Genetic analysis of the paired box transcription factor gene in a cohort of Polish patients with primary congenital hypothyroidism and dysgenetic thyroid glands

Małgorzata Kumorowicz-Czoch[1], Anna Madetko-Talowska[2], Adam Dudek[3], Dorota Tylek-Lemańska[4]

[1]Department of Pediatric and Adolescent Endocrinology, Chair of Pediatrics, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Cracow, Poland Head of Department: Professor Jerzy Starzyk M.D., Ph.D.
[2]Division of Medical Genetics, Chair of Pediatrics, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Cracow Head of Department: Professor Jacek J. Pietrzyk M.D., Ph.D.
[3]Pediatric Endocrinology Outpatient Clinic, Provincial Hospital No 2, Rzeszow
[4]Division of Mass Screening and Metabolic Diseases, University Children's Hospital of Cracow

Financial support: The study was supported by grant no. N N407 160640 from the National Science Centre

Disclosure: none

Context

The morphological and biochemical phenotype of *PAX8* mutation in patients with congenital hypothyroidism (CH) is variable. The contribution of mutations in *PAX8* gene in children with CH and dysgenetic thyroid glands still remains a subject of interest of researchers.

Objective

The aim of the study was screening for *PAX8* gene mutations in children with CH due to thyroid dysgenesis.

Patients

48 children (37 girls, 11 boys) with CH associated with: thyroid ectopy (n=22), agenesis (n=10), hypoplasia (n=6), or thyroid dysgenesis of unknown cause (n=10) were enrolled. The study participants were born in south-eastern Poland in the years 1993-2012 and selected in neonatal mass screening for CH. CH was confirmed, manager, and followed-up in two centers of pediatric endocrinology in southeastern Poland.
Based on the results of serum TSH and thyroxin/free thyroxin (T4/fT4) during the first confirmation of diagnosis, 38 cases were classified as having apparent hypothyroidism. Compensated hypothyroidism was diagnosed in 12 patients. In seven out of the 48 patients (14.5%) with TD, coexistent congenital diseases were detected.

Methods

Blood samples (2ml) were drawn from all the study participants. Subsequently, DNA was extracted with the use of MasterPure™ DNA Purification Kit for Blood Version II (Epicentre Biotechnologies). Concentration of DNA samples was measured with the use of a NanoDrop 1000 Spectrophotometer (Thermoscientific).

DNA was extracted from peripheral blood samples with the use of Master Pure DNA Purification Kit (Epicentre Biotechnologies).The 12 exons of the *PAX8* gene along with their exon-intron boundaries were amplified and sequenced by Sanger method. Capillary electrophoresis was run on ABI 3500 (Life Technologies-Applied Biosystems; Carlsbad, CA, USA).

Results

Coding regions and exon-intron boundaries of the *PAX8* gene were fully sequenced in all the 48 patients with CH and TD. Our study revealed the presence of two novel genetic variants and thirteen well-described SNPs in the *PAX8* gene in Polish CH and TD patients (Table 1.). A heterozygous transition in exon 3 of the *PAX8* gene: **c.68G>A** was detected in a 3-year-old girl with thyroid hypoplasia - Fig. 1, and was not observed in her clinically and biochemically euthyroid mother and father (Family 1) - Fig. 2. Additionally, a novel genetic variant in 3'UTR: **c.*416C>T** in exon 12 of the *PAX8* gene occurred in a 3-year-old boy with thyroid ectopy and concomitant congenital urogenital malformation. The patient's mother and two out of three examined brothers carried this genetic variation in one allele, while it was of a wild type in the father and the third brother. The thyroid function in all the members of the patient's family was normal.

Tab.1. Details of *PAX8* genetic changes detected in CH and TD patients.
Location of genetic variants according to the *PAX*8 transcript ENST00000429538.

