Evaluation of genotype–phenotype relationships in patients referred for endocrine assessment in suspected Pendred syndrome

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Abstract

Design: Patients with Pendred syndrome have genotypic and phenotypic variability, leading to challenges in definitive diagnosis. Deaf children with enlarged vestibular aqueducts are often subjected to repeated investigations when tests for mutations in *SLC26A4* are abnormal. This study provides genotype and phenotype information from patients with suspected Pendred syndrome referred to a single clinical endocrinology unit.

Methods: A retrospective analysis of 50 patients with suspected Pendred syndrome to investigate the correlation between genetic, perchlorate discharge test (PDT) and endocrine status.

Results: Eight patients with monoallelic *SLC26A4* mutations had normal PDT. Of the 33 patients with biallelic mutations, ten of 12 patients with >30% discharge developed hypothyroidism. In our cohort, c.626G>T and c.3-2A>G result in milder clinical presentations with lower median perchlorate discharge of 9.3% (interquartile range 4–15%) compared with 40% (interquartile range 21–60%) for the remaining mutations. Eight novel mutations were detected. All patients with PDT <30% remained euthyroid to date, although the majority are still under the age of 30. There was a significant correlation between PDT and goitre size ($R=0.61$, $P=0.0009$) and the age of onset of hypothyroidism ($R=-0.62$, $P=0.0297$). In our population, the hazard of becoming hypothyroid increased by 7% per percentage point increase in PDT ($P<0.001$).

Conclusion: There is a correlation between *SLC26A4* genotype and thyroid phenotype. If results hold true for larger patient numbers and longer follow-up, then for patients with monoallelic mutations, PDT could be unnecessary. Patients with biallelic mutations and PDT discharge >30% have a high risk of developing goitre and hypothyroidism, and should have lifelong monitoring.

European Journal of
Endocrinology
(2015) 172, 217–226

Introduction

From the first description by Pendred in 1896 to the large series by Fraser in 1965 (1), the diagnosis of Pendred syndrome was based on the clinical triad of deafness, goitre and thyroid dysfunction due to an iodide organification defect. In 1997, the disease-causing gene was identified as *SLC26A4* (2) (solute carrier family 26 (anion exchanger), member 4), coding for the anion transporter

protein pendrin. Over the last few decades, diagnostic procedures have evolved considerably with the introduction of the perchlorate discharge test (PDT) (3) and genetic testing. Today, the vast majority of patients are identified in infancy or childhood due to hearing impairment, and the detection of enlarged vestibular aqueducts (EVA) on CT scan leads to genetic testing for biallelic mutations

of the *SLC26A4* gene. Approximately, 180 mutations have been reported (<http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm>), which include mis-sense, nonsense, splice site, and frameshift mutations, as well as partial gene deletions (4, 5). Mutations in *FOXI1* (6) and *KCNJ10* (7) mutations have also been implicated in some cases of Pendred syndrome, although their role, if any, is likely to be minor (8, 9).

Clinically, thyroid hormone abnormalities and development of goitre have incomplete penetrance and may occur in later life, the variability may be partly accounted for by fluctuations in iodine deficiency in the region (10, 11). Phenocopies (co-incidental hearing impairment and thyroid dysfunction/goitre not due to Pendred syndrome) have also been described (12, 13). Recently, atypical presentation with thyroid dysgenesis has also been associated with *SLC26A4* mutations in a few patients (14). A number of studies have investigated the correlation of the clinical phenotype and genetic background (15, 16, 17, 18, 19), but the necessity of PDT for diagnostic purposes remains unclear.

With these uncertainties, individuals suspected to have Pendred syndrome often undergo a combination of genetic analysis and PDT, and if either result is abnormal, they are subjected to lifelong monitoring for thyroid dysfunction, which involves annual clinical assessment and examination as well as thyroid function tests. Anecdotally, physicians looking after such patients note that these seemingly innocuous investigations may result in significant psychological distress in this young population group.

We conducted a retrospective analysis of patients referred to our institution for suspected Pendred syndrome. The aim of the study was to address a number of hypotheses. Namely that genetic analysis would be sufficient to confirm the diagnosis of Pendred syndrome and that PDT may not be an essential evaluation for the diagnosis of all individuals suspected of having Pendred syndrome but that it may play a role in understanding thyroid prognosis. We also aimed to evaluate any genotype–phenotype correlation for thyroid dysfunction in Pendred syndrome.

