Mutations in the SLC26A4 (pendrin) gene in patients with sensorineural deafness and enlarged vestibular aqueduct

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ABSTRACT. Pendred syndrome and the enlarged vestibular aqueduct (EVA) are considered phenotypic variations of the same entity due to mutations in the SLC26A4 (pendrin) gene. Pendred syndrome consists in sensorineural deafness, goiter and impaired thyroid hormone synthesis while in EVA thyroid function seems to be preserved. The aim of this study was to evaluate thyroid function and morphology and to look for mutations in the SLC26A4 gene in patients presented with EVA. Among 57 consecutive patients with sensorineural deafness 15 with EVA, as assessed by magnetic resonance imaging (MRI), were identified and studied. A complete evaluation of thyroid function including thyroid echography and perchlorate discharge test was carried out in all patients with EVA; all exons of the SLC26A4 gene were amplified from peripheral leukocytes and directly sequenced, using specific intronic primers. Out of 15 patients with EVA, goiter was present in 8 (53%), hypothyroidism in 7 (47%), increased serum thyroglobulin levels in 8 (53%) and a positive perchlorate discharge test in 10 (67%). Nine alleles of the SLC26A4 gene were mutated: 2 novel mutations (L465W and G497R) and 4 already known mutations (T410M, R409H, T505N and IVS1001+1G>A) were found. Four subjects were compound heterozygous and 1 heterozygous (G497R/wt). All patients harbouring mutations in the SLC26A4 gene had goiter and a positive perchlorate discharge test: 3 were slightly hypothyroid and 2 euthyroid. The remaining 10 patients had no mutations in the SLC26A4 gene: 4 of them were hypothyroid, 2 with goiter and positive perchlorate discharge test, 2 without goiter and with negative perchlorate discharge test. Two patients without mutations were euthyroid with positive perchlorate discharge test. Patients with mutations in the SLC26A4 gene had larger thyroid volume (p<0.002), higher serum thyroglobulin (Tg) levels (p<0.002) and greater radioiodine discharge after perchlorate (p=0.09) than patients without mutations. The results of the present study lend support to the concept that all patients with mutated SLC26A4 gene have abnormalities of thyroid function tests.

Key-words: Pendred's syndrome, deafness, thyroid hormone synthesis, SLC26A4, pendrin.

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Accepted December 9, 2003.

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to hot-spots, but span throughout the gene. Correlation of genotype to phenotype led to postulate a specific role for some mutations. Campbell et al. (11) found that the type of temporal bone malformation is similar in subjects from multiplex families carrying the same mutation. Pendred syndrome and EVA are currently believed to be a continuum of the same disorder; the observation that mutations in the SLC26A4 gene were identified in patients with non-syndromic sensorineural deafness lends support to this concept. Li et al. (12) reported that in a large consanguineous Indian family 10 non-goitrous subjects with congenital deafness were homozygous for SLC26A4 gene mutation in exon 13. Kitamura et al. (13) studied 5 non-goitrous subjects with EVA and absence of goiter from 4 Japanese families and identified 2 mutations (H723R and T410M) located in the extracellular domain close to the C terminal: 2 patients were homozygous and 3 heterozygous; information on thyroid function in the patients of the above reported series was largely incomplete. Functional differences of the SLC26A4 gene product were identified in vitro. Scott et al. (14) compared the effects of common Pendred syndrome allele variants (L236P, T416P, E384G) with those reported only in individuals with non-syndromic hearing loss (V480D, V653A, I490/G497S): iodide transport activity was abolished in the former, but retained in the latter, although at a lower level with respect to wild type SLC26A4 gene product. At variance, Taylor et al. (15) reported that all mutations in the SLC26A4 gene affected iodide transport in transfected cells: because affected individuals had variable abnormalities in thyroid function, it was postulated that additional genetic or environmental factors are likely to contribute to changes in thyroid function in Pendred syndrome. A study of 9 Japanese patients with EVA (16) revealed a mutation (H723R) in 6 patients (3 homozygous and 3 heterozygous); goiter was absent in all patients but serum thyroglobulin (Tg) levels were found to be higher in homozygous than in heterozygous individuals. In addition, it should be pointed out that high iodine intake, as occurring in Japanese patients, might contribute to mitigate thyroid dysfunction. In summary, although evidence accumulated both on clinical and genetic grounds suggesting that Pendred syndrome and EVA may represent different aspects of the same syndrome, the genotype-phenotype relationship remains unclear. The aim of this study was a detailed audiological, endocrinological and genetic evaluation of 15 patients with EVA belonging to a group of 57 consecutive patients with sensorineural deafness referred to our Institution.

