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Germline mosaic transmission of a novel duplication of *PXDN* and *MYT1L* to two male half-siblings with autism

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Abstract

Objective—Autism is a neurodevelopmental disorder with a strong genetic component to susceptibility. Here, we report the molecular characterization of an apparent *de novo* 281 kb duplication of Chromosome 2p25.3 in two male half-siblings with autism.

Methods—The 2p25.3 duplication was first identified through a low-density microarray, validated with FISH, and duplication breakpoints were delineated using an Affymetrix 6.0 SNP microarray.

Results—FISH results validated the novel copy number variant and revealed the mother to be mosaic, with ~33% of her lymphoblast cells carrying the duplication. Therefore, the duplication was transmitted through the mechanism of germline mosaicism. Additionally, duplication breakpoints were refined and show that *PXDN* is fully duplicated, while seven exons of the terminal portion of the 25 exon gene *MYT1L* are within the duplicated region.

Conclusions—*MYT1L*, a gene predominately expressed in the brain, has recently been linked to other neuropsychiatric illness such as schizophrenia and depression. Results from this study indicate that the 2p25.3 duplication disrupting *PXDN* and *MYT1L* is a potential autism-causing variant in the pedigree reported here and should receive further consideration as a candidate gene for autism.

Keywords

MYT1L; autistic disorder; DNA copy number variations; mosaicism; genetics

INTRODUCTION

We describe molecular genetic characterization of two male half-siblings with autism and their psychiatrically normal mother. The genetic investigation of this family was performed with a high-density genome-wide SNP microarray, with confirmatory testing performed using targeted fluorescent in situ hybridization (FISH). These tests identified a novel 281 kb duplication of two genes on Chromosome 2p25.3, *PXDN* and *MYT1L* (partial duplication), in both half-siblings. Their common mother was somatic mosaic for the duplication.

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METHODS

Patient ascertainment and phenotypic characterization

The study participants were ascertained at the University of Iowa and provided written informed consent to a protocol approved by the University of Iowa IRB. Whole blood was obtained which was used to both establish lymphoblastoid cell lines and to extract DNA using salt precipitation.

Patients R1812 and R1813 were male half-siblings born to a common mother (R1811) by different fathers after uneventful pregnancies. Patient histories and physical exams did not identify evidence of any medical condition associated with autism (such as tuberous sclerosis) or any gross central nervous system injuries. Fragile X was ruled out by genetic testing and karyotype results were normal. Neither sibling had significant facial dysmorphism or other indication of syndromic autism.

Patient R1812 is non-verbal and scored well above the cut-off criteria for autism on both the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) (Lord, Rutter et al. 1989; Lord, Rutter et al. 1994). The Vineland Test of Adaptive Functioning (Sparrow and Cicchetti 1985) was administered to Patient R1812 at age 16 and indicated severe delays with age equivalent scores in both the daily living and communication domains of ~2.5 years. Patient R1813 is verbal with no clinically significant language delay, though he exhibits stereotypical autistic language (echolalia, neologisms, and pragmatic language problems). He was well above the cutoff criteria for autism on the ADI-R and met criteria in all domains of the ADOS with the exception of the communication domain. The Ravens Matrices was used to assign Patient R1813 an IQ of 118 (Raven 1956). The mother of Patients R1812 and R1813 had a Performance IQ of 109 and self-reports problems with spelling. As a child, she had normal language and reading abilities. Information was not available for either the father of Patient R1812 or the father of Patient R1813.

Initial identification of 2p25.3 duplication

The family was included in the original Autism Genome Project (AGP) study, which identified the 2p25.3 duplication using an Affymetrix 10K SNP microarray analyzed with dChip 2006 software for copy number variation (Szatmari, Paterson et al. 2007). The duplication was identified in both male half-siblings, but array results indicated that their common mother did not harbor the duplication. No other CNVs of clinical interest were identified.

Follow-up FISH and microarray studies

We used fluorescence in situ hybridization (FISH) to confirm the 2p25.3 duplication of *PXDN* and *MYTIL* with a series of eight probes designed from four BAC and four fosmid clones (Figure 1 and Table 1). Probes were selected to cover the gene and both upstream and downstream sequence, including the *PXDN* gene. FISH was performed on nuclei from lymphoblastoid cell line cells using standard conditions according to manufacturer's protocols. A minimum of 100 interphase nuclei per individual per probe were examined; at least 20 metaphase nuclei were examined to confirm the chromosomal location of each probe. Criteria for calling duplication were two independent probe intensity signals adjacent to each other (three signals total) in > 70% of interphase nuclei, combined with confirmation of correct chromosomal location through the metaphase signal.