Patients and methods

Patients

We conducted a retrospective analysis of 50 patients (27 females and 23 males) with suspected Pendred syndrome, referred to our university hospital for the evaluation

of thyroid on the basis of phenotype, between 2004 and 2012. The inclusion criteria were as follows. All patients had pre-lingual deafness, or apparently postlingual, but progressive hearing impairment; 46 also had EVA/endolymphatic sacs on CT/MRI scanning. Of the remaining four subjects, all had other reasons for suspecting Pendred syndrome: two were hypothyroid with hearing loss, and the other two subjects were deaf siblings, one of whom had a goitre, whose parents were first cousins. As a real-world study, all patients referred for evaluation of these criteria were included in the assessment and analysis of results. As part of routine clinical evaluation, all patients were assessed clinically, biochemically (free thyroxine₄, thyrotrophin (TSH) and thyroid peroxidase (TPO) antibodies), and also had undergone genetic analysis of the *SLC26A4* gene. Eighty percent of the patients (40 of 50 patients) agreed to undergo a PDT. Patients found to have biallelic mutations were monitored annually for thyroid hormone levels and goitre development, which was classified as large, moderate, small or none, based on clinical examination by their regular endocrine physician. The goitre size was noted in the clinical notes reviewed retrospectively for the purpose of this study. Ultrasound scanning of the thyroid was not routinely used for evaluation due to the lack of indications for this.

Genetic analysis

All patients gave consent for genetic testing. Genomic DNA of patients was extracted from peripheral blood by standard methods. Patients in whom a single mutation was identified all had complete bidirectional exon resequencing (see [Supplementary Table 1](#) and [Methods](#), see section on [supplementary data](#) given at the end of this article) using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Branchburg, NJ, USA). Exon resequencing was performed on all samples with a total reaction volume of 20 µl, consisting of 0.5 µl genomic DNA (ca. 250 µg/µl), 19 µl Megamix (Microzone Ltd, Hayward Heath, UK) and 0.5 µl of primer mix containing 10 pmol/µl forward and reverse primers. All exons were amplified using the Peltier thermal cycler (Bio-Rad tetrad 2 DNA engine) and PCR was performed under the following conditions: denaturation at 95 °C for 3 min (95 °C×30 s, 65 °C×30 s, and 72 °C×45 s) with decrease in annealing temperature by about 1 °C for further 14 cycles (95 °C×30 s, 50 °C×30 s, and 72 °C×45 s), for 20 cycles, followed by final extension at 72 °C for 5 min. All forward and reverse primers had a tailed sequence at the 5' of the primer for automated Sanger

sequencing. The sequence was analysed on an ABI3730 Genetic Analyser (Applied Biosystems) using standard protocols. The data were collated using Foundation Data Collection v3.0 and were analysed using the software programme Mutation Surveyor v.3.30 (Softgenetics, State College, PA, USA) and compared with the GenBank sequences of *SLC26A4* (Genomic reference: NM_000441, ENSG00000091137). The data were also analysed for reported variants within the gene using Alamut (Interactive Biosoftware, Rouen, France).

Perchlorate test

The patients were offered a perchlorate test on the basis of clinical judgement. The principles of the PDT are that radiolabelled iodine (as radiolabelled sodium iodide) is administered and the emittance of radioactivity is measured over the thyroid. Potassium perchlorate (a competitive inhibitor of iodide transport into the thyroid) is administered and then emittance of radioactivity is measured over the thyroid and compared with the initial result. In the normal thyroid, the amount of radiolabelled iodine in the thyroid remains relatively stable due to the rapid oxidation of iodide to iodine and its subsequent incorporation into thyroglobulin. In the presence of an iodine organification defect, the transport of iodine into the follicle (and therefore its incorporation into thyroglobulin) is delayed, resulting in the leakage of iodide into the bloodstream. This leakage is demonstrated by a decrease in radiolabelled iodine in the thyroid. There were minor variations in the protocol for PDT as the records spanned 8 years. Tracer $^{123}\text{I}^-$ was administered, 100–200 mg of i.v. sodium perchlorate was injected 30 min later and dynamic images were acquired up to 60 min.

