.50 ± 4.9	0.48	0.084	-5.5 ± 7.8	-5.7 ± 9.7	0.98	0.050
14. Femoral A.A. Left		4.0 ± 2.8	0.50 ± 3.5	0.39	0.10	-9.5 ± 3.5	-3.6 ± 11.0	0.48	0.10
15. Femoral N.S. Angle Right	135° ± 5°	141.5 ± 9.2	142.5 ± 3.5	0.90	0.051	146.0 ± 7.1	141.8 ± 6.2	0.39	0.13
16. Femoral N.S. Angle Left		146.5 ± 2.1	139.0 ± 22.6	0.69	0.060	142.5 ± 0.71	137.9 ± 6.8	0.37	0.13
17. Femoral M.A. Right	0° ± 5° varus	7.0 ± 1.4	2.0 ± 1.4	0.072	0.48	1.0 ± 5.7	-0.50 ± 5.9	0.75	0.060
18. Femoral M.A. Left		6.0 ± 4.2	0.50 ± 3.5	0.29	0.14	-5.0 ± 2.8	0.91 ± 6.0	0.21	0.22
19. Sharp's Right	35° ± 4°	37.0 (n=1)	46 (n=1)			37.5 ± 3.5	41.1 ± 5.7	0.42	0.12

Table 8.7.7.2. Limb Alignment by Gene and Mutation Type (continued)

Variable	Normal Values	EXT 1 MS (n=2)	EXT 2 MS (n=2)	P-value	Power	EXT 1 NS (n=2)	EXT 2 NS (n=12)	P-value	Power
20. Sharp's Left		33.0 (n=1)	41.0 (n=1)			41.0 ± 8.5	40.8 ± 4.6	0.96	0.050
21. Fibular Height Right	50 ± 10	52.5 ± 0.71	55.0 ± 4.2	0.49	0.081	59.0 ± 7.1	49.9 ± 11.5	0.31	0.16
22. Fibular Height Left		59.5 ± 10.6	35.5 ± 7.8	0.12	0.31	48.0 ± 22.6	49.5 ± 13.5	0.90	0.052
23. Ankle Joint Angle Right	0° ± 5°	-6.5 ± 0.71	0.0	0.084	0.44	-26.0 ± 7.1	1.0 ± 10.5	0.0055	0.89
24. Ankle Joint Angle Left		1.5 ± 0.71	0.0	0.33	0.11	-20.5 ± 19.1	2.4 ± 11.1	0.03	0.61
25. % Weightbear Right	50 ± 10	52.5 ± 45.9	58.5 ± 3.5	0.87	0.052	61.5 ± 10.6	45.7 ± 24.3	0.40	0.12
26. % Weightbear Left		75.5 ± 7.8	58.5 ± 9.2	0.18	0.22	53.0 ± 5.7	47.9 ± 22.8	0.77	0.059
Parameters beyond the normal range		**111**	**14**			**15**	**9**		

Table 8.7.7.2. Limb Alignment by Gene and Mutation Type (continued)

Variable	Normal Values	EXT 1 Splice Site (n=3)	EXT 2 Splice Site (n=2)	P-value	Power	EXT 2 FS (n=3)
1. Carpal Slip Right	5 ± 2mm	5.0 ± 2.6	2.0 ± 0.0	0.23	0.19	3.7 ± 1.2
2. Carpal Slip Left		3.0 ± 2.6	4.5 ± 2.1	0.56	0.076	3.7 ± 2.1
3. Radial Inclination Right	21° ± 2°	27.3 ± 0.58	27.5 ± 0.71	0.79	0.055	20.7 ± 9.5
4. Radial Inclination Left		33.7 ± 2.1	24.5 ± 4.9	0.057	0.54	25.7 ± 7.6
5. Ulnar Shortening Right	0 ± 1 mm	1.0 ± 1.7	3.0 ± 1.4	0.27	0.16	-4.0 ± 6.2
6. Ulnar Shortening Left		7.0 ± 4.6	2.5 ± 2.1	0.29	0.14	-4.7 ± 5.9
7. Radial Bow Right	10° ± 5°	8.3 ± 1.2	8.5 ± 0.71	0.87	0.052	7.3 ± 1.5
8. Radial Bow Left		13.5 ± 5.7	8.0 ± 0.0	0.28	0.15	9.3 ± 3.8
9. Radial Head Dislocation R		1 dislocation				0
10. Radial Head Dislocation L		1 dislocation				0
11 Elbow Joint Right	9° ± 3°	11.0 ± 12.2	-14.0 ± 2.8	0.073	0.46	-9.3 ± 10.0
12. Elbow Joint Left		0.33 ± 13.2	-17.5 ± 2.1	0.17	0.24	-7.3 ± 4.5
13. Femoral A.A. Right	7° ± 2° valgus	2.2 ± 2.0	-5.5 ± 0.71	0.016	0.89	-8.7 13.5
14. Femoral A.A. Left		0.0 ± 9.9	-4.5 ± 0.71	0.59	0.82	-4.7 ± 5.7
15. Femoral N.S. Angle Right	135° ± 5°	142.3 ± 29.4	134.5 ± 17.7	0.76	0.057	135.7 ± 11.0
16. Femoral N.S. Angle Left		149.0 ± 18.2	137.0 ± 16.9	0.51	0.083	132.3 ± 6.8
17. Femoral M.A. Right	0° ± 5° varus	10.8 ± 3.9	5.5 ± 3.5	0.29	0.14	-4.0 ± 8.0
18. Femoral M.A. Left		-4.0 ± 8.5	0.0 ± 0.0	0.57	0.070	3.0 ± 3.6
19. Sharp's Right	35° ± 4°	40.3 ± 3.9	39.0 ± 4.2	0.79	0.054	42.7 ± 8.0
20. Sharp's Left		38.8 ± 1.8	38.5 ± 7.8	0.97	0.050	44.3 ± 5.5

Table 8.7.7.2. Limb Alignment by Gene and Mutation Type (continued)

Variable	Normal Values	EXT 1 Splice Site (n=3)	EXT 2 Splice Site (n=2)	P-value	Power	EXT 2 FS (n=3)
21. Fibular Height Right	50 ± 10	44.5 ± 7.8	42.5 ± 20.5	0.91	0.051	62.2 ± 1.0
22. Fibular Height Left		49.0 ± 12.7	56.0 ± 9.9	0.60	0.067	68.3 ± 7.6
23. Ankle Joint Angle Right	0° ± 5°	3.0 ± 0.0	-1.5 ± 2.1	0.096	0.39	-13.0 ± 6.1
24. Ankle Joint Angle Left		2.5 ± 0.71	-4.5 ± 0.71	0.010	0.99	-11.3 ± 5.9
25. % Weightbear Right	50 ± 10	69.0 ± 1.4	54.5 ± 4.9	0.058	0.56	36.3 ± 29.0
26. % Weightbear Left		67.0 ± 9.9	50.5 ± 0.71	0.14	0.28	60.0 ± 20.2
Parameters beyond the normal range		**14**	**9**			**12**

Table 8.7.7.3. Segment Lengths and Percentile Height by Gene and Mutation Type

Variable	EXT 1 MS (n=2)	EXT 2 MS (n=2)	P-value	Power	EXT 1 NS (n=2)	EXT 2 NS (n=12)	P-value	Power
Total Leg Length-Right	73.5 ± 14.1	75.0 ± 19.1	0.94	0.050	80.5 ± 2.1	86.7 ± 8.4	0.34	0.15
Upper Leg – Right	36.3 ± 8.8	38.3 ± 10.3	0.85	0.052	38.5 ± 0.71	45.7 ± 5.0	0.075	0.42
Lower Leg – Right	30.0 ± 6.4	33.0 ± 9.2	0.74	0.057	32.0 ± 1.4	35.5 ± 3.2	0.17	0.25
Total Leg Length – Left	72.3 ± 14.5	74.8 ± 18.7	0.90	0.051	80.0 ± 1.4	85.9 ± 9.1	0.39	0.13
Upper Leg – Left	36.0 ± 9.9	38.5 ± 8.5	0.81	0.053	38.3 ± 1.1	44.6 ± 5.0	0.11	0.35
Lower Leg – Left	29.0 ± 4.9	32.0 ± 8.5	0.71	0.059	31.0 ± 1.4	37.2 ± 4.9	0.12	0.33
Total Arm Length – Right	44.0 ± 9.9	46.5 ± 11.3	0.84	0.053	43.0 ± 0.0	51.6 ± 5.4	0.051	0.51
Upper Arm – Right	26.5 ± 4.9	26.8 ± 6.0	0.97	0.050	28.0 ± 0.0	31.1 ± 3.8	0.29	0.17
Lower Arm – Right	20.3 ± 3.9	21.8 ± 6.0	0.79	0.054	19.5 ± 2.1	23.9 ± 3.1	0.084	0.40
Total Arm Length – Left	45.3 ± 10.3	45.3 ± 12.4		0.050	42.0 ± 1.4	52.0 ± 5.3	0.025	0.66
Upper Arm – Left	27.5 ± 7.8	25.3 ± 6.0	0.78	0.055	26.5 ± 0.71	31.8 ± 4.3	0.12	0.32
Lower Arm – Left	22.0 ± 3.5	22.8 ± 7.4	0.91	0.051	17.5 ± 3.5	24.5 ± 2.7	0.0065	0.87
Percentile Height	4.0 ± 1.4	39.0 ± 33.9	0.28	0.14	3.0 ± 0.0	54.3 ± 27.7	0.026	0.64

Table 8.7.7.3. Segment Lengths and Percentile Height by Gene and Mutation Type (continued)

