Copy number variation analysis and sequencing of the X-linked mental retardation gene TSPAN7/TM4SF2 in patients with autism spectrum disorder

Abdul Noora, Peter J. Gianakopoulosa, Bridget Fernandeze, Christian R. Marshallb, Peter Szatmari, Wendys, Stephen W. Scherer and John B. Vincenta

The study of copy number variations (CNVs) in autism has already begun to reap benefits. Recently, several groups have reported a significant contribution of CNVs in the etiology of autism with the identification of several candidate autism genes (Szatmari et al., 2007; Marshall et al., 2008). For instance, by genome-wide scan of over 1000 autism families using the 10K single nucleotide polymorphism (SNP) microarray (Affymetrix, Santa Clara, California, USA), the neurexin-1 gene on chromosome 2 was identified as disrupted in one family (Szatmari et al., 2007). In our own study, by performing a genome-wide scan of 427 unrelated autism probands using the 500 K SNP microarray (Affymetrix), we reported several autism candidate genes, including TSPAN7 on Xp11.4 (Marshall et al., 2008). Previously, by characterizing the X chromosome breakpoint of an X:2 balanced translocation, TSPAN7 (MIM 300096; also known as TM4SF2 and MRX58) was found to be disrupted in a patient with mental retardation (MR) (Zemni et al., 2000). Additional studies have also reported several mutations in TSPAN7 in nonsyndromic X-linked MR patients (Zemni et al., 2000; Abidi et al., 2002).

In a family from Newfoundland, we identified a 121-kb duplication spanning seven exons of TSPAN7 in an affected son that is inherited from an unaffected mother [Chromosomes X 38,250,331 to 38,371,333 (UCSC 2004, http://genome.ucsc.edu/cgi-bin/hgGateway)] flanked by SNPs rs5917211 and rs5917628 (Marshall et al., 2008). The duplication included 12 SNPs on the NspI and SspI Affymetrix microarray, spanned exons 2–8 of TSPAN7 and was not present in a control cohort of 1652 samples. In this study, we hypothesized that mutations in TSPAN7 may be causative for autism as well as for nonsyndromic X-linked MR. In addition, to ascertain a possible effect of this CNV on gene expression of TSPAN7, we amplified and sequenced TSPAN7 mRNA from lymphoblasts from the proband. Reverse transcriptase-PCR revealed a normal size transcript expressed in the patient, and no change in mRNA sequence was observed. These results indicated that this CNV gain does not seem to disrupt the expression or coding sequence of the TSPAN7 gene. To further investigate the potential involvement of TSPAN7 in the etiology of autism, we sequenced the entire coding region and exon–intron boundaries of TSPAN7 in a cohort of 250 (210 male and 40 female) unrelated autism probands. Details of these samples are published elsewhere (Moessner et al., 2007). Briefly, all the samples met autism diagnostic interview and autism diagnostic observation schedule criteria for autism. Institutional ethical review board approval was obtained for the study, and informed written consent was obtained for each participant. No coding mutations were identified among our probands.

In conclusion, our results indicate that coding mutations in TSPAN7 are not associated with our cohort of autism patients. However, the involvement of TSPAN7 mutations in a very small fraction of autism patients cannot be excluded. Moreover, the null result of the TSPAN7 CNV further emphasizes the importance of validating possible CNV effects at the mRNA level to establish a contribution to disease. Altogether, our findings exclude the involvement of TSPAN7 sequence variants in most cases of autism.

Acknowledgements
This work was supported by Genome Canada/Ontario Genomics Institute, and Autism Speaks. C.R.M. is supported by the SickKids Foundation and the National Alliance for Research on Schizophrenia and Depression (NARSAD). S.W.S. is an Investigator of the GlaxoSmithKline-CIHR Pathfinder Chair in Genetics and Genomics at...
SickKids and the University of Toronto. J.B.V. is supported by a NARSAD Independent Investigator Award.

References