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CASES SERIES AND REPORTS

Further characterisation of the recently described *SLC26A4* c.918+2T>C mutation and reporting of a novel variant predicted to be damaging

Caratterizzazione della mutazione SLC26A4 c.918+2T>C e report di una nuova variante potenzialmente a rischio

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SUMMARY

Pendred syndrome (PS) is the second most common type of autosomal recessive syndromic hearing loss (HL). It is characterised by sensorineural HL and goiter with occasional hypothyroidism. These features are generally accompanied by malformations of the inner ear, as enlarged vestibular aqueduct (EVA). In about 50% of probands, mutations in the *SLC26A4* gene are the cause of the disease. Here we report the case of a Portuguese female, aged 47, presenting with severe to profound HL and hypothyroidism. Her mother and sister, both deceased, had suffered from HL and goiter. By MRI and CT, an enlarged vestibular aqueduct and endolymphatic sac were observed. Molecular study of the patient included screening for *GJB2* coding mutations and *GJB6* common deletions followed by screening of all *SLC26A4* exons, as well as intronic regions 8 and 14. Mutation c.918+2T>C was found for the first time in homozygosity in the intronic region 7 of the *SLC26A4* gene. Whilst sequencing the control samples, a novel mutation c.821C>G was found in heterozygosity in the exon 7 of *SLC26A4* gene and was predicted to be damaging. This study thus led to the finding of two novel *SLC26A4* genotypes and provides new insight on the phenotypic features associated with PS.

KEY WORDS: Pendred syndrome (PS) • Hearing loss (HL) • Enlarged vestibular aqueduct (EVA) • Magnetic resonance imaging (MRI) • Computerised tomography (CT) • Videonystagmography (VNG) • Berkeley Drosophila Genome Project (BDGP)

RIASSUNTO

La sindrome di Pendred è, in ordine di frequenza, la seconda causa di ipoacusia su base genetica autosomica recessiva. Si manifesta con un ipoacusia accompagnata dalla presenza di un gozzo tiroideo con eventuale ipotiroidismo. Tali caratteristiche si accompagnano a malformazioni dell'orecchio interno, quali l'acquedotto vestibolare largo. Nel 50% dei casi vi è una mutazione del gene SLC26A4. Riportiamo nel presente lavoro il caso di una paziente portoghese di 47 anni affetta da ipoacusia di grado severo/profondo e ipotiroidismo. La madre e la sorella della paziente, entrambe decedute, erano a loro volta affette da ipoacusia associata a gozzo tiroideo. La risonanza magnetica e la TC hanno entrambe evidenziato un allargamento dell'acquedotto vestibolare e del sacco endolinfatico. La paziente è stata sottoposta a uno studio di GJB2 e GJB6 seguiti da uno screening di tutti gli esoni di SLC26A4 e delle regioni introniche 8 e 14. È stata rilevata, per la prima volta in omozigosi, una mutazione c.918 + 2T>C nella regione intronica 7 del gene SLC26A4. Sequenziando i campioni di controllo è stata rilevata una nuova mutazione c.821C>G presente in eterozigosi nell'estone 7 del gene SLC26A4, per la quale si è ipotizzato un ruolo dannoso. Il presente studio ha condotto alla scoperta di due nuovi genotipi di SLC26A4, e alla miglior definizione degli aspetti fenotipici associati alla sindrome di Pendred.

PAROLE CHIAVE: *Sindrome di Pendred • Ipoacusia • Sindrome dell'acquedotto vestibolare largo • Risonanza magnetica • Tac • Videonistagmografia • Berkeley Drosophila Genome Project*

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Introduction

Hereditary syndromic hearing loss (HL) includes about 400 syndromes, such as Pendred syndrome (PS). This syndrome is the second most common type of autosomal recessive syndromic HL worldwide [1], with an incidence estimated to be as high as 7.5 to 10 in 100,000 individuals [2 3].

