MIT AND DIT ARE PRE-CLINICAL BIOMARKERS OF HYPOTHYROIDISM IN DEHAL1 DEFICIENT MICE AND HUMANS

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Introduction DEHAL1 deiodinates mono- and di-iodotyrosines (MIT, DIT) and recycles iodide to sustain thyroid hormone synthesis. Human *DEHAL1* defects lead to severe hypothyroidism which seems undetectable at neonatal screening, implying a risk for mental retardation in children. The time of onset and triggering factors for such hypothyroidism are unknown. Identification of early biomarkers at pre-clinical phases would prevent delayed diagnosis of child hypothyroidism and derived intellectual deficits.

Objectives To establish the thyroid hormone and iodide metabolism in *Dehal1*$^{(-/-)}$ mice and investigate iodine deficiency as a triggering factor towards the identification of safe biomarkers that may predict the development of this hypothyroidism in mice and humans.

Methods *Dehal1*$^{(-/-)}$ and wild-type (wt) mice underwent normal (NID), low (LID) and very low (VLID) iodine diets containing 5.6, 1 and 0.25µgI/day, respectively. At days 0, 12 and 28, urinary iodine concentration (UIC) was determined by modified-Kolthoff. MIT, DIT, T3 and T4 were simultaneously measured by a novel mass spectrometry (LC/MS-MS) protocol in urine or plasma. UIC, MIT and DIT were also determined in members of a consanguineous family harboring a novel *DEHAL1* frameshift mutation (p.A57SfsX62).

Results Under **NID**, *Dehal1*$^{(-/-)}$ and wt mice were euthyroid on T4 (39.5±1.9ng/ml and 39.3±2.7ng/ml) and T3 (0.5±0.01ng/ml and 0.49±0.02ng/ml). However, urinary MIT/DIT were 20-fold higher in *Dehal1*$^{(-/-)}$ (MIT:6.07±1.2ng/ml; DIT:7.28±1.7ng/ml) than in wt mice (MIT:0.34±0.26ng/ml; DIT:0.15±0.07ng/ml) (***p<0.0001). UIC was also 2-fold higher in *Dehal1*$^{(-/-)}$ than in wt animals (38±4 *vs*. 21±4µgI/L) (*p<0.05). Under **LID**, T4 and T3 remained still normal in both genotypes. However, urinary MIT/DIT in *Dehal1*$^{(-/-)}$ progressively decreased to 50% in d28, while remaining practically unmeasurable in wt (***p<0.0001). UIC drastically dropped by 90% in both genotypes, but *Dehal1*$^{(-/-)}$ mice still continued to secrete significantly more iodide than wt mice (*p<0.05). Finally, under **VLID**, *Dehal1*$^{(-/-)}$ mice succumb to profound hypothyroidism (T4: 5.6ng/ml; T3: 0.14ng/ml) while wt mice only show mild T4 reduction (by 20%) and are fully euthyroid on T3 levels (***p<0.001). In knockouts, urinary DIT dramatically falls by 77% while MIT decay is milder, by 35%, under stringent iodine restriction. Iodide was virtually absent in the urine of both genotypes.

In human studies, six members of a Lebanese family with a deleterious *DEHAL1* mutation where clinically followed-up in Germany. Index patient (homozygote) developed overt goitrous hypothyroidism (TSH 14.1mUI/L; FT4 0.42ng/dl) at 14 years of age. Urinary MIT (316 ng/ml), DIT (129ng/ml) and UIC (261µgI/L) were sharply elevated compared to healthy siblings and parents (MIT/DIT: 0.8-1.7ng/ml) (UIC: 70-79µgI/L). Uniquely, an older brother of 18y (also homozygote) showed elevated MIT (66ng/ml) and DIT (120ng/ml) but was euthyroid (TSH: 0.63mUI/L, FT4: 1.22ng/dl). Interestingly, two years later, he developed hypothyroidism (TSH: 7.6mUI/L, FT4: 0.45ng/dl), supporting the predictive value of iodotyrosines in humans.

Conclusions Environmental iodine deficiency triggers hypothyroidism in *Dehal* deficiency. Iodotyrosines are pre-clinical biomarkers of the disorder in mice and humans. The results support the potential interest of determining iodotyrosines as part of the human neonatal screening for hypothyroidism.

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