F402L variant in *NLRP12* in subjects with undiagnosed periodic fevers and in healthy controls

Sirs.

Mutations in the *NLRP12* gene have been associated with an auto-inflammatory disorder similar to the Familial Cold-induced Auto-inflammatory Syndrome (FCAS), thus called FCAS-2 (1). Vitale *et al*. recently described a small group of patients complaining of cold-induced recurrent fevers in which two NLRP12 non-synonymous variants were found, likely representing low-penetrance gene mutations. In particular, the p.F402L variant was identified in five patients and in none of 72 origin-matched controls (2).

In a prospective study on 43 children with recurrent fevers we analysed the exons sequences of five auto-inflammation genes, including *MEFV*, *MVK*, *TNFRSF1A*, *NLRP3* and *NLRP12*. The study recruited patients consecutively referred to our Institute because of recurrent fevers not fulfilling the clinical criteria for any definite auto-inflammatory syndrome. In particular, patients with Periodic Fever Adenitis, Pharyngitis and Aphthae (PFAPA), were considered only if episodes recurred over tonsillectomy, if episodes failed to respond to glucocorticoids or if multi-system symptoms were prominent. In most patients, recurrent fever was associated with other manifestations, including abdominal pain, arthralgia, skin rashes, yet without the clinical pictures typical of other well defined hereditary periodic fevers, such as Familial Mediterranean Fever, Mevalonate Kinase Deficiency and TNF receptor associated periodic fever. Indeed, only one patient from this series was eventually diagnosed with Mevalonate Kinase Deficiency.

Based on our sequencing data, with a particular focus on *NLRP12* gene, nine patients resulted carriers of nucleotide variants on this gene. Specifically, one patient was heterozygous for the known missense variation rs141245482 (c.910C>T; p.H304Y) while the other eight children were heterozygous for the missense variant rs34971363 (c.1206C>G, p.F402L) previously described by Vitale *et al*., with a frequency of 18.6%. However, when we screened 94 healthy blood donors, we identified the F402L variant in 11 control samples (11.7%). Notably, one of the controls had the F402L variant in homozygosis, yet without any clinical auto-inflammatory symptom.

The comparison of our study with the report from Vitale *et al*. raises more questions than it answers.

First, they make reference to the F402L amino acid change but they did not give any indications about the nucleotide exchange, considering that two nucleotide variants (rs199985574:c.1204C>T; rs34971363:c.1206C>G) have been reported to give the same amino acid variation (NCBI dbSNP 138, http://www.ncbi.nlm.nih.gov/SNP/). It would be interesting to know to what nucleotide change they refer to because there is a great difference between the two polymorphisms frequencies in the general population. Specifically, the polymorphism rs34971363, that we identified in our patients, has a MAF of 0.05 while the second one is extremely rare with a MAF of 0.0002 (NHLBI-ESP, http://evs.gs.washington.edu/), albeit both polymorphisms result in the same amino acid variation.

Second, we cannot confirm a definite association between the F402L variant of NLRP12 and any auto-inflammatory phenotype in our series. Even though there was a trend toward an increased frequency of the F402L allele in subjects with recurrent fever, the difference was not significant (8/86 mutated alleles in patients compared to 12/188 in controls).

However, the lack of association may depend on the selection of our patients' series. In fact, in contrast to the report by Vitale *et al*., our study was not based only on patients with cold-induced fever and urticaria. In particular, cold-induced symptoms were not a prominent feature in our series, as the exposure to cold was identified as a trigger only in two patients. Moreover, the phenotype of our patients with F402L variant appeared quite heterogeneous. Tonsillitis, skin rash and abdominal pain was the most frequent symptoms. As concern to treatments most patients showed a poor response to steroids, colchicine was tested in two patients with no significant benefit.

Third, the different prevalence of the mutation in two close populations (12/188 *vs*

