Pathogenic Copy Number Variants (pCNVs) in Individuals diagnosed on the Autism Spectrum Disorder (ASD): A Closer look at Candidate Genes

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The genetic basis for autism spectrum disorders (ASDs) is well established, and its heterogenetic nature provides us with substantial evidence for the many chromosomal aberrations associated with this complex disorder. However, little is known about the genes that occupy the different chromosomal regions and the gene networks they participate in as they relate to phenotypes associated with ASDs. Here, the author reports candidate genes that may be implicated with the observed clinical phenotypes in 9 patients diagnosed with an ASD identified to have pathogenic copy number variants (pCNVs) through array-comparative genomic hybridization (CGH). Formal clinical assessments, which include a full physical examination, a medical history report, as well as a family history, were administered by a clinical geneticist unaware of the array-CGH results. Candidate genes were then compiled through the genome browser of the Database of Genomic Variants website and subsequently narrowed down utilizing the SUSPECTS database. Additional information on each candidate gene was obtained through the NCBI, iHOP, and metalife databases. The author's findings suggest a number of genes involved in neurodevelopment as well as craniofacial and systemic features that may account for the observed phenotypes in the 9 affected patients.

Abbreviations: ASD – Autism Spectrum Disorder; CGH – Comparative Genomic Hybridization; FISH – Fluorescence In Situ Hybridization; pCNVs – Pathogenic Copy Number Variants; qPCR – Quantitative Polymerase Chain Reaction

Keywords: Autism; Chromosome; Disorder; Genetics; Interactions; Networks; Phenotype

Introduction:

Autism Spectrum Disorders (ASDs) are neurodevelopmental disorders characterized by impairments in communication, social interaction, and repetitive behavior, and have a prevalence as high as 60 per 10,000 individuals by recent estimates (Baird *et al.,* 2000; Bertrand *et al.,* 2001; Chakrabarti *et al.,* 2001; Chakrabarti *et al.,* 2005). The heritability of ASD has also been well established over the years, particularly through comparing monozygotic (MZ) and dizygotic (DZ) concordance rates which, when taken collectively with family studies, clearly point to an important genetic liability for autism (Newschaffer *et al.,* 2007). Although current estimates show that clinical genetic evaluation can positively identify a specific etiology in up to 40% of individuals with an ASD (Shaefer and Mendelsohn, 2008), little is understood in terms of the genotype-phenotype correlations for the vast majority of genetic abnormalities found in affected individuals. Most candidate genes for specific clinical phenotypes and the possible gene networks and interactions they participate in have yet to be clearly determined. To address this, the current paper reports possible candidate genes associated with particular pathogenic copy number variants (pCNVs) in 3 pairs of individuals, 2 pairs of which had *de novo* changes, and a pair of brothers who inherited the same X-linked deletion from their unaffected mother. Three other individuals were also

identified to have unique copy number changes not shared with other affected individuals in the cohort. Array-CGH, FISH, and RT-qPCR data were gathered from previous experiments, as well as detailed phenotypic descriptions for these affected individuals. From these, candidate genes in each unbalanced region were compiled through the use of various databases so as to provide a genetic basis for their clinical presentation.

Methods and Materials:

Identification of Pathogenic Copy Number Variants (pCNVs)

A total of nine subjects were identified to have pCNVs by collaborators through various genetic testing methods. Low resolution array-CGH findings were confirmed by FISH and RT-qPCR methods which were also used to refine breakpoints and determine the origins of the changes when parents were available for testing.

Individuals identified to harbor pCNVs found at genomic loci are associated with other disorders, and they also fulfilled CNV criteria strongly suggesting their pathogenicity (Fan *et al.,* 2007; Lee *et al.,* 2007). Several criteria were used to distinguish benign CNVs (bCNVs) from potentially pathogenic ones (pCNVs), including: a *de novo* origin (or maternally inherited X-linked in male probands), the involvement of multiple genes not known to vary in databases, the overlap with a gene or region that leads to a clinical phenotype when unbalanced, or if the affected region is >1Mb and overlaps well characterized genes (Fan *et al.,* 2007; Lee *et al.,* 2007). This is in contrast to benign CNVs (bCNVs) which are found in at least 2 healthy individuals in independent studies found on the Database of Genomic Variants website [http://projects.tcag.ca/variation/].

