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

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) organization 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.(Glu17AspfTer77), in exon 2;
- c.819+44C>G (2.6%), and c.621_622delGG, p.(Glu207AspfTer11) (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%); rs17853780, c.208C>G, p.(Pro70Ala), exon 4 (5.1%), and a novel one c.1268G>A, p.Gly393ARG in exon 2 (6.2%), 3 were compound heterozygous carriers. 2 of the patients (2.6%) were carriers of heterogeneous deletions of all exons included in the MLPA kit (Fig 2.a-d).

Conclusion
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:

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NR CMN Sex Familial Ethnicity* NTS participation NTSH mut/ Delila Age (y) STSH mU/l FxNa mmol/l Age (y) sT4 pmol/l tSH g/mg Genotype Authors

HST 85 m no b yes 284 6 576 7.6 14 ND 479 hetero NM_000547.5:c.31_50dup, p.(Glu17AspfTer77) exam2 Bikker, H., 1994 (2)

HST 77a m no b none 339 2 24.9 ND 345 hetero NM_000547.5:c.621_622delGG, p.(Glu207AspfTer11) exam7 new

HST 100a b brother b yes 245 3 1260 11.6 22 0.97 412.5 hetero 1-17 deletion (MLPA) 1_17 exams new

HST 100b m CH b yes 144 4 >100 ND 12 4.33 ND 1-17 deletion (MLPA) 1_17 exams new

HST 133 m no b yes 329 3 373 11 10 2 249 hetero NM_000547.5:c.1522_1542del p.(Phe508_Leu544del) exam9 new

HST 87 f yes, mother b yes 199 2 705 8 12 1.3 210 hetero ex 14 rs76366277, CM032390 NM_000547.5:c.2422delT p.(Cys808AlafsTer24) exam14 Bakker, H. 2000 (3)

HST 83 m yes, sister r yes 145 4 107 ND 9 5.7 509 comp hetero rs76355996 NM_000547.5:c.819-44C>G, rs17662774, CM032390 NM_000547.5:c.2422delT p.(Cys808AlafsTer124) exam7,14 Bakker, H. 2000 (3)

HST 74 f yes, brother r none/Greece ND 211 1y6m 3.3 ND comp hetero ex 14 rs76355996 NM_000547.5:c.819+44C>G, rs17662774, CM032390 NM_000547.5:c.2422delT p.(Cys808AlafsTer124) exam7,14 Bakker, H. 2000 (3)

HST 121 f no b yes 297 4 1120 10.8 14 ND 547 homo NM_000547.5:c.5730+T>C, p.(Trp1915Cys) exam5 Stoever, L. 2015 (4)

HST 119 f no b yes 238 3 1130 9.2 22 ND 451 homo NM_000547.5:c.2422delT p.(Cys808AlafsTer124) exam4,14 Cangul, H. 2015 (5)

HST 108 f no b NTS >100 22 46 ND 22 homo NM_000547.5:c.11776-6T>G, p.(Gly393Arg) exam 8 new

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

Exon 7
Patient HST 77a

Exon 7
Patient HST 100a,b

Exon 8
Patient HST 119

1-17 deletion (MLPA)
Patients HST 100a,b

Exon 8
Patient HST 119

Exon 7
Patient HST 119

1-17 deletion (MLPA)
Patients HST 100a,b