Statistical analyses

Statistical analysis was performed using StatsDirect software (Addison-Wesley-Longman, Cambridge, UK). The Shapiro–Wilk test was used to assess normal distribution, Mann–Whitney *U* test to compare numerical variables, Pearson's correlation test for correlation and the hazard ratio for PDT was estimated using Cox's proportional hazards model. A value of $P < 0.05$ was considered statistically significant.

Results

Of the 50 patients with suspected Pendred syndrome referred for thyroid evaluation, 33 had biallelic mutations

in *SLC26A4*, 11 had a monoallelic mutation, while six had no detectable mutations. As all 50 patients had been referred to endocrinology based on clinical suspicion, all data are included in subsequent analysis although only those found to have biallelic mutations fit the criteria for a formal genetic diagnosis of Pendred syndrome. All patients were singleton cases with the exception of a pair of siblings. Three patients were homozygotes (subjects 4, 12, and 33) and 30 patients were compound heterozygotes. Results of the mutation analysis and the thyroid assessment (PDT results, thyroid function and clinical assessment of goitre) for patients with biallelic mutations are given in [Table 1](#). A total of 41 different mutations were detected, among which eight had not previously been described: c.73C>A (p.Pro25Thr), c.284G>C (p.Gly95Ala), c.454delG (p.Val152Phefs*2), c.1252G>A (p.Gly418Arg), c.1511T>C (p.Phe504Ser), c.1920G>A (p.Trp640*), c.2118C>A (p.Cys706*), and c.2174insTATC (p.Ala725Alafs*30).

PDT results are given in [Table 2](#). Among the 33 patients with biallelic mutations (ages 10–61 years at last follow-up), 26 underwent PDT. Patients with PDT <20% ($n=10$) had no goitre or hypothyroidism. Patients with PDT between 20 and 30% ($n=4$) often had goitre but not hypothyroidism. Patients who were 20 years or older with a PDT >30% ($n=10$) developed hypothyroidism, while a 17 years and a 19 years patients had >30% PDT and were euthyroid at the last follow-up ([Fig. 1](#)).

In contrast, none of the patients with a monoallelic mutation had an abnormal PDT result ($n=8$); PDT was not performed in three patients. All were euthyroid at the time of endocrine referral (age at PDT ranged from 15 to 48 years), but two patients subsequently developed hypothyroidism during follow-up due to Hashimoto's thyroiditis with positive TPO antibodies ([Supplementary Table 2](#), see section on [supplementary data](#) given at the end of this article). Two out of six patients with no detectable mutation showed >20% PDT results; both of these patients were known to be hypothyroid, one with established Hashimoto's thyroiditis and the other with a personal and family history of autoimmune thyroid disease (data given in [Supplementary Table 3](#)).

There was a significant positive correlation between the PDT result and the goitre size ($n=26$, correlation coefficient (R)=0.61, $P=0.0009$), rated as large, moderate or small, and there was a negative correlation between the PDT result and the age of onset of hypothyroidism ($R=-0.62$, $P=0.0297$). In our population, the hazard of becoming hypothyroid increased by 7% per percentage

Table 1 SLC26A4 mutations among patients with bilateral mutations. Mutations are reported in <http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm>. Additional mutations (marked with % sign) c.1541A>G, c.1615-2A>G, c.2090A>C, c.2186T>C have been reported separately by Choi *et al.* (21), Simoes-Teixeira *et al.* (26), Dai *et al.* (27) and Rendtorff *et al.* (28).

