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RAPID COMMUNICATION

Missense Mutation in the Transcription Factor NKX2–5: A Novel Molecular Event in the Pathogenesis of Thyroid Dysgenesis

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Context: Congenital hypothyroidism (CH) is a common endocrine disorder with an incidence of 1:3000–4000 at birth. In 80–85% of cases, CH is caused by defects in thyroid organogenesis, resulting in absent, ectopically located, and/or severely reduced gland [thyroid dysgenesis (TD)]. Mutations in genes controlling thyroid development have demonstrated that in a few cases, TD is a Mendelian trait. However, accumulating evidence supports the view that the genetics of TD are complex, possibly with a polygenic/multifactorial basis. A higher prevalence of congenital heart disease has been documented in children with CH than in the general population. Such an association suggests a possible pathogenic role of genes involved in both heart and thyroid development. *NKX2–5* encodes a homeodomain-containing transcription factor with a major role in heart development, and mutations affecting this gene have been reported in individuals with congenital heart disease.

Objective: In the present work we investigated the possible involvement of *NKX2–5* mutations in TD.

Results: Our results indicate that *Nkx2–5*$^{-/-}$ embryos exhibit thyroid bud hypoplasia, providing evidence that *NKX2–5* plays a role in thyroid organogenesis and that *NKX2–5* mutations contribute to TD. *NKX2–5* mutational screening in 241 patients with TD allowed the identification of three heterozygous missense changes (R25C, A119S, and R161P) in four patients with TD. Functional characterization of the three mutations demonstrated reduced DNA binding and/or transactivation properties, with a dominant-negative effect on wild-type NKX2–5.

Conclusion: Our results suggest a previously unknown role of *NKX2–5* in the pathogenesis of TD. **(*J Clin Endocrinol Metab* 91: 1428–1433, 2006)**

PRIMARY CONGENITAL HYPOTHYROIDISM (CH) is the most frequent endocrine-metabolic disease in infancy, with an incidence of about 1/3000–4000 newborns. In most cases (80–85%), primary permanent CH is caused by alterations in thyroid gland morphogenesis, with a gland that can be absent (athyreosis), ectopically located, and/or severely reduced in size (hypoplasia) (1). All these entities are grouped under the term thyroid dysgenesis (TD).

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Abbreviations: CAT, Chloramphenicol acetyltransferase; CH, congenital hypothyroidism; CHD, congenital heart disease; DIO2, type II deiodinase; E, embryonic day; Foxe1, Forkhead box E1 (thyroid transcription factor 2); FT_3, free T_3; Luc, luciferase; TD, thyroid dysgenesis; TG, thyroglobulin; TPO, thyroperoxidase; TSHR, TSH receptor; TTF2, thyroid transcription factor 2; WT, wild type.

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Accumulating evidence indicates that genetic factors are involved in the pathogenesis of TD. Mutations in *TITF1* [*NKX2–1* or thyroid transcription factor 1 (*TTF-1*)], Forkhead box E1 (*FOXE1*; *TTF-2*), *PAX8*, and *TSHR* have been demonstrated as causes of TD in animal models and in a small percentage of patients with TD (1).

Because congenital malformations, mostly cardiac, have higher frequency in children with CH than in the general population (2, 3), it is possible to hypothesize that the genes involved in heart organogenesis might also participate in thyroid development. Based on these findings, genes expressed during embryogenesis in precursors of thyroid and heart are promising candidate genes to be investigated for their involvement in the pathogenesis of TD.

One of these is the gene encoding for *NKX2–5* (*CSX* or *NKX2.5*), a homeodomain-containing transcription factor that plays a crucial role in heart morphogenesis (4–6). In the mouse, *Nkx2–5* expression precedes the onset of heart myogenic differentiation and continues in the cardiomyocytes of

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embryos, fetuses, and adults (4, 7, 8). In addition, on embryonic d 8.5–9.5 (E8.5–9.5), the *Nkx2–5* transcript has been demonstrated in the precursors of thyroidal cells in the pharyngeal floor (9). At later stages, *Nkx2–5* pharyngeal expression is limited to the area corresponding to the thyroid primordium (9).

Several mutations in the *NKX2–5* gene have been identified in patients with congenital heart disease (CHD) (10–13); however, no data on the thyroidal status of these patients are currently available.