MATERIALS AND METHODS

Subjects
During the years 2000-2002, 57 consecutive patients (32 male, 25 female, age range 9-56 yr) with progressive sensorineural deafness were referred to our Institution. All patients were submitted to complete audiological examination and high-resolution magnetic resonance imaging (MRI) of the inner ear. EVA was defined as an increase in the caliber of the aqueduct, measured midway between the common crus and the external aperture greater or equal to 1.5 mm. Patients with MRI evidence of EVA subsequently underwent a complete functional and morphological thyroid evaluation and mutational analysis of the SLC26A4 gene. A control group of 51 healthy controls (34 male, 17 female, age range 19-67 yr) was submitted to the same genetic analysis as the patients. The study was approved by the institutional review committee, and all patients gave their informed consent.

Audiological evaluation
Audiological evaluation included otomicroscopic examination, pure-tone audiometry or conditioned audiometry depending on the age of the subject, impedance audiometry and brainstem auditory evoked potentials.

Assessment of thyroid function and morphology
Serum FT$_3$, FT$_4$, FT$_4$/T$_3$, Free T$_3$, Free T$_4$, TSH, and TPO Ab were measured by commercial kits. Normal ranges were: FT$_3$, 8.4-23.2 pmoI/l; FT$_4$, 3.8-8.4 pmoI/l; TSH, 0.4-3.5 mU/l. Serum TSH-receptor (TR) antibody (Ab) was determined by radioreceptor assay (TRAK assay, BRAHMS Diagnostica, Berlin, Germany; normal values, less than 5 U/l). Serum Tg (Sorin Biomedica, Saluggia, Italy; normal values <3-30 µg/l), anti-Tg (TgAb, Sorin Biomedica; undetectable in normal controls), and anti-thyroid peroxidase (TPO) Ab were determined by commercial kits. Thyroid volume was measured by ultrasound using a 7.5 MHz linear transducer and calculated by the ellipsoid model: width x length x thickness x 0.52 for each lobe (17). Normal values in our area ranged from 5 to 16 ml. Urinary iodine excretion was measured using an autoanalyzer (Technicon, Rome, Italy). The median urinary iodine excretion in our area is 110 µg/l. Potassium perchlorate discharge test was performed as follows: 2 h after administration of a tracer dose of $^{131}$I (5 µCi), 1 g KClO$_3$ was administered, and discharge of radioiodine was assayed after 1 h. Normal value in our Department is a discharge <10% of the administered radioiodine dose.

DNA sequencing
DNA was extracted from peripheral leukocytes by standard methods (18). Exons 2 to 21 of the SLC26A4 gene were amplified by PCR using specific intronic primers, as described (4). The above mentioned primer sets amplified a part of the introns containing the splicing sequence. The PCR products were purified on 1% Nusieve® gel and with Wizard PCR prep DNA purification system (Promega, Madison, MA, USA). Both strands were sequenced directly after PCR amplification, using FS AmpliTaq DNA polymerase and fluorescein-dinucleotides. An ABI Prism 310 (Perkin Elmer) apparatus was employed. Sequence analyses were performed using a Sequencing Analysis 3.0 software.