First report of *MEFV* gene duplication in a patient with familial Mediterranean fever

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Familial Mediterranean fever (FMF [MIM249100]), which causative gene is *MEFV*, is the first-described and most frequent monogenic autoinflammatory disease, and affects people of Mediterranean descent. FMF is characterised by recurrent episodes of fever, abdominal pain due to peritonitis and pleuritis. It is classically a recessively inherited disease. However, a growing number of publications describe patients with only one *MEFV* mutation fulfilling clinical criteria. Most variants associated with FMF are missense mutations located in exon 10 and act as hypermorphic mutations that specifically decrease the activation threshold of the pyrin inflammasome.(1) Here we report and characterise the first duplication of the *MEFV* gene in a typical patient with FMF.

The index case was a 21-year-old woman, non-consanguineous, with a history of recurrent fever. Both parents were asymptomatic. The patient presented classical FMF features, starting from age 6 years. The fever attacks lasted 2 to 3 days, occurred 1 to 2 times a month, and were associated with arthralgia, abdominal pain and vomiting and elevated C-reactive protein level (102 mg/L). She responded well to colchicine. Sanger sequencing of the *MEFV* gene detected the known pathogenic p.Met694Val mutation inherited from her father and the variant of uncertain significance (VUS) p.Glu148Gln inherited from her mother (Fig. 1A). According to current guidelines, this genotype could be consistent with a clinical diagnosis of FMF (2, 3). The autoinflammatory targeted panel sequencing ruled out other SAID and demonstrated an allelic unbalance (1/3 or 2/3) for all *MEFV* variants (Fig. 1B) and we deduced the inheritance of each variant (Fig. 1C). Quantitative PCR (qPCR) analysis of multiple amplicons spanning from *ZNF200* to the 3' end of *MEFV* confirmed the presence of 3 copies of this region in the proband *versus* 2 copies in healthy controls (Fig. 1D). We confirmed that the patient's mother carried the duplication and the breakpoint of the duplication was successfully identified (Fig. 1E). Hence, we concluded that the rearrangement was a tandem duplication in direct orientation and was named chr16:g.3256171_3320350dup (hg19/GRCh37). It was not found in the Database of Genomic Variants (DGV; http://dgv.tcag.ca).