Given the foregoing, we considered *NKX2–5* as an excellent candidate gene involved in TD. In the present work, *NKX2–5* mutational screening was carried out in a group of 241 patients with TD, allowing identification of a heterozygous missense mutation in four cases. Functional characterization of the identified mutations showed a reduction in DNA binding and/or transactivation ability. These data suggest a possible pathogenic role for *NKX2–5* mutations in TD.

Patients and Methods

Embryonic expression of Nkx2–5 and analysis of thyroid phenotype in Nkx2–5$^{-/-}$ embryos

In situ hybridization on paraffin sections was performed as previously described (14) on staged *Nkx2–5*$^{-/-}$ embryos (gift from Dr. Seigo Izumo, Cardiovascular Division, Department of Medicine, Israel Deaconess Medical Center, Harvard Medical School, Boston, MA). The Nkx2–5 riboprobe was previously described (15).

Immunohistochemistry assays to detect the expression of Titf1, Pax8, and Foxe1 (TTF2) were carried out in *Nkx2–5*$^{-/-}$ and age-matched wild-type (WT) embryos using standard procedures (16).

Patient recruitment

Two hundred forty-one individuals with persistent CH (53 with athyreosis, 98 with thyroid ectopy, 15 with hypoplasia, and 75 with diagnosis of CH without goiter) were included in the study; 561 Italian volunteers with no thyroid-related disorders were also enrolled as controls. The study was approved by the ethical committees of the participating institutes. All individuals or parents of minors gave informed consent.

Mutation analysis

The entire *NKX2–5* coding region was screened for mutations on gnomic DNA extracted from peripheral blood lymphocytes. Oligonucleotide primers (sequences available upon request) were designed based on reported human *NKX2–5* cDNA and genomic sequences. PCRs were carried out in a 25-μl reaction volume with the following cycling parameters: 94 C for 8 min, 94 C for 45 sec, 64 C for 30 sec, and 72 C for 45 sec, for 33 cycles, then 72 C for 15 min.

PCR products were analyzed by denaturing HPLC, using the Wave 2100 DNA Fragment Analysis System (Transgenomics, Omaha, NE). Amplimers with abnormal denaturing profiles were sequenced bidirectionally.

Functional characterization of the mutant NKX2–5

Plasmids encoding murine NKX2–5 (17) and human type II deiodinase (DIO2)-chloramphenicol acetyltransferase (CAT) (18), thyroglobulin (TG)-CAT (19), and thyroperoxidase (TPO)-luciferase (Luc) (20) have been previously described.

Mutations were introduced by PCR-based mutagenesis with the QuikChange site-direct mutagenesis kit (Stratagene, La Jolla, CA) and were verified by sequencing.

Binding experiments, cotransfection, and Western blot experiments were performed as previously described (17). All experiments were performed in duplicate, and at least three separate transfections were carried out. Data (Luc/CAT or CAT/Luc ratios) are shown as the mean ± SD. Statistical analysis was performed using Student's *t* test.

Results

Thyroid phenotype in Nkx2–5$^{-/-}$ embryos

We examined the expression of *Nkx2–5* during thyroid development. Consistent with previous data (9), *in situ* hybridization demonstrated Nkx2–5 expression in the ventral region of the pharynx and in the thyroid bud on E8.5. Nkx2–5 was expressed in the thyroid primordium up to E11.5; thereafter, Nkx2–5 transcript was no longer detected in the thyroid bud, whereas it was present in the heart region (data not shown).

Because *Nkx2–5* mRNA was present in the thyroid primordium at an early stage of development, we investigated whether its expression was required for thyroid morphogenesis. To such a goal, we analyzed the phenotype of the thyroid primordium in *Nkx2–5*$^{-/-}$ mouse embryos by immunohistochemistry on E9.5, using the anti-Pax8, anti-Titf-1, and anti-Foxe1 antibodies (Fig. 1). At this stage, the thyroid primordium appeared as a midline epithelial thickening in the ventral wall of the primitive pharynx. The endodermal cells fated to become thyroid follicular cells were univocally identified by the expression of Nkx2–1, Pax8, and Foxe1. The

