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Familial PAX8 Small Deletion (c.989_992delACCC) Associated with Extreme Phenotype Variability

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The PAX8 gene, mapped on 2q12-q14, encodes for a transcription factor involved in thyroid cell proliferation and differentiation. Five mutations in PAX8 have been so far described in both sporadic and rare familial forms of thyroid dysgenesis with proposed autosomal dominant inheritance, all associated with thyroid hypoplasia and/or dysfunction.

Fifty-four subjects with congenital hypothyroidism detected during neonatal screening and associated with an ultrasound or scintiscan picture of thyroid dysgenesis were investigated for PAX8 mutations. The entire PAX8 coding region with exon-intron boundaries was amplified from genomic DNA, and a mutational screening was performed by denaturing HPLC followed by direct sequencing when denaturing HPLC elution abnormalities appeared.

A new heterozygous deletion (c.989_992delACCC) in exon 7 causing a frameshift with premature stop codon after codon 277 was identified in a subject with thyroid hypoplasia. This mutation is the only one so far identified that lies outside the paired domain. The predicted mutant protein completely lacks the C-terminal region but contains the paired box, octapeptide, and homeodomain. It retains the ability to bind a paired-domain sequence *in vitro* but is transcriptionally inactive. These results provide evidence that the C-terminal region is essential for transcriptional activity.

The new mutation has been inherited from the completely euthyroid mother. It was also present in a brother with slightly elevated TSH only. Thus, it is associated with thyroid dysgenesis in the proband and both euthyroidism and compensated hypothyroidism in her family. This suggests that other factors/genes may modulate phenotypic expression. (*J Clin Endocrinol Metab* 89: 5669–5674, 2004)

PAX PROTEINS ARE a family of transcription factors that play important roles in the formation of several tissues from all germ layers in the mammalian embryo (1). They recognize specific DNA sequences through a conserved element, the paired domain, and trigger early events of cell differentiation. Their gene expression is spatially and temporally restricted during development and in the adult. The PAX8 gene, mapped on 2q12-q14, is involved in thyroid development and in the proliferation and differentiation of thyroxine-producing follicular cells (2). In thyrocytes, it activates expression of the thyroid differentiation marker genes, such as thyroglobulin (Tg), thyroperoxidase (TPO), and sodium/iodide symporter genes (3–5). Five mutations in PAX8 have been so far described in both familial or sporadic forms of thyroid dysgenesis (6–10). All mutations are located within the paired box domain and disrupt both the ability to bind DNA and to activate transcription. All PAX8 mutated subjects had overt hypothyroidism due to thyroid hypoplasia, but in one familial case where mild hypothyroidism with elevated anti-TPO antibodies and thyroid ultrasound (US), discrete irregularities were present in the mother carrying the mutation (8).

In this study, we found a new familial PAX8 mutation associated with an extreme clinical heterogeneity during mutational screening of 54 patients with congenital hypothyroidism due to thyroid dysgenesis.

Abbreviations: DHPLC, Denaturing HPLC; fT_4, free T_4; n.v., normal value; Tg, thyroglobulin; TPO, thyroperoxidase; US, ultrasound.

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Patients and Methods

Patients

Fifty-four subjects with thyroid dysgenesis (16 agenesis, four hypoplasia, and 34 ectopy) were enrolled.

Congenital hypothyroidism was revealed by positive neonatal screening; decreased T_4 and elevated TSH values were subsequently confirmed by RIA analysis. Forty-three subjects showed clinical signs of hypothyroidism (jaundice, hoarse cry, muscular hypotonia, drowsiness, umbilical hernia, poor suckling, dry skin, and posterior fontanel > 0.5 cm). The other 11 subjects were asymptomatic.

Scintiscan showing reduced uptake in four patients, ectopic in 34, and absent in 16, together with US (performed in 20 subjects), disclosed thyroid dysgenesis.

Informed consent was obtained from all subjects or their families, and blood samples were collected. The study was approved by the Institutional Review Board of the Department of Pediatric Sciences, University of Torino (Torino, Italy).

