

Identification of two different mutations in the PDS gene in an inbred family with Pendred syndrome

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Abstract

Recently the gene responsible for Pendred syndrome (PDS) was isolated and several mutations in the PDS gene have been identified in Pendred patients. Here we report the occurrence of two different PDS mutations in an extended inbred Turkish family. The majority of patients in this family are homozygous for a splice site mutation (1143-2A→G) affecting the 3' splice site consensus sequence of intron 7. However, two affected sibs with non-consanguineous parents are compound heterozygotes for the splice site mutation and a missense mutation (1558T→G), substituting an evolutionarily conserved amino acid. The latter mutation has been found previously in two Pendred families originating from The Netherlands, indicating that the 1558T→G mutation may be a common mutation.

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Pendred syndrome (MIM 274600) is an autosomal recessive disorder with an estimated frequency of 1-8 per 100 000 characterised by prelingual deafness and goitre.¹ Additional abnormalities are an iodide organification defect that can be shown by the perchlorate

discharge test,² an abnormally developed cochlea (Mondini malformation), and a widened vestibular aqueduct.³⁻⁴ In 1996, the Pendred syndrome gene was localised on chromosome 7q31, initially in a region of 5.5 cM.⁵ This region was then refined^{6,7} and, recently, the Pendred syndrome gene (PDS) was isolated.⁸ The gene encodes a transmembrane protein, named pendrin, that is closely related to known sulphate transporters. The homology of pendrin to two other sulphate transporters implicated in human diseases, "down regulated in adenoma" (DRA) involved in congenital chloride diarrhoea,⁹ and a sulphate transporter (DTD) involved in diastrophic dysplasia,¹⁰ suggests that the sulphate transporter gene family is clinically important. However, the exact function of pendrin in the thyroid and its involvement in cochlear development remain to be elucidated. To date, 29 different mutations in the PDS gene have been reported in Pendred syndrome patients.^{8,11,12} Four PDS mutations are found to be recurrent, at least in the western European population (L236P, E384G, T416P, and 1001+1G→A).^{11,12} Interfamilial as well as intrafamilial clinical variability has been described in Pendred syndrome¹³ and recently a PDS mutation has been reported in a family with non-syndromic recessive deafness.¹⁴

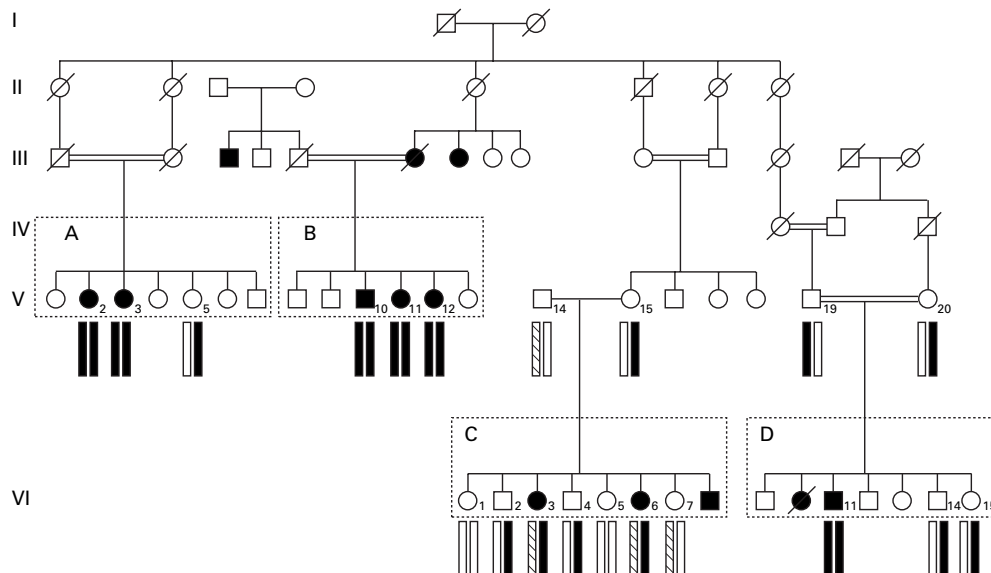


Figure 1 Pedigree of the inbred Pendred syndrome family. Only family members from whom DNA was obtained are numbered. Black bars beneath subjects represent the 1143-2A→G mutation, striped bars represent the 1558T→G mutation, and white bars represent wild type alleles.