Variable	EXT 1 Splice Site (n=3)	EXT 2 Splice Site (n=2)	P-value	Power	EXT 2 FS (n=3)
Total Leg Length-Right	83.2 ± 6.9	83.5 ± 8.5	0.96	0.050	85.7 ± 4.3
Upper Leg – Right	41.7 ± 2.0	42.3 ± 6.7	0.89	0.051	42.3 ± 1.9
Lower Leg – Right	34.2 ± 3.8	34.5 ± 3.5	0.93	0.051	34.7 ± 2.0
Total Leg Length – Left	82.5 ± 6.5	83.3 ± 8.1	0.92	0.051	84.8 ± 2.9
Upper Leg – Left	41.2 ± 2.4	42.8 ± 6.0	0.69	0.062	41.0 ± 0.9
Lower Leg – Left	35.7 ± 4.6	34.0 ± 4.2	0.71	0.060	36.7 ± 1.2
Total Arm Length – Right	46.7 ± 4.5	49.8 ± 8.1	0.61	0.069	50.7 ± 4.1
Upper Arm – Right	27.7 ± 2.1	31.8 ± 4.6	0.25	0.17	30.5 ± 2.2
Lower Arm – Right	22.3 ± 1.4	23.5 ± 3.5	0.63	0.068	23.5 ± 4.4
Total Arm Length – Left	46.8 ± 3.5	51.3 ± 6.0	0.36	0.12	51.0 ± 5.8
Upper Arm – Left	28.2 ± 2.0	32.8 ± 3.9	0.17	0.24	31.5 ± 1.8
Lower Arm – Left	20.2 ± 2.5	23.0 ± 2.8	0.32	0.14	23.0 ± 4.6
Percentile Height	17.0 ± 19.3	25.0 ± 0.0	0.62	0.069	9.7 ± 7.6

8.7.8 Gene and Severity

Table 8.7.8.1 Lesion Quality by Gene and Severity

Variable	EXT 1 Severe (n=5)	EXT 2 Severe (n=17)	P-value	Power	EXT 1 Mild (n=2)	EXT 2 Mild (n=2)	P-value	Power
Lesion Rank 1	9.6 ± 7.4	5.7 ± 4.4	0.16	0.28	8.0 ± 1.4	10.0 ± 1.4	0.29	0.14
% Rank 1	25.8 ± 11.6	26.5 ± 15.7	0.93	0.051	30.0 ± 7.1	66.5 ± 3.5	0.022	0.88
Lesion Rank 2	6.2 ± 3.3	4.7 ± 2.9	0.34	0.15	7.0 ± 2.8	1.0 ± 0.0	0.096	0.39
% Rank 2	17.0 ± 6.0	25.2 ± 12.1	0.16	0.27	25.5 ± 9.2	6.5 ± 0.71	0.10	0.37
Lesion Rank 3	5.4 ± 2.1	2.2 ± 1.9	0.0043	0.88	4.0 ± 1.4	1.0 ± 0.0	0.096	0.39
% Rank 3	16.4 ± 6.8	9.8 ± 7.0	0.079	0.41	15.0 ± 5.7	6.5 ± 0.71	0.17	0.24
Lesion Rank 4	13.8 ± 3.5	8.2 ± 5.1	0.035	0.57	8.0 ± 1.4	3.0 ± 0.0	0.038	0.72
% Rank 4	41.0 ± 10.1	38.3 ± 18.6	0.76	0.060	29.5 ± 3.5	20.0 ± 1.4	0.072	0.48
Small (%)	26.6 ± 13.7	26.9 ± 14.2	0.97	0.050	32.8 ± 4.5	64.3 ± 10.1	0.057	0.57
Medium (%)	29.3 ± 9.9	32.8 ± 13.4	0.59	0.080	*33.8 ± 0.71*	*14.0 ± 0.0*	*0.0007*	*1.0*
Large (%)	43.4 ± 14.5	38.3 ± 17.9	0.57	0.085	26.5 ± 14.8	21.4 ± 10.1	0.72	0.058
Average Number of Lesions	35.0 ± 11.8	20.9 ± 9.6	0.012	0.75	27.0 ± 1.4	15.0 ± 1.4	0.014	0.97
No. Pedunculated	9.2 ± 3.2	6.2 ± 4.5	0.19	0.24	7.5 ± 2.1	5.0 ± 1.4	0.29	0.14
% Pedunculated	26.6 ± 5.6	30.8 ± 14.3	0.53	0.092	27.6 ± 6.4	33.9 ± 12.6	0.59	0.068
No. Sessile	21.8 ± 10.8	13.8 ± 7.2	0.065	0.45	19.5 ± 0.71	10.0 ± 2.8	0.044	0.66
% Sessile	61.0 ± 11.3	65.5 ± 15.3	0.55	0.087	72.4 ± 6.4	66.1 ± 12.6	0.59	0.068
No. Distal	13.4 ± 6.1	8.4 ± 4.5	0.057	0.47	12.5 ± 2.1	5.5 ± 3.5	0.14	0.28
% Distal	37.6 ± 7.1	39.8 ± 13.7	0.75	0.061	46.6 ± 10.3	37.9 ± 27.1	0.72	0.058
No. Proximal	16.2 ± 5.4	9.4 ± 4.7	0.013	0.75	10.0 ± 2.8	9.0 ± 4.2	0.81	0.054
% Proximal	46.6 ± 6.9	45.2 ± 15.3	0.85	0.054	37.4 ± 12.4	58.9 ± 22.7	0.36	0.11
No. Pelvic	*4.2 ± 3.1*	*0.82 ± 1.5*	*0.0024*	*0.93*	1.5 ± 2.1	0.0 ± 0.0	0.42	0.095
% Pelvic	11.3 ± 7.5	3.2 ± 5.7	0.017	0.70	5.4 ± 7.6	0.0 ± 0.0	0.42	0.095
No Diaphyseal	1.6 ± 0.89	1.4 ± 1.3	0.77	0.059	2.5 ± 3.5	0.50 ± 0.71	0.51	0.078
% Diaphyseal	5.5 ± 5.3	8.7 ± 11.9	0.58	0.083	8.9 ± 12.6	3.1 ± 4.4	0.60	0.067
No. Flat Bone	*4.8 ± 3.1*	*0.94 ± 1.4*	*0.0007*	*0.98*	2.5 ± 0.71	0.0 ± 0.0	0.038	0.72
% Flat Bone	12.9 ± 7.1	3.9 ± 5.7	0.0081	0.81	9.2 ± 2.1	0.0 ± 0.0	0.026	0.85
No. Complex	5.8 ± 7.1	2.6 ± 2.2	0.11	0.34	2.5 ± 0.71	3.0 ± 1.4	0.69	0.059
% Complex	13.7 ± 12.3	13.0 ± 8.9	0.89	0.052	9.3 ± 3.1	20.5 ± 11.4	0.31	0.13
No. Simple	25.6 ± 6.5	17.9 ± 8.9	0.090	0.38	24.5 ± 2.1	12.0 ± 2.8	0.038	0.72
% Simple	75.1 ± 8.9	85.4 ± 9.4	0.042	0.54	90.7 ± 3.1	79.5 ± 11.4	0.31	0.13

Table 8.7.8.1 Lesion Quality by Gene and Severity (continued)

Variable	EXT 1 Severe (n=5)	EXT 2 Severe (n=17)	P-value	Power	EXT 1 Mild (n=2)	EXT 2 Mild (n=2)	P-value	Power
No. Flared	18.4 ± 11.8	6.6 ± 6.3	0.0068	0.83	3.5 ± 0.71	8.0 ± 2.8	0.16	0.25
% Flared	48.9 ± 29.3	28.5 ± 22.9	0.11	0.33	13.0 ± 3.3	52.7 ± 13.9	0.059	0.55
No. Not Flared	16.6 ± 8.5	14.1 ± 6.9	0.51	0.096	23.5 ± 2.1	7.0 ± 1.4	0.011	0.98
% Not Flared	51.1 ± 29.3	70.8 ± 23.0	0.13	0.31	86.9 ± 3.3	47.3 ± 13.9	0.059	0.55
No. Left	20.2 ±7.4	10.0 ± 5.5	0.0031	0.91	14.5 ± 0.71	10.5 ± 2.1	0.13	0.31
% Left	57.8 ± 8.5	46.3 ± 8.1	0.012	0.75	53.7 ± 0.19	69.6 ± 7.6	0.097	0.38
No. Right	15.0 ± 5.8	10.9 ± 4.8	0.12	0.32	12.5 ± 0.71	4.5 ± 0.71	0.0077	0.99
% Right	42.8 ± 9.3	53.7 ± 8.1	0.019	0.68	46.3 ± 0.19	30.4 ± 7.6	0.097	0.38

Table 8.7.8.2. Limb Alignment by Gene and Severity

Variable	Normal Values	EXT 1 Severe (n=5)	EXT 2 Severe (n=17)	P-value	Power	EXT 1 Mild (n=2)	EXT 2 Mild (n=2)	P-value	Power
1. Carpal Slip R	5 ± 2mm	5.0 ± 2.2	2.2 ± 3.8	0.17	0.26	5.5 ± 4.9	2.5 ± 0.71	0.49	0.083
2. Carpal Slip L		4.6 ± 2.9	3.3 ± 3.7	0.48	0.10	5.0 ± 4.2	1.5 ± 0.71	0.37	0.11
3. Radial Inclination R	21° ± 2°	27.8 ± 0.96	24.2 ± 5.2	0.19	0.23	28.5 ± 12.0	24.5 ± 4.9	0.71	0.059
4. Radial Inclination L		31.6 ± 5.6	26.5 ± 4.9	0.063	0.45	28.0 ± 8.5	26.0 ± 2.8	0.78	0.055
5. Ulnar Shortening R	0 ± 1 mm	-1.3 ± 4.7	-1.8 ± 5.1	0.85	0.054	-1.0 ± 2.8	-1.0 ± 2.8		0.050
6. Ulnar Shortening L		4.8 ± 5.1	-0.59 ± 5.1	0.050	0.50	-2.5 ± 4.9	-2.5 ± 3.5		0.050
7. Radial Bow R	10° ± 5°	9.0 ± 1.6	7.1 ± 2.1	0.12	0.33	9.0 ± 4.2	11.0 ± 1.4	0.59	0.068
8. Radial Bow Left		16.1 ± 9.5	7.5 ± 2.4	0.0020	0.94	9.5 ± 1.4	9.5 ± 0.71		0.050
9. Radial Head Dislocation R		1	0			0	1		
10. Radial Head Dislocation L		2	0			0	1		
11. Elbow Joint R	9° ± 3°	8.8 ± 10.9	-4.6 ± 13.3	0.079	0.41	-23.0 ± 1.4	-13.5 ± 0.71	0.014	0.97
12. Elbow Joint L		-1.2 ± 9.9	-8.4 ± 11.9	0.24	0.20	-10.5 ± 10.6	-9.5 ± 2.1	0.91	0.051
13. Femoral A.A. R	7° ± 2° valgus	-0.90 ± 5.9	-6.2 ± 9.4	0.25	0.19	-8.5 ± 12.0	-0.50 ± 4.9	0.48	0.084
14. Femoral A.A. L		-3.8 ± 8.9	-3.9 ± 9.4	0.99	0.050	4.0 ± 2.8	0.50 ± 3.5	0.39	0.10
15. Femoral N.S. Angle R	135° ± 5°	143.8 ± 21.2	139.8 ± 8.4	0.53	0.092	141.5 ± 9.2	142.5 ± 3.5	0.89	0.051
16. Femoral N.S. Angle L		146.4 ± 13.4	136.8 ± 7.8	0.053	0.49	146.5 ± 2.1	139.0 ± 22.6	0.69	0.060
17. Femoral M.A. R	0° ± 5° varus	5.9 ± 6.9	-0.46 ± 6.4	0.099	0.36	7.0 ± 1.4	2.0 ± 1.4	0.072	0.48
18. Femoral M.A. L		-4.5 ± 5.2	1.2 ± 5.2	0.066	0.45	6.0 ± 4.2	0.50 ± 3.5	0.29	0.14
19. Sharp's Right	35° ± 4°	38.9 ± 3.4	41.1 ± 5.7	0.47	0.11	37.0 (n = 1)	46.0 (n=1)		
20. Sharp's Left		39.9 ±5.2	41.1 ± 5.0	0.65	0.071	33.0 (n=1)	41 (n=1)		