In addition, Affymetrix 6.0 SNP arrays were used to more precisely delineate the duplication breakpoints.

RESULTS

Four probes, fosmids G248P82127D12 and G248P80381B4 and BACs RP11-755h23 and RP11-299i20, showed evidence for duplication (three signals in 75–90% of interphase nuclei) in both affected half-brothers. In the mother, 33–40% of each of these four probes was also found to be duplicated. Thus, the mother was mosaic in her lymphoblasts for the duplication. This supported the duplication arising through somatic mosaicism that also affected the maternal germline. Surrounding probes showed normal copy number in the mother and her children.

The Affymetrix array 6.0 data showed the 2p25.3 duplication to be approximately 281 kb with the distal breakpoint between 1.553 and 1.556 Mb and the proximal breakpoint between 1.834 and 1.838 Mb (Figure 2). Results showed *PXDN* to be fully duplicated while only 63 kb of the *MYTIL* terminus, containing 7 exons, was duplicated. The overlap between this duplication and structural variants reported in the Database of Genomic Variants (DGV; <http://projects.tcag.ca/variation/>) was only 2.4%, indicating that this duplication was a novel CNV.

Additionally, the Affymetrix 6.0 array data, in contrast to the 10k SNP data, did show evidence for the duplication in the mother, but with a similar signal intensity shift as that of her children. Thus, while the higher density array picked up the duplication, it did not distinguish the mosaic from the fully penetrant duplications. No other CNVs of apparent clinical significance were identified.

DISCUSSION

We report the characterization of a novel 281 kb duplication in two male half-siblings with autism on Chromosome 2p25.3, duplicating *PXDN* and partially duplicating *MYTIL*. Using FISH, we determined that their psychiatrically healthy mother was somatic mosaic, with 33–39% of her lymphoblastoid cells having the duplication. She also harbored the duplication in her germline, since she passed it on to her two sons. Mosaicism occurs when an early post-zygotic cell acquires a new mutation and is then transmitted to daughter cells. The percentage of cells affected and the tissue types composed of mutated cells are dependent on the stage at which the mutation occurs, and influence the resultant phenotype. For example, if a mutation occurs in one cell at the two-cell stage of development, it is expected that 50% of the cells of the body will have the mutation. For this mother, the mutation appears to have arisen in a cell that gave rise to lymphoblasts and germ cells, but not neural tissue, so that while she has no autism phenotype, at least some portion of her progeny do.

Our data also highlight the uncertain ability of microarray studies to identify mosaicism. The best chance for a microarray to detect mosaicism is if a CNV is present in two or more siblings but neither parent (making half-sibling pedigrees ideal for this task because it is apparent who the transmitting parent must be). Microarrays may also suggest CNV mosaicism if a series of contiguous probe signal intensities are intermediate between normal (2x) and either duplication (3x) or deletion (1x). This latter occurrence, however, requires a sensitivity and signal-to-noise ratio that many arrays lack. In our study, for example, the 10k SNP array failed to identify the 2p25.3 duplication in the mother, while the Affymetrix array 6.0 showed her CNV with the same average signal intensity as in her children. Similar occurrences in either direction have been reported in other disorders (Leon, Zou et al. 2010; Neill, Torchia et al. 2010; Scott, Cohen et al. 2010; Filho, Souza et al. 2011) highlighting the utility of additional molecular methodologies to detect mosaicism.

Of the two genes affected by the duplication in this family, *MYT1L* appears most likely to be relevant to autism. Based on *Drosophila* studies, *PXDN* is believed to function in immune defense by facilitating the removal of phagocytosed material and regulating reactive oxygen species (Horikoshi, Cong et al. 1999). It is ubiquitously expressed in the human body, including brain (Horikoshi, Cong et al. 1999), but to date, there are no data suggesting a role for *PXDN* in autism or other neurological disease.