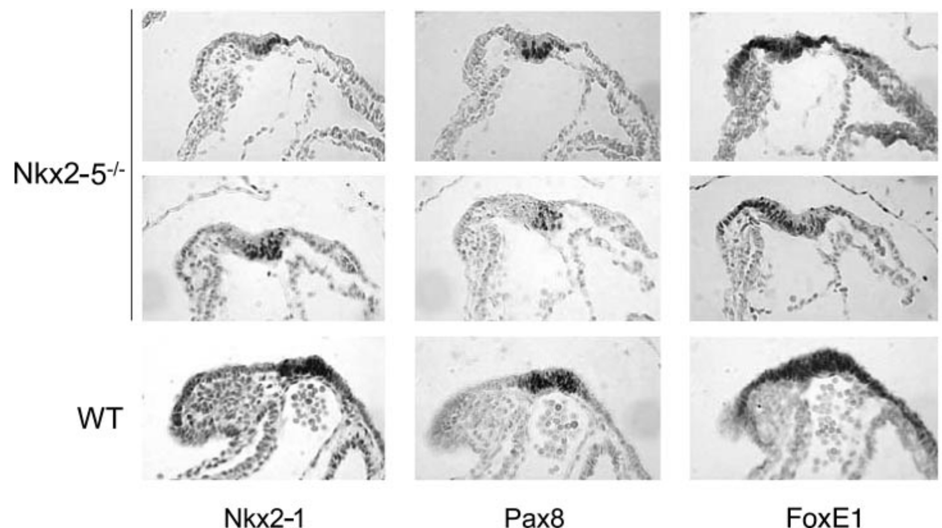

FIG. 1. Thyroid budding in *Nkx-5*$^{-/-}$ embryos. Immunohistochemistry with Nkx2–1, Pax8, and FoxE1 antibodies in two different *Nkx2–5*$^{-/-}$ and one WT embryos on E9.5. In *Nkx2–5*$^{-/-}$ mice, thyroid budding occurs normally, but the number of thyroid precursor cells is clearly reduced compared with WT.

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identification in the foregut *Nkx2–5*$^{-/-}$ embryos of a thyroid primordium as a small outgrowing bud of endodermal cells indicated that thyroid morphogenesis occurred, and thyroid transcription factors were normally expressed. However, serial sections of *Nkx2–5*$^{-/-}$ and WT embryos revealed that the number of cells stained with Nkx2–1 and Pax8 were strongly reduced (<50%). Foxe1-positive cells also appeared reduced; however, the presence of a positive signal in the entire pharynx makes a precise quantification of such a reduction difficult. Our findings indicate that the thyroid bud appears smaller in the *Nkx2–5*$^{-/-}$ embryo compared with the WT embryo, suggesting that *Nkx2–5* is required during thyroid development.

Search for mutations in the NKX2–5 gene

Given the role of Nkx2–5 in thyroid development, we screened for mutations in the coding region of the *NKX2–5* gene a cohort of 241 individuals affected by TD. Three distinct heterozygous missense mutations were identified in four subjects (Fig. 2): two of them (355G→T and 482G→C) had not previously been reported, whereas the third (73C→T) was previously described in patients with CHD (12). The 355G→T and 482G→C transversions, predicting, respectively, the A119S change, occurred a few residues upstream from the beginning of the homeodomain, and the R161P substitution occurred within the homeodomain. The third mutation (73C→T) changed an arginine residue to cysteine at position 25 (R25C).

The A119S mutation was observed in a 13-yr-old girl with ectopic thyroid and no documented congenital heart defect (patient 1). Her thyroid function at diagnosis indicated severe hypothyroidism with a TSH level greater than 150 mU/liter (normal range, 0.3–4.2), free T_3 (FT_3) of 1.7 pg/ml (normal range, 2.16–4.60), and FT_4 of 1.5 pg/ml (normal range, 7.6–17.2).

The R161P change was found in a 6-yr-old girl (patient 2) with thyroid ectopy and hypothyroidism (TSH, 47 mU/liter; FT_3, 2.8 pg/ml; FT_4, 1.2 pg/ml). Of note, this patient exhibited patent foramen ovale at birth that resolved spontaneously and minor mitral valve insufficiency.

