

ROLE OF NEXT GENERATION SEQUENCING IN THE AETIOLOGICAL DIAGNOSIS OF CONGENITAL HYPOTHYROIDISM WITH GLAND IN SITU

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INTRODUCTION

The pathogenic role of genetic factors in congenital hypothyroidism (CH) is now widely known.

The constant evolution of diagnostic methods in the field of medical genetics provides the opportunity to obtain an etiological diagnosis in CH patients with genetic defects in candidate genes.

AIM

To evaluate, by Next Generation Sequencing (NGS) of a panel of target genes, the frequency and type of potentially causative variants in a selected sample of CH patients with gland-in-situ (GIS).

METHOD

We performed genetic analysis by NGS of 18 candidate genes (*DUOX2*, *DUOX2A2*, *FOXE1*, *GLIS3*, *IGSF1*, *IYD*, *NKX2-1*, *NKX2-5*, *PAX8*, *SLC16A2*, *SLC26A4*, *SLC5A5*, *TG*, *THRA*, *THRB*, *TPO*, *TSHB*, *TSHR*) involved in both morphogenesis and thyroid function.

Inclusion criteria:

CH patients with GIS, born from January 2003 to December 2015 in Emilia-Romagna region, and:
- positive newborn screening (NBS);
- permanent CH emerging from the re-evaluation of the diagnosis.

Exclusion criteria:

- children with chromosomal abnormalities;
- absence of written informed consent.

The NGS analysis was carried out using the Ion Torrent S5 Life Technology instrument.

Allelic variants (AVs) with MAF <0.05 were included and classified using Varsome. Benign and probably benign AVs were excluded.

RESULTS

41 patients (25 males, 16 females) were enrolled. 36 AVs were detected in 23/41 patients (56%):
- 13 pts presented with 1 AV;
- 7 pts with 2 AVs;
- 3 pts with 3 AVs.
The remaining 18/41 were found to be wild-type (WT).

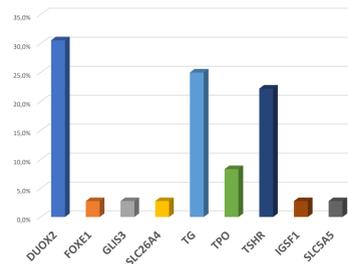
25/36 AVs were found in genes involved in thyroid hormone synthesis:
- 11 (30.5%) *DUOX2*;
- 9 (25%) *TG*;
- 3 (8.3%) *TPO*;
- 1 *SLC26A4*;
- 1 *SLC5A5*.
10/36 AVs in genes involved in thyroid morphogenesis:
- 8 (22.2%) *TSHR*;
- 1 *GLIS3*;
- 1 *FOXE1*.

1/36 AV in a gene (*IGSF1*) implicated in the hypothalamic-pituitary function.

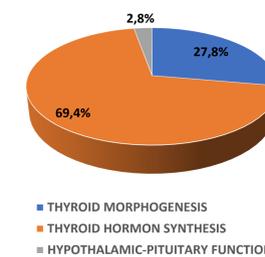
50% of the AVs (18/36) were classified as **VUS**, 28% (10/36) as **pathogenic** and 22% (8/36) as **likely pathogenic**, according to the guidelines of the ACMG.

In 7 patients (19,4%) we found an **oligogenic CH**.

Graph 1. AVs distribution in target genes



Graph 2. AVs distribution according to functional role of genes



Graph 3. AVs distribution according to pathogenetic classification

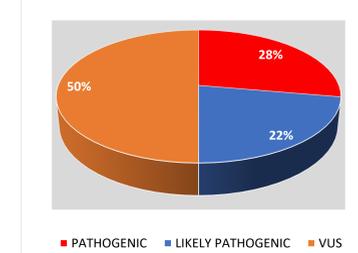


Table 1. Results of genetic analysis, screening test, and diagnostic confirmation in patients with one AV

| PT | SEX | NBS TSH (μIU/mL) | NEONATAL SERUM TSH (μIU/mL) | THYROID SIZE | LT4 DOSE AT 36 m (μg/kg/day) | GENE/AV |
|----|-----|------------------|-----------------------------|--------------|------------------------------|--------------------|
| 1 | F | 11 | 6.7 | N | 1.8 | TG: c.886C>T |
| 2 | M | 18 | 31.3 | N | 2.5 | TG: c.6674T>C |
| 3 | M | 15.3 | 20.3 | N | 2.5 | TG: c.7955C>G |
| 4 | M | 13 | 10.5 | ↓ | 2.4 | TG: c.7753C>T |
| 5 | M | 10.4 | 16.5 | ↓ | 2.8 | TSHR: c.93C>A |
| 6 | F | 11.2 | 9.3 | ↓ | 2.3 | SLC5A5: c.358-7C>A |
| 7 | M | 10.6 | 14.3 | N | 3 | TSHR: c.326G>A |
| 8 | F | 10.8 | 346.4 | N | 1.4 | DUOX2: c.2785C>T |
| 9 | F | 10 | 9.2 | N | 3.1 | DUOX2: c.3830C>G |
| 10 | F | 14.9 | 11.1 | N | 2.3 | TG: c.3218-2A>G |
| 11 | F | 11.8 | 24.5 | N | 1.7 | TSHR: c.1748T>C |
| 12 | M | 107.6 | 249.7 | N | 3.3 | DUOX2: c.1588A>T |
| 13 | F | 50 | 457 | N | 2.5 | DUOX2: c.1588A>T |

Table 2. Results of genetic analysis, screening test, and diagnostic confirmation in patients with more than one AV

| PT | SEX | NBS TSH (μIU/mL) | NEONATAL SERUM TSH (μIU/mL) | THYROID SIZE | LT4 DOSE AT 36 m (μg/kg/day) | GENE/AV |
|----|-----|------------------|-----------------------------|--------------|------------------------------|---|
| 1 | M | 32.2 | 83.3 | N | 2.9 | FOXE1: c.790G>T, TPO: c.317A>C, TSHR: c.861G>C |
| 2 | M | 36.9 | 152 * | N | 3,27 | DUOX2: c.1126C>T, c.4396T>G, TG: c.5923G>T |
| 3 | F | 11.4 | 10.9 | N | 2.4 | SLC26A4: c.1972C>T, TSHR: c.1556G>T |
| 4 | F | 110 | 398.5 * | N | 3.8 | DUOX2: c.4405G>A, TPO: c.1187_1188insGCCG c.1577C>T |
| 5 | M | 16 | 9.2 | N | 3 | DUOX2: c.367G>A, TSHR: c.1582C>T |
| 6 | M | 11 | 27.1 | ↓ | 1.7 | GLIS3: c.1872C>T, TG: c.886C>T |
| 7 | F | 11 | 11.3 | ↓ | 1.9 | DUOX2: c.3847+2T>C, IGSF1: c.365C>T |
| 8 | F | 10 | 34 | ↑ | 2,6 | TG: c.7955C>A, TSHR: c.1657G>A |
| 9 | F | 17 | 42 | N | 3.1 | TG: c.7955C>A, TSHR: c.1657G>A |
| 10 | M | 97.3 | >100 * | N | 2.7 | DUOX2: c.2905C>T, c.3155G>A |

* P = 0,017

CONCLUSIONS

Despite the preliminary nature of the results, in 56% of patients with CH and GIS we found at least 1 AV of probable pathogenic significance.

Further investigations through the search for copy number variations and the analysis of the regulatory regions, currently ongoing, will allow to increase the detection rate and elucidate the mechanisms underlying CH.

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