Array-CGH

PUREGENE DNA Isolation Kits (Gentra, Minneapolis, MN) were utilized to extract DNA from peripheral blood and were matched to normal male and female control DNAs (Promega, Madison, WI) as a reference. Both sample and reference DNAs were then subsequently hybridized using the 1-Mb BAC array (Tyson *et al.,* 2005) [Spectral Genomics, Houston, TX] through dye swap methods. Spectralware 2 software (Spectral Genomics) was then utilized for data analysis, and clones bearing a significant gain or loss were identified through the use of the established values of 1.2 and 0.8, respectively, as cut-offs.

FISH

Deletions and duplications of BAC DNA clones identified by array-CGH were confirmed through FISH analyses (Rajcan-Separovic *et al.,* 2007). A Zeiss Axioplan 2 fluorescence microscope and the MacProbe software (Applied Imaging, Santa Clara, CA) were then utilized to view the slides and capture the images, respectively.

Real-Time Quantitative PCR (RT-qPCR)

RT-qPCR (Qiao *et al.,* 2007) was employed to confirm the pCNVs. The ABI Prism 7900HT system (Applied Biosystems) using SYBR Green I detection was utilized to assess the RT-qPCR products. The primers used can be made available upon request.

Phenotypic Data

A spectrum of clinical characteristics modified from the De Vries scoring method were taken into account, and includes prenatal and postnatal growth abnormalities that are indicative of subtelomeric rearrangements, not in exclusion of other characteristics. A spectrum of clinical and physical characteristics were noted for each participant by a professional Clinical Geneticist (M.E.S.L.) blinded to the array results. These include micro- and macrocephaly, prenatal and postnatal growth abnormalities, craniofacial dysmorphisms, systemic anomalies, and the presentation of medical co-morbidities such as seizures, intellectual disabilities (ID), and gastro-intestinal (GI) problems. Pregnancy and postnatal

histories were also collected at the time of the appointment.

Identification of Candidate Genes

A general list of candidate genes were compiled for each proband through the Database of Genomic Variants website (http://projects.tcag.ca/variation/), and were subsequently narrowed down further using the SUSPECTS database (http://www.genetics.med.ed.ac.uk/suspects/). The major premise of SUSPECTS is that genes associated with complex traits will participate in the same gene networks and exhibit similar expression patterns, and the program achieves this by ranking candidate genes in their possible involvement with the trait of interest.

Information on each candidate gene identified by SUSPECTS was collected through the NCBI website (http://www.ncbi.nlm.nih.gov/), specifically looking at the Entrez Gene record and the database of Genotype and Phenotype (dbGaP) entry (when available) for each gene. Further information regarding genotype-phenotype relationships were gathered from the iHOP website (http://www.ihop-net.org/UniPub/iHOP/) which compiles a list of all known functions, interactions, and diseases the gene of interest is associated with. The metalife database (http://www.phenomicdb.de/) was also utilized to complement the gene ontology information collected from the iHOP website and gives an informative summary of all the research done on the gene of interest as well as linking them to various phenotypes associated with the gene.

Results:

Table 1 summarizes the candidate genes for each subject pair sharing the same pathogenic CNV, as well as the candidate genes for individuals bearing unique pCNVs. Known gene ontologies for each candidate gene are also listed in Table 1. A list of shared and unique phenotypes was also compiled in Table 2 for each pair and unique individuals bearing a particular pathogenic CNV. Figure 1 is a schematic diagram summarizing all of the candidate genes found on the various chromosomes.

Subjects A and B

The affected, unrelated pair share almost identical 2p15-16.1 deletions and both are of *de novo* origin. The affected region has been further defined to be from positions 56,800,000 to 63,200,000 for *subject A,* and from 55,500,000 to 63,400,000 for *subject B* on chromosome 2 confirmed through RT-qPCR and/or FISH methods.

Candidate genes for both subjects within the overlapping regions include *PEX13* and *OTX1,* which are involved in Peroxisome Biogenesis Disorders and brain and sensory organ development as well as inner ear morphogenesis, respectively.