Finally, the R25C substitution was observed in one case with thyroid ectopy (patient 3) and in one case with athyreosis of the gland (patient 4). The former was a 24-yr-old woman with no evidence of cardiac malformation (TSH, 300 mU/liter; FT_3, 2.74 pg/ml; FT_4, 5.4 pg/ml). Patient 4 was a 15-yr-old boy with athyreosis (TSH, 419 mU/liter; FT_3, 2,20 pg/ml; FT_4, 1.6 pg/ml) born at the 35th week of gestation. Since birth, the baby presented bilateral cortex atrophy and attention deficit hyperactivity disorder, but his intelligence quotient was normal, and no history of cardiac abnormalities was reported.

The 355G→T and 482G→C changes were not observed among 561 controls, whereas the 73C→T substitution was identified in a single, apparently healthy individual. Statistical analysis indicates a significant association between these defects and disease ($P = 0.0305$, by Fisher's exact test).

In all cases the mutation was inherited from one of the parents (Fig. 2). Electrocardiogram, thyroid function test, and cardiac and thyroidal ultrasounds were performed in all family members of the patients carrying the mutation. The results documented the presence of minor mitral valve insufficiency in the father transmitting the R161P change (family 2), whereas in the family segregating the A119S change, the mother transmitting the mutation exhibited autoimmune hypothyroidism and was on life-long treatment with L-T_4.

Functional characterization of the TD-associated NKX2–5 mutants

The transcriptional properties of WT and R25C, A119S, and R161P NKX2–5 mutants were investigated by cotrans-

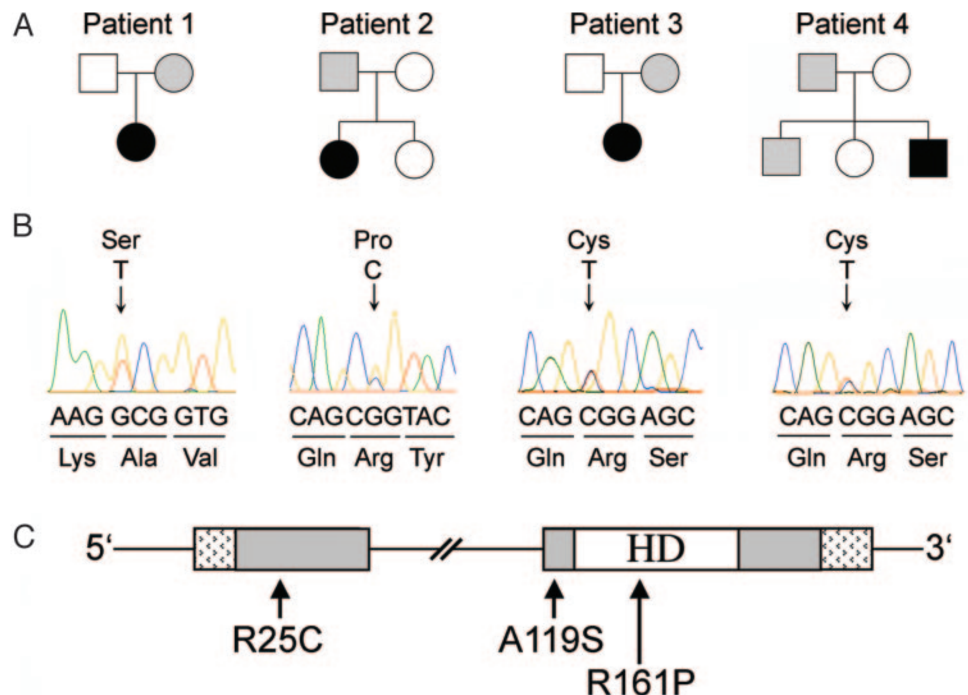

FIG. 2. *NKX2–5* mutations in patients with TD. A, Family pedigrees. Probands are shown in *black*; unaffected family members carrying the mutations are shown in *gray*. B, Electropherograms showing heterozygous missense mutations in patients with TD. C, Schematic representation of the human NKX2–5 gene. ▨, Coding region. HD, Homeobox region. Identified amino acid substitutions and positions of affected residues are shown.

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fection assays in the HeLa cells as previously described (15, 17). All NKX2–5 mutants were able to activate the reporter genes in a dose-dependent manner, although their activity was reduced compared with WT in all the assays performed (*DIO2*, *TG*, and *TPO* promoters) at all tested concentrations (10, 25, 50, and 100 ng vector; Fig. 3A). This effect was not related to a reduction in the mutant protein concentration, as confirmed by Western blot analysis (data not shown).