No.	Age (years)	Allele 1 cDNA	Allele 1 (protein level)	Novel mutation	Functional defect	Allele 2 cDNA	Allele 2 (protein level)	Novel mutation	Functional defect	Perchlorate discharge (% uptake)	Goitre	Hypothyroidism (age of onset/age at last follow-up)
1	17	c.1826T>G	p.Val609Gly	○	No data	c.2015G>A	p.Gly672Glu	○	Complete	77	Large	Yes (17)
2	14	c.1226G>A	p.Arg409His	○	Partial	c.1541A>G	p.Gln514Arg (%)	○	Partial complete for Gln514Lys	76	No	Yes (10)
3	15	c.1151A>G	p.Glu384Gly	○	Complete	c.1615-2A>G	p.?	○	No data	63	Mod	Yes (12)
4	16	c.2174insTATC	p.Ala725Alafs*30	●	No data	c.2174insTATC	p.Ala725Alafs*30	●	No data	60	Mod	Yes (15)
5	20	c.454delG	p.Val152Phefs*2	●	No data	c.1334T>G	p.Leu445Trp	○	No data	56	Mod	Yes (18)
6	19	c.412G>T	p.Val138Phe	○	Complete	c.919-2A>G	p.?	○	No data	50	Small	No (19)
7	19	c.707T>C	p.Leu236Pro	○	Complete	c.1151A>G	p.Glu384Gly	○	Complete	42	Small	Yes (17)
8	25	c.707T>C	p.Leu236Pro	○	Complete	c.1246A>C	p.Thr416Pro	○	Complete	41	Small	Yes (22)
9	21	c.1246A>C	p.Thr416Pro	○	Complete	c.1284delITGC	p.Ala429del	○	No data	40	Small	Yes (21)
10	49	c.412G>T	p.Val138Phe	○	Complete	c.1920G>A	p.Trp640*	●	No data	40	Small	Yes (36)
11	17	c.73C>A	p.Pro25Thr	●	No data	c.1790T>C	p.Leu597Ser	○	Polymorphism	40	Small	No (17)
12	24	c.1337A>G	p.Gln446Arg	○	Complete	c.1337A>G	p.Gln446Arg	○	No data	35	Small	Yes (11)
13	21	c.626G>T	p.Gly209Val	○	Partial	c.1151A>G	p.Glu384Gly	○	Complete	25	Small	No (21)
14	26	c.2T>C	p.Met1?	○	No data	c.1334T>G	p.Leu445Trp	○	No data	24	No	No (25)
15	27	c.2127delT	p.Phe709Leufs*12	○	No data	c.2T>C	p.Met1?	○	No data	24	Small	No (26)
16	41	c.1246A>C	p.Thr416Pro	○	Complete	c.1511T>C	p.Phe504Ser	●	No data	21	Large prior surgery	Yes (post surgery at 35)
17	28	c.2T>C	p.Met1?	○	No data	c.1334T>G	p.Leu445Trp	○	No data	19	No	No (27)
18	25	c.2T>C	p.Met1?	○	No data	c.412G>T	p.Val138Phe	○	Complete	15	No	No (24)
19	27	c.626G>T	p.Gly209Val	○	Partial	c.1003T>C	p.Phe335Leu	○	Partial	15	No	No (27)
20	18	c.626G>T	p.Gly209Val	○	Partial	c.1229C>T	p.Thr410Met	○	Complete	11	No	No (18)
21	22	c.85G>C	p.Glu29Gln	○	Partial	c.1229C>T	p.Thr410Met	○	Complete	10	No	No (21)
22	17	c.626G>T	p.Gly209Val	○	Partial	c.1489G>A	p.Gly497Ser	○	Partial	9	No	No (17)
23	15	c.3-2A>G	p.?	○	No data	c.898A>C	p.Ile300Leu	○	No data	4	No	No (13)
24	19	c.284G>C	p.Gly95Ala	●	No data	c.2118C>A	p.Cys706*	●	No data	4	No	No (18)
25	61	c.3-2A>G	p.?	○	No data	c.626G>T	p.Gly209Val	○	Partial	4	No	No (61)
26	27	c.3-2A>G	p.?	○	No data	c.2015G>A	p.Gly672Glu	○	Complete	3	No	No (24)
27	46	c.1151A>G	p.Glu384Gly	○	Complete	c.1541A>G	p.Gln514Arg	○	Partial (%)	Not available	Large	Yes (23)
28	15	c.626G>T	p.Gly209Val	○	Partial	c.2186T>C	p.Leu729Pro (%)	○	No data	Not available	Small	No (15)
29	14	c.716T>A	p.Val239Asp	○	No data	c.1181delITCT	p.Phe394del	○	No data	Not available	No	No (12)
30	14	c.85G>C	p.Glu29Gln	○	Partial	c.1151A>G	p.Glu384Gly	○	Complete	Not available	Small	No (13)
31	21	c.1536delAG	p.Arg512Serfs*14	○	No data	c.2090A>C	p.Asp697Ala (%)	○	No data	Not available	Mod	Yes (20)
32	13	c.1181delTCT	p.Phe349del	○	No data	c.1252G>A	p.Gly418Arg	●	No data	Not available	No	No (13)
33	10	c.716T>A	p.Val239Asp	○	No data	c.716T>A	p.Val239Asp	○	No data	Not available	No	No (10)