Subjects C and D

Subject D is the aunt of *subject C,* and both individuals share the same deletion at the 14q11.2 locus and duplication at the 15q11-12 locus. An unbalanced product of a reciprocal cryptic 14q/15q translocation represents this finding. A perfect overlap at the 14q11.2 and 15q11-12 regions between both subjects was found (19,570,792 to 20,341,734 and 20,536,416 to 30,830,821, respectively).

Of particular note, several candidate genes are implicated with nervous system development, which include *CYFIP1, NDN, UBE3A, APBA2, GREM1,* and *SCG5,* all of which are found on the shared 15q11-12 site. Several candidate genes found on the same locus are also implicated with Angelman syndrome as well as Prader-Willi syndrome, and includes the *NIPA1/NIPA2, SNRPN, UBE3A,* and *ATP10A* genes.

Subjects E and F

Subjects E and F are brothers who share the

Table 1

Subject(s)	Candidate Gene(s)[1]	Pathogenic CNV(s) and cytoband(s)[2]	Origin	Genomic Region (bp)[2]	Gene Ontology[3]
A, B	**1.**PEX13 **2.**OTX1	del 2p15-16.1	de novo	**A**=56,800,000/63,200,000 **B**=55,500,000/63,400,000	**1.**Peroxisome Biogenesis Disorders **2.**Brain and sensory organ development, Inner ear morphogenesis
C, D	**1a.**NP **2a.**NIPA1/NIPA2 **b.**CYFIP1 **c.**NDN **d.**SNRPN **e.**UBE3A **f.**ATP10A **g.**GABRB3 **h.**GABRA5 **i.**GABRG3 **j.**APBA2 **k.**CHRFAM7A **l.**GREM1 **m.**SCG5	**1.**del 14q11.2 **2.**dup 15q11-12	Both translocations	**1.** 19,570,792/20,341,734 **2.** 20,536,416/30,830,821	**1a.**Purine nucleoside phosphorylase activity **2a.**Prader-Willi/Angelman syndrome **b.**Nervous system development **c.**Neuron development **d.**RNA splicing, Prader-Willi Syndrome **e.**Brain development, Angelman Syndrome **f.**Angelman Syndrome **g,h,i.**GABA-A receptor activity **j.**Nervous system development **k.**Role in failure to thrive in infants **l.**Bone and Nervous System development **m.**Neuropeptide signaling, hormone secretion regulation
E, F	**1.**PHF8 **2.**WNK3	del Xp11.2 (highly skewed X-inactivation)	familial inherited	53,970,960 /54,326,640	**1.**X-linked mental retardation **2.**Protein amino acid phosphorylation
G	**2a.**SEMA5A **b.**CCT5 **c.**CTNND2	**1.**del 3p24.3-25 **2.**del 5p15.2	**1.**de novo **2.**de novo	**1.** 15,780,358 /15,940,642 **2.** 9,334,790 /11,738,791	**2a.**Axonal guidance, Nervous system development **b.**Chaperon protein binding **c.**Neuron adhesion, synaptic plasticity
H	**1.**GTF2I	dup 7q11.23	unknown origin	72,200,000/73,767,523	**1.**General transcription factor, Williams-Beuren syndrome
I	**1.** L3MBTL4	dup 18p11.3	de novo	5,910,725/6,063,460	**1.**Cell adhesion, platelet activation, integrin complex component

[1]Confirmed through Database of Genomic Variants website and subsequently narrowed down with SUSPECTS database
[2]Affected region confirmed by RT-qPCR and/or FISH
[3]Information gathered collectively from the NCBI, iHOP, and metalife databases