Table 8.7.8.2. Limb Alignment by Gene and Severity (continued)

Variable	Normal Values	EXT 1 Severe (n=5)	EXT 2 Severe (n=17)	P-value	Power	EXT 1 Mild (n=2)	EXT 2 Mild (n=2)	P-value	Power
21. Fibular Height R	50 ± 10	51.8 ± 10.3	51.2 ± 12.3	0.94	0.051	52.5 ± 0.71	55.0 ± 4.2	0.49	0.081
22. Fibular Height L		48.5 ± 15.0	53.8 ± 13.9	0.51	0.095	59.5 ± 10.6	35.5 ± 7.8	0.12	0.31
23. Ankle Joint Angle R	0° ± 5°	-11.5 ± 17.2	-1.9 ± 10.4	0.17	0.26	-6.5 ± 0.71	0.0 (n=1)		
24. Ankle Joint Angle L		-9.0 ± 17.3	-1.0 ± 10.9	0.26	0.19	1.5 ± 0.71	0.0 (n=1)		
25. % Weightbear R	50 ± 10	65.3 ± 7.5	45.0 ± 23.2	0.11	0.35	52.5 ± 45.9	58.5 ± 3.5	0.87	0.052
26. % Weightbear L		60.0 ± 10.4	50.5 ± 20.6	0.39	0.13	75.5 ± 7.8	58.5 ± 9.2	0.18	0.22
Number of parameters that fall beyond the normal range		**15**	**14**			**11**	**7**		

Table 8.7.8.3. Segment Lengths and Percentile Height by Gene and Severity

Variable	EXT 1 Severe (n=5)	EXT 2 Severe (n=17)	P-value	Power	EXT 1 Mild (n=2)	EXT 2 Mild (n=2)	P-value	Power
Total Leg Length-Right	82.1 ± 5.2	86.1 ± 7.5	0.28	0.18	73.5 ± 14.1	75.0 ± 19.1	0.94	0.050
Upper Leg – Right	40.4 ± 2.3	44.7± 4.8	0.071	0.43	36.3 ± 8.8	38.3 ± 10.3	0.85	0.052
Lower Leg – Right	33.3 ± 3.0	35.2 ± 2.9	0.22	0.21	30.0 ± 6.4	33.0 ± 9.2	0.74	0.057
Total Leg Length – Left	81.5 ± 4.8	85.4 ± 7.9	0.31	0.16	72.3 ± 14.5	74.8 ± 18.7	0.89	0.051
Upper Leg – Left	40.0 ± 2.4	43.8 ± 4.7	0.10	0.36	36.0 ± 9.9	38.5 ± 8.5	0.81	0.053
Lower Leg – Left	33.8 ± 4.2	36.7 ± 4.4	0.21	0.23	29.0 ±4.9	32.0 ± 8.5	0.71	0.059
Total Arm Length – Right	45.2 ± 3.8	51.2 ± 5.2	0.026	0.63	44.0 ± 9.9	46.5 ± 11.3	0.84	0.053
Upper Arm – Right	27.8 ± 1.5	31.1 ± 3.5	0.057	0.48	26.5 ± 4.9	26.8 ± 6.0	0.97	0.050
Lower Arm – Right	21.2 ± 2.1	23.8 ± 3.1	0.11	0.35	20.3 ± 3.9	21.8 ± 6.0	0.79	0.054
Total Arm Length – Left	44.9 ± 3.7	51.7 ± 5.1	0.012	0.76	45.3 ± 10.3	45.3 ± 12.4		0.050
Upper Arm – Left	27.5 ± 1.7	31.9 ± 3.8	0.023	0.65	27.5 ± 7.8	25.3 ± 6.0	0.78	0.055
Lower Arm – Left	19.1 ± 2.9	24.1 ± 2.9	0.0034	0.90	22.0 ± 3.5	22.8 ± 7.4	0.91	0.051
Percentile Height	11.4 ± 15.6	42.9 ± 29.6	0.035	0.57	4.0 ± 1.4	39.0 ± 33.9	0.28	0.14

8.7.9 Gender and Severity

Table 8.7.9.1 Lesion Quality by Gender and Severity

Variable	Males Severe (n=12)	Females Severe (n=10)	P-value	Power	Males Mild (n=2)	Females Mild (n=2)	P-value	Power
Lesion Rank 1	8.7 ± 6.2	4.2 ± 2.3	0.044	0.52	10.0 ± 1.4	8.0 ± 1.4	0.29	0.14
% Rank 1	27.8 ± 16.6	24.6 ± 12.4	0.63	0.075	52.0 ± 24.0	44.5 ± 24.6	0.79	0.054
Lesion Rank 2	5.8 ± 3.3	4.2 ± 2.6	0.24	0.19	3.0 ± 2.8	5.0 ± 5.7	0.69	0.059
% Rank 2	19.7 ± 9.2	27.8 ± 12.7	0.099	0.36	12.5 ± 9.2	19.5 ± 17.7	0.67	0.061
Lesion Rank 3	3.9 ± 2.4	1.7 ± 1.7	0.025	0.64	3.0 ± 2.8	2.0 ± 1.4	0.69	0.059
% Rank 3	12.5 ± 6.7	9.9 ± 8.2	0.44	0.11	12.5 ± 9.2	9.0 ± 2.8	0.66	0.062
Lesion Rank 4	11.6 ± 5.1	7.0 ± 4.6	0.040	0.54	5.0 ± 2.8	6.0 ± 4.2	0.81	0.054
% Rank 4	40.0 ± 16.2	37.6 ± 18.4	0.74	0.062	23.0 ± 5.7	26.5 ± 7.8	0.66	0.062
Small (%)	28.6 ± 15.0	24.6 ± 12.5	0.51	0.096	53.7 ± 25.0	43.4 ± 19.4	0.69	0.060
Medium (%)	29.6 ± 8.9	35.0 ± 15.9	0.32	0.15	24.3 ± 14.2	23.8 ± 13.5	0.97	0.050
Large (%)	38.4 ± 16.5	40.7 ± 18.5	0.76	0.060	15.1 ± 1.3	32.8 ± 6.0	0.056	0.58
Average Number of Lesions	29.9 ± 11.6	17.1 ± 6.9	0.0061	0.84	21.0 ± 7.1	21.0 ± 9.9		0.050
No. Pedunculated	8.3 ± 5.3	5.2 ± 2.2	0.097	0.34	5.0 ± 1.4	7.5 ± 2.1	0.29	0.14
% Pedunculated	30.0 ± 14.4	33.2 ± 12.6	0.64	0.073	24.0 ± 1.4	37.5 ± 7.6	0.13	0.29
No. Sessile	19.6 ± 8.9	10.9 ± 5.2	0.014	0.74	16.0 ± 5.7	13.5 ± 7.8	0.75	0.056
% Sessile	64.8 ± 15.2	60.2 ± 11.1	0.49	0.099	75.9 ± 1.4	62.5 ± 7.6	0.13	0.29
No. Distal	11.7 ± 5.2	7.0 ± 4.1	0.032	0.59	8.5 ± 2.1	9.5 ± 1.5	0.88	0.051
% Distal	39.7 ± 14.2	38.8 ± 10.6	0.88	0.053	36.3 ± 24.8	48.2 ± 12.6	0.61	0.067
No. Proximal	13.6 ± 5.5	7.8 ± 3.9	0.011	0.76	12.0 ± 0.0	7.0 ± 1.4	0.038	0.72
% Proximal	46.5 ± 8.5	43.9 ± 20.3	0.73	0.062	60.6 ± 20.4	35.7 ± 10.1	0.26	0.16
No. Pelvic	2.3 ± 2.9	0.80 ± 1.1	0.16	0.28	0.0 ± 0.0	1.5 ± 2.1	0.42	0.095
% Pelvic	5.9 ± 7.7	4.0 ± 6.1	0.55	0.087	0.0 ± 0.0	5.4 ± 7.6	0.42	0.095
No Diaphyseal	1.2 ± 1.1	1.8 ± 1.3	0.24	0.20	0.50 ± 0.71	2.5 ± 3.5	0.51	0.078
% Diaphyseal	5.6 ± 8.6	12.8 ± 14.0	0.22	0.22	3.1 ± 4.4	8.9 ± 12.6	0.60	0.067
No. Flat Bone	2.4 ± 3.0	1.1 ± 1.4	0.22	0.21	1.0 ± 1.4	1.5 ± 2.1	0.81	0.054
% Flat Bone	6.4 ± 7.6	5.4 ± 6.5	0.75	0.061	3.8 ± 5.4	5.4 ± 7.6	0.84	0.052
No. Complex	3.8 ± 2.5	2.0 ± 1.3	0.091	0.38	2.5 ± 0.71	3.0 ± 1.4	0.69	0.059
% Complex	13.7 ± 8.4	11.3 ± 5.8	0.51	0.095	12.0 ± 0.68	17.9 ± 15.2	0.64	0.064
No. Simple	23.9 ± 9.0	14.6 ± 5.7	0.010	0.78	18.5 ± 6.4	18.0 ± 11.3	0.96	0.050
% Simple	84.4 ±10.1	83.6 ± 6.4	0.84	0.054	87.9 ± 0.68	82.1 ± 15.2	0.64	0.064
No. Flared	14.3 ± 9.3	3.2 ± 3.2	0.0018	0.94	7.0 ± 4.2	4.5 ± 2.1	0.53	0.075
% Flared	46.1 ± 24.2	17.4 ± 16.8	0.0049	0.87	38.9 ± 33.3	26.8 ± 22.7	0.71	0.058
No. Not Flared	15.6 ± 8.7	13.6 ± 4.9	0.53	0.091	14.0 ± 11.3	16.5 ± 12.0	0.85	0.052