PS is characterised by sensorineural HL, goiter and a partial defect in iodide organification. These features are generally accompanied by malformations of the inner ear, ranging from enlarged vestibular aqueduct (EVA) to Mondini dysplasia [4]. The clinical features observed in PS typically result from biallelic (homozygote/compound heterozygote) mutations in the *SLC26A4* gene. According to the Human Gene Mutation Database more than 260 mutations in the *SLC26A4* gene have been identified to date [5], including splice site aberrations, frame shift and nonsense mutations, as well as large deletions (rare cases) and a relatively common mutation, c.-103 T > C, in a regulatory element of the promoter region of the *SLC26A4* gene [6 7]. The mutation spectrum of *SLC26A4* varies widely among ethnic groups, with certain mutations demonstrating a higher prevalence in specific populations [8-11].

This gene, containing 21 exons, localises to chromosome 7 (7q22.3-q31.1) and encodes the multifunctional anion exchanger pendrin [4 12]. Pendrin is a 73 kDa membrane protein that belongs to the SLC26 anion transporter family. It is comprised of 780 amino acids and is predicted to have 12 putative transmembrane domains, with both the amino- and carboxy-termini located on the cytosol [13 14]. In the C-terminus region a STAS domain (Sulfate Transporter Antagonist of Anti-Sigma Factor) is located,which probably plays an important role in the biosynthesis, function and regulation of this transporter [15 16]. The *SLC26A4* gene is expressed in specific areas of the endolymphatic compartment in the cochlea known to play a role in the endolymph reabsorption [17]. Moreover, in the absence of pendrin, profound prenatal endolymphatic hydrops are observed along with the destruction of many of the epithelial cells surrounding the scala media [17]. Regarding the thyroid organ, pendrin is involved in iodide metabolism as it transports intracellular iodide to the follicular lumen [18] where the normal processes of iodide accumulation, oxidation and organification into thyroglobulin, leading to the production of the thyroid hormone, take place [18]. Patients with PS present a dysfunctional pendrin protein, and the thyroid gland is unable to accumulate and maintain iodide in the follicular lumen, place where thyroglobulin is kept and incorporates iodide to synthesise thyroid hormone [19]. Due to a defect in the synthesis of thyroid hormone, pathologies such as compensatory goiter and hypothyroidism may be present in these patients [19]. Herein, we report the case of a Portuguese female, aged 47, presenting with severe to profound HL and hypothyroidism.

Materials and methods

A Portuguese female presenting with severe to profound HL (Fig. 1) and hypothyroidism was referred for genetic analysis. This patient later reported that her mother and sister, both deceased, had also suffered from HL and goiter.

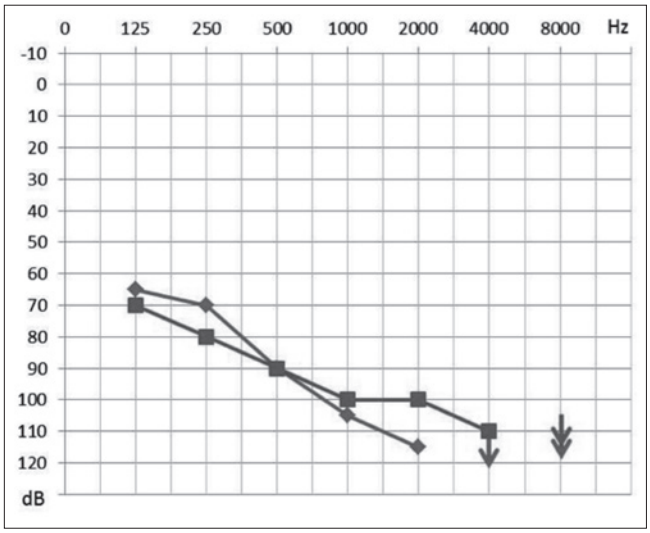

Fig. 1. Audiogram of the patient. Squares in red represent the right ear; diamonds in blue represent the left ear.

Hearing levels were determined by pure-tone audiometry. Imaging study of the ear was performed by magnetic resonance imaging (MRI), computed tomography (CT) and videonystagmography (VNG). A complete clinical history was taken to exclude aetiologies for HL such as infection, acoustic trauma, or ototoxic drugs. The patient reported no familial consanguinity, although this possibility cannot be excluded.

Blood samples were collected after written informed consent was obtained. Total genomic DNA was extracted from peripheral blood using the JetQuick Blood and Cell Culture Kit (Genomed).