Table 2

[1]ID = Intellectual Disability

Subject(s)	Shared Phenotypes	Unique Phenotypes
A, B	Severe ID[1], microcephaly (<2%), craniofacial dysmorphisms (short forehead, high and broad nasal root), other systemic dysmorphisms (bilaterally tight heel cords, oral motor dysfunction), abnormal brain imaging	**A**=Prenatal growth retardation, **B**=Seizure disorder, postnatal small stature (<5%)
C, D	Craniofacial dysmorphisms (strabismus, flat occiput, prominent alar cartilage), no other systemic dysmorphisms	**C**=Moderate ID, respiratory distress and poor suck and feeding difficulties (postnatal) **D**=Mild ID, seizure disorder, floppy infant (postnatal)
E, F	Moderate ID, craniofacial dysmorphisms (flat occiput, coarse asymmetric face [right side > left side fullness], micrognathia, unilateral cleft lip), other systemic dysmorphisms (pes planus, bone anomalies)	**E**=Prominent metopic suture, prominent finger pads **F**=Long slender fingers, macrocephaly at birth, mild hyperoptic refractive error
G	N/A	**G**=Moderate ID, seizure disorder, macrocephaly (>98%), postnatal large stature (>98%), craniofacial dysmorphisms (coarse facial features, frontal bossing, prominent supra-orbital ridge), other systemic dysmorphsims (prominent finger pads, bilateral tight heel cords, slight toe walking)
H	N/A	**H**=Moderate ID, craniofacial dysmorphisms (plagiocephaly, brachycephaly, prognathia), other systemic dysmorphisms (GI: unusual dark stool colour, walks on heels, occasional enuresis), normal brain imaging
I	N/A	**I**=Mild ID, hightened blood pressure (during pregnancy), craniofacial dysmorphisms (mild bilateral epicanthal folds, ears bilaterally protuberant, slight malar flattening), other systemic dysmorphisms (slight metatarsus varus when walking)

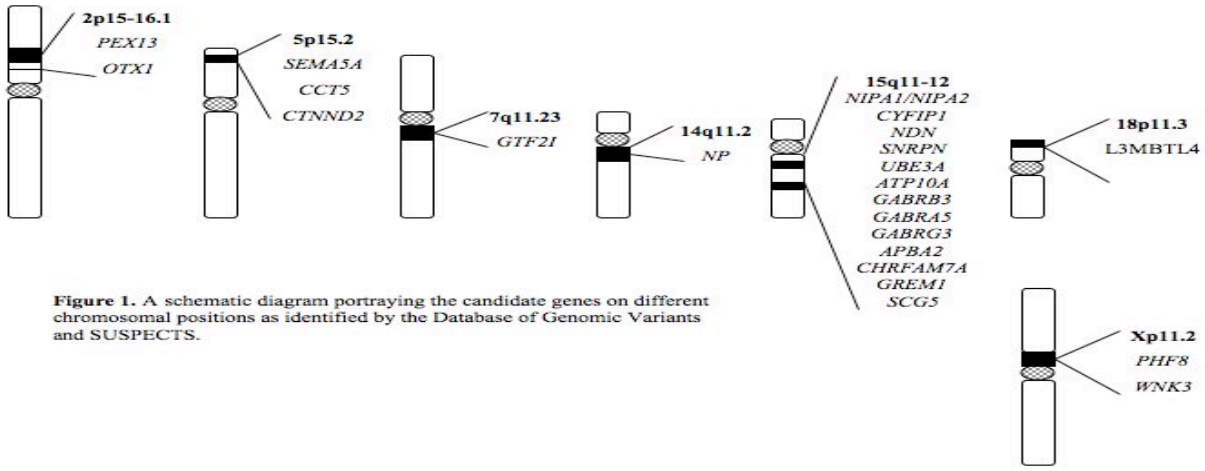

Figure 1. A schematic diagram portraying the candidate genes on different chromosomal positions as identified by the Database of Genomic Variants and SUSPECTS.

same Xp11.2 deletion that is familial inherited, and the precise genomic region was found to be from 53,970,960 to 54,326,640 on the X chromosome. Of particular note, the mother was confirmed to have a very skewed X-inactivation, perhaps implying dosage imbalance problems in both siblings. Potential candidate genes in the affected region of both *subjects E and F* include *PHF8,* which is associated with X-linked mental retardation, as well as the *WNK3* gene which is involved in protein amino acid phosphorylation.

Subject G

The affected individual harbors two deletions at the 3p24.3-25 and 5p15.2 regions, with both regions of *de novo* origin. The precise affected region for the chromosome 3 deletion was found to be from 15,780,358 to 15,940,642, and the chromosome 5 deletion is from 9,334,790 to 11,738,791, as confirmed by FISH and/or RT-qPCR.

In relation to the observed phenotypes, several candidate genes that may play a role in the pathogenesis of classical autism includes *SEMA5A* and *CTNND2,* both of which are located on chromosome 5 and are involved in axonal guidance and neuron adhesion and synaptic plasticity, respectively. *CCT5* was also identified by SUSPECTS to be another candidate gene on the same chromosome, and is involved with chaperon protein binding in neurons.