Novel mutations (filled circles ● indicate novel mutations, while empty circles ○ indicate known mutations). Functional studies ("Complete" indicates complete loss of function demonstrated in previous studies, while "Partial" indicates partial loss of function).

Table 2 Perchlorate discharge test results according to genetic analysis for patients with suspected Pendred syndrome.

Perchlorate discharge result	Number of patients			
	Biallelic mutations	Monoallelic mutation	No mutations	Total
Strongly positive >30%	12	0	1	13
20–30%	4	0	1	5
10–20%	4	0	1	5
Negative (<10%)	6	8	3	17
Result not available	7	3	0	10
Total	33	11	6	50

point increase in perchlorate discharge (hazard ratio 1.07, 95% CI 1.03–1.11, $P < 0.001$).

Genotype–phenotype correlations

Eight novel mutations were identified in our cohort, of which four were frameshift or truncating mutations and therefore highly likely to be pathogenic: c.2164ins TATC (p.Ala725Alafs*30), c.454delG (p.Val152Phefs*2), c.1920G>A (p.Trp640*) and c.2118C>A (p.Cys706*). *In silico* analyses of the novel missense mutations were performed using Alamut, and c.284G>C (p.Gly95Ala), c.1252G>A (p.Gly418Arg), and c.1511T>C (p.Phe504Ser) were identified as likely to be pathogenic, while the predicted significance of c.73C>A (p.Pro25Thr) was unknown (summarised in Table 3). It is not reported in exome databases, although c.74C>T (p.Pro25Leu; rs367907345) has been reported in one in 12 871 alleles (<http://evs.gs.washington.edu/EVS/>). It affects a highly conserved amino acid and is predicted to be possibly damaging by Polyphen and deleterious by SIFT (Sorting Intolerant from Tolerant).

Discussion

The patient cohort exhibited a number of *SLC26A4* mutations previously described, as well as eight novel mutations. The likely pathogenicity was presumed for frameshift and truncating mutations. The potential pathogenicity of missense mutations c.73C>A (p.Pro25Thr) and c.1252G>A (p.Gly418Arg) is paralleled by data from Pera *et al.* (20) where functional assay of *in silico*-designed mutations showed that impairment of transport function could occur when an amino acid bearing a fixed charge was missing or introduced. This includes aspartic acid (Asp[−]), glutamic acid (Glu[−]), lysine

(Lys⁺), arginine (Arg⁺), and histidine (His⁺). Furthermore, the loss or inclusion of proline may result in structural disruption to regular secondary structures such as α -helices or β -sheets. Replacing proline with another residue does not automatically alter local secondary structure, but when there is structural disruption to pendrin, this can be detrimental to transport function. Pendrin variants result in pathogenicity if they impair anion transport (iodide, chloride and bicarbonate) or if they disrupt polypeptide trafficking and stability, because the gene product must migrate to the cell periphery consistent with plasma membrane localisation (21). *In vitro* functional assays of missense mutations (radioactive flux studies and localisation assays using intracellular fluorescent indicators to sense changes in halide and/or proton amounts) (22) have helped to classify mutations as full or partial loss-of-function, although some mutations show inconclusive results (Table 4). In our 33 patients with biallelic mutations, ten patients had well-characterised mutations, nine patients harboured known mutations where only one out of two had been characterised, seven patients had one or two novel mutations, and seven patients had mutations for which functional studies were not available, thus genotype–phenotype analyses are incomplete.

Figure 1

Perchlorate discharge test results according to thyroid status and age in patients with biallelic *SLC26A4* mutation. A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-14-0679>

Table 3 *In silico* analysis of novel missense mutations.