Because NKX2–5 binds to DNA and promotes transcription as a dimer (8), we evaluated a possible dominant-negative effect of the three NKX2–5 mutants to WT NKX2–5. To this aim, we performed cotransfection experiments in HeLa cells with WT and mutant NKX2–5 plasmids. The results are shown in Fig. 3B. Coexpression of WT with the same amount of the three mutated proteins resulted in a reduction of luciferase activity, suggesting a dominant-negative effect of the mutants.

DNA binding properties of the three NKX2–5 mutants were also analyzed by EMSAs using a short DNA stretch corresponding to the NKX2–5-binding site within the *DIO2* (oligo D) (17). The results are shown in Fig. 4. A significant reduction in DNA binding was observed for both A119S and R161P mutants, whereas the R25C mutation apparently did not interfere with the binding, as previously reported (21). WT, R25C, A119S, and R161P NKX2–5 protein levels were similar, as demonstrated by Western blots with the same extracts used for the EMSA assays.

Discussion

In this study we provided evidence for the contribution of *NKX2–5* gene mutations to the pathogenesis of TD. Specifically, we showed that although thyroid budding occurs normally in $Nkx2\text{–}5^{-/-}$ embryos, the number of thyroid precursor cells is clearly reduced compared with that in WT cells. Consistently, the expression of *Titf1*, *Foxe1*, and *Pax8* in the thyroid primordium does not seem to be impaired in $Nkx2\text{–}5^{-/-}$ embryos.

Mutational screening of the entire *NKX2–5* coding sequence in a cohort of 241 patients allowed the identification of three mutations, R25C, A119S, and R161P, in four subjects with TD (three patients with thyroid ectopy and one with athyreosis). Functional studies demonstrated that these mutants exhibited a significant functional impairment, with reduction of transactivation properties and dominant-negative effect, which was associated with reduced DNA binding in the A119S and R161P mutants. The R25C amino acid change was previously characterized, showing a defect only in dimer formation, without any significant effect on DNA binding as monomer or transactivation of the atrial natriuretic factor (*ANF*) promoter (21). Our results indicate that although the R25C NKX2–5 mutant normally also binds the *DIO2* promoter, its activity on the *DIO2*, *TG*, and *TPO* promoters is significantly impaired. In addition the R25C NKX2–5 exerts a dominant-negative action on the activity of WT NKX2–5 measured on *DIO2* and *TG* promoters. The observed discrepancies from previously published data could be the con-

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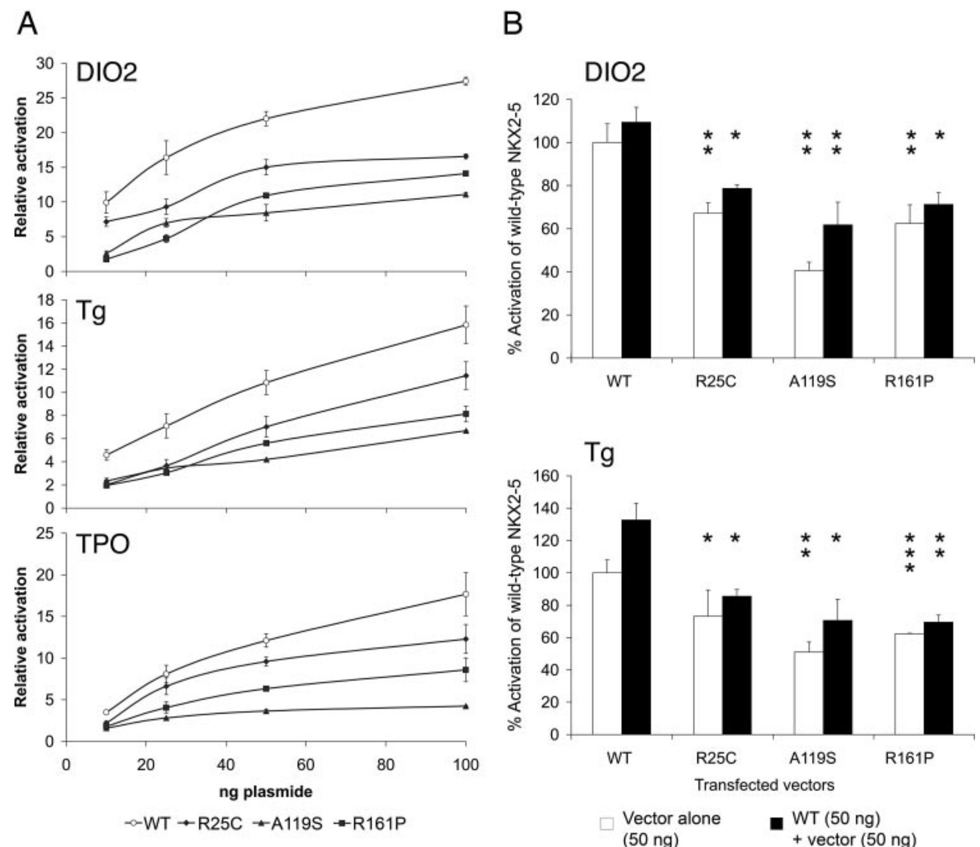