Mutation detection

PAX8 gene analysis was performed on genomic DNA isolated from peripheral blood leukocytes with a DNA extraction kit, Genomic DNA

Isolation Kit Puregene (Gentra Systems, Inc., Minneapolis, MN). The whole PAX8 coding sequence and intron/exon boundaries were amplified with previously described PAX8-specific primers (6, 7). The amplification reaction was performed with 500 ng of genomic DNA included in a 50-μL PCR mixture containing 25 pM each primer, 500 μM each dNTP, 2.5 mM magnesium chloride, 50 mM potassium chloride, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, and 1 IU AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). After an initial denaturation at 95 C for 2 min, 35 cycles of PCR amplification were performed. Each cycle consisted of 60 s at 95 C for denaturation, 60 s at an annealing temperature ranging from 58–68 C depending upon the specific primer pair used, and 60 s at 72 C for DNA extension. The PCR product was analyzed on 3% agarose gels, stained with ethidium bromide, and visualized with UV light. Mutational screening was performed with denaturing HPLC (DHPLC) analysis (11). To enhance heteroduplex formation, 5–7 μl PCR products was denatured at 95C for 5 min followed by gradual reannealing up to 40 C over 30 min. Samples were then analyzed in Transgenomic Wave DHPLC (Transgenomic Inc., Santa Clara, CA). The gradient was formed by mixing buffer A (0.1 mM tri-ethyl-ammonium-acetate) and buffer B (0.1 M tri-ethyl-ammonium-acetate, 25% acetonitrile), and the analysis was carried out at a flow rate of 0.9 ml/min and a buffer B gradient increase of 2% per min. Start and end concentrations of buffer B were adjusted according to the size of PCR products. Oven temperature for optimal heteroduplex separation under partial DNA denaturation was determined for each amplified fragment using the DHPLC Melt software (http://med.stanford.edu/labs/branimir_sikic). The principal criteria applied for assigning the presence of a sequence alteration in each DHPLC fragment were the number and the shape of the elution peaks in comparison with a wild-type subject elution profile used as reference. Sequence reactions were carried out for each abnormal elution profile. Genomic DNA was reamplified with the same DHPLC primers, and the PCR products were sequenced in both directions using the Genetic Analyzer 3100 Sequencer (Applied Biosystems). Sequences were compared with the human full-length cDNA PAX8 (PAX8a isoform) sequence reported by Poleev *et al.* (1).

To avoid potential artifacts due to AmpliTaq Gold DNA Polymerase, each sequence alteration was confirmed by sequencing both DNA strands of three independent PCR products.

Direct sequencing of the specific PCR product was also used to trace segregation of the identified DNA changes in the mutated family and test 50 unrelated normal individuals.

EMSAs

Double-stranded oligonucleotide C derived from the Tg promoter (5′-CACTGCCCAGTCAAGTGTTCTTGA-3′) was labeled with [γ-^{32}P] ATP and T_4 polynucleotide kinase and used as probe.

The binding reactions were carried out in a buffer containing 10 mM HEPES (pH 7.9), 10% glycerol, 0.1 mM EDTA, 8 mM MgCl2, 1 mM dithiothreitol, and 0.15 μg/ml of poly (dI-dC) for 30 min at room temperature. DNA-protein complexes were resolved on a 6% nondenaturing polyacrylamide gel and visualized by autoradiography.

Plasmids

To generate pCMV5-PAX8/277del, two specific primers (all sequences 5′–3′, CGGCGAATTCATGCCTCACAACTCCATCAGA and GCTCTAGATCAGGCCTTCCCGTCGTCCAG) were used to amplify by PCR a fragment of the coding region of human PAX8a spanning from codons 1–277. They were designed to introduce in the amplified fragment *Eco*RI and *Xba*I sites for subcloning into the expression vector and deletion of the ACCC nucleotides and stop codon (as found in the patient). The fragment was subcloned into the corresponding sites of the expression vector pCMV5 (12).

The plasmids used in transient transfection experiments have been described previously, namely: CP5-CAT (13) and pCMV5-H29a (14). To generate CP5CAT, the Gal4 polymerized binding sites of G5E1b (15) were replaced with a pentamer of the Pax8 binding sequence that was inserted between the *Pst*I and *Xba*I restriction sites upstream the E1b TATA box, which is followed by the CAT coding region. CMV-LUC plasmid was used as the internal control.

Cell culture and transfection

The HeLa cell line has been described previously (16). HeLa cells were grown in DMEM supplemented with 10% fetal calf serum. For transient transfection experiments, cells were plated at a density of 3×10^5 cells/60-mm tissue culture dish, 5–8 h before transfection. Transfections were carried out with the FuGENE 6 reagent (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's directions. The DNA/FuGENE ratio was 1:2 in all experiments.

Cell extracts were prepared 48 h after transfection to determine either the levels of CAT protein with CAT enzyme-linked immunosorbent assay kit (Roche Diagnostics) or LUC activities as described previously (17). The experiments were done in duplicate and repeated three or more times.