Table 8.7.9.1 Lesion Quality by Gender and Severity (continued)

Variable	Males Severe (n=12)	Females Severe (n=10)	P-value	Power	Males Mild (n=2)	Females Mild (n=2)	P-value	Power
% Not Flared	53.9 ± 24.2	81.3 ± 17.9	0.0075	0.82	61.1 ± 33.3	73.2 ± 22.7	0.71	0.058
No. Left	15.6 ± 7.8	8.4 ± 4.2	0.017	0.70	13.0 ± 1.4	12.0 ± 4.2	0.78	0.055
% Left	47.6 ± 8.3	48.9 ± 9.1	0.75	0.061	64.4 ± 14.9	58.9 ± 7.6	0.69	0.060
No. Right	14.3 ± 5.3	8.8 ± 3.4	0.0095	0.80	8.0 ± 5.7	9.0 ± 5.7	0.88	0.051
% Right	52.4 ± 8.3	51.4 ± 9.1	0.82	0.055	35.6 ± 14.9	41.1 ± 7.6	0.69	0.060

Table 8.7.9.2 Limb Alignment by Gender and Severity

Variable	Normal Values	Males Severe (n=12)	Females Severe (n=10)	P-value	Power	Males Mild (n=2)	Females Mild (n=2)	P-value	Power
1. Carpal Slip R	5 ± 2mm	2.9 ± 4.4	2.6 ± 3.0	0.84	0.054	6.0 ± 4.2	2.0 ± 0.0	0.31	0.13
2. Carpal Slip L		3.6 ± 3.6	3.6 ± 3.6	0.99	0.050	5.0 ± 4.2	1.5 ± 0.71	0.37	0.11
3. Radial Inclination R	21° ± 2°	25.3 ± 3.4	24.5 ± 6.2	0.72	0.063	32.5 ± 6.4	20.5 ± 0.71	0.12	0.33
4. Radial Inclination L		28.4 ± 5.9	26.7 ± 4.9	0.48	.10	31.0 ± 4.2	23.0 ± 1.4	0.13	0.31
5. Ulnar Shortening R	0 ± 1 mm	-2.5 ± 5.1	-0.90 ± 4.9	0.49	0.098	-3.0 ± 0.0	1.0 ± 0.0	--	--
6. Ulnar Shortening L		0.30 ± 5.7	0.92 ± 5.5	0.79	0.057	-5.5 ± 0.71	0.50 ± 0.71	0.014	0.97
7. Radial Bow R	10° ± 5°	7.4 ± 2.3	7.7 ± 2.1	0.77	0.059	12.0 ± 0.0	8.0 ± 2.8	0.18	0.22
8. Radial Bow Left		10.3 ± 6.9	8.6 ± 4.5	0.52	0.094	8.8 ± 0.35	10.3 ± 0.35	0.051	0.61
9. Radial Head Dislocation R		1 dislocation	0			0	1 dislocation		
10. Radial Head Dislocation L		1 dislocation	1 dislocation			0	1 dislocation		
11. Elbow Joint R	9° ± 3°	-1.4 ± 15.1	-2.9 ± 12.9	0.81	0.056	-17.5 ± 6.4	-19.0 ± 7.1	0.84	0.052
12. Elbow Joint L		-8.4 ± 12.6	-4.9 ± 10.9	0.49	0.099	-5.5 ± 3.5	-14.5 ± 4.9	0.17	0.23
13. Femoral A.A. R	7° ± 2° valgus	1.6 ± 7.1	-0.11 ± 6.6	0.58	0.082	5.5 ± 3.5	3.5 ± 3.5	0.63	0.065
14. Femoral A.A. L		-5.5 ± 8.5	-1.9 ± 9.7	0.38	0.13	2.5 ± 0.71	2.0 ± 5.7	0.91	0.051
15. Femoral N.S. Angle R	135° ± 5°	141.0 ± 8.8	140.4 ± 15.4	0.91	0.051	140.0 ± 7.1	144.0 ± 5.7	0.59	0.068
16. Femoral N.S. Angle L		138.4 ± 6.9	139.7 ± 12.9	0.77	0.059	135.0 ± 17.7	150.0 ± 7.1	0.39	0.10
17. Femoral M.A. R	0° ± 5° varus	1.6 ± 7.1	-0.11 ± 6.6	0.58	0.082	5.5 ± 3.5	3.5 ± 3.5	0.63	0.065
18. Femoral M.A. L		-1.1 ± 5.8	1.4 ± 5.2	0.32	0.15	3.5 ± 7.8	3.5 ± 0.0	0.94	0.050
19. Sharp's Right	35° ± 4°	39.3 ± 3.7	42.3 ± 6.6	0.21	0.22	Data not available	Data not available		
20. Sharp's Left		41.3 ± 4.8	40.5 ± 5.3	0.69	0.066	Data not available	Data not available		
21. Fibular Height R	50 ± 10	54.4 ± 10.3	48.0 ± 12.8	0.22	0.21	55.5 ± 3.5	52.0 ± 0.0	0.29	0.14
22. Fibular Height L		52.4 ± 11.2	53.1 ± 17.3	0.92	0.051	48.5 ± 26.2	46.5 ± 7.8	0.93	0.050

Table 8.7.9.2 Limb Alignment by Gender and Severity (continued)

Variable	Normal Values	Males Severe (n=12)	Females Severe (n=10)	P-value	Power	Males Mild (n=2)	Females Mild (n=2)	P-value	Power
23. Ankle Joint Angle R	0° ± 5°	-3.9 ± 15.7	-3.8 ± 8.3	0.99	0.050	-7.0 ± 4.3	-7.0 (n=1)	0.58	0.065
24. Ankle Joint Angle L		-2.4 ± 15.5	-2.9 ± 8.9	0.94	0.051	0.50 ± 0.71	2.0 (n=1)	0.33	0.11
25. % Weightbear R	50 ± 10	52.1 ± 25.5	46.1 ± 19.7	0.56	0.085	70.5 ± 20.5	40.5 ± 28.9	0.35	0.11
26. % Weightbear L		51.8 ± 17.5	53.1 ± 21.7	0.88	0.052	66.5 ± 20.5	67.5 ± 3.5	0.95	0.050
Number of parameters that fall beyond the normal range		**10**	**9**			**11**	**12**		

Table 8.7.9.3. Segment Lengths and Percentile Height by Gender and Severity

Variable	**Males Severe (n=12)**	**Females Severe (n=10)**	**P-value**	**Power**	**Males Mild (n=2)**	**Females Mild (n=2)**	**P-value**	**Power**
Total Leg Length-Right	86.5 ± 5.9	83.8 ± 8.6	0.39	0.13	86.0 ± 3.5	62.5 ± 1.4	0.013	0.98
Upper Leg – Right	43.3 ± 4.3	44.2 ± 5.3	0.69	0.066	44.0 ± 2.1	30.5 ± 0.71	0.013	0.97
Lower Leg – Right	35.3 ± 2.9	34.2 ± 3.1	0.39	0.13	37.0 ± 3.5	26.0 ± 0.71	0.049	0.62
Total Leg Length – Left	85.9 ± 6.6	82.9 ± 8.5	0.37	0.13	85.3 ± 3.9	61.8 ± 0.35	0.014	0.97
Upper Leg – Left	42.5 ± 4.0	43.5 ± 5.2	0.62	0.076	43.8 ± 1.1	30.8 ± 2.5	0.021	0.90
Lower Leg – Left	36.8 ± 4.7	35.2 ± 4.2	0.43	0.12	35.3 ± 3.9	25.8 ± 0.35	0.075	0.47
Total Arm Length – Right	50.0 ± 5.5	49.6 ± 5.7	0.86	0.054	52.8 ± 2.5	37.8 ± 1.1	0.016	0.95
Upper Arm – Right	31.0 ± 3.3	29.5 ± 3.5	0.28	0.17	30.5 ± 0.71	22.8 ± 0.35	0.0052	1.0
Lower Arm – Right	23.4 ± 3.3	22.9 ± 2.9	0.71	0.065	24.5 ± 2.1	17.5 ± 0.0	0.043	0.67
Total Arm Length – Left	50.5 ± 5.9	49.8 ± 5.4	0.78	0.058	53.3 ± 1.1	37.3 ± 1.1	0.0044	1.0
Upper Arm – Left	31.3 ±3.8	30.4 ± 4.2	0.62	0.076	31.3 ± 2.5	21.5 ± 0.71	0.033	0.77
Lower Arm – Left	23.0 ± 3.9	22.9 ± 3.2	0.92	0.051	26.3 ± 2.5	18.5 ± 1.4	0.062	0.54
Percentile Height	36.0 ± 28.8	35.5 ± 32.9	0.97	0.050	10.0 ± 7.1	33.0 ± 42.4	0.53	0.076