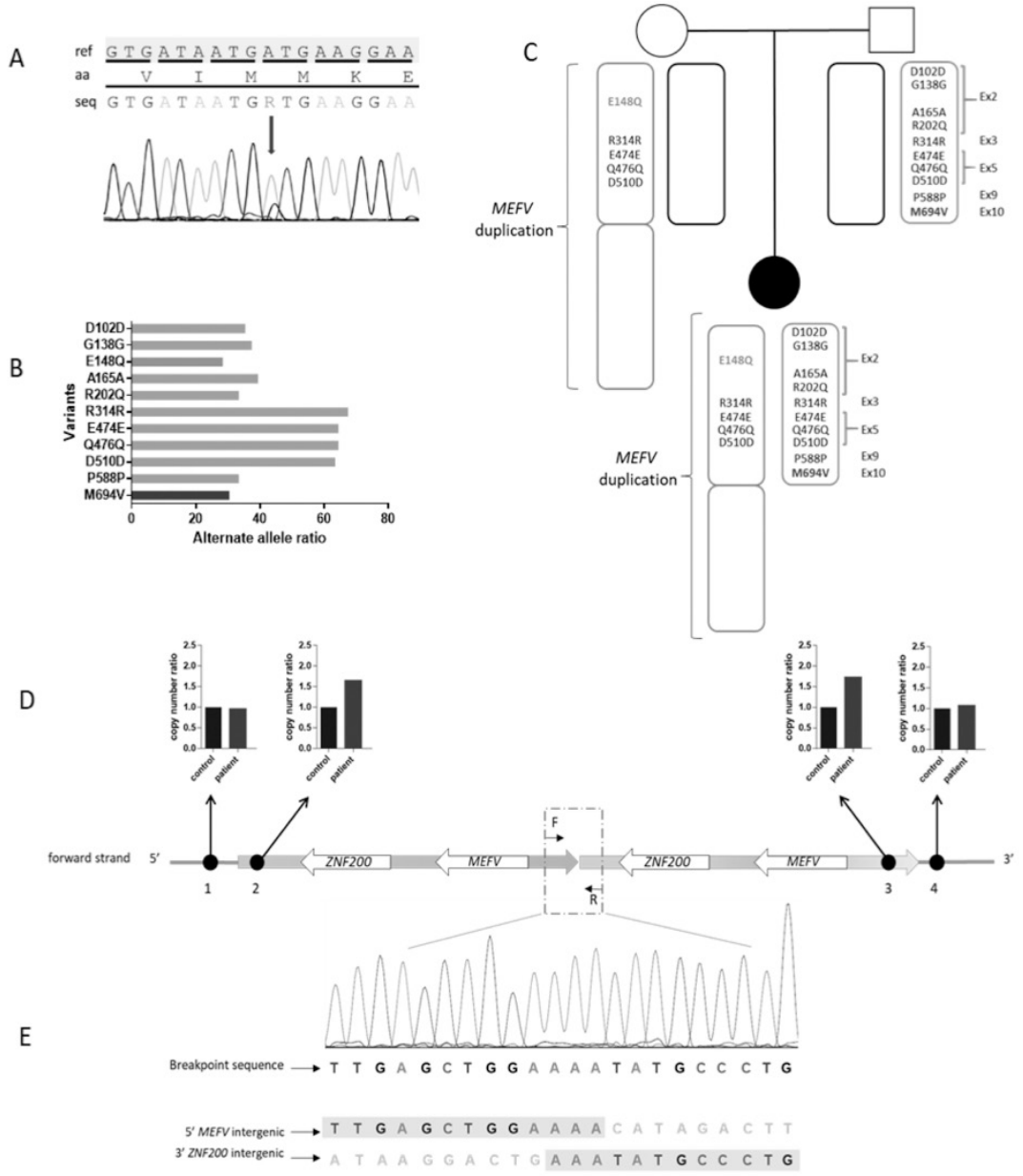

Fig. 1. *MEFV* duplication characterisation.

A: *MEFV* Sanger electropherograms showing the unbalanced allele ratio in the proband for the p.Met694Val mutation. The arrows indicate the mutations. ref: reference nucleotide sequence; aa: reference amino acid sequence; seq: patient nucleotide sequence.

B: Quantitative evaluation of proband alleles by next-generation sequencing, showing a 1/3 or 2/3 ratio. For clarity, in tables, we used a short usual protein denomination, such as M694V, for p.Met694Val.

C: Pedigree with the pathogenic mutation (in bold), the variant of uncertain significance (in bold light grey) and the synonymous variants (in black).

D: Quantitative PCR (qPCR): the large arrows symbolising the tandem duplication and the white arrows representing the *MEFV* and *ZNF200* genes (both located on the reverse strand). The black circles indicate the regions studied by qPCR assay: 1 and 4 are located outside the duplication and show a patient's copy number ratio of 1 (2 copies); 2 and 3 are located inside the duplication and show a patient's copy number ratio of 1.5 (3 copies).

E: Zoom at the breakpoint on the Sanger sequence obtained with primers forward and reverse. Alignment of the wild-type *5'MEFV* and the 3' *ZNF200* intergenic sequences reveals an overlap at a polyA site.

The 2 following hypotheses can be proposed regarding the impact of the genotype on the phenotype of our patient. On the basis of the recessive inheritance pattern and familial segregation, the inherited maternal chromosome should bear one pathogenic *MEFV* variant. The p.Glu148Gln VUS is frequent in the general population and controversial as to whether it is a benign polymorphism or may have a non-specific pro-inflammatory effect. (4) Therefore, the implication of the *MEFV* duplication, alone or combined with p.Glu148Gln, as the second pathogenic allele in trans of p.Met694Val, cannot be ruled out. Whether this duplication could lead to *MEFV* deregulation remains unclear. The familial segregation and the rarity of the duplication advocate for this hypothesis.

Alternatively, the duplication could be an epiphenomenon not related to the pathogenesis in our patient. FMF patients bearing only 50% pathogenic mutation such as p.Met694Val have been well described. However, our patient would be the first with an FMF disease caused by only 1/3 of the pathogenic mutation and, if assumed as a susceptibility factor, 1/3 of the p.Glu148Gln variant. Why individuals with less than 2 pathogenic variants express the disease whereas most carriers, such as her father, do not is largely not understood and likely involves other genetic or environmental factors.

In conclusion, this report highlights the complexity of the genetic pathophysiology in FMF, which does not always follow the "pure" monogenic pattern. The involvement of p.M694V is clearly associated with the disease, but the duplication could play a role in our patient phenotype via a gene dosage effect or *MEFV* deregulation.

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