Results

PAX8 DHPLC analysis revealed an abnormality in the PCR fragment encompassing exon 7 from one patient. Direct sequencing then showed a heterozygous deletion of ACCC nucleotides at codon 277 (c.989_992delACCC) causing a frameshift and the creation of a stop codon immediately after the deleted nucleotides (Fig. 1). This mutation was not found in 50 healthy controls; hence, it is not a common polymorphism. It predicts a truncated protein that completely lacks the C-terminal region but contains the paired domain, the octapeptide, and the homeodomain homology region (Fig. 2).

Exon 7 direct sequencing of the patient's parents, brother, and sister, all asymptomatic, showed that the mutation was present in the mother and the brother.

PAX8 DHPLC analysis also revealed an abnormality in the PCR fragment encompassing exon 5 DHPLC elution profile from 17 patients. Direct sequencing disclosed a new polymorphism involving a C to G substitution at nucleotide +47 in intron 5 (IVS + 47C>G).

The inheritance of each parental allele among the siblings was determined by looking for the IVS5 + 47C>G polymorphism. It was present in heterozygosity in both mutated siblings and their father and absent in the mother and the unaffected sibling homozygous for the wild-type sequence; thus, the two mutated siblings share the same paternal allele.

DNA-binding properties and transcriptional activity of PAX8 truncated protein

In this study, we have identified a new PAX8 mutation resulting in a truncated protein that lacks the c-terminal domain but contains the paired domain, the octapeptide, and the homeodomain homology region. We call the mutant PAX8/277del and the full-length human PAX8 protein PAX8a.

It is well known that different PAX8 splicing isoforms containing an intact paired domain bind known paired domain recognition sequences (14, 18). All known PAX8 isoforms bind oligonucleotides CT and C containing the TATA-box proximal Pax8-binding site of the TPO and the Tg promoters, respectively (14, 19). To evaluate the binding ability of PAX8/277del protein, we carried out EMSA experiments using protein extracts prepared from HeLa cells transiently transfected with expression vectors encoding either PAX8a or PAX8/277del proteins (Fig. 3A). The results clearly show that PAX8a and PAX8/277del bind to oligonucleotide C with comparable affinities. Thus, deletion of the