Name of variant	Location	Frequency in NHLBI exome sequencing project (all alleles)	Conservation of amino acid	Grantham score (0–215 based on chemical properties between amino acids – polarity and molecular volume)	Polyphen (possible impact of substitution on structure and function of protein using physical and comparative considerations)	SIFT (conservation based. <0.05 predicted to be deleterious; > 0.05 predicted to be tolerated)
c.73C>A (p.Pro25Thr)	Exon 2	–	Highly conserved	38	Possibly damaging 0.918	0
c.74C>T (p.Pro25Leu) ^a	Exon 2	1/12871	Highly conserved	98	Probably damaging 0.408	0
c.284G>C (p.Gly95Ala)	Exon 3	–	Highly conserved	60	Probably damaging 1.0	0
c.284G>A (p.Gly95Arg) ^b	Exon 3	–	Highly conserved	125	Probably damaging 1.0	0
c.1252G>A (p.Gly418Arg)	Exon 10	–	Highly conserved	125	Probably damaging 1.0	0
c.1511T>C (p.Phe504Ser)	Exon 13	–	Highly conserved	155	Damaging 0.992	0
c.2186T>C (p.Leu729Pro) ^c	Exon 19	–	Moderately conserved	98	Possibly damaging 0.77	0.01

^aMutation of same amino acid reported in NHLBI dataset.^bMutation of same amino acid reported in Ensembl.^cNow reported as a mutation by Rendhorff *et al.* 2013 (28).

In general, these *in vitro* experimental findings correlate with our clinical data on individual patients, although correlation is not perfect (Tables 1 and 4). It should be remembered that functional assays are performed on limited model systems which may not always fully represent the situation *in vivo*. Among patients with known partial loss-of-function mutations, only one out of nine patients is hypothyroid (a compound heterozygote with the likely more severe mutation p.Gln514Arg) while the remaining eight patients are euthyroid; seven out of eight have perchlorate discharge <20%. In contrast, among patients with complete loss of function in at least one allele, nine of 16 patients are hypothyroid (one following surgery) and ten of 14 patients have >30% perchlorate discharge while only two of 14 have normal perchlorate discharge.

Milder clinical phenotypes were reflected in the patients with mutations c.626G>T (p.Gly209Val) and c.3-2A>G which appear to have potentially mild effects. Patients with these mutations who had a PDT ($n=7$) showed a median perchlorate discharge of 9.3% (interquartile range 4–15%), while the median perchlorate discharge for other mutations was 40% (interquartile range 21–60; $n=19$), resulting in a statistically significant difference ($P=0.0002$, Mann–Whitney U test). Low pathogenicity is supported by functional data from Taylor *et al.* (23) which showed that c.626G>T (p.Gly209Val) confers a cell membrane protein distribution similar to that of WT protein, and although there was a deleterious effect on iodine efflux, there was a retained ability to partially efflux iodide, albeit at a much reduced rate. The detailed clinical information from these patients was not outlined in that study. Similarly, although c.3-2A>G affects a canonical splice acceptor site, it is located upstream of the translation start codon in exon two, and incomplete or absent removal of intron 1 in the splicing process may still result in some functional transcripts (21). One patient who is a compound heterozygote for both c.626G>T (p.Gly209Val) and c.3-2A>G has remained euthyroid with no goitrous enlargement at 61 years of age.

In contrast, although trafficking studies have been performed on c.1541A>G (p.Gln514Arg) (21), and these showed an intermediate pattern with prominent endoplasmic reticulum (ER) and post-ER punctate staining that extended to the cell periphery suggestive of residual partial function, no efflux studies were performed, and data suggest that this mutation results in complete loss of function. Two compound heterozygotes with this mutation (subjects 2 and 27) exhibited thyroid

Table 4 Summary of functionally characterized missense allelic variants of *SLC26A4* (adapted from (22)).