Molecular study of the proband included screening of *GJB2*, *GJB6* and *SLC26A4* genes. The most common *GJB6* deletions were screened by multiplex PCR, using the method described by del Castillo [20]. Automated sequencing was performed for the coding exon of the *GJB2* gene [21], and for all exons, as well as intronic regions 7 and 14, of the *SLC26A4* gene (Table I).

Two hundred control chromosomes, from 100 self-reported normal hearing individuals from the Portuguese population, were sequenced for intronic region 7 and exon 7 of the *SLC26A4* gene.

All PCR products were purified using a Jetquick PCR Product Purification Spin Kit (Genomed). The electrophoretograms from bidirectional sequencing were evaluated by visual inspection and pairwise alignment to reference sequences using NCBI's BLAST [22].

The Berkeley Drosophila Genome Project (BDGP) [23] splice site prediction program was used to predict the effect of the splicing mutation found in the patient. The SIFT prediction software [24] was used to predict the effect of a new variant, c.821C > G (p.Ala274Gly), identified in an individual of the control sample.

Table I. *SLC26A4* exons and intronic regions studied.

Primer name	Region	Primer sequence (5'-3')	Amplified region (bp)
SLC26A4 2F	Exon 2	GGCTGCAGCTAACAGGTGATC	432
SLC26A4 2R		GAGGACCGGAGACCGAAAGTC	
SLC26A4 3F	Exon 3	ACAGTTCTTGGCAAAAGCATGG	411
SLC26A4 3R		GAAGGGTAAGCAACCATCTGTCAC	
SLC26A4 4F	Exon 4	TTTGCATCATCATAAAGGCAAAGTC	419
SLC26A4 4R		TGAAATCCCATTTCCCTGACAA	
SLC26A4 5F	Exon 5	CTCAGCTTCTTTCGTGAACAAAC	439
SLC26A4 5R		TTTGGGTTCCAGGAAATTACTTTGT	
SLC26A4 6F	Exon 6	GTGCTATAGGCAGGCTACTAGTGTT	364
SLC26A4 6R		CCTGGCCCAGACTCAGAGAAT	
SLC26A4 7/8F	Exons 7 and 8	TGGGAAGATTCATATGAGAATTGATTG	581
SLC26A4 7/8R		TGGTTGTTTCTTCCAGATCACA	
SLC26A4 IVS8F	Intron 8 (partial)	GTGTGCGTGTAGCAGCAGG	502
SLC26A4 IVS8R		GGACTATTGAAGGAGTATCAGTG	
SLC26A4 9F	Exon 9	CATGTGAAATGGCATGGATGG	583
SLC26A4 9R		GGTCTGGTGAAAGAATCCAACC	
SLC26A4 10F	Exon 10	CGCAGAGTAGGCATGGGAGTTT	314
SLC26A4 10R		TTGTCCTGCTAAGCTCGGTGC	
SLC26A4 11/12F	Exons 11 and 12	AGACAGGGAAGTATGAAGTGTG	555
SLC26A4 11/12R		TTTCTCCTCTGGAGTTCCCAAA	
SLC26A4 13F	Exon 13	AGGTAGTTATCACATGATGGTACCTG	501
SLC26A4 13R		GAGCACAGCAGTAGAGGACAT	
SLC26A4 14F	Exon 14	AAACACCAGAATGATGGGCTC	338
SLC26A4 14R		GTCAGAAGGTGCACTGGATC	
SLC26A4 IVS14F	Intron 14 (partial)	GTTGAGTGCTGCTACCCAGCTCCTC	185
SLC26A4 IVS14R		AGGTAGTAATAACTATGCCAGAC	
SLC26A4 15F	Exon 15	CTACCCAGCTCCTCTGACAA	329
SLC26A4 15R		GCCCTACACAAAGGGAAGAGGG	
SLC26A4 16F	Exon 16	ACCCTTTGAGAAATAGCCTTTCCAG	357
SLC26A4 16R		CCACTCCCGCTTGCCTATAA	
SLC26A4 17F	Exon 17	AGTTTGGGCTGAGGTGAAACC	486
SLC26A4 17R		CAAAGCCCATGTATTTGCCCTG	
SLC26A4 18F	Exon 18	CGCTGGATGTTGCCTCTCT	357
SLC26A4 18R		GGCCTTCAGACATAATGTGCCA	
SLC26A4 19F	Exon 19	TTTCTTAGCTGGGCATGGTAGG	705
SLC26A4 19R		GGAATTTATGTACACAAATCCCAGATCAC	
SLC26A4 20F	Exon 20	AGAAGCACCAGGAAAGCTTCA	283
SLC26A4 20R		GGGAATTATGTTCCCTGACAGTTC	
SLC26A4 21F	Exon 21	CCTAAGATGAGTAGCAGTAAGCA	354
SLC26A4 21R		GCTGCCAAATCGTCTGAATAATTC	

