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

Acta Otorhinolaryngol Ital 2016;36:233-238

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, with an incidence estimated to be as high as 7.5 to 10 in 100,000 individuals.
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. 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, 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. The mutation spectrum of SLC26A4 varies widely among ethnic groups, with certain mutations demonstrating a higher prevalence in specific populations.

This gene, containing 21 exons, localises to chromosome 7 (7q22.3-q31.1) and encodes the multifunctional anion exchanger pendrin. 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. 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. The SLC26A4 gene is expressed in specific areas of the endolymphatic compartment in the cochlea known to play a role in the endolymph reabsorption.

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.

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. Automated sequencing was performed for the coding exon of the GJB2 gene, and for all exons, as well as intrinsic 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 intrinsic region 7 and exon 7 of the SLC26A4 gene.

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

The Berkeley Drosophila Genome Project (BDGP) splice site prediction program was used to predict the effect of the splicing mutation found in the patient. The SIFT prediction software was used to predict the effect of a new variant, c.821C > G (p.Ala274Gly), identified in an individual of the control sample.
Further characterisation of the recently described SLC26A4 c.918+2T > C mutation

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

| Primer name  | Region                  | Primer sequence (5'-3') | Amplified region (bp) |
|--------------|-------------------------|-------------------------|-----------------------|
| SLC26A4 2F   | Exon 2                  | GGCTGCAAGCTAACAGGTTGATC | 432                   |
| SLC26A4 2R   |                         | GAGGACGGAGGACGAAAGAGTC  |                       |
| SLC26A4 3F   | Exon 3                  | ACAGTTCTTGCCAAAAGCATGG | 411                   |
| SLC26A4 3R   |                         | GAGGTTAGCAACATCGTTGAC   |                       |
| SLC26A4 4F   | Exon 4                  | TTTGATCTCATATAAGGCAAAGTC | 419                   |
| SLC26A4 4R   |                         | GAAATCCTTTTCCCTGCAAA    |                       |
| SLC26A4 5F   | Exon 5                  | CTGACGTCCTTGGTAACCAAC   | 439                   |
| SLC26A4 5R   |                         | TTTGATGTCGGAATATTCTTTGT |                       |
| SLC26A4 6F   | Exon 6                  | GTCTCATAAGGCAAGATCGTGT  | 364                   |
| SLC26A4 6R   |                         | CCGTCGCCAGACTGAGAAT     |                       |
| SLC26A4 7/8F | Exons 7 and 8           | TGAGGAGATTGTATGAGATGTG  | 581                   |
| SLC26A4 7/8R |                         | TCGTGTTTTTCTCAAGATCA    |                       |
| SLC26A4 IVS9F| Intron 8 (partial)      | GGACTATTGAAGGATACAGATG  | 502                   |
| SLC26A4 9F   | Exon 9                  | CATCGTAAATGGCATGGATG    | 583                   |
| SLC26A4 9R   |                         | GGTCTGGTAAAGATTCAACCC   |                       |
| SLC26A4 10F  | Exon 10                 | CGCAGATTAGGCGATGGGATTT  | 314                   |
| SLC26A4 10R  |                         | TTGTCCTGCTAAGCTGGTGTC   |                       |
| SLC26A4 11/12F| Exons 11 and 12         | AGACAGGGAAGATGAACTGGTG  | 555                   |
| SLC26A4 11/12R|                      | TTTCTCTCTGAGATCTGCCAAA  |                       |
| SLC26A4 13F  | Exon 13                 | AGTGAATTATCATGATGGATCTG | 501                   |
| SLC26A4 13R  |                         | GACACAGAGAGTAGGACAT     |                       |
| SLC26A4 14F  | Exon 14                 | AAACACGAGAATGATGGGCTC   | 338                   |
| SLC26A4 14R  |                         | GTAGAGAGGTGACACTGATC    |                       |
| SLC26A4 IVS14F| Intron 14 (partial)     | GTGAGGCTGCTACCAAGCTCCTC | 185                   |
| SLC26A4 IVS14R|                      | CGATGTAATGAACTATGCAAGAC |                       |
| SLC26A4 15F  | Exon 15                 | CTACCAGCTCCTCTGCAAA     | 329                   |
| SLC26A4 15R  |                         | GCCCTACAAGGAAAGAGGGGAGG |                       |
| SLC26A4 16F  | Exon 16                 | ACCCTTTGGAAATAGCTCGCTGAC | 357                   |
| SLC26A4 16R  |                         | CCCTCCCAGCTGCTACTTATAA  | 486                   |
| SLC26A4 17F  | Exon 17                 | AGTTGGGCTAGGGTAACCCGAA | 486                   |
| SLC26A4 17R  |                         | CAAGGCCCATGATTGTTGCCCTG | 357                   |
| SLC26A4 18F  | Exon 18                 | CGTGGATGTGTGGCTTCTCT    |                       |
| SLC26A4 18R  |                         | GGCCTGCAGATAATGGTGGCA   |                       |
| SLC26A4 19F  | Exon 19                 | TTCTAAGCTGCTGCCATGAGTG  | 705                   |
| SLC26A4 19R  |                         | GGAATTTAGTCAATACCCAGATCAC | 283                   |
| SLC26A4 20F  | Exon 20                 | AGAAAGCAAACGAAAGCTCACA  | 283                   |
| SLC26A4 20R  |                         | GGAATTTAGTCTCCCTGACAGTTC |                       |
| SLC26A4 21F  | Exon 21                 | CCTAGATGAGTACGTTAGAGGA  | 354                   |
| SLC26A4 21R  |                         | GCCGAAATCCGCTGAAATTT    |                       |
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.

### 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.
Further characterisation of the recently described SLC26A4 c.918+2T > C mutation

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) 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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Received: October 22, 2015 - Accepted: November 28, 2015

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