

Short Communication

Modulation of expressivity in *PDGFRB*-related infantile myofibromatosis: a role for *PTPRG*?

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ABSTRACT. Infantile myofibromatosis is a rare genetic disorder characterized by the development of benign tumors in the skin, muscle, bone, and viscera. The molecular pathogenesis is still incompletely known. An autosomal dominant form had been reported as causally related with mutations in the gene for platelet-derived growth factor receptor beta (*PDGFRB*). We report here two siblings with infantile myofibromatosis and with a *PDGFRB* mutation identified by exome sequence analysis. However, the unaffected mother also had the same

PDGFRB mutation. We showed that both children had also inherited from their healthy father a heterozygous mutation in the gene for receptor protein tyrosine phosphatase gamma (*PTPRG*), an enzyme known to dephosphorylate *PDGFRB*. We suggest that in this family, the additional mutation in *PTPRG* may explain the full phenotypic penetrance in the siblings affected, in comparison with the unaffected mother.

Key words: Infantile myofibromatosis; *PDGFRB* gene; *PTPRG* gene; Incomplete penetrance; Whole-exome sequence analysis

INTRODUCTION

Infantile myofibromatosis (IM; MIM 228550) is a rare disorder characterized by the development of benign tumors in the skin, striated muscles, bones, and in exceptional cases, visceral organs (Orbach, 2013). Recently, two studies suggested that mutations in *PDGFRB* (gene for platelet-derived growth factor receptor, beta polypeptide; MIM 173410) were causally related to autosomal dominant IM (Cheung et al., 2013; Martignetti et al., 2013). In one of these studies, Cheung et al. (2013) identified the same c.1681C>T (p.Arg561Cys) mutation in *PDGFRB* in all the 11 affected individuals from four distinct families with IM. None of the unaffected parents had the mutation. In the second and larger study, Martignetti et al. (2013) performed whole-exome sequencing in 11 affected members of nine unrelated IM families. In eight of these they identified one of two pathogenic mutations in *PDGFRB*: c.1681C>T (p.Arg561Cys) or c.1978C>A (p.Pro660Thr).

While trying to establish the culpable gene in cases of autosomal recessive infantile myofibromatosis, we studied a French family with two affected children and their healthy non-consanguineous parents, previously described in detail (Puzenat et al., 2009). Whole-exome sequencing identified a *PDGFRB* mutation in both siblings with IM but, to our surprise, also in the unaffected mother. This is the first documentation of incomplete penetrance in *PDGFRB*-related IM.

MATERIAL AND METHODS

Whole-exome sequencing and analysis

Whole-exome sequencing was performed by the Centre for Applied Genomics, Hospital for Sick Children, Toronto, Canada, using the Agilent SureSelect Human All Exon V4 kit (Agilent Technologies, Santa Clara, CA, USA) and the SOLiD 5500xl platform (Applied Biosystems, Foster City, CA, USA).

All data were aligned via BFAST and BWA aligner to the hg19/GRCh37 reference genome. Variants were quality trimmed using Genome Analysis Toolkit (GATK 1.1.28) and they were annotated for functional effect by SnpEff 2.0.5. Alignment, calling and annotation of the variants against databases such as 1000 Genomes (April 2012 release), NHLBI Exome Sequencing Project (ESP6500) and Single Nucleotide Polymorphism database (dbSNP137) were done using a software developed in-house called Mendel, MD (RGCCCL Cardenas and SDJ Pena, manuscript in preparation).

Sanger sequencing

Sanger sequencing was performed for validation of the variants of interest identified in exome analysis using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and the Applied Biosystems (ABI) 3730 Genetic Analyzer. Sequencing data was analyzed using the Sequencher version 4.1.4 software (Genes Code Corporation, Ann Arbor, MI, USA).

RESULTS

Over 100,000 variants were identified in each individual by exome sequencing. After filtering for common polymorphisms and non-pathogenic variants, we identified, in both siblings with IM, the same mutation c.1681C>T (p.Arg561Cys; NM_002609.3) in *PDGFRB*, which had been previously described by Cheung et al. (2013) and Martignetti et al. (2013). Sanger sequencing confirmed the presence of the heterozygous mutation (Figure 1A). However, to our surprise, we found that the unaffected mother also showed the same *PDGFRB* c.1681C>T heterozygous mutation.

