CLINICAL STUDY

Greater prevalence of PROKR2 mutations in Kallmann syndrome patients from the Maghreb than in European patients

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Abstract

Context: Kallmann syndrome (KS) is a genetically heterogeneous developmental disorder that associates hypogonadotropic hypogonadism and anosmia. Various causative genes have been identified, but their respective involvement in different world regions is poorly documented.

Objective: We aimed to compare the prevalence of mutations in five routinely analyzed KS genes between Maghrebian and European patients.

Methods: Blood samples from 120 presumably unrelated Maghrebian patients were collected for DNA sequencing by the Sanger technique. The prevalence of the non-synonymous mutations in KAL1, FGFR1, FGF8, PROKR2, and PROK2 was determined for each gene, and compared with those previously obtained from the analysis of 712 European patients.

Results: Diverse mutations in PROKR2, a gene involved both in monogenic recessive and digenic/oligogenic KS transmission modes, were found in 23.3% of the Maghrebian patients, but only in 5.1% of the European patients (Fisher’s exact test, \[ P < 0.001 \]), whereas mutations in each of the other four KS genes were present either at similar frequencies in the Maghrebian and European patients (KAL1, PROK2, FGF8, from 6.6 to 0.8%; Fisher’s exact test, \[ P > 0.4 \] for all comparisons) or at a lower frequency in Maghrebian patients (FGFR1, 5.0 vs 11.7%; Fisher’s exact test, \[ P < 0.05 \]). Homozygosity resulting from consanguineous marriages was not sufficient to account for the greater prevalence of PROKR2 mutations in the Maghrebian patients.

Conclusions: The great prevalence of PROKR2 mutations in Maghrebian patients has practical consequences for molecular diagnosis of the disease and genetic counseling in the Maghrebian population.

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Introduction

Kallmann syndrome (KS) associates congenital hypogonadotropic hypogonadism, due to gonadotropin-releasing hormone (GNRH) deficiency and anosmia or hyposmia, related to the absence of the olfactory bulbs and tracts (1). KS results from a pathological sequence whereby early developmental failure of the peripheral olfactory system disrupts the embryonic migration of neuroendocrine GNRH-cells from the olfactory epithelium to the brain (2). Patients usually present with the absence of spontaneous puberty. KS is genetically heterogeneous and several causative genes have already been identified including PROKR2 and PROK2, which encode prokineticin receptor 2 and prokineticin 2, respectively (1). PROKR2, a seven-transmembrane domain G-protein-coupled receptor, is one of the two identified receptors to prokineticins 1 and 2, a pair of bioactive peptides, which are considered as pleiotropic chemokine-like cytokines (3, 4, 5, 6). In KS patients, PROKR2 and, less frequently, PROK2 mutations have been found in the heterozygous condition, homozygous, or compound heterozygous states (7, 8, 9, 10, 11, 12, 13, 14). These genes are likely to be involved both in monogenic recessive and complex transmission modes of the KS (1). A digenic/oligogenic mode of transmission in heterozygous patients carrying PROKR2 mutations has indeed been put forward based on identifications in patients homozygous for the same mutations and in unaffected heterozygotes in the general population (7, 8, 11, 13 and Exome Variant Server website URL: http://evs.gs.washington.edu/EVS/), but digenic disease transmission has so far been validated in only a few patients (7, 10, 13, 15).

Here, we aimed to compare the prevalence of mutations in five routinely analyzed KS genes between...
Maghrebian and European patients, and report on the unexpectedly large prevalence of PROKR2 mutations in KS patients from the Maghreb.

Subjects and methods

This study has been approved by the local ethics committee. Informed consent was obtained from all the adult individuals who participated in the study and from the parents of the minor participants. Genomic DNAs were obtained directly from blood samples or from lymphoblastoid cell lines using a standard extraction procedure. The exons and exon–intron boundaries of the five genes routinely analyzed for KS molecular diagnosis, namely KAL1 (MIM# 300836), encoding the extracellular matrix glycoprotein anosmin-1, FGFR1 (MIM# 136350), encoding fibroblast growth factor receptor 1, FGF8 (MIM# 600483), encoding fibroblast growth factor 8, PROKR2 (MIM# 607123), and PROK2 (MIM# 607002), were PCR-amplified and sequenced in every patient, using standard PCR and Sanger sequencing conditions (7, 16, 17, 18). The mutations were confirmed by sequencing two independent PCR products on both DNA strands. In addition, mutations in PROKR2 were sought in a control group of 164 unrelated Maghrebian individuals by PCR-amplification and sequencing of the corresponding PCR products.

