Apparent genotype–phenotype mismatch in a patient with *MYH9*-related disease: When the exception proves the rule

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Dear Sirs,

MYH9-related disease (*MYH9*-RD) is one of the most frequent forms of inherited thrombocytopenia and is characterised by enlarged platelets and Döhle body-like granulocyte inclusions. Mutations of *MYH9*, the gene coding for non-muscle myosin heavy chain-IIA (myosin-9), are responsible for the disease (1). In addition to congenital thrombocytopenia, patients are at risk of developing nephropathy, deafness, cataracts and alterations of liver enzymes during life depending on which domain of myosin-9 is altered. Indeed, there is a strong correlation between genotype and phenotype, as mutations affecting the head domain of myosin-9 are associated with a higher incidence and a more precocious appearance of kidney damage and deafness than those hitting the tail domain (2). In particular, individuals with missense mutations at position 702 of the motor domain, the most frequently altered residue, have invariably severe thrombocytopenia associated with all the other syndromic features of the disease, often becoming fully manifest at a young age (3).

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Here, we report a case of apparent mismatch between genotype and phenotype in a family with the p.R702C mutation. The proband was a 10-year-old boy who was referred to us for a persistent, severe thrombocytopenia diagnosed three years earlier. He had rare epistaxes, easy bruising and occasional gum bleedings associated with a pronounced bilateral sensorineural deafness, proteinuria (0.2 g/24 hours), microhaematuria with jaline cylinders and mildly elevated liver enzymes (AST 1.28 and ALT 1.46, upper normal level) (4). Automatic and microscopic platelet counts were 19 x 10^9/l and 30 x 10^9/l, respectively, the MPV was 13.4 fl (normal value 8-11 fl), with giant platelets on a peripheral blood smear (5). Members of his family were also studied revealing a mild macrothrombocytopenia in his father (86 x 10^9/l at cell counter and 92 x 10^9/l at microscopy; MPV (11.7 fl); normal platelets were found in the other members. Despite being 37 years old, the father did not present any renal, auditive or ocular symptoms (►Figure 1A) and had never exhibited bleeding problems, neither spontaneous nor after minor surgery.

The proband's leukocytes had typical Döhle body-like inclusions, and immunocytochemical staining confirmed the abnormal distribution of myosin-9; which was present in numerous aggregates of small size, in all leukocytes, leading to the diagnosis of *MYH9*-RD (6). Myosin-9 aggregates were observed also in his father's leukocytes but in only about half of the cells.

Direct sequence analysis of genomic DNA from peripheral blood cells revealed a heterozygous c.2104C>T mutation in *MYH9* affecting residue 702 (p.R702C) in both the proband and his father. However, the father's electropherograms, at variance with those from the son, showed the mutant "T" peak of a lower intensity than that of the corresponding wild-type C nucleotide (►Figure 1B). This observation, together with his phenotype limited to mild thrombocytopenia, at odds with the mutation affecting the motor domain, led us to suspect mosaicism.

To confirm our hypothesis, we first checked for the presence of the c.2104C>T allele using restriction fragment length polymorphism (RFLP) analysis (►Figure 1C). RFLP confirmed that the proband's father carried the mutation in peripheral blood cells. However, although this analysis is not strictly quantitative, the mutant fragment (159 bp) appeared to be of a lesser intensity than the wild-type fragment (144 bp). Then, we tested urine sediment exfoliated cells, where a faint mutant RFLP fragment was detected (►Figure 1D). The scarcity of the "T" peak was confirmed by sequencing analysis (►Figure 1E), suggesting that only a few cells of the urinary tract carried the c.2104C>T mutation. The mutant allele was barely detectable even from buccal mucosa and hair bulb cells (►Figure 1E), supporting the hypothesis that the amount of the mutant allele in the father's non-haematological tissues is minimal.

Taken together, the phenotype limited to mild macrothrombocytopenia, the presence of myosin-9 aggregates in only half of the leukocyte population, the lower height of the mutant peak in chromatograms, the faint intensity of the mutant fragment in RFLP analysis of peripheral blood and urinary sediment cells, and the absence of the mutation in hair bulbs and buccal mucosa, support the presence of somatic mosaicism in the father. Therefore, we assume that the c.2104C>T mutation occurred during the father's early embryonic development in a single progenitor cell from which both germline cells and other mesodermal tissues, such as blood and urinary system, were derived. Given the limited presence of the mutant allele in cells from the urinary tract, the proband's father is unlikely to develop renal failure and probably also the other syndromic manifestations of *MYH9*-RD.