Subject H

Subject H is an individual with a unique 7q11.23 duplication of unknown origin that is not found in other subjects presenting with pathogenic CNVs. The affected genomic region was found to be from 72,200,000 to 73,767,523 through FISH and/or RT-qPCR methods. Of particular note, the GTF2I candidate gene was identified by the SUSPECTS database which is known to be as a general transcription factor, as well as being implicated with Williams-Beuren syndrome.

Subject I

This affected individual was identified to have a duplication of *de novo* origin at the 18p11.3 region which was further refined to be from 5,910,725 to 6,063,460 on chromosome 18. *L3MBTL4* was the sole candidate gene identified that may account for the observed phenotypes, and is known to be involved in cell adhesion, platelet activation, as well as being a component of the integrin complex.

Discussion:

Subjects A and B: 2p15-16.1 deletion

Several characteristics shared by both subjects include intellectual disability as well as poor oral motor skills and poor muscle tone,

which overlap with certain Peroxisome Biogenesis Disorder (PBD) phenotypes, thus making the *PEX13* gene on chromosome 2 a possible culprit for the observed phenotypes. Since both subjects are still alive, Refsum's disease is the most likely out of all the other PBDs as its prognosis is the most hopeful in terms of living past the early childhood years. However, normal laboratory evidence of phytanic acid and long chain fatty acids, both of which are found in elevated levels in affected individuals due to faulty enzymes during the alpha oxidation of phytanic acid and fatty acid oxidation, were found for both individuals harboring the overlapping 2p15-16.1 deletion (Rajcan-Separovic *et al.,* 2007). Nonetheless, the possibility of *PEX13* contributing to part of the observed phenotypes, in particular those involved with neurodevelopment (in keeping with microcephaly in both subjects) and poor muscle tone, cannot be completely ruled out, as well as other candidate genes in the deleted region.

Another candidate gene that may be responsible for neurodevelopment as well as sensory organ development is *OTX1*, a transcription factor that was recently found to be essential in cerebellum development (de Haas *et al.,* 2006). The observed large ears, relative to the microcephaly in both subjects, may also be in part due to the loss of function of the *OTX1* gene. Furthermore, evidence suggests that the *OTX1* gene dictates the segregation of the saccule and the utricle during inner ear morphogenesis (Beisel *et al.,* 2005), and its loss of function due to the deletion may perhaps indeed be responsible for the hyperacusis observed in *subject A* as well as the bilateral sensorineural loss (mild to moderate in the left ear and slight to mild in the right ear) observed in *subject B*.

Subjects C and D: 14q11.2 deletion and 15q11-12 duplication

Both subjects present strikingly similar characteristics, most notably sharing several craniofacial dysmorphisms (strabismus, flat occiput, prominent alar cartilage) and presentation of intellectual disability (*subject C* has moderate ID while *subject D* has mild ID). These findings suggest an underlying genomic basis that may be responsible for the shared pathogenesis, and indeed, both a deletion and a duplication arising through a reciprocal cryptic 14q/15q translocation were found whose affected genomic areas were found to have perfect overlap between the two relatives (Table 1).

Candidate genes that may be implicated with cephalic development were found in the duplicated 15q11-12 region and as such, dosage effects may be at play here in terms of over-expression of a particular gene and/or altered regulation of a gene that may exert its effects on adjacent genes involved in the same gene network. The highly conserved *CYFIP1* gene may be one such gene. Through co-localization experiments, there is evidence that the products of the *CYFIP1* gene do indeed interact with *FMRPs* [Fragile X mental retardation proteins] (Schenck *et al.,* 2001). Although the functions of the *CYFIP1* proteins are currently unknown, the extraction of *CYFIP1* proteins at the synaptosome of the distal portion of dendrites suggests that they also interact with the small GTPase *Rac1* where it also localizes (Schenck *et al.,* 2001). *Rac1* is known to be essential for dendritic spine maturation as well as maintenance (Nakayama *et al.,* 2000), and a duplication of the *CYFIP1* gene with whom it interacts may have direct or indirect effects on the maturation and maintenance of these structures. One possibility is that improper dosage of the *CYFIP1* gene product could directly alter expression levels of *Rac1* and other genes in the network involved in dendrite formation. This would have major implications for neurodevelopment and could thus be responsible for the shared ID and ASD observed in both subjects.