Results

We sought mutations in the coding regions of KAL1, responsible for the X chromosome-linked form of KS (KAL1), FGFR1 and FGF8, involved in the autosomal dominant form of the disease (KAL2), and PROKR2 and PROK2, involved both in the autosomal recessive form (KAL3) and in a digenic/oligogenic mode of KS transmission, in 120 presumably unrelated Maghrebian patients (91 males and 29 females) originating from Morocco, Algeria, and Tunisia. Most patients were diagnosed with KS in their second decade because of the absence of spontaneous puberty and low sex hormone and gonadotropin blood levels, together with a defective sense of smell (many patients, however, did not undergo formal olfactory testing), but in a few male cases, the presence of a microphallus and/or bilateral cryptorchidism at birth led to earlier diagnosis. A few patients displayed additional non-reproductive, non-olfactory clinical abnormalities, namely hearing impairment (three patients), pes cavus (two patients), cleft lip and palate (one patient), unique upper incisor (one patient), or ichthyosis (one patient). A list of the 22 different non-synonymous mutations identified is provided in Table 1. Only one of these patients had mutations in more than one KS gene, namely missense mutations in KAL1 (p.S396L) and PROKR2 (p.P290S/+).

We compared, for each KS gene, the prevalence of non-synonymous mutations in the Maghrebian patients and in 712 previously analyzed non-Maghrebian KS patients (510 males and 202 females) mainly of European origin ([13] and C Fouveaut, C Leroy and C Dodé 2012, unpublished results). Much to our surprise, we found that 28 (23 males and five females) out of 120 Maghrebian patients (23.3%) carried PROKR2 mutations, whereas only 36 (24 males and 12 females) out of 712 non-Maghrebian patients (5.1%) did (Fisher’s exact test, P < 0.001). In contrast, the prevalence of the mutations in each of the other four KS genes analyzed was either not statistically different between non-Maghrebian and Maghrebian patients (KAL1, FGF8, PROK2; Fisher’s exact test, P > 0.4 for all comparisons) or lower in the Maghrebian patients (FGFR1; Fisher’s exact test, P < 0.05) (Table 2). In addition, the most prevalent PROKR2 mutations were different between the two groups of patients. The main PROKR2 mutation in the European patients was the ancient founder mutation, c.518T>G (p.L173R), which was present in 17/43 (39.5%) mutated alleles (19), whereas c.868C>T (p.R290S) (15/36 mutated alleles, 41.7%), c.253C>T (p.P290S) (8/36 mutated alleles, 22.2%), and c.802C>T (p.R268C) (6/36 mutated alleles, 16.7%) were the most frequent (three out of seven) different PROKR2 mutations found in the Maghrebian patients (Tables 3 and 4). These three mutations were also identified, in the heterozygous state, in a total of six out of 164 (3.7%) unrelated control individuals from the Maghreb population. Other PROKR2 mutations were present in one Maghrebian patient each (three patients for p.L173R), and were not found in the control group (Tables 3 and 4).