FIG. 3. Functional characterization of the NKX2.5 mutants. A, Transactivation of the human DIO2, TG, and TPO promoters. The relative activations obtained on the three promoters used with increasing amounts (10, 25, 50, and 100 ng) of WT, R25C, A119S, or R161P NKX2–5 proteins are shown. On all examined promoters and at each examined concentration, protein activity was significantly lower for all mutants compared with WT ($P < 0.05$ in all comparisons). B, Dominant-negative effect of the R25C, A119S, and R161P NKX2–5 mutants on transactivation of the human DIO2 and TG promoters. Experiments were performed using 50 ng of each construct (□) or 50 ng WT NKX2–5 plus 50 ng of each construct (■). Results are expressed as the percentage of activation on both DIO2 and TG promoters compared with 50 ng WT NKX2–5. Coexpression of WT protein and each mutant resulted in a significant reduction in the transactivation activity as a consequence of a dominant-negative effect. Statistical significance was calculated with Student's *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

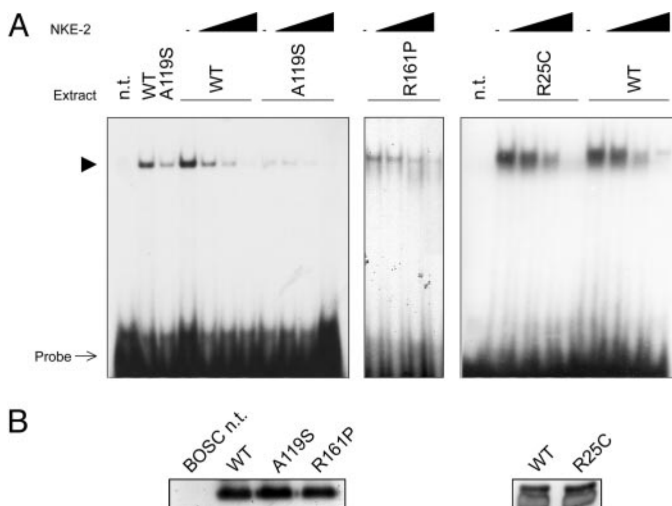

FIG. 4. DNA binding ability of the R25C, A119S, and R161P NKX2–5 mutants. A, EMSA performed with nuclear extracts of BOSC cells transfected with WT, R25C, A119S, and R161P NKX2–5 constructs. *Arrowheads* indicate the bound NKX2–5. n.t., Nontransfected cells. Extracts were also incubated with increasing amounts of cold NKE-2 oligo, which contains the high-affinity NKX2–5-binding site from the proximal atrial natriuretic factor (ANF) promoter, as competitor. Increasing amounts of NKE-2 DNA produced a significantly higher displacement of bound oligo D in the case of mutant proteins compared with WT. B, Western blots performed with the same extract as that used for EMSA. Gels were hybridized with the anti-Flag antibody as previously described (15). Levels of expressions were similar for both mutants and WT NKX2–5.

sequence of the different experimental models used, and they support the hypothesis that NKX2–5 regulates the expression of target genes through complex mechanisms. These mechanisms can be different in various tissues and at each developmental stage and can be mediated via the interaction with other proteins. In this scenario, it has been recently demonstrated that the expression of a dominant-negative Nkx2–5 (N188K) isoform can interfere with Titf1 action (22).