Several other candidate genes that may be responsible for the shared dysmorphisms include *UBE3A* and *APBA2*. Most notably, maternally derived duplications of the 15q11-13 region results in changes to *UBE3A* expression observed in autistic individuals, whereas the duplication is not present in normal individuals (Veenstra-VanderWeele *et al.,* 1999). In addition, *GREM1* may also be the culprit gene for the observed bone fractures in *subject D* and the ligamentous laxity in *subject C,* as over-expression of the gene in transgenic mice analogous to that of a duplication event resulted in a 20-30% reduction in bone mineral density as well as formation of bone fractures (Gazzerro *et al.,* 2005). It has been well established that

deletions in the 15q11-13 region result in Prader-Willi syndrome (PWS) as well as Angelman syndrome (AS) (Dittrich *et al.,* 1992) in which several of the phenotypes overlap with those present in both subjects. These include reduced fetal movements, respiration and feeding difficulties, strabismus, and intellectual disability (Holm *et al.,* 1993). *NIPA1/NIPA2, NDN, SNRPN, UBE3A, ATP10A,* and the Gamma acid receptor family (*GABA)* were identified by SUSPECTS in the duplicated 15q11-12 region to be implicated in autistic disorder in both subjects. There is evidence that a marker in the gene for the gamma aminobutyric acid receptor subunit of *GABRB3* was found to have linkage disequilibrium with autistic disorder, making this gene as well as other members of the gene family a prime candidate gene (Cook *et al.,* 1998). Furthermore, the role of benzodiazepine as a *GABA* receptor agonist in treating autistic phenotypes such as anxiety disorders and seizures suggest a potential role of the *GABA* gene family in the presentation of these phenotypes beyond the normal inhibitory neurotransmitter *GABA* function (Cook *et al.,* 1998). Additional studies need to be conducted in the future so as to precisely dissect the roles of the *GABA* gene family as well as others that exhibit linkage disequilibrium with autistic disorders.

Subjects E and F: Xp11.2 deletion

Both brothers share the same familial inherited Xp11.2 deletion, and a possible candidate gene that may account for the shared moderate ID is *PHF8.* The *PHF8* gene encodes a PHD finger protein which has been shown through truncation mutation experiments to cause X-linked mental retardation (XLMR) with or without cleft lip/cleft palate presentation (Laumonnier *et al.,* 2005). The PHD finger protein has also been thought to regulate and modify chromatin structure (Jensen *et al.,* 2005), which has major implications in terms of altered transcription levels and neurodevelopment in individuals with mutations or deletions of the *PHF8* gene.

Another candidate gene that may be implicated with the observed moderate ID in both subjects is *WNK3,* as it has been shown to occupy the critical linkage region on Xp11.2, and thus may also play a critical role in neurodevelopmental disorders such as XLMR (Ropers *et al.,* 2003). However, future studies need to be conducted so as to determine whether the *WNK3* deletions could account for the differences in autism occurrence in the cases presented here in comparison to the *PHF8* deletion cases, or whether deletion size differences affect interaction with neighboring genes.

Subject G: 3p24.3-25 and 5p15.2 deletions

Subject G is an affected individual identified to harbor unique deletions at the 3p24.3-25 and 5p15.2 regions. Several phenotypes unique to *subject G* in relation to the deletions include moderate ID, macrocephaly (>98%), postnatal large stature (>98%), and several craniofacial dysmorphisms (coarse facial features, frontal bossing). *SEMA5A,* a candidate gene identified by SUSPECTS, may account for the observed moderate ID and macrocephaly as it is known to be involved in axonal guidance and nervous system development (Adams *et al.,* 1996). The gene occupies the 5p15.2 region and experimental evidence shows that axonal development and formation of synapses may be affected by changes in *SEMA5A* expression (Jones *et al.,* 2002). Furthermore, deletions from the 5p band are also implicated with the Cri-du-chat phenotype, and haploinsufficiency of *SEMA5A* may be responsible for the intellectual disability in individuals exhibiting the phenotype (Simmons *et al.,* 1998). A deletion at the 5p15.2 region may thus have major implications in *SEMA5A* expression levels as not enough products are made to maintain proper axonal development and synapse formation, possibly leading to the observed macrocephaly and moderate ID in *subject G.*