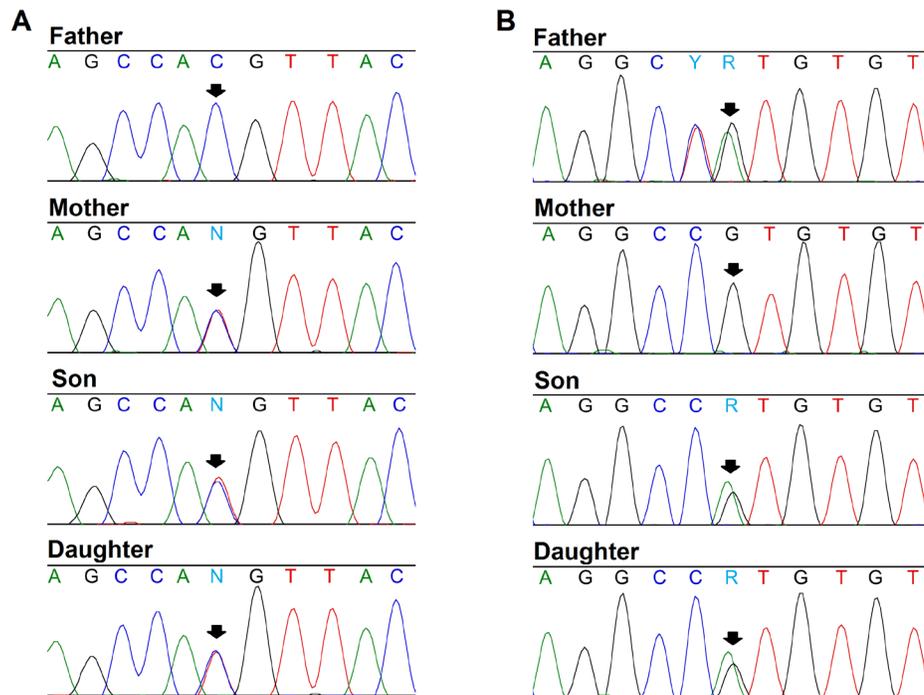


Figure 1. Sanger sequencing. **A.** Fragments of sequence chromatograms of *PDGFRB* from the healthy father (normal homozygous), the healthy mother (heterozygous mutation), and the affected son and daughter (heterozygous mutation). Black arrows indicate the position of the variant c.1681C>T (p.Arg561Cys). **B.** Fragments of sequence chromatograms are shown of *PTPRG* from the healthy father (heterozygous mutation), the healthy mother (normal homozygous), and the affected son and daughter (heterozygous mutation). Black arrows indicate the position of the variant c.1276G>A (p.Val426Met).

We know that some variants may require the presence of additional pathogenic variants at other loci for full penetrance (Cooper et al., 2013; Schaffer, 2013). We thus searched the affected siblings of our family for paternal mutations in genes known to interact with *PDGFRB*. Indeed, we found that both siblings had inherited from their healthy father a heterozygous mutation in *PTPRG* (protein tyrosine phosphatase, receptor type, gamma; MIM 176886), which codes for an enzyme known to dephosphorylate PDGFRB, reducing its activity (Barr et al., 2009). We believe that the *PTPRG* mutation might have contributed to the phenotype penetrance in the children and non-penetrance in the mother. The existence of the *PTPRG* mutation (NM_002841.3; p.Val426Met; c.1276G>A; rs139041779) was confirmed by Sanger sequencing (Figure 1B). According to the NHLBI Exome Sequencing Project (ESP6500), it has an allele frequency of 0.0001. The mutation is located in a region well conserved throughout evolution in *PTPRG* orthologues (Figure 2). It was predicted to be deleterious by SIFT (score 0.00), probably damaging by PolyPhen-2 (score 0.979) and disease causing by Mutation Taster (P value of 0.999).