8.7.10 Gender and Mutation Type

Table 8.7.10.1 Lesion Quality by Gender and Mutation Type

Variable	Males Missense (n=2)	Females Missense (n=2)	P-value	Power	Males Nonsense (n=8)	EXT 2 Nonsense (n=6)	P-value	Power
Lesion Rank 1	10.0 ± 1.4	8.0 ± 1.4	0.29	0.14	10.8 ± 6.7	3.3 ± 2.5	0.025	0.65
% Rank 1	52.0 ± 24.0	44.5 ± 27.6	0.79	0.054	33.7 ± 17.7	25.2 ± 16.1	0.37	0.13
Lesion Rank 2	3.0 ± 2.8	5.0 ± 5.7	0.69	0.059	4.9 ± 3.2	3.7 ± 1.6	0.41	0.12
% Rank 2	12.5 ± 9.2	19.5 ± 17.7	0.67	0.061	15.7 ± 6.9	29.2 ± 12.9	0.026	0.64
Lesion Rank 3	3.0 ± 2.8	2.0 ± 1.4	0.69	0.059	3.8 ± 2.3	1.0 ± 1.1	0.020	0.69
% Rank 3	12.5 ± 9.2	9.0 ± 2.8	0.66	0.062	11.9 ± 7.4	7.7 ± 7.8	0.32	0.16
Lesion Rank 4	5.0 ± 2.8	6.0 ± 4.2	0.81	0.054	11.0 ± 4.8	4.8 ± 3.1	0.018	0.72
% Rank 4	23.0 ± 5.7	26.5 ± 7.8	0.66	0.062	38.9 ± 19.6	37.6 ± 22.9	0.91	0.051
Small (%)	53.7 ± 25.0	43.4 ± 19.4	0.69	0.060	33.1 ± 15.8	25.2 ± 16.0	0.38	0.13
Medium (%)	24.3 ± 14.2	23.8 ± 13.5	0.97	0.050	26.5 ± 7.7	36.4 ± 18.8	0.20	0.23
Large (%)	15.1 ± 1.3	32.8 ± 6.0	0.056	0.58	35.9 ± 19.8	39.1 ± 22.2	0.78	0.058
Average Number of Lesions	21.0 ± 7.1	21.0 ± 9.9	--	0.050	30.4 ± 12.1	12.8 ± 3.1	0.0050	0.89
No. Pedunculated	5.0 ± 1.4	7.5 ± 2.1	0.29	0.14	8.5 ± 5.6	4.3 ± 0.82	0.098	0.37
% Pedunculated	24.0 ± 1.4	37.5 ± 7.6	0.13	0.29	29.8 ± 14.1	39.1 ± 14.5	0.36	0.13
No. Sessile	16.0 ± 5.7	13.5 ± 7.8	0.75	0.056	19.8 ± 9.3	7.7 ± 2.4	0.0093	0.82
% Sessile	75.9 ± 1.4	62.5 ± 7.6	0.13	0.29	64.8 ± 16.8	53.2 ± 6.1	0.23	0.19
No. Distal	8.5 ± 7.8	9.5 ± 2.1	0.88	0.051	13.4 ± 4.9	4.5 ± 2.1	0.0013	0.98
% Distal	36.3 ± 24.8	48.2 ± 12.6	0.61	0.067	46.1 ± 12.1	34.4 ± 10.7	0.084	0.39
No. Proximal	12.0 ± 0.	7.0 ± 1.4	0.038	0.72	13.1 ± 5.4	6.2 ± 2.9	0.015	0.74
% Proximal	60.6 ± 20.4	35.7 ± 10.1	0.26	0.16	43.2 ± 8.7	45.5 ± 29.2	0.87	0.052
No. Pelvic	0.0 ± 0.0	1.5 ± 2.1	0.42	0.095	2.4 ± 3.5	0.33 ± 0.82	0.19	0.24
% Pelvic	0.0 ± 0.0	5.4 ± 7.6	0.42	0.095	5.9 ± 8.9	2.8 ± 6.8	0.49	0.098
No Diaphyseal	0.50 ± 0.71	2.5 ± 3.5	0.51	0.078	0.75 ± 0.89	1.7 ± 1.6	0.20	0.23
% Diaphyseal	3.1 ± 4.4	8.9 ± 12.6	0.60	0.067	1.7 ± 2.4	17.4 ± 19.3	0.11	0.34
No. Flat Bone	1.0 ± 1.4	1.5 ± 2.1	0.81	0.054	2.6 ± 3.6	0.50 ± 0.84	0.18	0.24
% Flat Bone	3.8 ± 5.4	5.4 ± 7.6	0.84	0.052	6.7 ± 8.9	4.1 ± 6.9	0.55	0.086
No. Complex	2.5 ± 0.71	3.0 ± 1.4	0.69	0.059	5.8 ± 5.5	1.0 ± 0.63	0.061	0.47
% Complex	12.0 ± 0.68	17.9 ± 15.2	0.64	0.064	15.9 ± 9.8	11.3 ± 7.4	0.46	0.10
No. Simple	18.5 ± 6.4	18.0 ± 11.3	0.96	0.050	23.5 ± 8.5	11.2 ± 2.5	0.0051	0.89
% Simple	87.9 ± 0.68	82.1 ± 15.2	0.64	0.064	84.1 ± 9.8	83.1 ± 9.7	0.89	0.052
No. Flared	7.0 ± 4.2	4.5 ± 2.1	0.53	0.075	15.3 ± 7.7	2.2 ± 2.3	0.0019	0.96
% Flared	38.9 ± 33.3	26.8 ± 22.7	0.71	0.058	50.9 ± 18.2	17.1 ± 20.5	0.0067	0.86
No. Not Flared	14.0 ± 12.0	16.5 ± 12.0	0.85	0.052	15.1 ± 8.6	10.7 ± 3.6	0.26	0.19
% Not Flared	61.6 ± 33.3	73.2 ± 22.7	0.71	0.058	49.0 ± 18.2	82.9 ± 20.5	0.0067	0.86
No. Left	13.0 ± 1.4	12.0 ± 4.2	0.78	0.055	17.1 ± 8.5	5.5 ± 1.9	0.0068	0.86
% Left	64.4 ± 14.9	58.9 ± 7.6	0.69	0.060	52.0 ± 6.8	42.6 ± 7.1	0.081	0.41
No. Right	8.0 ± 5.7	9.0 ± 5.7	0.88	0.051	13.3 ± 4.5	7.3 ± 1.2	0.0087	0.83
% Right	35.6 ± 14.9	41.1 ± 7.6	0.69	0.060	47.9 ± 6.8	57.4 ±7.1	0.081	0.41

Table 8.7.10.1 Lesion Quality by Gender and Mutation Type (continued)

Variable	Males Splice Site (n=2)	Females Splice Site (n=3)	P-value	Power	Males FS (n=2)	Females FS (n=1)	P-value	Power
Lesion Rank 1	5.0 ± 0.0	5.0 ± 0.0	--	--	4.0 ± 2.8	7.0	0.55	0.069
% Rank 1	14.5 ± 0.71	22.0 ± 5.2	0.15	0.27	17.4 ± 0.92	29.2	0.060	0.59
Lesion Rank 2	10.0 ± 1.4	3.3 ± 2.3	0.038	0.67	5.0 ± 1.4	10.0	0.21	0.18
% Rank 2	29.0 ± 5.7	20.3 ± 11.1	0.39	0.11	26.4 ± 13.7	41.7	0.53	0.071
Lesion Rank 3	5.5 ± 3.5	3.3 ± 2.1	0.44	0.098	3.0 ± 2.8	1.0	0.67	0.058
% Rank 3	15.5 ± 9.2	16.3 ± 7.6	0.92	0.051	11.5 ± 3.5	4.2	0.34	0.11
Lesion Rank 4	14.0 ± 0.0	11.7 ± 4.7	0.56	0.076	11.5 ± 10.6	6.0	0.75	0.055
% Rank 4	40.5 ± 2.1	41.7 ± 10.6	0.89	0.051	44. ± 11.9	25.0	0.41	0.090
Small (%)	13.8 ± 0.0	22.6 ± 6.7	0.18	0.23	25.8 ± 10.7	27.7	0.91	0.051
Medium (%)	36.2 ± 4.0	28.3 ± 10.2	0.39	0.11	35.3 ± 14.5	47.0	0.63	0.061
Large (%)	47.7 ± 3.3	49.0 ± 8.0	0.85	0.053	39.0 ± 3.8	25.0	0.21	0.19
Average Number of Lesions	34.5 ± 2.1	23.3 ± 7.6	0.15	0.27	23.5 ± 17.7	24.0	0.99	0.050
No. Pedunculated	5.5 ± 2.1	7.3 ± 3.2	0.54	0.079	10.5 ± 7.8	4.0	0.62	0.062
% Pedunculated	15.8 ± 5.2	30.9 ± 5.1	0.049	0.59	44.9 ± 0.75	16.7	0.021	0.99
No. Sessile	25.5 ± 4.9	14.3 ± 4.2	0.071	0.47	13.0 ± 9.9	20.0	0.67	0.058
% Sessile	74.5 ± 18.9	61.8 ± 2.9	0.30	0.14	55.1 ± 0.75	83.3	0.021	0.99
No. Distal	11.5 ± 0.71	10.3 ± 4.2	0.73	0.058	5.0 ± 4.2	12.0	0.41	0.091
% Distal	33.5 ± 4.1	43.9 ± 7.9	0.19	0.22	20.2 ± 2.9	50.0	0.074	0.49
No. Proximal	17.5 ± 4.9	9.7 ± 5.0	0.19	0.23	11.5 ± 7.8	12.0	0.97	0.050
% Proximal	50.4 ± 11.2	39.9 ± 10.5	0.36	0.12	50.9 ± 5.2	50.0	0.91	0.050
No. Pelvic	3.5 ± 0.71	1.0 ± 1.0	0.058	0.53	0.50 ± 0.71	3.0	0.21	0.18
% Pelvic	10.2 ± 2.7	3.8 ± 3.3	0.11	0.35	1.4 ± 1.9	12.5	0.14	0.28
No Diaphyseal	2.0 ± 1.4	2.0 ± 1.0	--	0.050	2.0 ± 1.4	2.0	-	0.050
% Diaphyseal	5.9 ± 4.5	9.7 ± 6.1	0.51	0.084	15.0 ± 17.3	4.0	0.69	0.057
No. Flat Bone	3.5 ± 0.71	1.7 ± 2.1	0.33	0.13	0.50 ± 0.71	3.0	0.21	0.18
% Flat Bone	10.2 ± 2.7	5.8 ± 6.3	0.44	0.098	1.4 ± 1.9	12.5	0.14	0.28
No. Complex	4.0 ± 1.4	2.3 ± 1.5	0.31	0.14	1.5 ± 0.71	4.0	0.21	0.18
% Complex	11.5 ± 3.4	9.5 ± 3.9	0.61	0.069	10.5 ± 10.9	16.7	0.72	0.055
No. Simple	27.5 ± 3.5	19.7 ± 6.4	0.23	0.19	22.0 ± 18.4	20.0	0.94	0.050
% Simple	80.2 ± 15.2	84.2 ± 0.84	0.65	0.065	89.5 ± 10.9	83.3	0.72	0.055
No. Flared	16.0 ± 18.4	4.3 ± 4.9	0.34	0.13	9.0 ± 11.3	6.0	0.86	0.051
% Flared	44.8 ± 50.5	15.6 ± 13.7	0.38	0.11	28.2 ± 26.9	25.0	0.94	0.050
No. Not Flared	18.5 ± 16.3	19.0 ± 2.6	0.96	0.050	14.5 ± 6.4	15.0	0.96	0.050
% Not Flared	55.2 ± 50.5	84.4 ± 13.7	0.38	0.11	71.9 ± 26.9	62.5	0.82	0.052
No. Left	16.5 ± 2.1	13.0 ± 2.6	0.22	0.19	8.5 ± 6.4	12.0	0.73	0.055
% Left	47.7 ± 3.2	57.0 ± 6.1	0.15	0.27	36.2 ± 0.19	50.0	0.011	1.0
No. Right	18.0 ± 0.0	10.7 ± 5.5	0.17	0.24	15.0 ± 11.3	12.0	0.86	0.051
% Right	52.3 ± 3.2	44.0 ± 7.9	0.27	0.16	63.8 ± 0.19	50.0	0.011	1