NKX2–5 is essential for normal heart morphogenesis, myogenesis, and function (6), and several loss of function mutations in *NKX2–5* have been described in patients with CHD (10–13). The coinheritance of *NKX2–5* mutations and different CHD suggests that this transcription factor is involved in diverse developmental processes. In our patients a very mild (patient 2) or a completely absent (patients 1, 3, and 4) cardiac phenotype was observed. This observation raises the question of whether the effect of the mutations could be specifically critical for thyroid development rather than that of the heart. Analyses of animal models with tissue-specific gene targeting will probably address this point. In fact, other genes of the NK2 family, including *Titf1* or *Nkx2–6* and *Nkx2–3*, are expressed in addition to *Nkx2–5* in the endodermal layer of the developing pharynx and in the thyroid anlage, with possible redundant functions (6, 23, 24).

In all cases, mutations were not *de novo*, but were transmitted by one of the parents. Of note, only the father in family 2 presented a minor mitral valve insufficiency. In addition, of 561 controls, one normal subject presented the same R25C mutation observed in two of our patients. The identification of one control carrying the mutation does not change the significant statistical association between TD and a mutation in NKX2–5; over all, these data suggest that *NKX2–5* mutations have a variable penetrance and clinical significance, as previously demonstrated in both humans (10–12) and mice (5, 6), where a strong influence of the genetic background has been demonstrated (5). The incomplete penetrance and variable expressivity of mutations at both thyroidal and cardiac levels strongly suggest that other genes are expected to contribute to the phenotype and support the view that TD is a polygenic disorder (25) or that stochastic epigenetic mechanisms can play a relevant role (26).

In conclusion, we identified *NKX2–5* as a novel gene involved in the pathogenesis of congenital hypothyroidism with ectopy or athyreosis of the thyroid gland. The variable phenotypes in patients with loss of function *NKX2–5* mutations suggest that other factors/genes may play a role modulating penetrance and expressivity at both the cardiac and thyroidal levels.

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The authors have no conflict of interest.

References

1. **De Felice M, Di Lauro R** 2004 Thyroid development and its disorders: genetics and molecular mechanisms. Endocr Rev 25:722–746
2. **Roberts H, Moore C, Fernhoff P, Brown A, Khoury M** 1997 Population study of congenital hypothyroidism and associated birth defects, Atlanta, 1979–1992. Am J Med Genet 71:29–32
3. **Olivieri A, Stazi MA, Mastroiacovo P, Fazzini C, Medda E, Spagnolo A, De Angelis S, Grandolfo ME, Taruscio D, Cordeddu V, Sorcini M** 2002 A population-based study on the frequency of additional congenital malformations in infants with congenital hypothyroidism: data from the Italian Registry for Congenital Hypothyroidism (1991–1998). J Clin Endocrinol Metab 87:557–562
4. **Komuro I, Izumo S** 1993 Csx: a murine homeobox-containing gene specifically expressed in the developing heart. Proc Natl Acad Sci USA 90:8145–8149
5. **Biben C, Weber R, Kesteven S, Stanley E, McDonald L, Elliott DA, Barnett L, Koentgen F, Robb L, Feneley M, Harvey RP** 2000 Cardiac septal and valvular dysmorphogenesis in mice heterozygous for mutations in the homeobox gene Nkx2–5. Circ Res 87:888–895
6. **Tanaka M, Schinke M, Liao HS, Yamasaki N, Izumo S** 2001 Nkx2.5 and Nkx2.6, homologs of *Drosophila* tinman, are required for development of the pharynx. Mol Cell Biol 21:4391–4398
7. **Lyons I, Parsons LM, Hartley L, Li R, Andrews JE, Robb L, Harvey RP** 1995 Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2–5. Genes Dev 9:1654–1666
8. **Kasahara H, Bartunkova S, Schinke M, Tanaka M, Izumo S** 1998 Cardiac and extracardiac expression of Csx/Nkx2.5 homeodomain protein. Circ Res 82: 936–946
9. **Lints TJ, Parsons LM, Hartley L, Lyons I, Harvey RP** 1993 Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development 119:419–431
10. **Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP, Maron**