Mutation	Protein level	Pathology	Localisation of the protein	Function	References
Complete loss of function c.412G>T c.707T>C	p.Val138Phe p.Leu236Pro	Pendred syndrome – goitre and raised PDT Pendred syndrome – hearing loss and goitre	ER ER	Loss of iodide efflux Loss of iodide efflux	(23) (29) For clinical data (30)
c.1151A>G	p.Glu384Gly	Pendred syndrome – hearing loss and goitre	ER	Loss of chloride and iodide efflux	(29) (31)
c.1229C>T c.1246A>C	p.Thr410Met p.Thr416Pro	EVA EVA – goitre and normal PDT Pendred syndrome – hearing loss and goitre	Intracellular ER ER	Loss of $\text{Cl}^-/\text{HCO}_3^-$ exchange activity Loss of Cl^-/I^- exchange activity Loss of iodide efflux Loss of chloride and iodide uptake	(21) (32) (23) (29) (31)
c.1337A>G c.1540C>A	p.Gln446Arg p.Gln514Lys	EVA – no goitre EVA/Pendred syndrome – no goitre but raised perchlorate discharge EVA, Pendred syndrome compound het with two different mutations in two patients; one patient with normal PDT and one raised PDT	Intracellular ER	Loss of $\text{Cl}^-/\text{HCO}_3^-$ exchange activity Loss of Cl^-/I^- exchange activity Loss of iodide efflux	(21, 32) For clinical data (30) (23)
c.1541A>G c.2015G>A Partial loss of function c.85G>C c.626G>T	p.Gln514Arg p.Gly672Glu p.Glu29Gln p.Gly209Val	EVA – one patient with normal PDT but compound heterozygote for G209V Pendred syndrome – goitre EVA Pendred syndrome no goitre but raised perchlorate discharge, EVA EVA Pendred syndrome – goitre and raised PDT	Intermediate Partial PM PM PM	Nil Loss of iodide efflux Reduction of the chloride and iodide transport Reduction of iodide efflux	(21) (23) (20) (23)
c.1003T>C c.1226G>A	p.Phe335Leu p.Arg409His	EVA Pendred syndrome – goitre and raised PDT	PM	Loss of $\text{Cl}^-/\text{HCO}_3^-$ and Cl^-/I^- exchange activity Loss of iodide efflux	(21) (33) For clinical data (30)
c.1489G>A	p.Gly497Ser	EVA	Intracellular	Strong reduction of chloride and iodide uptake Loss of $\text{Cl}^-/\text{HCO}_3^-$ exchange activity	(29, 32)
Incomplete functional characterisation c.2T>C c.1334T>G	p.Met1Thr p.Leu445Trp	Pendred syndrome – goitre and raised PDT Pendred syndrome EVA	Intracellular Intracellular	Nil Nil	(21) (21)
c.1790T>C	p.Leu597Ser	EVA – goitre normal PDT	PM	Cl^- and I^- transport similar to WT Reduction of Cl^- efflux rate constant Reduction of Cl^-/I^- exchange activity	(20) (21)

Pendred syndrome, Pendred syndrome (deafness, goitre/abnormal PDT); EVA, enlarged vestibular aqueduct; ER, endoplasmic reticulum; PM, plasma membrane.

dysfunction at an early age. In addition, anion transfer studies have been performed on a closely related mutation variant c.1540C>A, Gln514Lys (20), which showed a complete loss of function, suggesting that a mutation at this codon site is highly significant.

Limited studies confined to polypeptide trafficking have also been performed on c.2T>C (p.Met1Thr) (21), although no functional assays on anion transport have been done. The mutated protein displayed an intracellular reticular pattern consistent with ER retention, which is likely to result in complete loss of function. However, this is not fully supported by our clinical data, as none of the four compound heterozygote patients in our cohort harbouring this variant demonstrated hypothyroidism or >30% PDT (PDT range 15–24%). In addition, three of our patients with this variant but with an incompletely characterised mutation on the other allele had no goitre, while the fourth patient with a truncating mutation on the other allele had a small goitre. Therefore, the clinical data are consistent with partial function for the c.2T>C (p.Met1Thr) change.