Table 8.7.10.2. Limb Alignment by Gender and Mutation Type

Variable	Normal Values	Males Missense (n=2)	Females Missense (n=2)	P-value	Power	Males Nonsense (n=8)	EXT 2 Nonsense (n=6)	P-value	Power
1. Carpal Slip Right	5 ± 2mm	6.0 ± 4.2	2.0 ± 0.0	0.31	0.13	1.8 ± 5.2	2.3 ± 3.9	0.88	0.052
2. Carpal Slip Left		5.0 ± 5.2	1.5 ± 0.71	0.37	0.11	3.8 ± 4.4	3.3 ± 4.2	0.86	0.053
3. Radial Inclination Right	21° ± 2°	32.5 ± 6.4	20.5 ± 0.71	0.12	0.33	24.5 ± 3.1	25.3 ± 5.1	0.74	0.061
4. Radial Inclination Left		31.0 ± 4.2	23.0 ± 1.4	0.13	0.31	28.5 ± 6.0	25.5 ± 3.1	0.29	0.17
5. Ulnar Shortening Right	0 ± 1 mm	-3.0 ± 0.0	1.0 ± 0.0	--		-4.6 ± 5.3	-0.50 ± 4.3	0.18	0.25
6. Ulnar Shortening Left		-5.5 ± 0.71	0.50 ± 0.71	0.014	0.97	1.1 ± 5.1	-1.2 ± 4.4	0.39	0.12
7. Radial Bow Right	10° ± 5°	12.0 ± 0.0	8.0 ± 2.8	0.18	0.22	7.4 ± 2.8	6.9 ± 2.4	0.73	0.062
8. Radial Bow Left		8.8 ± 0.35	10.3 ± 0.35	0.051	0.61	10.9 ± 8.4	6.2 ± 1.5	0.20	0.23
9. Radial Head Dislocation R		0	1 dislocation			0	0		
10. Radial Head Dislocation L		0	1 dislocation			1 dislocation	0		
11 Elbow Joint Right	9° ± 3°	-17.5 ± 6.4	-19.0 ± 7.1	0.84	0.052	1.1 ± 14.9	-4.8 ± 13.3	0.46	0.10
12. Elbow Joint Left		-5.5 ± 3.5	-14.5 ± 4.9	0.17	0.23	-9.3 ± 13.1	-3.1 ± 12.5	0.39	0.13
13. Femoral A.A. Right	7° ± 2° valgus	1.5 ± 2.1	-10.5 ± 9.2	0.21	0.19	-5.8 ± 10.9	-5.5 ± 8.3	0.95	0.050
14. Femoral A.A. Left		2.5 ± 0.71	2.0 ± 5.7	0.91	0.051	-7.4 ± 7.9	-0.50 ± 12.6	0.24	0.20
15. Femoral N.S. Angle Right	135° ± 5°	140.0 ± 7.1	144.0 ± 5.7	0.59	0.068	144.6 ± 6.3	139.3 ± 5.0	0.12	0.33
16. Femoral N.S. Angle Left		135.5 ± 17.6	150.0 ± 7.1	0.39	0.10	139.4 ± 5.3	137.5 ± 8.2	0.61	0.076
17. Femoral M.A. Right	0° ± 5° varus	5.5 ± 3.5	3.5 ± 3.5	0.63	0.065	1.4 ± 6.3	-2.3 ± 4.8	0.27	0.18
18. Femoral M.A. Left		3.5 ± 7.8	3.0 ± 0.0	0.94	0.050	-0.71 ± 6.2	0.83 ± 6.2	0.66	0.069
19. Sharp's Right	35° ± 4°	Data not available	Data not available			39.1 ± 3.8	42.5 ± 7.2	0.28	0.18
20. Sharp's Left		Data not available	Data not available			40.6 ± 4.8	41.2 ± 5.4	0.83	0.055
21. Fibular Height Right	50 ± 10	55.5 ± 3.5	52.0 ± 0.0	0.29	0.14	51.9 ± 11.1	50.3 ± 12.5	0.80	0.056
22. Fibular Height Left		48.5 ± 26.2	46.5 ± 7.8	0.93	0.050	48.3 ± 10.3	50.8 ± 19.9	0.76	0.059
23. Ankle Joint Angle Right	0° ± 5°	-7.0 (n=1)	-3.0 ± 4.2	0.58	0.065	-2.9 ± 18.8	-3.5 ± 7.2	0.94	0.051
24. Ankle Joint Angle Left		2.0 (n=1)	0.50 ± 0.71	0.33	0.11	-0.57 ± 18.5	-1.8 ± 9.2	0.89	0.052

Table 8.7.10.2. Limb Alignment by Gender and Mutation Type (continued)

Variable	Normal Values	Males Missense (n=2)	Females Missense (n=2)	P-value	Power	Males Nonsense (n=8)	EXT 2 Nonsense (n=6)	P-value	Power
25. % Weightbear Right	50 ± 10	70.5 ± 20.5	40.5 ± 28.9	0.35	0.11	55.9 ± 25.2	39.1 ± 18.7	0.21	0.22
26. % Weightbear Left		66.5 ± 20.5	67.5 ± 3.5	0.95	0.050	51.6 ± 19.5	45.3 ± 23.9	0.61	0.076
Parameters beyond the normal range									

Table 8.7.10.2. Limb Alignment by Gender and Mutation Type (continued)

Variable	Normal Values	Males Splice Site (n=2)	Females Splice Site (n=3)	P-value	Power	Males FS (n=2)	Females FS (n=1)	P-value	Power
1. Carpal Slip Right	5 ± 2mm	5.0 ± 4.2	3.0 ± 1.0	0.46	0.093	4.0 ± 1.4	3.0	0.67	0.058
2. Carpal Slip Left		4.0 ± 1.4	3.3 ± 3.1	0.79	0.055	2.5 ± 0.71	6.0	0.15	0.25
3. Radial Inclination Right	21° ± 2°	27.5 ± 0.71	27.3 ± 0.58	0.79	0.055	25.5 ± 6.4	11.0	0.31	0.12
4. Radial Inclination Left		30.0 ± 2.8	30.0 ± 7.9	-	0.050	26.5 ± 10.6	24.0	0.88	0.051
5. Ulnar Shortening Right	0 ± 1 mm	2.0 ± 2.8	1.7 ± 1.5	0.87	0.052	-0.50 ± 2.1	-11.0	0.15	0.25
6. Ulnar Shortening Left		4.5 ± 4.9	5.7 ± 4.7	0.81	0.054	-3.5 ± 7.8	-7.0	0.78	0.053
7. Radial Bow Right	10° ± 5°	8.0 ± 1.4	8.7 ± 0.58	0.49	0.086	6.5 ± 0.71	9.0	0.21	0.18
8. Radial Bow Left		9.5 ± 2.1	12.5 ± 6.5	0.59	0.072	8.5 ± 4.9	11.0	0.75	0.054
9. Radial Head Dislocation R		1 dislocation	0			0			
10. Radial Head Dislocation L		0	1 dislocation			0			
11 Elbow Joint Right	9° ± 3°	3.5 ± 21.9	-0.67 ± 16.6	0.82	0.054	-15.0 ± 2.8	2.0	0.13	0.30
12. Elbow Joint Left		-3.5 ± 21.9	-9.0 ± 10.4	0.72	0.059	-9.5 ± 3.5	-3.0	0.37	0.099
13. Femoral A.A. Right	7° ± 2° valgus	-0.50 ± 6.4	-1.2 ± 4.5	0.89	0.051	-8.5 ± 19.1	-9.0	0.99	0.050
14. Femoral A.A. Left		3.8 ± 10.9	-5.5 ± 0.87	0.21	0.20	-7.0 ± 5.7	0.0	0.49	0.075
15. Femoral N.S. Angle Right	135° ± 5°	138.0 ± 12.7	140.0 ± 31.2	0.94	0.050	129.5 ± 3.5	148.0	0.15	0.26
16. Femoral N.S. Angle Left		144.5 ± 6.4	144.0 ± 23.3	0.98	0.050	128.5 ± 2.1	140.0	0.14	0.27
17. Femoral M..A. Right		8.0 ± 0.0	8.3 ± 7.4	0.097	0.050	-4.0 ± 11.3	-4.0	-	0.050

Table 8.7.10.2. Limb Alignment by Gender and Mutation Type (continued)