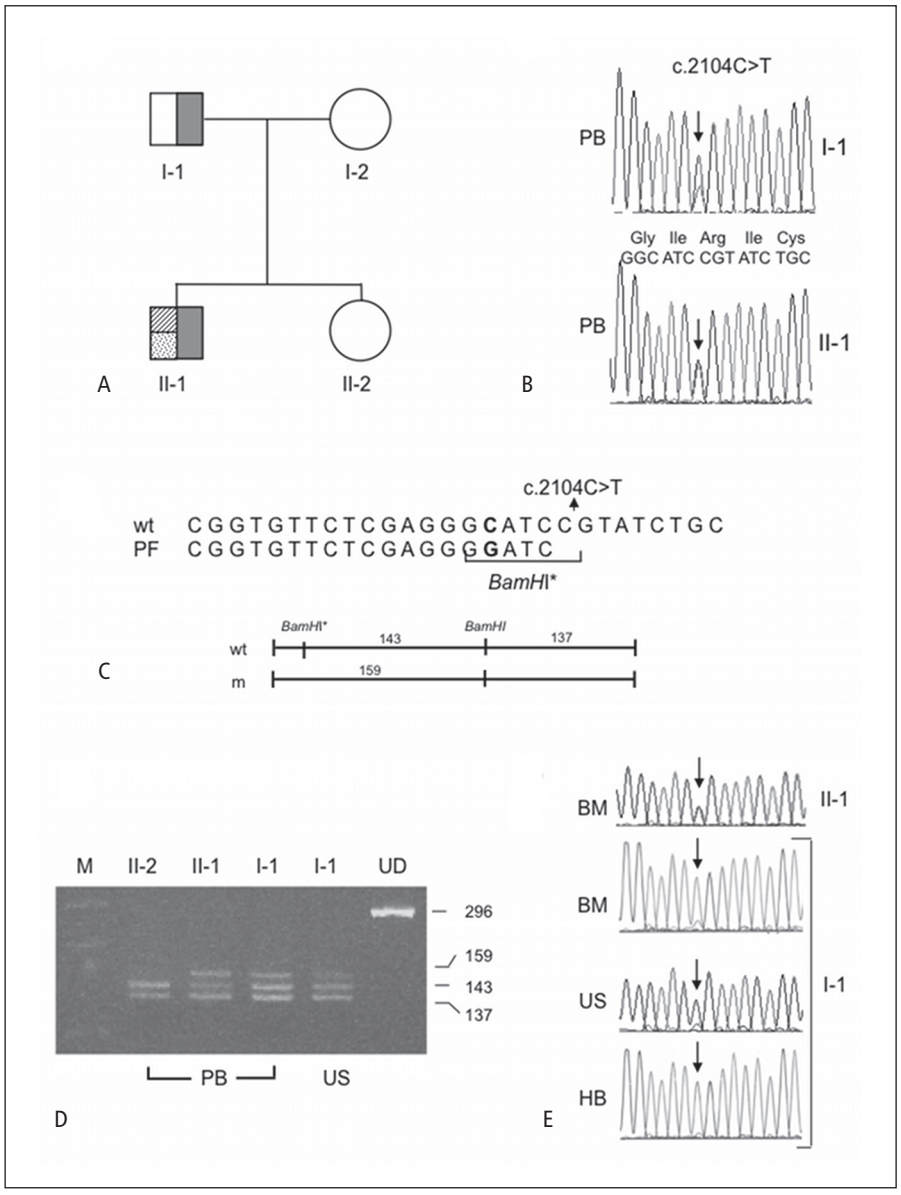

Figure 1: Genetic analysis of the family. A) Pedigree of the family. The proband (II-1) had macrothrombocytopenia (grey rectangle), bilateral sensorineural deafness (crosshatched square), proteinuria and haematuria (dotted square). The father (I-1) had only mild macrothrombocytopenia. B) Identification of the c.2104C>T (p.R702C) in genomic DNA extracted from two different peripheral blood (PB) cell samples of I-1 and II-1. The mutation was not detected in the other two family members. In I-1 the peak "T" of the mutant allele is smaller than in II-1. C) Strategy for restriction full-length polymorphism (RFLP) analysis. The wild-type (wt) sequence of *MYH9* (NM_002473.3) is indicated. The sequence of the forward primer (FP) used for the amplification reactions is shown below. The fourth from last base (bold) of the oligonucleotide was mutated from C to G, allowing the generation of a restriction site for BamHI in the wild-type amplified products. Below, schematic representation of the digestion pattern obtained from wt and mutant (m) amplification products. D) RFLP analysis of products (296 bp) amplified from genomic DNA of peripheral blood (PB) cells and urine sedimentary (US) cells using primer FP (exon 16) and the reverse primer 5'-GATGATGACCTGCATTTCGG-3' (intron 16). After BamHI digestion, wild-type products generated two detectable fragments of 137 and 143 bp whereas mutant products generated bands of 137 and 159 bp. Biological samples of family members I-1, II-1 and II-2 were tested. M, marker; UD, undigested PCR product. E) Sequencing analysis of PCR products amplified from genomic DNA samples of buccal mucosa (BM), urine sediment exfoliated cells (US), and hair bulb (HB). In patient I-1 the mutant "T" peak from all the three tissues is very small as compared with that obtained from buccal mucosa cells of patient II-1.

MYH9-RD is an autosomal dominant disease with 35% of sporadic cases occurring as *de novo* mutations (3). Despite frequent *de novo* events, only two cases of mosaicism, one somatic and one germinal, have been previously described (7, 8). These cases may escape diagnosis due to milder clinical presentation depending on the level of mosaicism. Indeed, clinical presentation varies according to somatic mosaicism frequency and tissue distribution (9). A mutation affecting only tissues of mesodermal origin, as in our patients, will lead to the involvement of haematopoietic cells but not of the other organs and tissues typical of the fully expressed *MYH9*-RD. This observation confirms the importance of molecular genetic testing for the correct diagnosis, prognostic assessment and treatment of *MYH9*-RD patients (2, 3, 10, 11).

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Conflicts of interest
None declared.

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