Localization	Genetic variants	Reference polymorphism accession number	Frequencies of *PAX8* genetic changes in screened patients
Intron 2-3	**c.25+24T>C**	rs1867763	29/48 (60%)
Exon3	**c.68G>A p.G23D**	-	1/48 (2%)
Exon5	**c.404A>G p.K135R**	rs190431939	2/48 (4%)
Intron 6-7	**c.601+51C>G**	rs4849186	13/48 (27%)
Exon 9	**c.906T>C p.F329L**	rs3188996	1/48 (2%)
Exon 12	**c.*367G>A**	rs144113497	1/48 (2%)
	c.*416C>T	-	1/48 (2%)
	c.*1077T>C	rs1049137	11/48 (23%)
	c.*1083A>C	rs1479	18/48 (37.5%)
	c.*1121A>C	rs1478	19/48 (39.5%)
	c.*1842G>A	rs144041400	4/48 (8%)
	c.*1991C>G	rs874898	18/48 (37.5%)
	c.*2223A>G	rs895412	36/48 (75%)
	c.*2386A>G	rs1077855	46/48 (96%)
	c.*2555C>T	rs2019137	33/48 (69%)

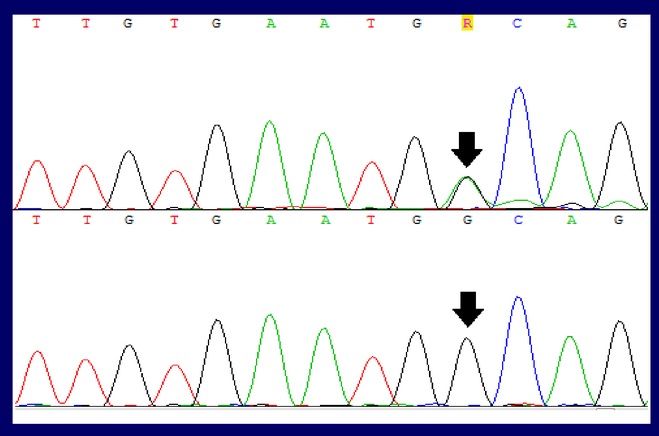

Fig. 1. Sequencing electropherogram: heterozygous transition G to A at 68 nucleoitide of *PAX8* gene.

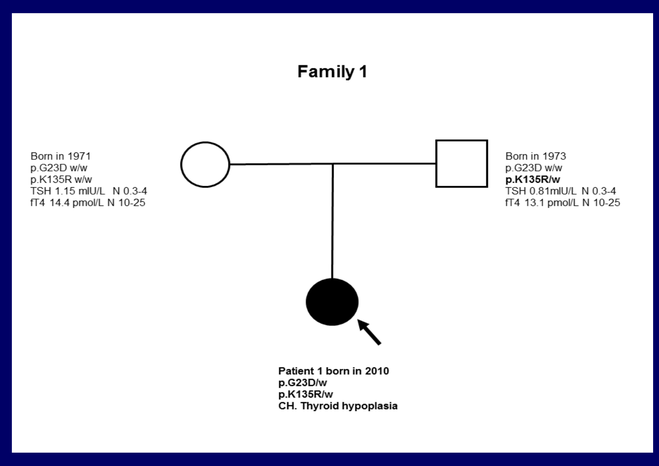

Fig. 2. Pedigree of Family 1.

Conclusions

The study reports the occurrence of two novel heterozygous substitutions in the *PAX8* gene. De novo genetic variant detected in a girl with thyroid hypoplasia might have an impact on her thyroid development, and in consequence, be responsible for thyroid hormones deficiency. However, indisputability of this statement calls for further functional analysis.

Acknowledgements

We appreciate greatly the cooperation of all patients and their families.
We express our gratitude to Teresa Nizankowska-Blaz, Elzbieta Golebiowska and Katarzyna Dolezal-Oltarzewska for the referral of patients, to Teresa Ochman and Ewa Samborska for excellent laboratory assistance, and to Joanna Szydlowska for English proofreading and corrections.
We would like to thank our families for supporting us in all aspects.

Contact

Malgorzata Kumorowicz-Czoch M.D., Ph.D.
Collegium Medicum Jagiellonian University
Św. Anny 12, Cracow, Poland
e-mail: malgorzata.kumorowicz-czoch@uj.edu.pl