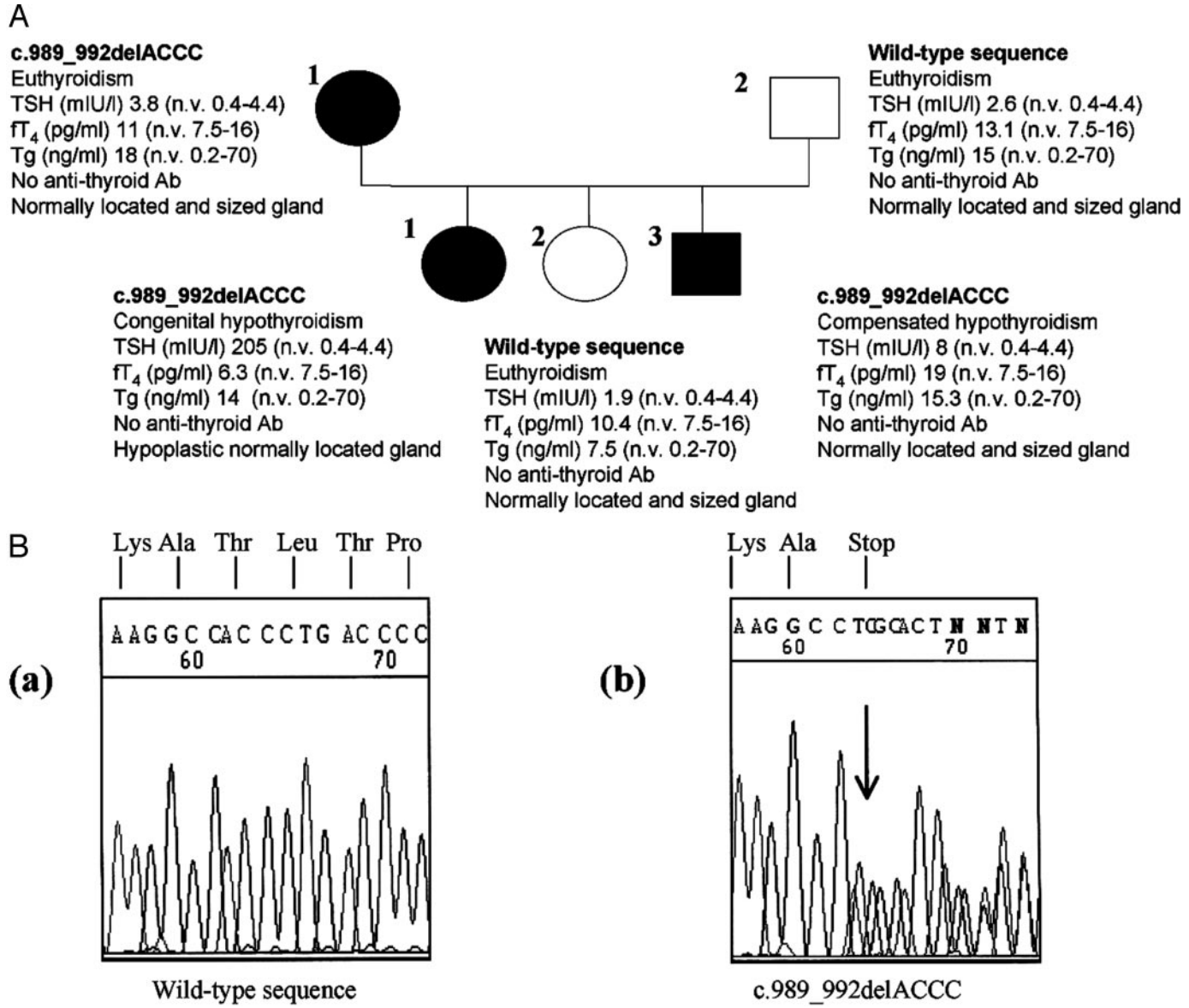


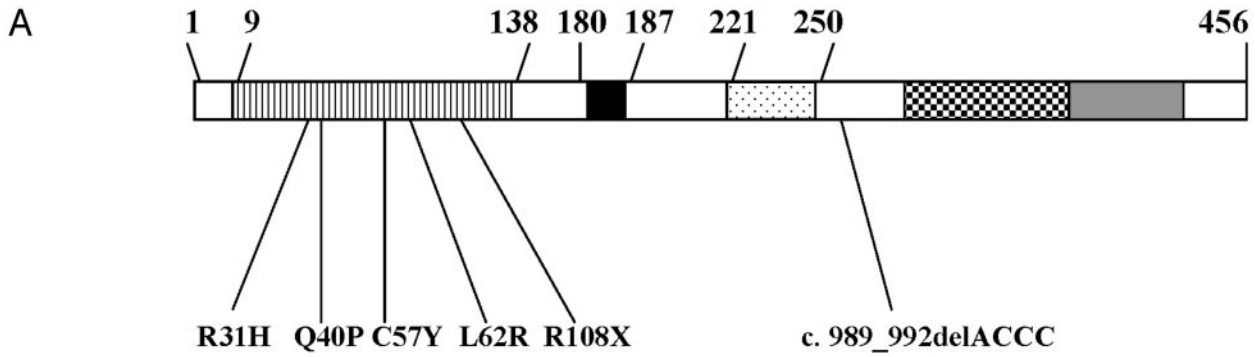
FIG. 1. A, Pedigree of the proband's family. PAX8 analysis in her parents, sister, and brother revealed the deletion in both the euthyroid mother (I-1) and the brother (II-3), whose TSH was only slightly elevated. B, Electropherograms of part of PAX8 exon 7 showing the wild-type sequence (a) and the heterozygous ACCC deletion (b) that causes the subsequent frameshift. The corresponding amino acid sequence is shown above each electropherogram.

C-terminal domain in PAX8/277del does not affect the ability of the protein to bind to a paired domain recognition sequence.

The predicted PAX8/277del protein completely lacks the C-terminal domain as a consequence of the deletion of four nucleotides causing a frameshift with the generation of a premature stop codon (Fig. 2). Previous studies have demonstrated that the activation domain of PAX8 is located in the C-terminal portion (20). Therefore, we looked to see whether the ability of PAX8/277del to activate transcription was different from that of the wild-type protein. We transiently transfected HeLa cells with expression vectors encoding either PAX8a or PAX8/277del proteins together with the reporter plasmid CP5-CAT (13), containing a PAX8-dependent promoter. As expected, PAX8/277del was not capable of activating transcription from the CP5-CAT reporter construct (Fig. 3B). These results indicate that the sequence downstream amino acid 276 is required for PAX8 transcriptional activity. They also demonstrate that the new deletion identified in this study generates a transcriptionally inactive PAX8 protein.

