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RESISTANCE TO LEVOTHYROXINE: DIO1 PATHOGENIC MUTATIONS INCREASE SERUM RT3 WHICH IMPAIRS D2 ACTIVITY IN THE PITUITARY GLAND

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Resistance to Exogenous Thyroid Hormone (RETH) may occur in patients with hypothyroidism undergoing levothyroxine (L-T4) substitution. It is characterized by the inability to normalize TSH levels using regular doses of L-T4. Many patients may experience iatrogenic hyperthyroidism by the use of excessive L-T4. The etiology of RETH and the mechanism(s) leading to these features are unknown.

AIM To identify a possible genetic component of RETH in LT4-treated patients with primary hypothyroidism with elevated TSH/fT4 and low T3/T4, T4/rT3 and T3/rT3 ratios.

PATIENTS & METHODS DNA from 32 RETH patients were processed for NGS thyroid-targeted panel or exome. Rare missense variants in *DIO1* with pathogenic *in silico* profiles were investigated *in vitro*. *DIO1* cDNA plasmids were processed for site-directed mutagenesis for the *DIO1* variants, transiently expressed in HEK293 cells and tested for deiodination of ^{125}I-T4 compared to the wild type enzyme. D1 enzyme kinetics were studied by the Vmax and Km. The effect of rT3 exposure on D2 activity (T3 production from ^{125}I-T4) was assayed in anterior-pituitary explants from mice exposed to 0, 40 and 60 ng/dl rT3 for 48 hours.

RESULTS Three heterozygous mutations in *DIO1* (Trp108*, Met169Lys, Ala210Thr) were identified in 5 RETH patients from 4 unrelated families. Hypothyroidism occurred by thyroid ^{131}I-ablation for Graves´s disease, thyroidectomy for differentiated thyroid cancer or hyperthyrotropinemia with the gland *in situ*. Consistent with the role played by D1 in rT3 clearance, all patients showed increased serum rT3 (1.09±0.3 nmol/L: RR: 0.14-0.54) and largely decreased T3/rT3 ratio (1.55±0.35, RR: 7.6-8.4). L-T4 doses received varied from 0.35-1.9 µg/kg/day.
Met169Lys and Ala210Thr mutants showed equally reduced D1 activity compared to wild type (40% vs. 70% T4-fraction conversion/µg protein/h; $p<0.05$). Vmax/Km for Met169Lys and Ala2010Thr were also reduced by 48% and 39% compared to wild type D1 (2.04±0.28 and 2.4±0.15 *versus* 3.92±0.06 fmols-T3/mg protein/min/µM-T4; $p<0.05$), respectively, showing *DIO1* variants are pathogenic. In pituitary glands, D2-mediated T3 production was significantly decreased by 14% and 47% when incubated for 48 h with 40 and 60 ng/dl rT3, respectively.

CONCLUSIONS *DIO1* mutations are present in 12% of patients with resistance to levothyroxine in our RETH cohort. D1 mutants increase rT3 by reducing its clearance while increased rT3 levels impair D2 activity in the pituitary. The work supports a targeted and genetically-guided T3+T4 therapy for hypothyroidism under D2-activity restrain by rT3 elevation. Serum TSH does not stand as universal index of thyroid function if not informed with concomitant rT3 levels.