Results

Clinical and audiologic evaluation

The patient had multinodular goiter at the time of diagnosis. Thyroid function was studied and revealed a slight increase in thyroid-stimulating hormone (TSH) levels, while serum thyroxine levels were below normal values. Thyroid microsomal antibodies were negative.

Hearing levels, determined by pure-tone audiometry, revealed severe to profound HL, as referred. After MRI and CT, enlargement of the vestibular aqueduct and the endolymphatic sac were observed (Fig. 2). VNG examination revealed bilateral hyporeflexia.

Molecular analysis

The mutation c.918 + 2T > C (Fig. 3), previously reported

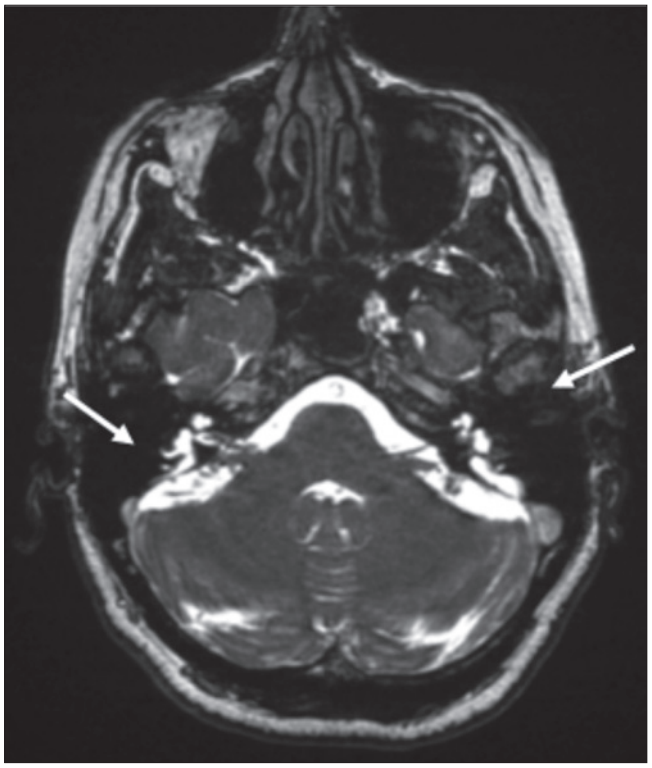

Fig. 2. Axial section MRI (FIESTA). Enlarged vestibular aqueduct (arrows).

by Chai et al. (2013) [25], was found in homozygosity in the intronic region between exons 7 and 8 of the *SLC26A4* gene. We sequenced 200 Portuguese control chromosomes to determine the allelic frequency of this mutation in the Portuguese population. The variant was not found in any of the control samples. No mutations were found in *GJB2* or *GJB6* genes. Regarding its functional effect, c.918 + 2T > C abolishes a donor splicing site, since the first two nucleotides of the intron 7 in the wild-type sequence, a guanine (G) and a thymine (T), respectively, are predicted to be a donor splicing site, with a cut-off of 0.9 and a score of 0.99 (according to the BDGP splice site prediction program). Thus, the presence of the transition T > C leads to the loss of this donor splicing site, thus skipping exon 8 and forming a non-functional protein product.

Whilst checking whether the mutation c.918 + 2T > C found in the PS patient was present in any of the 100 normal hearing control individuals, a new variant, c.821C > G (p.Ala274Gly), was found in heterozygosity in the exon 7 of *SLC26A4* gene (Fig. 4). This mutation changes alanine to glycine at position 274 and is predicted to impair protein function by SIFT software, with a score of 0.04 and a median conservation of 2.24. This variant was not found in any of the other Portuguese controls in the study and is not reported in 1000 Genomes, HGDM, ClinVar, or Pendred/BOR databases from Hereditary Hearing Loss Homepage [26]. Since this individual was a random control from the Portuguese population, no information concerning phenotype was available.