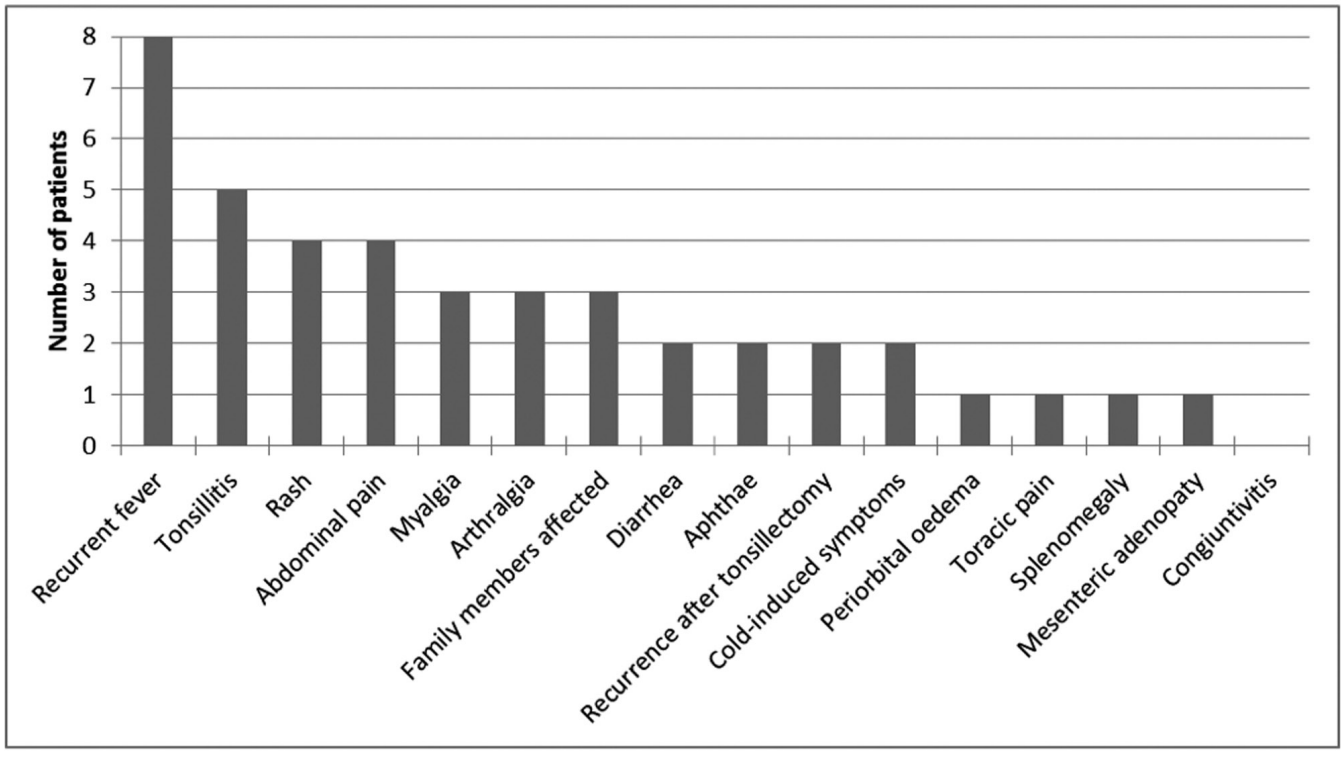

Fig. 1. Clinical features of the patients.

0/144 alleles, *p* 0.002) has no clear explanation and may reflect some bias in the selection of the control groups in the two studies. In any case, we should be prepared for the new challenges of genetic diagnostics in patients with periodic fever syndromes, while novel techniques are making more affordable the sequencing of multiple candidate genes (3). In particular, the relative frequency of possible low-penetrance genetic variants associated with auto-inflammation in the population should raise the question whether some cases of periodic fever actually have a multifactorial rather than a monogenic cause (4, 5).

C. DE PIERI[1]
J. VUCH[1,2]
E. ATHANASAKIS[3]
G.M. SEVERINI[3]
S. CROVELLA[2,3]
A.M. BIANCO[1]
A. TOMMASINI[1]

[1]Department of Paediatrics, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy;
[2]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy;
[3]Department of Advanced Diagnostics and Clinical Trials, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy.

Address correspondence to: Dr C. De Pieri, Department of Paediatrics, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Via dell'Istria 65/1, 34137 Trieste Trieste, Italy.
E-mail: carlodepieri@gmail.com

Competing interests: none declared.

References

1. JERU I, DUQUESNOY P, FERNANDES-ALNEMRI T *et al.*: Mutations in NALP12 cause hereditary periodic fever syndromes. *Proc Nat Acad Sci USA* 2008; 105: 1614-9.
2. VITALE A, RIGANTE D, MAGGIO MC *et al.*: Rare NLRP12 variants associated with the NLRP12-autoinflammatory disorder phenotype: an Italian case series. *Clin Exp Rheumatol* 2013; 31 (Suppl. 77): 155-6.
3. MARCUZZI A, PISCIANZ E, KLEINER G *et al.*: Clinical genetic testing of periodic fever syndromes. *BioMed Res Int* 2013; 2013: 501305.
4. JERU I, CHARMION S, COCHET E *et al.*: Involvement of the same TNFR1 residue in mendelian and multifactorial inflammatory disorders. *PloS One* 2013; 8: e69757.
5. CICCARELLI F, DE MARTINIS M, GINALDI L: An update on autoinflammatory diseases. *Curr Med Chem* 2013; 21: 261-9.