Table 1 Non-synonymous mutations of KAL1, FGFR1, FGF8, PROKR2, and PROK2 in the Maghrebian KS patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
</tr>
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<tbody>
<tr>
<td>KAL1</td>
<td>c.1187C&gt;T</td>
<td>p.S396L</td>
</tr>
<tr>
<td></td>
<td>c.1711T&gt;A</td>
<td>p.W571R</td>
</tr>
<tr>
<td></td>
<td>c.1735_1738del</td>
<td></td>
</tr>
<tr>
<td>FGFR1</td>
<td>c.424_427del</td>
<td>Frameshift</td>
</tr>
<tr>
<td></td>
<td>c.1907_1908del</td>
<td>Frameshift</td>
</tr>
<tr>
<td></td>
<td>IVS12−2A&gt;G</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IVS14+1G&gt;A</td>
<td></td>
</tr>
<tr>
<td>FGF8</td>
<td>c.506C&gt;T</td>
<td>p.A169V</td>
</tr>
<tr>
<td></td>
<td>c.253C&gt;T</td>
<td>p.R85C</td>
</tr>
<tr>
<td>PROKR2</td>
<td>c.253C&gt;G</td>
<td>p.R85G</td>
</tr>
<tr>
<td></td>
<td>c.254G&gt;A</td>
<td>p.R85H</td>
</tr>
<tr>
<td></td>
<td>c.518T&gt;G</td>
<td>p.L173R</td>
</tr>
<tr>
<td>PROK2</td>
<td>c.137G&gt;A</td>
<td>p.C46Y</td>
</tr>
<tr>
<td></td>
<td>c.301C&gt;T</td>
<td>p.R101W</td>
</tr>
<tr>
<td></td>
<td>c.364C&gt;T</td>
<td>p.R122X</td>
</tr>
</tbody>
</table>


Of the five KS genes analyzed, only PROKR2 and PROK2 have been implicated in the autosomal recessive form of the disease (KAL3). Thus, we asked whether the greater prevalence of PROKR2 mutations in Maghrebian patients than in European patients could simply reflect a greater proportion of KAL3 cases in the Maghrebian patients due to the higher frequency of inbreeding in Maghrebian countries, which has been estimated at ~30% of the unions (20). The proportion of the Maghrebian patients born to consanguineous parents is unknown, but the ratio of KS patients who carry PROKR2 mutations in homozygous state to the total number of patients carrying mutations in the gene, 7/28 (25%), may provide an estimate (see Table 3). Because the proportion of patients carrying PROKR2 mutations in the non-homozygous (i.e., heterozygous or compound heterozygous) state was still much larger in the Maghrebian cohort (17.5%) than in the non-Maghrebian cohort (4.6%; Fisher’s exact test, P < 0.01), we were able to conclude that consanguineous marriages are not sufficient to account for the greater prevalence of PROKR2 mutations in Maghrebian KS patients.

### Discussion

By sequencing the exons and exon–intron junctions of the five routinely analyzed KS genes in 120 KS patients originating from Maghrebian countries, we found that the prevalence of non-synonymous mutations in one of these genes, PROKR2, was four to five times greater than in European patients. All the PROKR2 mutations identified in the Maghrebian patients are missense mutations that have already been shown to have deleterious effects on PROKR2-signaling in transfected cells (12). The third most frequent mutation, p.R268C, however deserves special discussion because the activity of the mutated receptor in transfected cells was wildly impaired (12) Oualid Sbai and C Dode 2013, unpublished results), and the mutation has been found in ~8% of the African-American general population, both in the heterozygous state (7.9%) and in the homozygous state (0.3%), whereas each of the other six mutations identified in the patients has been reported, only in the heterozygous state, in <1% (<0.05% for the two most prevalent mutations) of the European-American and African-American populations (see Exome Variant Server website URL: http://evs.gs.washington.edu/EVS/). In addition, the p.R268C mutation and a clearly pathogenic mutation, p.G234D, have recently been found

### Table 2 Frequencies of non-synonymous mutations in five KS genes in Maghrebian and non-Maghrebian KS patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Non-Maghrebian patients (M + F) with mutations</th>
<th>Mutated alleles in non-Maghrebian patients</th>
<th>Maghrebian patients (M + F) with mutations</th>
<th>Mutated alleles in Maghrebian patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAL1</td>
<td>47 (47 + 0)/712 (6.6%)</td>
<td>47/712</td>
<td>5 (5 + 0)/120 (4.2%)</td>
<td>5/120</td>
</tr>
<tr>
<td>FGFR1</td>
<td>83 (53 + 30)/712 (11.7%)</td>
<td>83/1424</td>
<td>6 (4 + 2)/120 (5.0%)</td>
<td>6/240</td>
</tr>
<tr>
<td>FGFR8</td>
<td>7 (6 + 1)/712 (1.0%)</td>
<td>7/1424</td>
<td>1 (0 + 1)/120 (0.8%)</td>
<td>2/240</td>
</tr>
<tr>
<td>PROKR2</td>
<td>36 (24 + 12)/712 (5.1%)</td>
<td>44/1424</td>
<td>28 (23 + 5)/120 (23.3%)</td>
<td>36/240</td>
</tr>
<tr>
<td>PROK2</td>
<td>14 (11 + 3)/712 (2.0%)</td>
<td>15/1424</td>
<td>3 (0 + 3)/120 (2.5%)</td>
<td>3/240</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>181 (137 + 44)/712 (25.4%)</td>
<td>181/712</td>
<td>42 (31 + 11)/120 (35.0%)</td>
<td>42/120</td>
</tr>
</tbody>
</table>