In contrast, an increasing number of studies suggest *MYT1L* as a candidate for psychiatric and neurological disease. *MYT1L* is a developmentally expressed and neuronal-specific member of the Cys-Cys-His-Cys family of zinc finger proteins that shares significant sequence homology with *MYT1*, a transcription factor that binds to the promoter of the most abundantly expressed myelin gene, *Proteolipid protein (PLP)*, on the X-Chromosome (Kim and Hudson 1992). Two distinct *Myt1l* transcripts are expressed in neurons, and not glia, of the developing rat brain (Kim, Armstrong et al. 1997). Vierbuchen and colleagues (2010) identified *Myt1l* as one of three factors necessary to convert mouse fibroblasts into functional neurons *in vitro*. Although *Myt1l* was not essential to induce differentiation, its presence was required for neuronal cell maturation and functionality (Vierbuchen, Ostermeier et al. 2010).

In genetic studies of individuals with neuropsychiatric disorders, Gruchy et al. (2007) and Bonaglia, et al. (2008) both reported large, karyotypically detectable abnormalities that affect *MYT1L* in addition to other genes in 3 individuals with mental retardation, autistic-like conduct, and/or developmental delay (Gruchy, Jacquemont et al. 2007; Bonaglia, Giorda et al. 2009). Zou et al. described a female child diagnosed with mental retardation and PDD NOS harboring a 2p25.3 deletion proximal to *MYT1L*, and Vrijenhoek, et al. (2008) identified two overlapping partial duplications of *MYT1L* in patients with schizophrenia (Zou, Van Dyke et al. 2007; Vrijenhoek, Buizer-Voskamp et al. 2008). Additionally, a schizophrenia risk allele (rs1344706) of the gene *ZNF804A* is predicted to maintain an intronic binding site for *MYT1L* (Riley, Thiselton et al. 2010). Most recently, an association between *MYT1L* SNP rs3748989 and the development of major depressive disorder was reported in the Chinese Han population (Wang, Zeng et al. 2010).

In conclusion, we describe the germline mosaic transmission of a novel duplication affecting the genes *PXDN* and *MYT1L* to two male half-siblings with autism from their psychiatrically healthy mother. The data, taken together with previous publications, identify *MYT1L* as a high interest genetic candidate in the etiology of autism.

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References

- Armstrong RC, Migneault A, et al. High-grade human brain tumors exhibit increased expression of myelin transcription factor 1 (MYT1), a zinc finger DNA-binding protein. *J Neuropathol Exp Neurol.* 1997; 56(7):772–781. [PubMed: 9210873]
- Bonaglia MC, Giorda R, et al. A familial inverted duplication/deletion of 2p25.1–25.3 provides new clues on the genesis of inverted duplications. *Eur J Hum Genet.* 2009; 17(2):179–186. [PubMed: 18813332]
- Cook EH Jr, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature.* 2008; 455(7215):919–923. [PubMed: 18923514]