Clinical findings in the mutated family

The c.989_992delACCC deletion was found in a female patient born at term from unrelated parents after a normal pregnancy (birth weight 3100 g). Her familial history was negative for thyroid diseases. In the neonatal period, she displayed jaundice, hoarse cry, and poor suckling. Neonatal screening for congenital hypothyroidism was positive with



B

Wild-type PAX8

1 MPHNSIRSGH GGLNQLGGAF VNGRPLPEVV RQRIVDLAHQ GVRPCDISRQ LRVSHGCVSK
61 ILGRYYETGS IRPGVIGGSK PKVATPKVVE KIGDYKRQNP TMFAWEIRDR LLAEGVCDND
121 TVPSVSSINR IIRTKVQQPF NLPMDSCVAT KSLSPGHTLI PSSAVTPPES PQSDSLGSTY
181 SINGLLGIAQ PGSDKRKMDD SDQDSCRLSI DSQSSSSGPR KHLRTDAFSQ HHLEPLECPF
241 ERQHYPEAYA SPSHTKGEQG LYPLPLLNST LDDGKATLTP SNTPLGRNLS THQTYPVVAD
301 PHSPFAIKQE TPEVSSSSST PSSLSSSAFL DLQQVGSGVP PFNAFPHAAS VYGQFTGQAL
361 LSGREMVGPT LPGYPPHIPT SGQGSYASSA IAGMVAGSEY SGNAYGHTPY SSYSEAWRFP
421 NSSLLSSPYY YSSTSRPSAP PTTATAFDHL

PAX8/277del

1 MPHNSIRSGH GGLNQLGGAF VNGRPLPEVV RQRIVDLAHQ GVRPCDISRQ LRVSHGCVSK
61 ILGRYYETGS IRPGVIGGSK PKVATPKVVE KIGDYKRQNP TMFAWEIRDR LLAEGVCDND
121 TVPSVSSINR IIRTKVQQPF NLPMDSCVAT KSLSPGHTLI PSSAVTPPES PQSDSLGSTY
181 SINGLLGIAQ PGSDKRKMDD SDQDSCRLSI DSQSSSSGPR KHLRTDAFSQ HHLEPLECPF
241 ERQHYPEAYA SPSHTKGEQG LYPLPLLNST LDDGKA

FIG. 2. Location of published PAX8 mutations (A). The paired domain is depicted in the *striped box*, the octopeptide in the *black filled box*, the homeodomain in the *dotted box*, the repressor domain in the *squared box*, and the activating domain in the *gray filled box* (19). Codons delimiting each domain are numbered in the upper part of the figure. All the previous mutations are located in the paired domain (6–10). The mutation analyzed in this study is a small deletion that results in a frameshift and predicts a protein truncated after codon 276 (B). Functional analyses demonstrate that this protein binds to the Tg promoter but cannot activate transcription. The C-terminal region is thus essential for transcription.

TSH values of 76 mIU/liter [normal value (n.v.), <30 mIU/liter] on Guthrie card at 3 d of life and 215 mIU/liter at 18 d. At 20 d, RIA showed 205 mIU/liter TSH (n.v., 0.3–4.5 mIU/liter), 6.3 pg/ml free T_4 (fT_4) (n.v., 7.5–16 pg/ml), and 2.9 pg/ml free T3 (n.v., 2.6–5.2 pg/ml). Tg values were 14 ng/ml (n.v., 0.2–70). A Tc^{125} thyroid scintiscan revealed a normally located thyroid gland with reduced uptake, especially in the left lobe. The thyroid US confirmed thyroid hypoplasia involving the left lobe. Hormone therapy was started soon after diagnosis. The proband is now 3 yr old and 94 cm tall. Her weight is 13.5 kg, and she displays good clinical and metabolic control.

The mother displays an euthyroid state: TSH values were 3.8 mIU/liter (n.v., 0.4–4.4), fT_4 11 pg/ml (n.v., 7.5–16), Tg 18 ng/ml (n.v., 0.2–70), with a normally located and sized thyroid gland on US scan. Her thyroid function was reevaluated after 6 months, and TSH values were 3.2 mIU/liter, fT_4 13 pg/ml, Tg 19 ng/ml, and US scan confirmed a normally located and sized thyroid gland. The brother showed slightly elevated TSH values only, without thyroid volume or site abnormalities on US scan (Fig. 1).