Predictive value of PDT and relationship to hypothyroidism and goitre

In our cohort, patients with biallelic mutations and with PDT positivity >30% all developed hypothyroidism, except two individuals (aged 17 and 19 years respectively), who will need prospective monitoring to determine whether they will develop thyroid dysfunction in the future. For patients with biallelic mutations and PDT demonstrating <30% discharge, none had hypothyroidism ($n=14$), and for those with <20% ($n=10$), none had evidence of goitre development on clinical examination (USS was not performed unless indicated clinically). In a recent study in France, ultrasound imaging resulted in the diagnosis of goitre in up to 80% of the patients (24); however, this finding may have been influenced by the iodine deficiency typical of this area in France. In addition, the size of goitre may not have clinical significance for the patient. Patient 16 (with a known and a novel mutation) was unusual, in that her perchlorate discharge was mildly positive at 21%, but she developed a significant goitre at 35 years, requiring total thyroidectomy. She was euthyroid until then.

There was a strong positive correlation between the PDT result and goitre size, and there was a significant inverse correlation between the degree of PDT positivity and age at onset of hypothyroidism.

Among patients with monoallelic mutations, eight underwent PDT, and the results were all negative (all patients had <8% discharge). If these results hold true for larger numbers of patients and continued follow-up, it could be argued that the PDT may no longer be necessary in those with monoallelic mutations, given the enhanced mutation detection rate of modern molecular techniques. One may conclude in the light of the negative PDTs that in such cases, in the absence of biallelic mutations, the subjects do not have a formal diagnosis of Pendred syndrome, but rather, isolated EVA/enlarged endolymphatic sac. It may also be reasonable to carry out PDT in the setting of hearing impairment with other evidence of thyroid dysfunction, as another condition combining hearing impairment with EVA but no thyroid dysfunction may exist. This would be due to causes distinct from *SCL26A4* mutations. In patients with biallelic mutations, however, the need for PDT is highlighted by the observation that there is a high proportion of patients with a positive result who later develop goitre and, more importantly, hypothyroidism, especially when the PDT result is >30%.

In the patients with no mutations, two patients had a raised perchlorate discharge, but both patients were assessed because they had a clinically evident goitre and hearing loss at presentation, one of whom was found to have Hashimoto's thyroiditis with positive TPO antibodies and the other who was found to be biochemically hypothyroid with a family history of hypothyroidism. Neither had EVA. In these two patients, deafness and hypothyroidism may have independent aetiologies. This underlines the need for testing of autoantibodies in those found to have a raised perchlorate discharge.

The limitation of our data is the young age and short duration of follow-up in a large proportion of cases. In addition, the age at which patients underwent PDT varied widely depending on when they were referred and, in some cases, when they agreed to have the investigation. There are no data available on the durability of PDT results across time in patients predicted to have iodine organification defects. Furthermore, studies performed in different world regions suggest that the prevalence of goitre and thyroid dysfunction in these patients may be influenced by the levels of iodine deficiency in the general population (24). The prevalence of iodine deficiency may also affect the frequency of congenital hypothyroidism in Pendred's syndrome, which is not seen in our cohort but is more frequent in regions with iodine deficiency (24, 25). As such, it may be too early to conclude that a negative or borderline perchlorate discharge result is necessarily reassuring of long-term euthyroidism.

Conclusions

Data from our cohort suggests that there is a correlation between *SLC26A4* genotype and thyroid phenotype if no other confounding factors, such as autoimmune thyroid disease, are present. An obvious caveat remains relating to the heterogeneity of timing of referral and PDT in such a real-world clinical cohort. If our current analysis is borne out by longer follow-up with greater numbers, it may be that performing a PDT in the subgroup of patients with monoallelic mutations, with its attendant risk of radiation, becomes no longer clinically justifiable. On the other hand, the degree of perchlorate positivity in those with biallelic mutations may correlate with the subsequent development of clinically important goitre and hypothyroidism: in our cohort this cut-off was established as >30%. However, longer follow-up on a large group of patients is needed to establish if indeed a PDT cut-off at >30% could be used to confidently predict the development of goitre and hypothyroidism.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-14-0679>.

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.

Funding

L M Soh was supported by the Academic Medicine Development Award from National University Health System, Singapore.

Author contribution statement

L M Soh and M Druce as well as M Bitner-Glindzic and M Korbonits contributed equally to this work.

Acknowledgements

The authors are grateful for Jonathan Bestwick (Wolfson Institute, Barts and the London School of Medicine) for expert statistical advice.

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Received 10 August 2014

Revised version received 13 October 2014

Accepted 13 November 2014