Variable	Normal Values	Males Splice Site (n=2)	Females Splice Site (n=3)	P-value	Power	Males FS (n=2)	Females FS (n=1)	P-value	Power
18. Femoral M.A. Left		-5.0 ± 7.1	1.0 ± 1.4	0.36	0.11	1.5 ± 3.5	6.0	0.49	0.076
19. Sharp's Right	35° ± 4°	39.3 ± 3.9	38.8 ± 3.7	0.91	0.051	38.5 ± 4.9	51.0	0.29	0.13
20. Sharp's Left		42.0 ± 2.8	36.8 ± 3.5	0.19	0.22	43.0 ± 7.1	47.0	0.72	0.055
21. Fibular Height Right	50 ± 10	55.0 ± 2.8	39.0 ± 11.0	0.15	0.27	62.8 ± 0.35	61.0	0.15	0.25
22. Fibular Height Left		59.5 ± 4.9	49.0 ± 9.0	0.24	0.18	63.9 ± 1.3	77.0	0.075	0.49
23. Ankle Joint Angle Right	0° ± 5°	16.0 ± 22.6	1.0 ± 3.5	0.31	0.14	-9.5 ± 0.71	-20.0	0.052	0.66
24. Ankle Joint Angle Left		8.5 ± 17.7	0.0 ± 4.4	0.45	0.095	-8.0 ± 1.4	-18.0	0.11	0.35
25. % Weightbear Right	50 ± 10	63.5 ± 17.7	65.3 ± 6.4	0.87	0.052	39.5 ± 40.3	30.0	0.88	0.051
26. % Weightbear Left		45.5 ± 7.8	61.3 ± 12.1	0.21	0.20	52.5 ± 21.9	75.0	0.56	0.067
Parameters beyond the normal range									

Table 8.7.10.3. Segment Lengths and Percentile Height by Gender and Mutation Type

Variable	Males Missense (n=2)	Females Missense (n=2)	P-value	Power	Males Nonsense (n=8)	EXT 2 Nonsense (n=6)	P-value	Power
Total Leg Length-Right	86.0 ± 3.5	62.5 ± 1.4	0.013	0.98	86.9 ± 6.4	84.4 ± 10.4	0.59	0.078
Upper Leg – Right	44.0 ± 2.1	30.5 ± 0.71	0.013	0.97	44.2 ± 4.7	45.3 ± 6.4	0.73	0.062
Lower Leg – Right	37.0 ± 3.5	26.0 ± 0.71	0.049	0.62	35.3 ± 3.4	34.5 ± 3.3	0.66	0.069
Total Leg Length – Left	85.3 ± 3.9	61.8 ± 0.35	0.014	0.97	86.7 ± 7.6	83.0 ± 10.3	0.45	0.11
Upper Leg – Left	43.8 ± 1.1	30.8 ± 2.5	0.021	0.90	43.3 ± 4.7	44.3 ± 6.1	0.71	0.064
Lower Leg – Left	35.3 ± 3.9	25.8 ± 0.35	0.075	0.47	37.1 ± 5.3	35.3 ± 5.2	0.53	0.089
Total Arm Length – Right	52.8 ± 2.5	37.8 ± 1.1	0.016	0.95	50.4 ± 6.2	50.3 ± 6.0	0.96	0.050
Upper Arm – Right	30.5 ± 0.71	22.8 ± 0.35	0.0052	1.0	31.7 ± 3.6	29.3 ± 3.5	0.23	0.20
Lower Arm – Right	24.5 ± 2.1	17.5 ± 0.0	0.04	0.67	23.3 ± 3.7	23.2 ± 3.1	0.94	0.051
Total Arm Length – Left	53.3 ± 1.1	37.3 ± 1.1	0.0044	1.0	50.5 ± 6.8	50.7 ± 5.6	0.96	0.050
Upper Arm – Left	31.3 ± 2.5	21.5 ± 0.71	0.033	0.77	31.5 ± 4.5	30.4 ± 4.7	0.67	0.068
Lower Arm – Left	26.3 ± 2.5	18.5 ± 1.4	0.062	0.54	23.1 ± 4.4	24.0 ± 2.7	0.68	0.067
Percentile Height	10.0 ± 7.1	33.0 ± 42.4	0.53	0.076	43.4 ± 31.9	51.7 ± 33.3	0.65	0.071

Table 8.7.10.3. Segment Lengths and Percentile Height by Gender and Mutation Type (continued)

Variable	Males Splice Site (n=2)	Females Splice Site (n=3)	P-value	Power	Males FS (n=2)	Females FS (n=1)	P-value	Power
Total Leg Length-Right	83.3 ± 8.1	83.3 ± 7.1	0.99	0.050	88.0 ± 2.1	81.0	0.23	0.17
Upper Leg – Right	40.5 ± 4.2	42.8 ± 3.8	0.57	0.075	42.8 ± 2.5	41.5	0.75	0.054
Lower Leg – Right	34.8 ± 3.9	34.0 ± 3.6	0.84	0.053	35.8 ± 1.1	32.5	0.24	0.16
Total Leg Length – Left	82.0 ± 6.4	83.3 ± 7.4	0.85	0.053	86.5 ± 0.0	81.5	<0.0001	1.0
Upper Leg – Left	40.3 ± 2.5	42.8 ± 4.3	0.51	0.084	41.5 ± 0.0	40.0	<0.0001	-
Lower Leg – Left	35.3 ± 6.0	34.8 ± 3.8	0.93	0.051	37.0 ± 1.4	36.0	0.67	0.058
Total Arm Length – Right	45.5 ± 2.1	49.5 ± 6.9	0.50	0.085	53.0 ± 0.71	46.0	0.078	0.47
Upper Arm – Right	27.8 ± 1.1	30.3 ± 4.5	0.50	0.085	31.8 ± 0.35	28.0	0.073	0.50
Lower Arm – Right	21.3 ± 0.35	23.8 ± 2.3	0.22	0.19	26.0 ± 1.4	18.5	0.14	0.27
Total Arm Length – Left	47.3 ± 0.35	49.5 ± 6.3	0.66	0.064	53.8 ± 4.6	45.5	0.38	0.097
Upper Arm – Left	29.3 ± 1.1	30.5 ± 4.8	0.75	0.057	32.3 ± 1.8	30.0	0.49	0.076
Lower Arm – Left	20.0 ± 1.4	22.2 ± 3.3	0.46	0.092	25.5 ± 2.1	18.0	0.21	0.18
Percentile Height	32.0 ± 9.9	12.3 ± 11.4	0.14	0.28	10.5 ± 10.6	8.0	0.88	0.051

8.7.11 Gene and Mutation Location

Table 8.7.11.1 Lesion Quality by Gene and Mutation Location

Variable	EXT 1 Early (n=2)	EXT 2 Early (n=17)	P-value	Power	EXT 1 Late (n=2)	EXT 2 Late (n=17)	P-value	Power
Lesion Rank 1	16.5 ± 7.8	6.3 ± 4.9	0.019	0.69	6.2 ± 1.8	5.0 ± 0.0	0.41	0.11
% Rank 1	37.0 ± 7.1	33.2 ± 21.1	0.81	0.056	23.0 ± 8.4	20.0 ± 7.1	0.69	0.065
Lesion Rank 2	7.0 ± 4.2	3.5 ± 2.2	0.076	0.42	6.2 ± 2.9	6.5 ± 6.4	0.93	0.051
% Rank 2	15.5 ± 4.9	21.2 ± 12.9	0.55	0.086	21.0 ± 8.2	32.5 ± 0.71	0.12	0.33
Lesion Rank 3	5.0 ± 2.8	1.9 ± 1.9	0.058	0.48	5.0 ± 1.9	2.0 ± 1.4	0.10	0.36
% Rank 3	11.0 ± 2.8	9.5 ± 7.4	0.79	0.058	18.0 ± 5.9	10.0 ±1.4	0.13	0.30
Lesion Rank 4	15.0 ± 1.4	6.5 ± 4.4	0.018	0.70	11.0 ± 4.3	12.0 ± 2.8	0.78	0.057
% Rank 4	36.5 ± 14.8	35.9 ± 20.2	0.97	0.050	38.2 ± 9.8	37.0 ± 7.1	0.88	0.052
Small (%)	39.7 ± 10.4	31.9 ±18.5	0.58	0.082	23.9 ± 9.5	20.8 ± 9.8	0.71	0.062
Medium (%)	22.5 ±15.3	31.2 ±14.2	0.42	0.12	33.8 ± 0.78	27.8 ± 15.8	0.36	0.13
Large (%)	37.8 ± 25.7	34.8 ± 18.2	0.84	0.054	38.9 ± 14.2	50.5 ± 7.1	0.34	0.14
Average Number of Lesions	43.5 ± 13.4	18.3 ± 8.6	0.0021	0.95	28.4 ± 6.1	25.5 ± 10.6	0.65	0.068
No. Pedunculated	11.5 ± 2.1	6.2 ± 4.5	0.13	0.31	7.6 ± 2.4	5.0 ± 1.4	0.22	0.19
% Pedunculated	26.9 ±3.5	32.2 ±13.8	0.61	0.077	26.8 ± 6.3	22.7 ± 14.9	0.60	0.074
No. Sessile	27.5 ± 17.7	12.6 ± 6.1	0.015	0.73	18.6 ± 3.4	20.0 ± 12.7	0.81	0.055
% Sessile	59.8 ± 22.2	64.5 ± 14.5	0.68	0.067	66.1 ± 6.9	74.5 ± 18.9	0.38	0.12
No. Distal	17.0 ± 8.5	7.8 ±4.6	0.023	0.66	11.6 ± 3.1	10.5 ± 2.1	0.68	0.065
% Distal	37.9 ± 7.8	39.5 ± 15.2	0.88	0.052	41.1 ± 9.3	43.2 ± 9.6	0.80	0.055
No. Proximal	18.0 ± 5.7	9.4 ± 4.6	0.024	0.65	13.0 ± 5.2	9.5 ± 6.4	0.48	0.095
% Proximal	41.3 ± 0.24	48.0 ± 16.1	0.58	0.083	44.9 ± 10.6	35.1 ± 10.4	0.31	0.15
No. Pelvic	7.5 ± 0.71	0.59 ± 1.2	<0.001	1.00	1.8 ± 1.3	2.0 ± 2.8	0.89	0.052
% Pelvic	18.4 ± 7.3	1.8 ± 4.9	<0.001	0.99	6.1 ± 4.0	6.1 ± 8.6	0.99	0.050

Table 8.7.11.1 Lesion Quality by Gene and Mutation Location (continued)