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BJ, Seidman CE, Seidman JG 1998 Congenital heart disease caused by mutations in the transcription factor NKX2–5. Science 281:108–111
11. **Benson DW, Silberbach GM, Kavanaugh-McHugh A, Cottrill C, Zhang Y, Riggs S, Smalls O, Johnson MC, Watson MS, Seidman JG, Seidman CE, Plowden J, Kugler JD** 1999 Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J Clin Invest 104:1567–1573
12. **McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E** 2003 NKX2.5 mutations in patients with congenital heart disease. J Am Coll Cardiol 42:1650–1655
13. **Hirayama-Yamada K, Kamisago M, Akimoto K, Aotsuka H, Nakamura Y, Tomita H, Furutani M, Imamura S, Takao A, Nakazawa M, Matsuoka R** 2005 Phenotypes with GATA4 or NKX2.5 mutations in familial atrial septal defect. Am J Med Genet A 135:47–52
14. **Dathan N, Parlato R, Rosica A, De Felice M, Di Lauro R** 2002 Distribution of the titf2/foxe1 gene product is consistent with an important role in the development of foregut endoderm, palate, and hair. Dev Dyn 224:450–456
15. **Dentice M, Luongo C, Elefante A, Romina R, Ambrosio R, Vitale M, Rossi G, Fenzi G, Salvatore D** 2004 Transcrption factor Nkx-2.5 induces sodium/iodide symporter (NIS) gene expression and partecipates in retinoic acid- and lactation-induced transcription mammary cells. Mol Cell Biol 24:7863–7877
16. **Parlato R, Rosica A, Rodriguez-Mallon A, Affuso A, Postiglione MP, Arra C, Mansouri A, Kimura S, Di Lauro R, De Felice M** 2004 An integrated regulatory network controlling survival and migration in thyroid organogenesis. Dev Biol 276:464–475
17. **Dentice M, Morisco C, Vitale M, Rossi G, Fenzi G, Salvatore D** 2003 The different cardiac expression of the type 2 iodothyronine deiodinase gene between human and rat is related to the differential response of the Dio2 genes to Nkx-2.5 and GATA-4 transcription factors. Mol Endocrinol 17:1508–1521
18. **Gereben B, Salvatore D, Harney JW, Tu HM, Larsen PR** 2001 The human, but not rat, dio2 gene is stimulated by thyroid transcription factor-1 (TTF-1). Mol Endocrinol 15:112–124
19. **Sinclair AJ, Lonigro R, Civitareale D, Ghibelli L, Di Lauro R** 1990 The tissue-specific expression of the thyroglobulin gene requires interaction between thyroid-specific and ubiquitous factors. Eur J Biochem 193:311–318
20. **Francis-Lang H, Price M, Polycarpou-Schwarz M, Di Lauro R** 1992 Cell-type expression of the rat thyroperoxidase promoter indicates common mechanisms for thyroid-specific gene expression. Mol Cell Biol 12:576–588
21. **Kasahara H, Lee B, Schott JJ, Benson DW, Seidman JG, Seidman CE, Izumo S** 2000 Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease. J Clin Invest 106:299–308
22. **Dentice M, Luongo C, Elefante A, Ambrosio R, Salzano S, Zannini M, Nitsch R, Di Lauro R, Rossi G, Fenzi G, Salvatore D** 2005 Pendrin is a novel in vivo downstream target gene of the TTF-1/Nkx-2.1 homeodomain transcription factor in differentiated thyroid cells. Mol Cell Biol 25:10171–10182
23. **Reecy JM, Yamada M, Cummings K, Sosic D, Chen CY, Eichele G, Olson EN, Schwartz RJ** 1997 Chicken Nkx-2.8: a novel homeobox gene expressed in early heart progenitor cells and pharyngeal pouch-2 and -3 endoderm. Dev Biol 188:295–311
24. **Biben C, Wang CC, Harvey RP** 2002 NK-2 class homeobox genes and pharyngeal/oral patterning: Nkx2–3 is required for salivary gland and tooth morphogenesis. Int J Dev Biol 46:415–422
25. **Amendola E, De Luca P, Macchia PE, Terracciano D, Rosica A, Chiappetta G, Kimura S, Mansouri A, Affuso A, Arra C, Macchia V, Di Lauro R, De Felice M** 2005 A mouse model demonstrates a multigenic origin of congenital hypothyroidism. Endocrinology 146:5038–5047
26. **Vassart G, Dumont J** 2005 Thyroid dysgenesis: multigenic or epigenetic, or both? Endocrinology 146:5035–5037

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