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SHORT REPORT

Unexpected high frequency of P46L *TNFRSF1A* allele in sub-Saharan West African populations

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TNF receptor-associated periodic syndrome (TRAPS) is an autosomal dominant disorder characterized by recurrent attacks of fever and serositis. To date, more than 30 mutations have been reported in *TNFRSF1A*, the responsible gene. In Caucasian populations, the P46L (c.224C > T) *TNFRSF1A* sequence variation is considered as a low-penetrance mutation because its allele frequency is similar in patients and controls (∼1%). Whereas the spectrum of *TNFRSF1A* gene mutations has been well established in Caucasian and several Mediterranean populations, it remains unknown in sub-Saharan African populations. In this study, we found an unexpected high P46L allele frequency (∼10%) in two groups from West Africa – a group of 145 patients with sickle cell anaemia and a group of 349 healthy controls. These data suggest that the P46L variant is rather a polymorphism than a TRAPS causative mutation. We propose that the P46L high frequency in West African populations could be explained by some biological advantage conferred to carriers.

Introduction

TNF receptor-associated periodic syndrome (TRAPS, MIM#142680) is the most frequent autosomal dominant disorder characterized by recurrent attacks of fever, abdominal pain, acute synovial and cutaneous inflammation. More than 30 mutations have been so far reported in *TNFRSF1A*, the causative gene. Most of these mutations are clustered in the region spanning exons 2–4 of the genomic sequence, encoding cysteine-rich extracellular domains of the protein. Upon cellular activation, impaired cleavage of the *TNFRSF1A* ectodomain with correlative decrease in the plasma concentration of the soluble receptor has been

proposed as one of the mechanisms underlying the hyper-inflammatory response in TRAPS.[1] The P46L (c.224C > T) mutation was initially reported in one patient with TRAPS, but was also found in 1.23% of Caucasian and African American control chromosomes.[2] As the authors also demonstrated *in vitro* that P46L reduced *TNFRSF1A* shedding in monocytes, they concluded that P46L is a low-penetrance mutation. A broader study including patients and controls of Caucasian, Arab, Armenian and Sephardic Jewish extractions confirmed previously reported results by demonstrating P46L similar allele frequencies in patients (1.6%) and controls (1.3%).[3] Whereas the spectrum of *TNFRSF1A* gene mutations has been well established in several Caucasian and Mediterranean populations, it remains unknown in sub-Saharan African populations. In this study, we investigated P46L allele frequency in 494 unrelated African individuals not known to have any symptoms compatible with TRAPS.

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Materials and methods
Population analysis
The studied population consisted of two groups. The first group was composed of 145 patients (71 males and 74 females, average age 33.9 years) with typical sickle cell anaemia caused by homozygous SS beta-globin gene mutation; all originated from different African sub-Saharan countries (Benin, Cameroon, Congo, Ivory Coast, Togo and Zaire) and living in France. The second group was composed of 349 healthy women living in Togo and Ivory Coast (average age 26.1 years). Informed consent was obtained from the subjects according to the French Ethics Committee recommendations. The expected genotype distributions were calculated assuming a Hardy–Weinberg equilibrium. The observed and expected genotype distributions and allele frequencies were compared using χ^2 test.

Mutational analysis
The P46L mutation creates a *Stu*I restriction site. After genomic DNA extraction, exon 3 of *TNFRSF1A* gene was amplified by PCR using the following oligonucleotides: forward primer 5′-GGGCTCCTTCCTTGTGTTCT-3′ and reverse primer 5′-CTGACTCTCCTGCCTGTGC-3′, and digested for 4 h at 37°C with *Eco*147I (*Stu*I) (Fermentas, MBI, USA). PCR products were visualized on 2.5% agarose gels stained with ethidium bromide.

Results
In the group of sickle cell anaemia patients of different African origins (see Materials and methods for details) and without any suspicion of TRAPS ($n = 145$), 13 P46L heterozygous and one P46L homozygous individual were found among men and nine heterozygotes were found among women (total allele frequency 8.3%, Table 1). We found 48 heterozygotes and two homozygotes for P46L among 264 symptom-free women living in Togo (allele frequency 9.8%) and 17 heterozygotes and two homozygotes among 85 healthy women from Ivory Coast (allele frequency 12.4%). The P46L frequency was comparable in studied groups (8.3 *vs* 9.8%, $P = 0.45$; 8.3 *vs* 12.4%, $P = 0.16$; 9.8 *vs* 12.4%, $P = 0.35$). We did not find any significant difference between observed and expected

genotype distributions according to the Hardy–Weinberg equilibrium in the studied population (not shown). In addition, we did not find any individual carrying the P46L allele among 328 healthy control subjects of Caucasian ($n = 88$), Sephardic Jewish ($n = 176$) and Arab origins ($n = 64$).

Discussion
The P46L variant was first described as a TRAPS-causative mutation because it was found in one patient suffering from periodic fevers and because it reduced *TNFRSF1A* shedding in monocytes.[2] The defective *TNFRSF1A* shedding, proposed as the mechanism underlying the hyper-inflammatory response in TRAPS,[1] has been demonstrated in monocytes from patients with P46L.[2,4] However, the defective shedding is neither correlated with the presence of *TNFRSF1A* gene mutations nor necessary for the development of TRAPS. Indeed, an impaired *TNFRSF1A* shedding was not present in TRAPS patients with C30S, T37I, R42Q, del42, C52R and N65I *TNFRSF1A* mutations,[2,5,6] but it was observed in affected members of three *TNFRSF1A* mutation-negative families.[6] Supplementary investigations must be performed to understand the exact molecular mechanisms of TRAPS and the significance of an impaired *TNFRSF1A* shedding.

Until now, P46L was considered as a low-penetrance mutation. It was found in 0.6% of Caucasian control chromosomes and in 1.9% of African American control chromosomes.[2] A recent study[3] showed similar P46L allele frequencies in patients (1.6%) and controls (1.3%) of Caucasian, Arab, Armenian and Sephardic Jewish extractions. The present study highlighted an unexpected high P46L allele frequency (9.8%) in sub-Saharan African populations. Surprisingly, none of the P46L carriers identified in our study displayed symptoms compatible with TRAPS. Thus, this observation strongly suggests that the P46L allele may not be a deleterious mutation since it is not sufficient for TRAPS development.

The P46L frequency discrepancy observed between African and non-African populations may have no biological significance. Indeed, a founder effect and a genetic drift could not be excluded, thus a larger study implicating

Table 1 P46L allele distribution in West African populations

Population	P46L heterozygotes	P46L homozygotes	P46L allele frequency
SCA[a] patients[b] ($n = 145$)	22	1	8.3%
Togo healthy controls ($n = 264$)	48	2	9.8%
Ivory Coast healthy controls ($n = 85$)	17	2	12.4%
Total ($n = 494$)	87	5	9.8%

[a]SCA: sickle cell anaemia.
[b]See Materials and methods for detailed patients origins.

other sub-Saharan African populations and a haplotype study of the P46L carrier chromosomes must be undertaken. Nevertheless, we propose that P46L is a functional variant that may have a broader influence on susceptibility to inflammation. Indeed, this allele was found only in 1.9% (3/156) of African American control chromosomes,[2] whereas in our study it was found in 9.8% (97/988, $P<0.005$) of West Africa native's chromosomes. This difference prompts us to speculate that the P46L allele may provide some biological advantage to carriers living in West Africa. A few infectious diseases actually cause selective genetic pressure on host allele frequencies[7] and the implication of TNFα and its receptors in infectious diseases has already been suggested.[8–10] Further investigations must be performed to test this hypothesis.

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