Variable	EXT 1 Early (n=2)	EXT 2 Early (n=17)	P-value	Power	EXT 1 Late (n=2)	EXT 2 Late (n=17)	P-value	Power
No Diaphyseal	1.5 ± 0.71	1.2 ± 1.3	0.74	0.062	2.0 ± 2.0	2.5 ± 0.71	0.76	0.058
% Diaphyseal	3.4 ± 0.59	7.9 ± 12.1	0.61	0.076	7.8 ± 8.1	10.1 ± 1.4	0.71	0.062
No. Flat Bone	8.0 ± 0.0	0.71 ± 1.2	<0.001	1.00	2.6 ± 1.1	2.0 ± 2.8	0.68	0.065
% Flat Bone	19.3 ± 5.9	2.6 ± 5.1	<0.001	0.99	8.8 ± 2.9	6.1 ± 8.6	0.50	0.090
No. Complex	9.5 ± 12.0	2.7 ± 2.2	0.023	0.66	3.0 ± 1.6	2.5 ± 0.71	0.69	0.063
% Complex	18.5 ±21.9	14.2 ± 9.6	0.61	0.078	10.0 ± 3.8	10.1 ± 1.4	0.98	0.050
No. Simple	29.5 ± 7.8	16.7 ± 8.5	0.059	0.47	23.6 ± 3.9	22.5 ± 10.6	0.83	0.054
% Simple	68.3 ± 3.2	84.5 ± 9.9	0.038	0.56	84.0 ± 8.8	87.1 ± 5.4	0.67	0.065
No. Flared	25.5 ± 0.71	7.4 ± 6.1	<0.001	0.98	9.6 ± 11.3	2.0 ± 1.4	0.41	0.11
% Flared	61.3 ± 17.3	33.5 ± 24.1	0.14	0.29	29.6 ± 29.8	7.3 ± 2.5	0.36	0.13
No. Not Flared	18.0 ± 12.7	11.5 ± 5.9	0.21	0.22	18.8 ± 7.1	23.5 ± 9.2	0.49	0.093
% Not Flared	38.7 ± 17.3	66.5 ± 24.1	0.14	0.29	70.4 ± 29.8	92.7 ±2.5	0.36	0.13
No. Left	27.5 ± 4.9	9.7 ± 5.4	<0.001	0.99	15.0 ± 2.2	13.0 ± 2.8	0.36	0.13
% Left	64.5 ± 8.6	48.2 ± 10.9	0.060	0.46	53.5 ± 4.1	53.2 ± 11.1	0.97	0.050
No. Right	16.0 ± 8.5	9.9 ± 4.8	0.13	0.31	13.6 ± 4.0	12.5 ± 7.8	0.80	0.055
% Right	35.5 ± 8.6	51.8 ± 10.9	0.060	0.46	47.1 ± 4.9	46.7 ± 11.1	0.94	0.050

Table 8.7.11.2. Limb Alignment by Gene and Mutation Location

Variable	Normal Values	EXT 1 Early (n=2)	EXT 2 Early (n=17)	P-value	Power	EXT 1 Late (n=2)	EXT 2 Late (n=17)	P-value	Power
1. Carpal Slip R	5 ± 2mm	5.0	2.2 ± 3.8	0.49	0.099	5.2 ± 3.1	2.0 ± 0.0	0.23	0.19
2. Carpal Slip L		7.0 ± 1.4	2.9 ± 3.6	0.15	0.29	3.8 ± 3.0	4.5 ± 2.1	0.78	0.057
3. Radial Inclination R	21° ± 2°	29.0	23.8 ± 5.2	0.94	0.14	27.8 ± 6.1	27.5 ± 0.71	0.95	0.050
4. Radial Inclination L		28.5 ± 9.2	26.6 ± 4.8	0.64	0.073	31.4 ± 5.5	24.5 ± 4.9	0.18	0.24
5. Ulnar Shortening R	0 ± 1 mm	-8.0	-2.3 ± 4.8	0.27	0.18	0.20 ± 2.2	3.0 ±1.4	0.16	0.26
6. Ulnar Shortening L		1.5 ± 4.9	-1.2 ± 5.0	0.49	0.10	3.2 ± 6.6	2.5 ± 2.1	0.89	0.052
7. Radial Bow R	10° ± 5°	11.0	7.4 ± 2.5	0.18	0.25	8.6 ± 2.3	8.5 ± 0.71	0.96	0.050
8. Radial Bow Left		20.0 ± 15.6	7.7 ± 2.5	0.0019	0.95	11.9 ± 4.6	8.0 ± 0.0	0.31	0.15
9. Radial Head Dislocation R		0	1			1	1		
10. Radial Head Dislocation L		1	1			1	1		
11. Elbow Joint R	9° ± 3°	2.0	-4.6 ± 13.3	0.64	0.073	-2.6 ± 20.5	-14.0 ± 2.8	0.49	0.092
12. Elbow Joint L		-3.5 ± 4.9	-7.5 ± 11.5	0.64	0.072	-4.0 ± 12.3	-17.5 ±2.1	0.20	0.22
13. Femoral A.A. R	7° ± 2° valgus	-5.5 ± 7.8	-5.6 ± 9.7	0.99	0.050	-2.1 ± 8.5	-5.5 ± 0.71	0.62	0.072
14. Femoral A.A. L		-9.5 ± 3.5	-3.3 ± 9.5	0.38	0.13	1.6 ± 7.5	-4.5 ± 0.71	0.33	0.14
15. Femoral N.S. Angle R	135° ± 5°	146.0 ± 7.1	140.8 ± 6.9	0.33	0.15	142.0 ± 21.3	134.5 ± 17.7	0.68	0.065
16. Femoral N.S. Angle L		142.5 ± 0.71	137.1 ± 8.6	0.39	0.13	148.0 ± 13.0	137.0 ± 16.9	0.39	0.12
17. Femoral M.A. R	0° ± 5° varus	1.0 ± 5.7	-0.84 ± 5.9	0.69	0.067	8.9 ± 3.2	5.5 ± 3.5	0.30	0.15
18. Femoral M.A. L		-5.0 ± 2.8	1.3 ± 5.3	0.12	0.32	1.0 ± 7.9	0.0 ± 0.0	0.88	0.052
19. Sharp's Right	35° ± 4°	37.5 ± 3.5	41.7 ± 5.9	0.35	0.14	38.5 ± 3.0	39.0 ± 4.2	0.87	0.052
20. Sharp's Left		41.0 ± 8.5	41.5 ± 4.7	0.90	0.052	37.6 ± 3.3	38.5 ± 7.8	0.84	0.053

Table 8.7.11.2. Limb Alignment by Gene and Mutation Location (continued)

Variable	Normal Values	EXT 1 Early (n=2)	EXT 2 Early (n=17)	P-value	Power	EXT 1 Late (n=2)	EXT 2 Late (n=17)	P-value	Power
21. Fibular Height R	50 ± 10	59.0 ± 7.1	52.7 ± 10.8	0.44	0.11	49.4 ± 5.9	42.5 ± 20.5	0.47	0.096
22. Fibular Height L		48.0 ± 22.6	51.2 ± 15.1	0.79	0.058	54.6 ± 9.8	56.0 ± 9.9	0.87	0.052
23. Ankle Joint Angle R	0° ± 5°	-26.0 ± 7.1	-1.9 ± 10.8	0.0085	0.82	5.0 ± 15.8	-1.5 ± 2.1	0.61	0.073
24. Ankle Joint Angle L		-20.5 ± 19.1	-0.50 ± 11.2	0.041	0.55	5.8 ± 8.5	-4.5 ± 0.71	0.17	0.25
25. % Weightbear R	50 ± 10	61.5 ±10.6	45.5 ± 23.4	0.36	0.14	63.8 ± 25.4	54.5 ± 4.9	0.65	0.068
26. % Weightbear L		53.0 ± 5.7	51.5 ± 20.9	0.92	0.051	65.0 ± 15.9	50.5 ± 0.71	0.28	0.17
Number of parameters that fall beyond the normal range									

Table 8.7.11.3. Segment Lengths and Percentile Height by Gene and Mutation Location

Variable	**EXT 1 Early (n=2)**	**EXT 2 Early (n=17)**	**P-value**	**Power**	**EXT 1 Late (n=2)**	**EXT 2 Late (n=17)**	**P-value**	**Power**
Total Leg Length-Right	80.5 ± 2.1	85.1 ± 9.4	0.51	0.096	79.3 ± 10.1	83.5 ± 8.5	0.63	0.070
Upper Leg – Right	38.5 ± 0.71	44.2 ± 5.6	0.18	0.25	39.5 ± 5.5	42.3 ± 6.7	0.59	0.075
Lower Leg – Right	32.0 ± 1.4	35.0 ± 3.7	0.28	0.18	32.5 ± 4.8	34.5 ± 3.5	0.62	0.071
Total Leg Length – Left	80.0 ± 1.4	84.4 ± 9.7	0.54	0.090	78.4 ± 10.3	83.3 ± 8.1	0.58	0.076
Upper Leg – Left	38.3 ± 1.1	43.3 ±5.2	0.20	0.23	39.1 ± 5.9	42.8 ± 6.0	0.49	0.091
Lower Leg – Left	31.0 ± 1.4	36.5 ± 4.9	0.15	0.29	33.0 ± 5.5	34.0 ± 4.2	0.83	0.054
Total Arm Length – Right	43.0 ± 0.0	50.8 ± 5.7	0.077	0.41	45.6 ± 6.1	49.8 ± 8.1	0.48	0.094
Upper Arm – Right	28.0 ± 0.0	30.5 ± 3.8	0.39	0.13	27.2 ± 2.9	31.8 ± 4.6	0.16	0.26
Lower Arm – Right	19.5 ± 2.1	23.6 ± 3.4	0.13	0.32	21.5 ± 2.5	23.5 ± 3.5	0.42	0.11
Total Arm Length – Left	42.0 ± 1.4	51.0 ± 6.2	0.059	0.47	46.2 ± 5.8	51.3 ± 6.0	0.35	0.13
Upper Arm – Left	26.5 ± 0.71	30.9 ± 4.5	0.19	0.24	27.9 ± 4.2	32.8 ± 3.9	0.22	0.21
Lower Arm – Left	17.5 ± 3.5	24.0 ± 3.4	0.021	0.67	20.9 ± 2.7	23.0 ± 2.8	0.39	0.12
Percentile Height	3.0 ± 0.0	44.6 ± 30.1	0.074	0.42	11.8 ± 15.4	25.0 ± 0.0	0.30	0.15