Discussion

Congenital hypothyroidism affects about 1:3000–4000 newborns and comprises a heterogeneous group of diseases with different pathogenesis. Dysgenesis, characterized by abnormal development of the gland leading to thyroid agenesis, hypoplasia, or ectopy, is observed in 80–90% of cases (21, 22). The molecular bases of these disorders have only been elucidated in a few cases. The mutations in the TSHβ-subunit, TSH receptor, Gsα protein, and PAX8 gene (6–10) described in several subjects with thyroid hypoplasia underscore the importance of the role of these genes in the proliferation or survival of differentiated thyroid cell populations. Five PAX8 mutations have been reported in two sporadic and three familial cases with thyroid dysgenesis. All PAX8 mutated subjects displayed overt hypothyroidism due

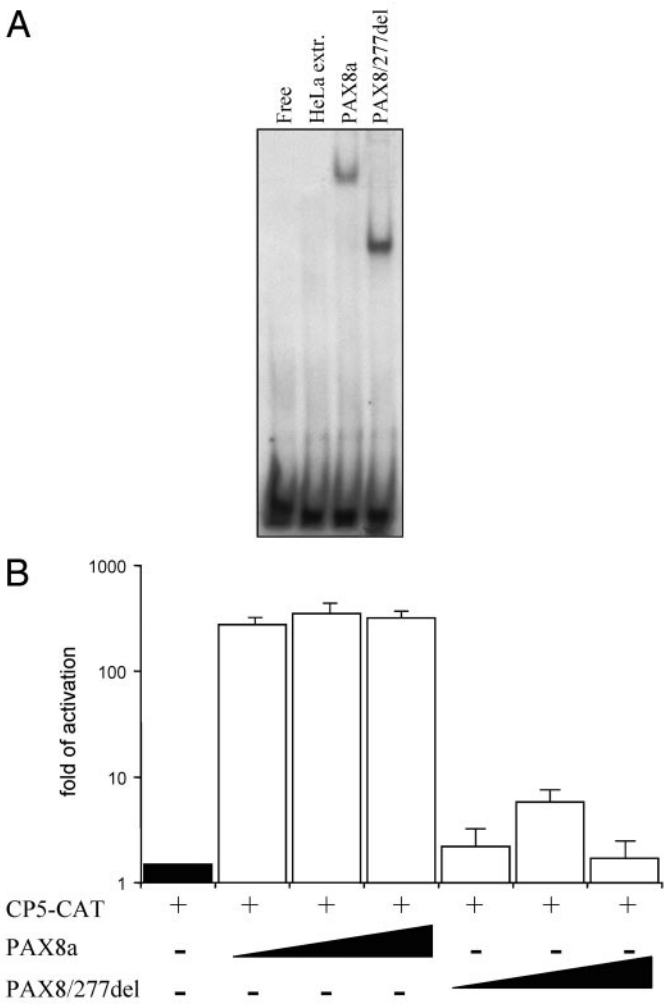


FIG. 3. Functional analysis of PAX8/277del. A, EMSA was performed with protein extracts prepared from HeLa cells transfected with PAX8a or PAX8/277del, using double-stranded oligonucleotide C as probe. Both proteins are able to form a low-mobility complex with oligo C. No retarded bands are present in the extract prepared from nontransfected HeLa cells used as negative control. B, HeLa cells were transiently transfected with the reporter plasmid CP5-CAT, the expression vector encoding PAX8a, and the deletion mutant PAX8/277del. Folds of activation are considered as ratio between values obtained with and without cotransfection of the expression vectors. CMV-LUC was added as internal reference, and CAT values were normalized to the LUC activity. Values are mean of the least three independent experiments (SD values are shown).

to thyroid hypoplasia, but in one familial case, the mother carrying the mutation showed mild hypothyroidism, elevated anti-TPO antibodies, and US normal thyroid location and size with discrete irregularities (8).

In this study, we report the results of PAX8 mutational screening on 54 subjects with thyroid dysgenesis. A new mutation was identified in a patient with thyroid hypoplasia. This expands the spectrum of PAX8 loss-of-function mutations in determining congenital hypothyroidism associated with thyroid dysgenesis. However, as indicated by previous studies in which three mutations were found in 145 (6) and one in 49 congenital hypothyroid patients (7), respectively, they account for very few cases of thyroid dysgenesis.

This new mutation is the first illustration of PAX8 small deletion. Nonsense-mediated mRNA decay could be expected from this mutation and thus lead to loss of function (23). On the other hand, if translated it results in a truncated protein that lacks the C-terminal domain but contains the paired domain, the octapeptide, and the homeodomain homology region.