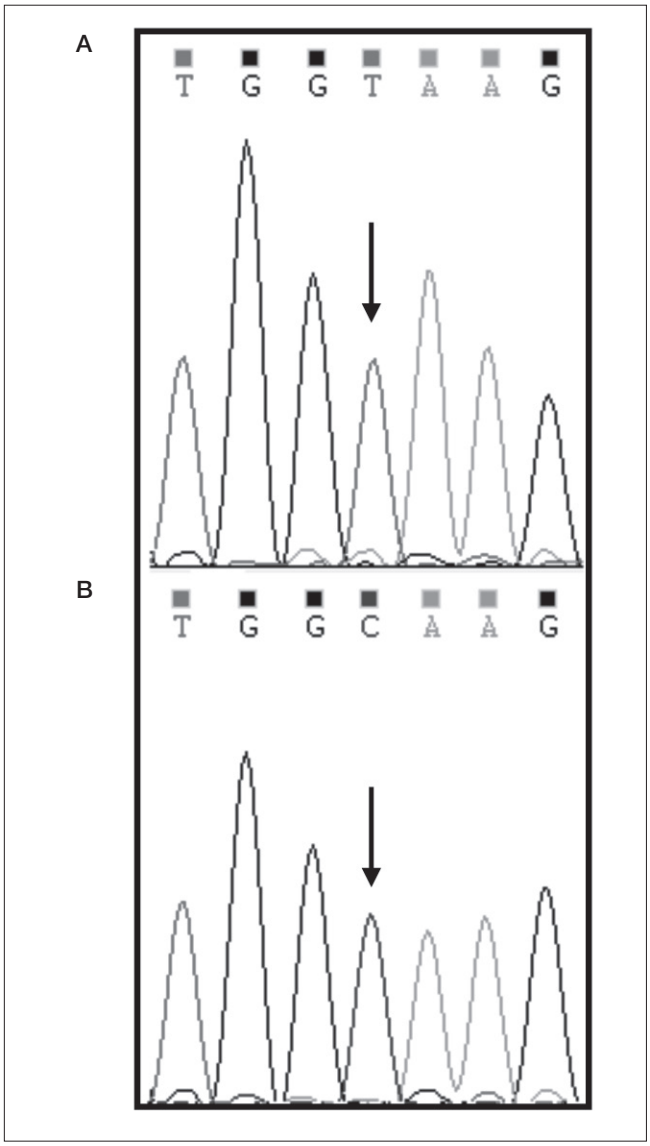

Fig. 3. Electrophoretograms showing: A - wild-type sequence; B - SLC26A4 novel mutation c.918 + 2T > C in homozygosity.

Discussion

Since its discovery, many studies have been performed to better understand the genetics of PS, possible genotype-phenotype correlations and the pathologies associated with this syndromic condition [27-30].

Previously, we found a novel splice site mutation in the *SLC26A4* gene, in a consanguineous Portuguese family [31]. Herein, we report the case of a Portuguese female diagnosed with PS and found to be homozygous for the donor splice site c.918 + 2T > C mutation in the *SLC26A4* gene. This mutation was recently reported by Chai et al. (2013) [25] in a Chinese child. The authors found this mutation in compound heterozygosity with another *SLC26A4* variant, c.919 - 2A >G, and described the patient as a non-syndromic severe to profound HL individual, presenting bilateral enlargement of the vestibular aqueduct [25].