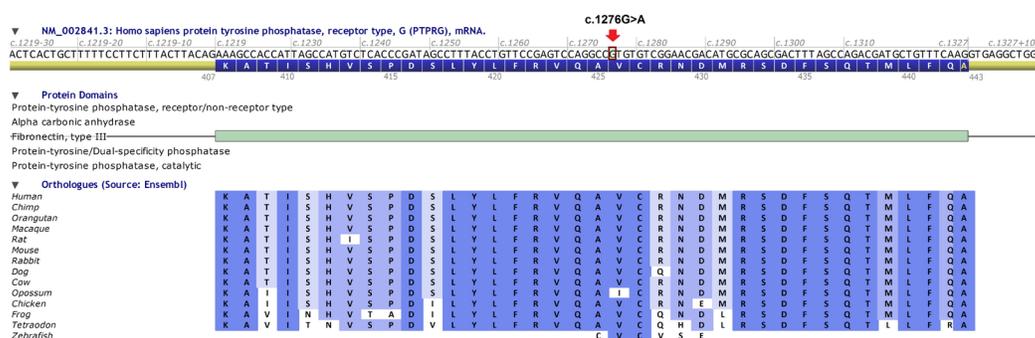


Figure 2. Comparative analysis using PTPRG orthologues. Part of the PTPRG protein showing that the variant is located in a region well conserved throughout evolution. The position of the variant was indicated by the red arrow. Alignments of PTPRG orthologues were made with the Alamut 2.4 software. Fully conserved amino acids are marked in dark blue and less-conserved amino acids are in lighter blue colors. Species name are as follows: *Homo sapiens* (human), *Pan troglodytes* (chimpanzee), *Pongo pygmaeus* (orangutan), *Macaca mulatta* (macaque), *Rattus norvegicus* (rat), *Mus musculus* (mouse), *Oryctolagus cuniculus* (rabbit), *Canis familiaris* (dog), *Bos Taurus* (cow), *Monodelphis domestica* (opossum), *Gallus gallus* (chicken), *Xenopus tropicalis* (frog), *Tetraodon nigroviridis* (tetradon), *Danio rerio* (zebrafish).

DISCUSSION

Incomplete penetrance has been previously proposed to occur in families with the autosomal dominant form of IM (Bracko et al., 1992; Kulkarni et al., 2012). In fact, in two of the families described by Martignetti et al. (2013) [family IM-4 and the previously published family IM-6 (Zand et al., 2004)], there might have been non-penetrance in individuals I-1 and I-2 from both pedigrees.

It is imaginable that the *PDGFRB* and *PTPRG* mutations in the children described here are additive events that could cooperatively lead to tumorigenesis through the Ras/MAPK pathway and cause IM. The p.Arg561Cys mutation appears to lead to increased PDGFRB kinase activity after stimulation by the ligand (Cheung et al., 2013). The *PTPRG* mutation seen

in our patients would probably cause a decrease in its efficiency to dephosphorylate PDGFRB, thus enhancing its activation and stimulating downstream signaling pathways that may induce cellular hyperproliferation (Figure 3). It remains to be established whether the *PTPRG* mutation is a private feature of our family, or whether it is a more general phenomenon. Thus, it will certainly be of interest to investigate the integrity of *PTPRG* in the cases described by Cheung et al. (2013) and Martignetti et al. (2013).

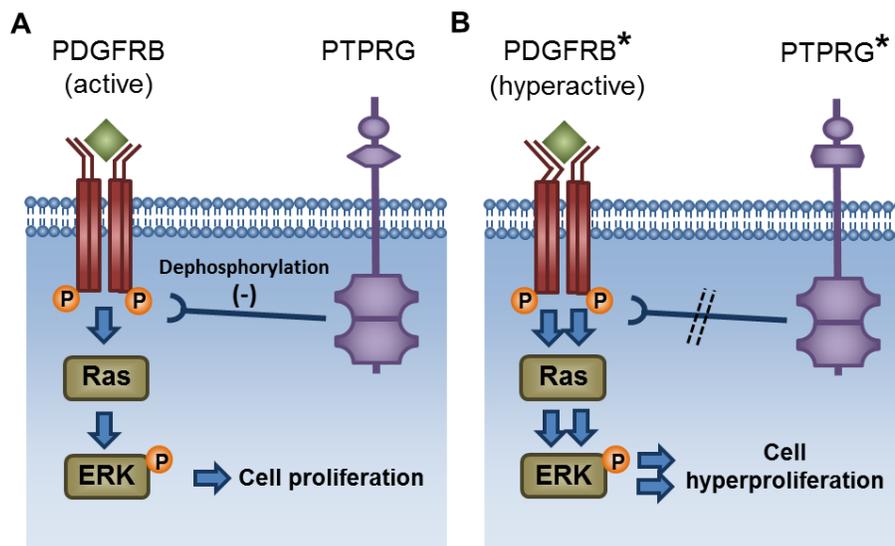


Figure 3. Schematic representation of the mechanisms of interaction of PDGFRB and PTPRG. Mutant proteins are indicated by an asterisk. **A.** Normal individual. Upon binding by PDGF, PDGFRB dimerizes and is autophosphorylated in multiple specific tyrosine residues, leading to the initiation of signal transduction pathways such as Ras/MAPK pathway. PTPRG modulates PDGFRB activation by dephosphorylation. **B.** Our patients. Mutant PDGFRB is excessively activated. Mutant PTPRG has decreased dephosphorylation activity, enhancing PDGFRB activation. This leads to cell hyperproliferation through Ras/MAPK pathway.

In conclusion, our data confirm that mutations in *PDGFRB* cause IM. Additionally, we suggest that a mutation in *PTPRG* may act as an aggravating factor, and we believe this explains the phenotypic penetrance in the two children studied here. Mutations in other interacting genes, especially *PTPRG*, as shown in the present study, may modulate the degree of expressivity of autosomal dominant infantile myofibromatosis.

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