Previous studies have indicated that different PAX8 splicing isoforms with an intact paired domain bind known paired-domain recognition sequences (18, 14), particularly oligonucleotides CT and C containing the TATA-box proximal Pax8-binding site of the TPO and the Tg promoters, respectively (14, 19). Our evaluation of the binding ability of PAX8/277del protein clearly showed that deletion of its C-terminal domain does not affect its ability to bind to a paired-domain recognition sequence. However, the PAX8/277del is not capable of activating transcription; thus, the C-terminal region downstream amino acid 276 is essential for PAX8 transcriptional activity. These data corroborate earlier demonstrations that used deletional mutants to show that the activation domain of PAX8 is localized in the region encoded by exons 10 and 11, and its amino acid sequence is conserved among Pax2, Pax5, and Pax8 (20).

The deletion was inherited from the mother and is also present in a brother, in line with the dominant model of inheritance proposed for the rare cases of familial thyroid dysgenesis due to PAX8 mutations described so far (6–8).

This loss-of-function mutation is associated with thyroid dysgenesis, compensated hypothyroidism, and euthyroidism in the same family. Such a broad spectrum of expression is strong evidence of the importance of the genetic background in phenotype establishment, as observed by Congdon (8), who found mild hypothyroidism in the mutated mother of a proband with congenital hypothyroidism associated with thyroid dysgenesis, although the mother's dysfunction may have been due to an autoimmune thyroid disease, as suggested by the presence of elevated antithyroid autoantibodies and discrete thyroid irregularities on US (8). Additional factors may modulate the phenotypic expression of PAX8 mutations. Assessment of the expression profile of normal human thyroid tissue using serial analysis of gene expression generated a collection of mRNA transcripts, of which 70% could not be attributed to a known human gene and may thus correspond to novel genes putatively involved in thyroid function (24). Although several genes involved in thyroid development and function have been identified, still more remain to be elucidated to explain thyroid physiology.

Haploinsufficiency, imprinting, and dominant-negative properties of the mutated allele have been proposed to explain the pathogenetic mechanism of PAX8 mutations (6–8). The fact that the mother and the brother did not have a phenotype argues against simple haploinsufficiency. Maternal imprinting seems to be ruled out because both siblings who have inherited the mutation from their mother display thyroid dysfunction. A dominant-negative effect of the PAX8 mutant allele seems excluded by the fact that the mother has normal thyroid function.

Moreover, phenotype variability due to differential allelic expression is unlikely because the two affected siblings share the same paternal PAX8 allele. Possible explanations for

these intrafamilial variable phenotypes include a polygenic etiology or stochastic expression of the PAX alleles, as documented for PAX5 (25).

Close monitoring of thyroid function in asymptomatic mutated patients is mandatory to disclose a possible late-onset thyroid dysfunction.

In conclusion, this study reports the first PAX8 gene mutation located outside the paired box domain. It also shows that the PAX8 C-terminal region is essential for transcriptional activity. The very marked differences in the phenotypes of the three subjects with the loss-of-function PAX8 mutation underlines that penetrance/expressivity are variable and suggests that other factors/genes may modulate phenotypic expression.

