Loss-of-function NNT mutations impair antioxidants mechanisms and decrease cortisol secretion in patients with familiar glucocorticoid deficiency


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Background

• Mitochondrial Nicotinamide Nucleotide Transidrogenase (NNT) is essential in the antioxidant defense mechanisms (Figure 1).
• Recently, mutations in the Nicotinamide Nucleotide Transidrogenase (NNT) gene were described in few Familial glucocorticoid deficiency (FGD) patients.

Aims

To characterize how mutations in NNT gene impair adrenal steroidogenesis resulting in familial glucocorticoid deficiency

Patient

• Case Report: A boy with 18-months old boy presenting with:
  - ACTH pg/ml > 1.250
  - Cortisol ug/ml < 1.2
  - 17-OH progesterone ng/dl < 3.9
  - Androstenedione ng/dl < 3.9
  - Testosterone ng/dl > 10
  - Plasma Renin Activity ng/ml/h 4.2
  - • WES analysis revealed few final candidate genetic variants, including a homozygous exon 17 transition (c.2597G>A, p.G866D) in NNT gene.

Methods

Molecular Analysis

Genomic DNA was evaluated by whole exome sequencing (WES). Candidate genetic variants were analyzed in silico and confirmed by Sanger sequencing.

Functional in vitro Genotype-phenotype (p.G866D)

Basal and Sh H2O2 stimulated ROS in mononuclear blood cells
• ROS intracellular production (DCFDA)
• Reduced glutathione (GSH; GSH-Glo Assay)
• Mitochondrial Mass (MitoTracker)

Adrenal Effect

H295 cell line
siRNA NNT gene knockdown
• ROS intracellular production (DCFDA)
• Mitochondrial Mass (MitoTracker)
• Cortisol secretion (RIA)

Results

• WES analysis revealed few final candidate genetic variants, including a homozygous exon 17 transition (c.2597G>A, p.G866D) in NNT gene.
• The novel mutation p.G866D, was validated by direct sequencing (Sanger; Figure 2).

Figure 2. Family pedigree analysis confirmed segregation of this homozygous variant c.2597G>A (ENST00000264663 - p.G866D) with the phenotype and asymptomatic parents and his younger brother were heterozygous carriers. c.2597G>A (ENST00000264663 - p.G866D).

Conclusion

This study confirms the association of the homozygous NNT p.G866D variant with the phenotype of FGD.

In vitro, this loss-of-function NNT variant significantly impairs antioxidants mechanisms and affects the glutathione reductase systems resulting in increased ROS accumulation. In adrenal cells, NNT impairment results in significant reduction of the steroidogenesis, as shown by decreased production of cortisol.