CCT5 is another candidate gene that was identified by SUSPECTS to be likely involved in neurodevelopment as the chaperon protein product of *CCT5* was found to have an additional role in polymerization as well as maintenance of cytoskeletal proteins in neurons (Bourke *et al.,* 2002). Deletions in the 5p15.2 region would thus have profound effects on proper neurodevelopment due to a lack of *CCT5* chaperone proteins essential for proper neuron functioning. The *CTNND2* gene, which is also found on 5p15.2, was also identified as a possible candidate for the shared macrocephaly

and moderate ID phenotypes. Mutational experiments suggest a specialized role for the *CTNND2* protein as mutations in the gene result in learning and synaptic plasticity deficits (Israely *et al.,* 2004). Furthermore, a strong correlation between a hemizygous deletion of the *CTNND2* gene and severe mental retardation in individuals with Cri-du-chat syndrome (CDCS) was found (Medina *et al.,* 2000), further underlying the critical role of *CTNND2* regarding intellectual disability in subjects manifesting a deletion in the 5p15.2 region associated with CDCS. Moreover, delta catenin (the protein product of *CTNND2*) was also found to co-bind with kaiso to the promoter sites of rapsyn [a synapse protein necessary for segregating acetylcholine receptors at the neuromuscular area] (Rodova *et al.,* 2004).

Deletion of the *CTNND2* gene on 5p15.2 would thus have adverse effects on proper rapsyn functioning, and this may attribute to the seizures, delays in motor milestones, as well as failure to thrive observed in the affected individual.

Subject H: 7q11.23 duplication

Subject H is an isolated case that does not share any pathogenic CNVs or cytogenetic bands with the other cases here, but several others have been reported. A unique 7q11.23 duplication of unknown origin was found, and *GTF2I* was the sole candidate gene identified by SUSPECTS that may be implicated with the unique phenotypes associated with the affected genomic region. Structural features of 7q11.23 render this region susceptible to genomic rearrangement and deletions, yielding various CNVs that are also involved with Williams-Beuren syndrome [WBS] (Cuscó et al., 2008). Several characteristics are shared by the subject with WBS which include intellectual disability, hyperacusis, as well as genito-urinary problems (Sammour *et al.,* 2006) [subject has a history of enuresis]. Furthermore, *GTF2I* was also shown to be involved in tooth development at the bud and early bell stage (Ohazama and Sharpe, 2007), and thus may also account for the carious as well as early loss of teeth in the subject. Moreover, hemizygosity of *GTF2I* was found to be sufficient to account for a number of features associated with WBS, including visuospatial deficits (Edelmann *et al.,* 2007), which may contribute to the astigmatism and myopia observed in *subject H.*

Subject I: 18p11.3 duplication

The affected subject harbors a unique duplication at the 18p11.3 region of *de novo* origin and presents several phenotypes that may be associated with the duplication. Of particular note, the candidate *L3MBTL4* gene is known to be involved in platelet activation, and a dosage imbalance arising through a duplication may have direct or indirect consequences with regards to the observed heightened blood pressure during pregnancy. Furthermore, the role of the *L3MBTL4* gene in cell adhesion, in particular as a component of the integrin complex which is known to be involved in mediating various intracellular signals, may indeed account for the craniofacial and systemic dysmorphisms as well as the mild ID observed in *subject I.* The implications of dosage effects through duplication of the 18p11.3 region need to be further investigated as well as the specific roles of *L3MBTL4,* as research on this region and its genes is limited to make any conclusive statements regarding genotype-phenotype relationships at this time.

Conclusions:

The identification of candidate genes on various chromosomes provide further insight as to how changes occurring at the gene level affects the phenotype at the organismal level. Autism spectrum disorders present an especially daunting task as its variability between different individuals suggests a multitude of genes that may interact with other candidate genes involved in the same or different gene networks. Additional experiments need to be conducted in the future so as to uncover the functions of these genes, how their expression is regulated, and what gene networks they participate in. Only through understanding these finer details at the gene level can we then unravel the genetic bases of some of the phenotypes associated with classical autism.

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