RESULTS OF THE hTPO MUTATIONAL SCREENING IN BULGARIAN PATIENTS WITH CONGENITAL HYPOTHYROIDISM

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INTRODUCTION

Congenital hypothyroidism (CH) is a partial or complete loss of function of the thyroid gland resulting in absent or decreased synthesis and secretion of thyroid hormones (TH) affecting infants since birth. Mutations of the hTPO gene are associated with autosomal recessive forms of CH. Based on our TSH screening results, the number of children with eutopic primary CH is increasing. TPO is mediating two central steps of TH synthesis: 1) organification of iodid to iodinated tyrosyl residues and 2) coupling of MIT and DIT to T3 and T4. The start of the hTPO mutational screening was in 1997 (1).

AIM

To set up a multistep mutational strategy in CH patients with eutopic thyroid glands, starting by the analysis of the hTPO gene.

MATERIAL AND METHODS

Selection of patients suitable for hTPO molecular analysis using the candidate gene approach (permanent CH, eutopic thyroid, elevated Tg). Thirty nine patients from 32 families were included. Molecular analysis on genomic DNA was done by SANGER sequencing and MLPA.

RESULTS

Seven different mutations were found by SANGER sequencing (Fig 1, Table 1):

- c.31_50dup, p.(Glu17AspfsTer77), in exon 2;
- c.819+4A>C (2.6%), and c.621_622delGG,: p.(Glu207AspfsTer11) (1.3%) – both in exon 7, the second one is novel;
- c.1430_1450del, p.(Ala477Asn483del), in exon 9 (1.3%), and one whole gene deletion detected by MLPA analysis.

In 8 of the 39 patients (20.5%) the phenotype could be explained by the genotype: 3 of all patients showed homozygous mutations - rs76366277:c.2422delT p.(Cys808AlafsTer24), exon 14 (6.4%); rs17855780, c.208C>G, p.(Pro70Ala), exon 4 (5.1%), and a novel one c.1268G>A, p.Gly393ARG in 8 exon (2.6%), 3 were compound heterozygous carriers. 2 of the patients (2.6%) were carriers of heterozygous deletions of all exons included in the MLPA kit (Fig.2 a-d).

Table 1: Phenotype – genotype data in patients with hTPO mutations

Nr CMM	Sex	familial	Ethnicity*	NTS participation	NTSH mu/l Delfia	age /d/	sTSH mU/l	sT4 nmol/l	age /d/	sfT4 pmol/l	Tg ng/ml	Genotype		Authors
HST 85	m	no	b	yes	284	6	576	7.6	14	ND	479	hetero	NM_000547.5:c.31_50dup, p.(Glu17AspfsTer77)	exon2	Bikker, H. 1994 (2)
HST 77a	m	no	b	none			339		46	2.9	ND	hetero	NM_000547.5:c.621_622delGG, p.(Glu207AspfsTer11)	exon7	new
HST 100a	m	brother CH	b	yes	245	3	1260	11.6	22	0.97	412.5	hetero	1-17 deletion (MLPA)	1_17 exons	new
HST 100b	m	brother CH	b	yes	144	4	>100	ND	12	4.33	ND	hetero	1-17 deletion (MLPA)	1_17 exons	new
HST 133	m	no	b	yes	329	3	373	11	10	2	497	hetero	NM_000547.5:c.1522_1542del p.(Phe508_Leu514del)	exon 9	new
HST 87	f	yes, mother	b	yes	199	2	705	8	12	1.3	210	hetero	ex 14 rs763662774, CM032390 NM_000547.5:c.2422delT p.Cys808AlafsTer24	exon14	Bakker, B. 2000 (3)
HST 83	m	yes, sister	r	yes	145	4	107	ND	9	5.7	509	comp hetero	rs575359996 NM_000547.5:c.819+4A>C, rs763662774, CM032390 NM_000547.5:c.2422delT p.(Cys808AlafsTer24)	exons7;14	Bakker, B. 2000 (3)
HST 74	f	yes, brother	r	none/Greece	ND		211	ND	1y6m	3.3	ND	comp hetero	rs575359996 NM_000547.5:c.819+4A>C, rs763662774, CM032390 NM_000547.5:c.2422delT p.(Cys808AlafsTer24)	exons7;14	Bakker, B. 2000 (3)
HST 121	f	no	b	yes	297	4	1120	10.8	14	ND	547	homo	NM_000547.5:c.573G>T, p.(Trp191Cys)	exon5	Stoeva, I. 2015 (4)
HST 119	m	no	b	yes	238	3	1130	9.2	22	ND	491	homo	ex 14 rs763662774, CM032390 NM_000547.5:c.2422delT p.(Cys808AlafsTer24)	exon14	Cangul, H. 2016 (5)
HST 108	f	no	b	none/before NTS			>100	22	46	ND	22	homo	NM_000547.5:c.1177G>T, p.(Gly393Arg)	exon 8	new

*(b-bulgarian, r-roma)

Fig. 1 Schematic drawing of TPO gene with the mutations (red) of the patients and their allele frequency