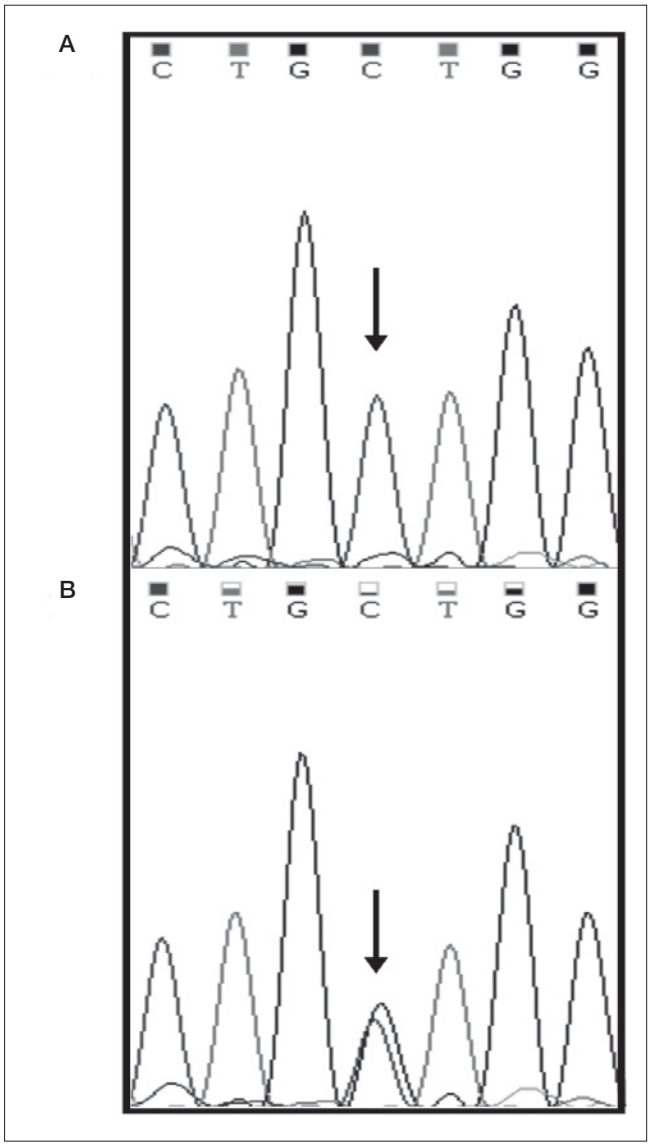

Fig. 3. Electrophoretograms showing: A - wild-type sequence; B - SLC26A4 novel mutation c.918 + 2T > C in homozygosity.

In the present study, we describe for the first time the c.918 + 2T > C mutation in homozygosity in a PS individual and also provide new insight on its phenotypic characterisation. The severe to profound HL phenotype, enlargement of the vestibular aqueduct and endolymphatic sac along with goiter and hyporeflexia are all compatible with features affecting PS patients.

The patient here considered reported that mother and sister, both deceased, had suffered from HL and goiter. This feature does not fit with the recessive pattern of PS inheritance. Due to the lack of additional familial information, the apparently dominant HL and goiter within this family remains to be explained. Since Chai et al. (2013) [25] reported the c.918 + 2T > C mutation in compound heterozygosity with another *SLC26A4* mutation in a child presenting features compatible with PS, we may also consider the hypothesis that the mother could have harboured this mutation in compound heterozygosity, thus giving rise to the HL and goiter phenotype. Although excluded by the patient, we cannot exclude consanguinity in this family, which would better explain the homozygous genotype observed in the patient and the HL and goiter phenotype of her deceased sister. Unfortunately, no information was provided regarding the father.

Conclusions

Having into account that: no alteration was found in all other exons of the *SLC26A4* gene or in the *GJB2* and *GJB6* genes; the c.918 + 2T > C mutation abolishes a donor splicing site and occurs in homozygosity, affecting both alleles; this mutation was not present in any of the 200 Portuguese control chromosomes analysed (allelic frequency < 0.99%), the *SLC26A4* genotype [c.918 + 2T >C + c.918 + 2T > C] could be pointed as the likely cause for the PS phenotype presented by the patient.

Considering the novel variant, c.821C > G (p.Ala274Gly), found in heterozygosity in a control individual, it is predicted to be probably damaging and it was not found in any of the remaining Portuguese control individuals. Further genotyping of Portuguese PS patients might eventually lead to the identification of this allele in a compound heterozygous patient. Moreover, since the mutation spectrum of *SLC26A4* has been shown to vary widely among ethnic groups, future determination of the mutation spectrum of *SLC26A4* gene in the Portuguese population might reveal some interesting specificities.

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