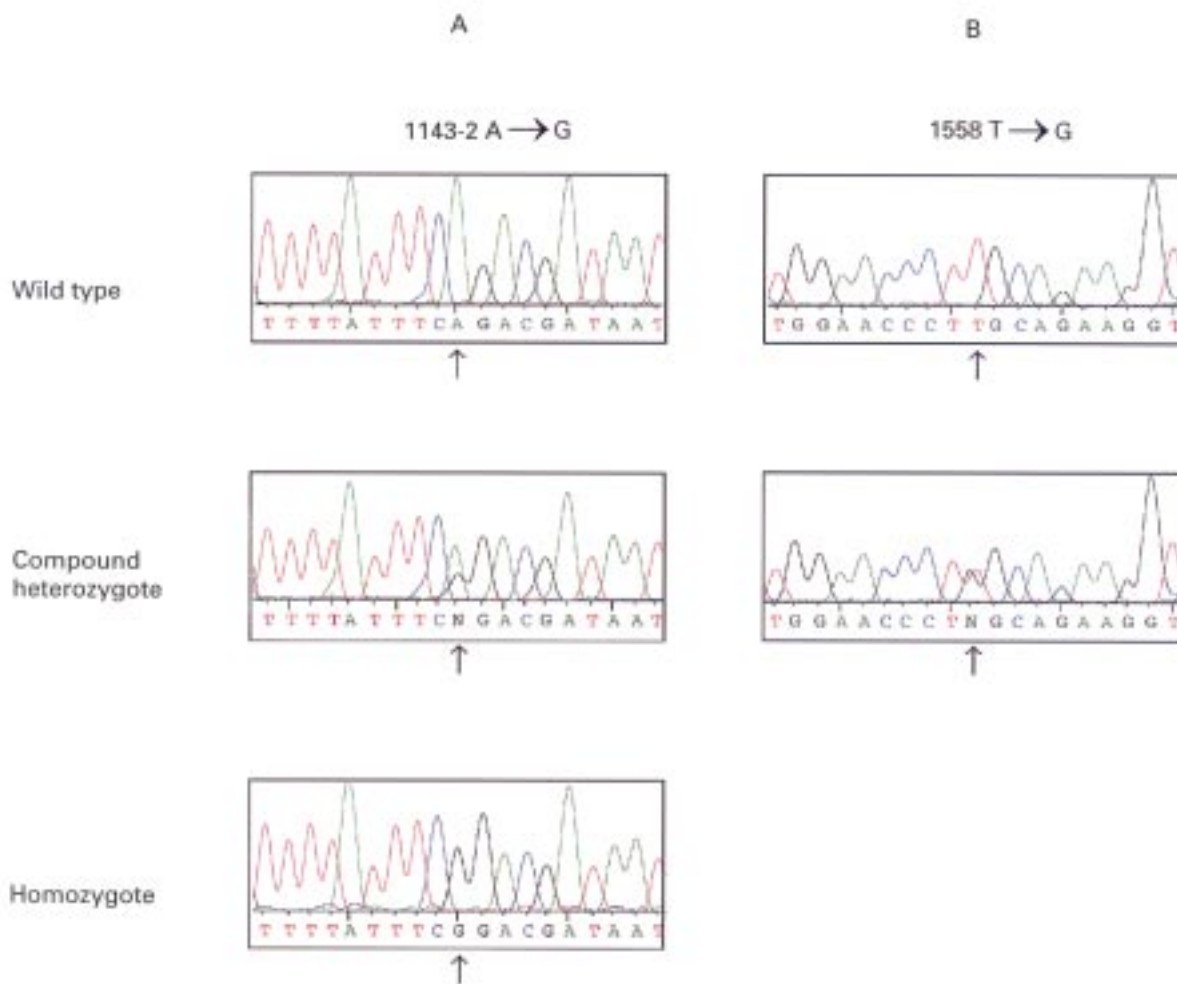


Figure 2 Sequence analysis of the region flanking the 1143-2A→G and the 1558T→G mutations. The arrows point to the variant nucleotide. (A) To illustrate the 1143-2A→G mutation, the sequence from control DNA, a compound heterozygote, and a homozygote for the mutation are shown. (B) To illustrate the 1558T→G mutation, the sequence from a control and a compound heterozygote are shown.