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References

1. **Poleev A, Fickenscher H, Mundlos S, Winterpacht A, Zabel B, Fidler A, Gruss P, Plachov D** 1992 PAX8, a human paired box gene: isolation and expression in developing thyroid, kidney and Wilms' tumors. Development 116:611–623
2. **Mascia A, Nitsch L, Di Lauro R, Zannini M** 2002 Hormonal control of the transcription factor PAX8 and its role in the regulation of thyroglobulin gene expression in thyroid cells. J Endocrinol 172:163–176
3. **Fabbro D, Pellizzari L, Mercuri F, Tell G, Damante G** 1998 Pax8 protein levels regulate thyroglobulin gene expression. J Mol Endocrinol 21:347–354
4. **Esposito C, Miccadei S, Salardi A Civitareale D** 1998 PAX8 activates the enhancer of the human thyroperoxidase gene. Biochem J 331:37–40
5. **Ohno M, Zannini S, Levy O, Carrasco N, Di Lauro R** 1999 The paired-domain transcription factor Pax8 binds to the upstream enhancer of the rat sodium/iodide symporter gene and participates in both thyroid specific and cyclic-AMP-dependent transcription. Mol Cell Biol 19:2051–2060
6. **Macchia PE, Lapi P, Krude H, Pirro MT, Missero C, Chiovato L, Souabni A, Baserga M, Tassi V, Pinchera A, Fenzi G, Gruters A, Busslinger M, Di Lauro R** 1998 PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nat Genet 19:83–86
7. **Vilain C, Rydlewski C, Duprez L, Heinrichs C, Abramowics M, Malvaux P, Renneboog B, Parma J, Costagliola S, Vassart G** 2001 Autosomal dominant transmission of congenital thyroid hypoplasia due to loss of function mutation of PAX8. J Clin Endocrinol Metab 86:234–238
8. **Congdon T, Nguyen LQ, Nogueira CR, Habiby RL, Medeiros-Neto G, Kopp P** 2001 A novel mutation (Q40P) in PAX8 associated with congenital hypothyroidism and thyroid hypoplasia: evidence for phenotypic variability in mother and child. J Clin Endocrinol Metab 86:3962–3967
9. **Tell G, Pellizzari L, Esposito G, Pucillo C, Macchia PE, Di Lauro R, Damante G** 1999 Structural defects of PAX8 mutant that give rise to congenital hypothyroidism. Biochem J 341:89–93
10. **Kopp P** 2002 Perspective: genetic defects in the etiology of congenital hypothyroidism. Endocrinology 143:2019–2024
11. **Wenzhong X, Oefner PJ** 2001 Denaturing high-performance liquid chromatography: a review. Hum Mutat 17:439–474
12. **Andersson S, Davis DL, Dahlback H, Jornvall H, Russell DW** 1989 Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. J Biol Chem 264:8222–8229
13. **Missero C, Cobellis G, De Felice M, Di Lauro R** 1998 Molecular events involved in differentiation of thyroid follicular cells. Mol Cell Endocrinol 140:37–43
14. **Poleev A, Wendler F, Fickenscher H, Zannini MS, Yaginuma K, Abbott C, Plachov D** 1995 Distinct functional properties of three human paired-box-protein, PAX8, isoforms generated by alternative splicing in thyroid, kidney and Wilms' tumors. Eur J Biochem 228:899–911
15. **Martin KJ, Lillie JW, Green MR** 1990 Evidence for interaction of different eukaryotic transcriptional activators with distinct cellular targets. Nature 346:147–152
16. **Berlingieri MT, Portella G, Grieco M, Santoro M, Fusco A** 1988 Cooperation between the polyomavirus middle-T-antigen gene and the human c-myc oncogene in a rat thyroid epithelial differentiated cell line: model of in vitro progression. Mol Cell Biol 8:2261–2266
17. **Zannini M, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, Taylor BA, Phillips SJ, Simeone A, Di Lauro R** 1997 TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation. EMBO J 16:3185–3197
18. **Kozmik Z, Kurzbauer R, Dorfler P, Busslinger M** 1993 Alternative splicing of Pax-8 gene transcripts is developmentally regulated and generates isoforms with different transactivation properties. Mol Cell Biol 13:6024–6035
19. **Zannini M, Francis-Lang H, Plachov D, Di Lauro R** 1992 Pax-8, a paired domain-containing protein, binds to a sequence overlapping the recognition site of a homeodomain and activates transcription from two thyroid-specific promoters. Mol Cell Biol 12:4230–4241
20. **Poleev A, Okladnova O, Musti AM, Schneider S, Royer-Pokora B, Plachov D** 1997 Determination of functional domains of the human transcription factor PAX8 responsible for its nuclear localization and transactivating potential. Eur J Biochem 247:860–869
21. **Toublanc JE** 1992 Comparison of epidemiological data on congenital hypothyroidism in Europe with those of the other parts of the world. Horm Res 138:230–235
22. **Delbert AF** 2002 Disorder of the thyroid in the newborn and infant. In: Sperling J, ed. Pediatric endocrinology. Philadelphia: Saunders; 161–185
23. **Cheng J, Fogel-Petrovic M, Maquat LE** 1990 Translation to near the distal end of the penultimate exon is required for normal levels of spliced triosephosphate isomerase mRNA. Mol Cell Biol 10:5215–5225
24. **Pauws E, Moreno JC, Tijssen M, Baas F, De Vijlder JJM, Ris-Stalpeers C** 2000 Serial analysis of the gene expression as a tool to assess the human thyroid expression profile and to identify novel thyroidal genes. J Clin Endocrinol Metab 85:1923–1927
25. **Nutt SL, Vambrie S, Steinlein P, Kozmik Z, Rolink A, Weith A, Busslinger M** 1999 Independent regulation of the two Pax5 alleles during B-cell development. Nat Genet 21:390–395

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