Research Letter

Does the P172H Mutation at the TM4SF2 Gene Cause X-Linked Mental Retardation?

To the Editor:

The TM4SF2 gene mapped at Xp11.4 has recently been associated with non-syndromic X-linked mental retardation (MRX) by the analysis of the breakpoint of a t(X;2) balanced translocation in a female patient with mild mental retardation and minor autistic features [Zemni et al., 2000]. To date, three TM4SF2 mutations have been identified in three familial cases of MRX: the G218X (family L28), the P172H (family T15) [Zemni et al., 2000], and, more recently, a deletion of 2bp (564delGT) in exon 5 (family MRX58) [Abidi et al., 2002]. Two of these changes create a premature stop codon and it has been predicted that the MRX in these cases results from the deficiency of the TM4SF2 product.

The family T15, first described by Zemni et al. [2000], was recently reevaluated [Gomot et al., 2002]. Neuropsychological evaluation showed that five out of seven males and one of three women had mild to moderate mental retardation. The mutation was present in all mentally impaired individuals and also in one male reported to be normal. Further segregation analysis of markers surrounding the TM4SF2 gene suggested that in this family the disease gene should be localized between loci DXS556 (Xp11.4) and DXS441 (Xq13.2), thus excluding the TM4SF2 gene. The authors put forward two alternative explanations: (1) the TM4SF2 P172H mutation would be the cause of MR in association with intra-familial phenotypic variability or (2) this would be a rare mutation not detrimental to the functioning of the TM4SF2protein, and therefore not causative of the phenotype [Gomot et al., 2002].

As part of an X-linked mental retardation (XLMR) candidate gene testing, we screened the seven exons of the TM4SF2 gene in 105 males with mental retardation (25 familial and 80 isolated cases), who were not carriers of the fragile X mutation. Blood samples were collected after informed consent. All the coding exons of the TM4SF2 gene were amplified using the primers and PCR conditions reported elsewhere [Zemni et al., 2000], and the amplification products were analyzed by SSCP (single strand conformation polymorphism) according to Splendore et al. [2000]. One sample showed an abnormal migration pattern for the fragment corresponding to exon 5 (data not shown). Sequencing in both directions was performed using the BigDye Terminator Cycle Sequencing Kit (PE Biosystems, Foster City, CA) in an ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA). A C→A transversion was found at nucleotide c.515 leading to the Pro→His substitution of amino acid 172 (P172H) (Fig. 1). This result was confirmed by the single-nucleotide primer extension assay (SNuPe, Amersham Biosciences, Piscataway, USA), a method that precisely localizes a SNP site.

The patient with the P172H was examined at 21 years of age and presented with mild to moderate mental retardation. He is able to write and read only simple texts. Developmental milestones had been slightly delayed, but speech acquisition was severely impaired, since he started talking at 3½ years. Dysmorphic features included: a long narrow face with pronounced oral hypotonia; short forehead and filtrum; high palate; teeth malocclusion; abnormally folded ears; depressed sternum; hypoplastic nails; somewhat scarce hair with alopecia spots. Ophthalmological findings included nystagmus and myopia. His mother and sister, both with normal intelligence, were found to be carriers of the P172H mutation, but not his mentally normal brother (Fig. 2).

This mutation was not detected in the SNuPe assay screening of additional 320 unrelated X chromosomes from normal males. The finding of the mutation in a cohort of 105 individuals with mental retardation but not in 320 normal men with a similar ethnic background further supports the hypothesis that this might be a pathogenic mutation. A possibility of this being a common polymorphism has also been ruled out by Zemni et al. [2000] as they did not find it in 100 normal chromosomes. Gomot et al. [2002] advanced the possibility that this mutation was not the cause of mental retardation in family T15 because they found one mentally normal male carrier, an individual who has been previously

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considered to be of borderline intelligence [Zemni et al., 2000]. Indeed, the small number of carriers of TM4SF2 mutations hereto described does not allow the proper delineation of the range of clinical variability, and the normal carrier in family T15 might well represent a non-penetrant or a benign case.

Since the female patient who carried the TM4SF2 gene truncated by a t(X;22) had mild mental retardation and minor autistic features, we speculated if mutations in this gene could be associated with autism. The negative finding of the P172H mutation in our investigation of 76 boys with autism shows that it does not seem to be the case for this mutation.

The TM4SF2 gene encodes a member of the tetraspanin family of cell-surface proteins that spans the membrane and form two extracellular domains [Maecker et al., 1997]. These proteins not only associate with one another but also interact with B1-integrins and class I and class II HLA proteins. Interaction with B1-integrins might activate Rac and RhoGTPases and mediate cellular processes, such as the regulation of actin cytoskeleton dynamics, the activation of signaling pathways, and cell proliferation, adhesion, and migration [Maecker et al., 1997]. The P172H mutation leads to the substitution of a non-polar by a polar amino acid in the putative 2nd extracellular domain, the large extracellular loop (LECL) of the protein, which is the site of interaction with integrins and other tetraspanin proteins [Berditchevski, 2001]. The LECLs of most tetraspanins, including the TM4SF2, contain two motifs: the Cys-Cys-Gly sequence (CCG motif) and the Pro-x-x-Cys-Cys sequence (PxxCC motif) in which “x” represents any amino acid at positions 172 and 173 [Berditchevski, 2001]. The alignment of the residue sequences of all the members of the tetraspanin family revealed just eight distinct residues in the second position of the PxxCC motif, but never a histidine. In addition, proline at the position 172 is conserved in both man and mouse, although in Drosophila tyrosine, an apolar amino acid is at this position instead of proline.

Alternatively, the C–A transversion could be involved with the activation of a cryptic splice site, leading to the transcription of an isoform with a premature stop codon. Increasing evidence points to such an effect of nonsense, missense, and silence mutations [Liu et al., 2001; Cartegni et al., 2002]. This hypothesis could also explain the presumptive variability in the degree of mental impairment associated with the mutation. Unfortunately, we were unable to obtain an mRNA sample of the patient to test this hypothesis.

In summary, the P172H mutation found in two unrelated patients with mental retardation is a very rare substitution still not detected in population-ascertained chromosomes, and it is located in a position that seems to be conserved at least in vertebrates. The presumably normal carrier of this mutation who belongs to a mental retardation family might well represent one of the extremes of the spectrum of phenotype variability associated with the P172H mutation. Therefore, at this point the P172H mutation cannot be ruled out as a cause of mental impairment.

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