Here we report two different PDS mutations, one novel and one previously reported, segregating in an extended inbred Pendred syndrome family originating from a small and isolated Turkish village.⁶ The family includes more than 13 affected subjects, all having prelingual hearing loss and a palpable goitre.¹⁵ In a previous study, linkage analysis with markers flanking the PDS gene showed two different disease haplotypes.⁶ Six affected subjects, all with consanguineous parents, were homozygous for the closest flanking markers, whereas two patients with non-consanguineous parents were heterozygous. Several possibilities could explain these results. Firstly, two different mutations segregating on distinct haplotypes could be responsible for Pendred syndrome in this family. Secondly, a single mutation could be responsible for Pendred syndrome, originating from a very distant common ancestor and with different haplotypes evolving from numerous recombinations. A third possibility is the independent occurrence of the same mutation on distinct haplotypes, perhaps as the result of a mutation hot spot.

To identify the PDS mutations in this family, DNA from two patients (V.2 and VI.3) of the pedigree (fig 1) was analysed. Patient V.2, a member of consanguineous branch A, was expected to be homozygous for a PDS mutation, whereas patient VI.3, whose parents are not consanguineous, was expected to be a compound heterozygote (assuming the presence of two different mutations).

Mutation detection of the PDS gene was performed by direct sequencing of PCR products using the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, CA). Genomic DNA was used as template for PCR amplification of DNA segments containing the PDS exons, as described previously.⁸ A homozygous A to G substitution was observed at the 3' splice site of intron 7 (1143-2A→G) in patient V.2 (fig 2A). Patient VI.3 was found to be a compound heterozygote for this splice site mutation and a T to G substitution at position 1558 in exon 11 (1558T→G) (fig 2B).

Linkage analysis in this family showed that the 1143-2A→G mutation segregated with Pendred syndrome. All the patients from the

consanguineous branches of the family were homozygous for this mutation (fig 1), whereas the two patients in branch C (VI.3 and VI.6) were heterozygous. As the 1143-2A→G mutation changes a 100% conserved nucleotide of the 3' splice site consensus sequence,¹⁶ the mutation most probably affects splicing of the PDS gene. However, as the PDS gene is only expressed in thyroid, kidney, and brain,⁸ and as none of these tissues was available from this family, we were unable to analyse the effect of the mutation at the mRNA level. Even with nested PCR, we failed to amplify fragments of the PDS gene from EBV transformed B cells (data not shown).

The presence of the 1558T→G mutation was analysed by sequence analysis in branch C, the non-consanguineous part of the pedigree (fig 1), and found to be present in patients VI.3 and VI.6 and also in VI.7, which is consistent with the previously described haplotype analysis.⁶ The mutation leads to the predicted substitution of Leu for Trp at position 445 of the pendrin protein (L445W). Alignment of the PDS sequence with four other homologous genes (human DRA, human DTD, mouse DTD, and rat sulphate anion transporter (SAT1)) showed that the L445W mutation affects a conserved amino acid, and thus most probably results in pendrin protein dysfunction. The possibility that the 1558T→G mutation represents a polymorphism in the Turkish population, rather than a disease causing mutation, could not formally be excluded as not enough DNA samples from this Turkish community were available to us. However, the 1558T→G mutation has also been found in two other Pendred syndrome families originating from The Netherlands, whereas it was not found in 50 independent controls from western Europe.^{3, 12} These data suggest that the 1558T→G mutation is disease causing. Anamnestically, no familial relationships between the three families are known. Furthermore, a different haplotype, including the intragenic marker D7S2459, is segregating with the disease at least for the Turkish and one Dutch families. The genetic relationship between the two Dutch families could not be investigated, as DNA from one of the families was not available in our laboratory. This suggests that this mutation has independently arisen several times, which is in contrast with the other frequent PDS mutations, for which founder effects have been shown.^{11, 12} However, the nucleotide sequences surrounding the 1558T→G mutation are devoid of obvious elements that might explain an increased mutation rate at this position.

It might seem surprising that two different mutations are present in this highly inbred family. However, similar findings have been

reported for several other disorders,¹⁷⁻¹⁹ and it has been suggested that multiple mutations in a single gene may be a relatively common phenomenon in inbred communities.²⁰

The identification of two different mutations in this consanguineous family indicates that great care should be taken when inbred families of this type are used for linkage analysis by homozygosity mapping.

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