- Desmond JC, Raynaud S, et al. Discovery of epigenetically silenced genes in acute myeloid leukemias. *Leukemia*. 2007; 21(5):1026–1034. [PubMed: 17330099]
- Filho AB, Souza J, et al. Somatic/gonadal mosaicism in a syndromic form of ectrodactyly, including eye abnormalities, documented through array-based comparative genomic hybridization. *Am J Med Genet A*. 2011; 155A(5):1152–1156. [PubMed: 21485001]
- Gruchy N, Jacquemont ML, et al. Recurrent inverted duplication of 2p with terminal deletion in a patient with the classical phenotype of trisomy 2p23-pter. *Am J Med Genet A*. 2007; 143A(20): 2417–2422. [PubMed: 17853488]
- Horikoshi N, Cong J, et al. Isolation of differentially expressed cDNAs from p53-dependent apoptotic cells: activation of the human homologue of the *Drosophila* peroxidase gene. *Biochem Biophys Res Commun*. 1999; 261(3):864–869. [PubMed: 10441517]
- Kim JG, Armstrong RC, et al. Myelin transcription factor 1 (Myt1) of the oligodendrocyte lineage, along with a closely related CCHC zinc finger, is expressed in developing neurons in the mammalian central nervous system. *J Neurosci Res*. 1997; 50(2):272–290. [PubMed: 9373037]
- Kim JG, Hudson LD. Novel member of the zinc finger superfamily: A C2-HC finger that recognizes a glia-specific gene. *Mol Cell Biol*. 1992; 12(12):5632–5639. [PubMed: 1280325]
- Leon E, Zou YS, et al. Mosaic Down syndrome in a patient with low-level mosaicism detected by microarray. *Am J Med Genet A*. 2010; 152A(12):3154–3156. [PubMed: 21108401]
- Liu Y, Carson-Walter EB, et al. Vascular gene expression patterns are conserved in primary and metastatic brain tumors. *J Neurooncol*. 2010; 99(1):13–24. [PubMed: 20063114]
- Lord C, Rutter M, et al. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. *J Autism Dev Disord*. 1989; 19(2):185–212. [PubMed: 2745388]
- Lord C, Rutter M, et al. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord*. 1994; 24(5):659–685. [PubMed: 7814313]
- Neill NJ, Torchia BS, et al. Comparative analysis of copy number detection by whole-genome BAC and oligonucleotide array CGH. *Mol Cytogenet*. 2010; 3:11. [PubMed: 20587050]
- Raven, JC. The coloured progressive matrices. London: H.K. Lewis; 1956.
- Riley B, Thiselton D, et al. Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. *Mol Psychiatry*. 2010; 15(1):29–37. [PubMed: 19844207]
- Scott SA, Cohen N, et al. Detection of low-level mosaicism and placental mosaicism by oligonucleotide array comparative genomic hybridization. *Genet Med*. 2010; 12(2):85–92. [PubMed: 20084009]
- Sparrow SS, Cicchetti DV. Diagnostic uses of the Vineland Adaptive Behavior Scales. *J Pediatr Psychol*. 1985; 10(2):215–225. [PubMed: 4020603]
- Szatmari P, Paterson AD, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat Genet*. 2007; 39(3):319–328. [PubMed: 17322880]
- Vierbuchen T, Ostermeier A, et al. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010; 463(7284):1035–1041. [PubMed: 20107439]
- Vrijenhoek T, Buizer-Voskamp JE, et al. Recurrent CNVs disrupt three candidate genes in schizophrenia patients. *Am J Hum Genet*. 2008; 83(4):504–510. [PubMed: 18940311]
- Wang T, Zeng Z, et al. Common SNPs in myelin transcription factor 1-like (MYT1L): association with major depressive disorder in the Chinese Han population. *PLoS One*. 2010; 5(10):e13662. [PubMed: 21048971]
- Zou YS, Van Dyke DL, et al. Microarray comparative genomic hybridization and FISH studies of an unbalanced cryptic telomeric 2p deletion/16q duplication in a patient with mental retardation and behavioral problems. *Am J Med Genet A*. 2007; 143(7):746–751. [PubMed: 17345620]

Figure 1.

Chromosome 2p25.3 targeted FISH results for affected patients R1812 and R1813 and their unaffected mother R1811. Fosmids are italicized and the * takes the place of the prefix *G248P*. Fosmids *G248P82127D12* and *G248P80381B4* and BACs *RP11-755h23* and *RP11-299i20* are duplicated in both patients and in ~33% of the mother's cells. Flanking fosmids and BAC probes have normal copy numbers.

Figure 2.

Chromosome 2p25.3 duplication detected by Nexus software analysis of Affymetrix 6.0 data for patient R1813. The green bar at the top of the image represents the duplication. This figure includes cytoband, genes within and flanking the duplication, CNVs detected in Nimblegen's 42M-probe control set, signal intensity data for each array probe, and chromosomal location.

Table 1

Genes and FISH probes

Name	Feature	Start	Stop	Length
PXDN	gene	1,635,660	1,748,291	112,631
MYT1L	gene	1,792,887	2,335,045	542,158
G248P800757B11	fosmid	1,459,471	1,499,455	39,984
G248P85079E10	fosmid	1,574,074	1,611,311	37,237
G248P82127D12	fosmid	1,607,869	1,644,203	36,334
G248P80381B4	fosmid	1,705,150	1,744,952	39,802
RP11-755h23	BAC	1,804,281	1,896,430	92,149
RP11-299i20	BAC	1,896,431	2,060,854	164,423
RP11-352j11	BAC	2,271,126	2,411,010	139,884
RP11-297i3	BAC	2,062,850	2,271,326	208,476