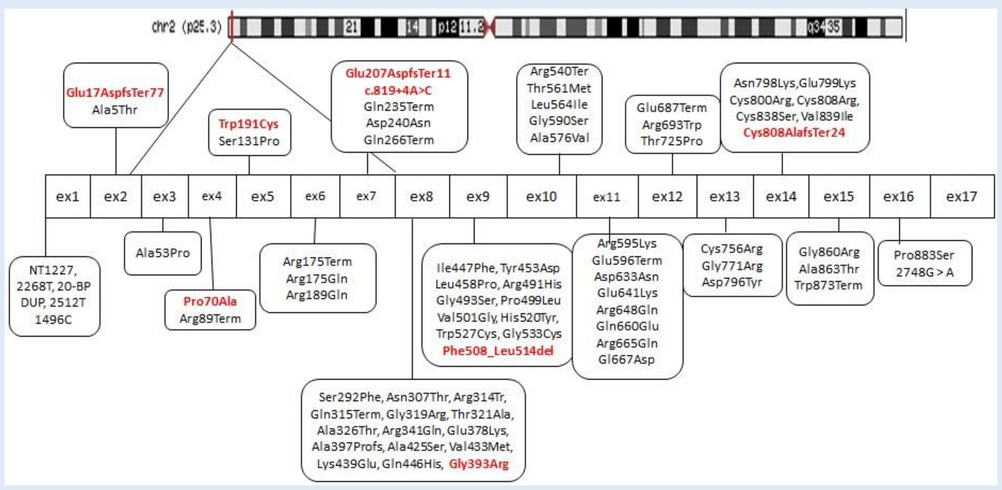
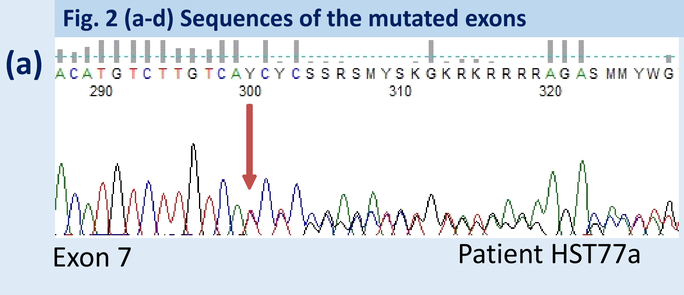
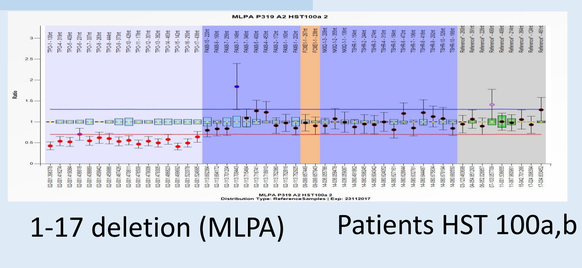
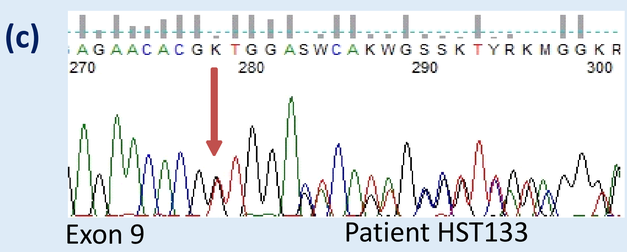
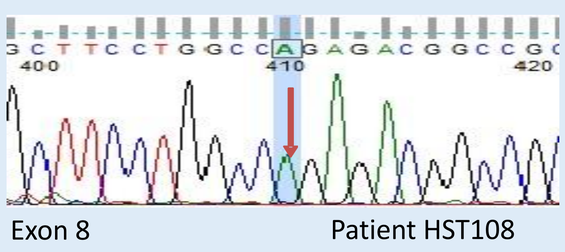


Fig. 2 (a-d) Sequences of the mutated exons









CONCLUSIONS

There is considerable heterogeneity among the hTPO gene mutations in the screened population and novel mutations were found. Some patients with large eutopic glands, high Tg and severe CH were negative in the present mutation screen, therefore targeted gene panel NGS is the next step of analysis that could establish the genetic causes of CH in Bulgarian patients in a higher percentage.

References: 1.Stoeva, I., P. Ambrugger, H. Biebermann et al. First results of the mutational screening in the hTPO gene in Bulgarian children with congenital hypothyroidism.- *Horm Res*., **53**, 2000, Suppl.2., p. 107; 2. Bikker, H. , MT den Hartog, F. Baas et al. A 20 basepair duplication in the human thyroid peroxidase gene results in a total iodide organification defect and congenital hypothyroidism. J Clin Endocrinol Metab. 1994, 79, 248-252. 3. Bakker, B., H. Bikker, T. Vulsma et al.- Two decades of screening for congenital hypothyroidism in the Netherlands: TPO gene mutations in total iodide organification defects (an update). J Clin Endocrinol Metab, 2000, 85, 3708-12.; 4. Stoeva, I., B. Stoilov, P. Ambrugger et al.- Clinical course in a girl with hTPO mutation R161I in exon 5:18 years of follow up. Horm Res Ped 2015, 82(S1) ; 5. Cangul, H., M. Dogan, Y. Saglan et al.-One Base Deletion (c.2422delT)in the TPO Gene Causes Severe Congenital Hypothyroidism. Jclin.Res.Endocrinol.2014 6(3), 169-73.

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