M and F denote the number of males and females respectively.
together, both in the homozygous state, in two KS siblings (14). Therefore, the pathogenic effect of the p.R268C mutation is questionable, but even if we exclude this particular sequence variant, the proportion of the Maghrebian KS patients who carried presumably pathogenic mutations in PROKR2 (19.2%) was still much greater than in the European patients (Fisher’s exact test, \( P < 0.01 \)).

What could be the reason for the prevalence of PROKR2 pathogenic mutations being greater in Maghrebian KS patients than in European patients, as the larger ratio of consanguineous unions in the Maghreb is not sufficient to explain the difference? Population bottleneck or founder effects, arising from basic processes in demographic history, may sometimes account for the greater prevalence of DNA sequence variants in human populations. In many respects, these two effects are similar as both arise from a small ancestral population, followed by rapid expansion. Population bottleneck refers to the reduction in size of a single, previously larger, population and a loss of prior diversity, while founder effect relates to the process of colonization by a few original founders, who carry only a small fraction of the total genetic diversity of the source population. Here, at least two independent population bottleneck or founder events, both having an impact on sequence variants of the same gene (PROKR2), would be required to explain on their own (i.e., without an additional positive selection pressure, see below) the two different PROKR2 mutations most prevalent in the Maghrebian patients (p.P290S, p.R85C), which may be considered rather unlikely. Another possible explanation is balancing selection (22), whereby some positive selection pressure would exert on the PROKR2 loss-of-function mutations in the heterozygous state, for instance because of the suspected involvement of prokineticin-signaling in preterm labor and premature birth (23, 24), or because this prokineticin receptor might also be a cell surface receptor to specific infectious agents, as shown for another G protein-coupled receptor, the CC chemokine receptor-5, and human immunodeficiency virus type 1 infection (25, 26). In most reported examples of balancing selection, however, the frequency of individuals heterozygous for the advantageous mutations is higher than the frequency of PROKR2 heterozygotes we observed in the Maghrebian population, i.e. 3.7% (or 1.8% if we exclude individuals carrying the p.R268C mutation). In addition, one would still have to explain why such a positive selection pressure specifically or preferentially exerts in this particular population. Finally, a third possibility could involve another gene of the PROKR2 signaling pathway and some advantageous allele of that gene enhancing prokineticin signaling through the receptor. Large enough prevalence of this particular, as yet unidentified allele in the Maghrebian population, which could result from only one founder (or population bottleneck) event, would therefore partially ‘relax’ the negative, purifying selection pressure that continuously exerts on the PROKR2 pathogenic mutations because KS strongly affects reproductive fitness. As a corollary, one would expect to find a larger proportion of heterozygous carriers of pathogenic PROKR2 mutations in the Maghrebian general population than in the European one, for which this proportion has previously been estimated at 3/250 (1.2%) (7). This value is not statistically different from the greater value we found in the group of 164 Maghrebian controls (1.8%), but this could be due to the relatively small size of the samples.

In conclusion, the great prevalence of PROKR2 mutations in KS patients originating from the Maghreb not only has practical consequences for the molecular diagnosis of this developmental disease and genetic counseling in the Maghrebian population, but also led us to elaborate on the concept of partial relaxation of the purifying selection pressure, which may also be relevant to other genetic diseases affecting reproduction with complex patterns of inheritance. The hypothetical relaxation on PROKR2 mutations in the Maghrebian population has still to be documented at the molecular level. In this respect, whole exome sequencing strategies followed by in vitro functional studies should be instrumental to characterize non-neutral sequence variants of genes involved in prokineticin-signaling.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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