RNA-seq identifies a novel cryptic intronic mutation in a new ATP6AP1-CDG patient

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RNA-seq analysis in CDG patients without molecular diagnosis

Next-generation sequencing (NGS) techniques: 25-50% of patients with Mendelian disorders still lack a genetic diagnosis (difficulties in the interpretation of non-coding variants and VUS).

RNA sequencing (RNA-seq) increases the diagnosis rate by 8-36% → assessment of variants affecting gene expression and splicing.

A high number of congenital disorders of glycosylation (CDG) has been discovered using NGS in the last decade. However, the value of RNA-seq in identification of pathogenic variants in undiagnosed patients suspected to have a CDG has not been widely explored yet.

We report a new ATP6AP1-CDG patient in whom the genetic cause of the disease was identified exclusively by RNA-seq followed by functional validation.

CDG patient with hyposialylation of transferrin and ApoC-III and negative WES analysis

Clinical presentation
- Cognitive impairment (borderline)
- Seizures
- Abnormality of the nose
- Large fleshy ears

Laboratory findings
- Abnormal sialotransferrin isoform profile (type II CDG)
- Abnormal Apo-CIII glycosylation
- Suggestive of a combined N- and O-glycosylation deficiency (Defect at Golgi level)

Initial molecular studies
- Trio-Whole exome sequencing (WES): negative for known CDG genes and other putative genes involved in glycosylation

17-year old boy from non-consanguineous parents
RNA-seq analysis showed aberrant splicing and reduced expression of ATP6AP1 gene in fibroblasts

a) Expression analysis showed a 33% left of ATP6AP1 transcript when compared to controls.
b) Aberrant splicing analysis showed a new splicing event in intron 2 of ATP6AP1.
c) Expression of the wild-type transcript of ATP6AP1 in patient’s fibroblasts and controls (n=2) by qPCR.

Intron exonization in ATP6AP1 as the underlying cause

Fibroblasts cDNA molecular studies

We have demonstrated an intron exonization before exon 3 of ATP6AP1 gene in the cDNA, which also contains a rare intronic hemizygous variant not covered by WES: chrX:153659943C>T (NM_001183.6: c.289-233C>T)

X-linked inheritance and variant segregation in the family

The carrier status in the mother was confirmed while the father showed the wild-type allele, which was consistent with the X-linked inheritance expected for ATP6AP1.
Functional studies demonstrated the pathogenicity of the c.289-233C>T variant

Splicing assay incorporating wild-type and c.289-233C>T minigene Exontrap vector system (Mobitec, Göttingen, Germany). Abbreviations are as follows: E1, Exontrap exon 1; E2, Exontrap exon 2; ATP6AP1 exon 3, exon 3 and the flanking intronic regions (left panel). RT-PCR using vector specific primers in HAP1 cells transfected with these vectors showed a defective splicing as a consequence of c.289-233C>T mutation (right panel).

A longer transcript of approximately 400 bp was observed in cells transfected with the vector containing the mutation, consistent with that observed in the patient and therefore confirming the pathogenicity of this variant.

ATP6AP1-CDG

- ATP6AP1: accessory protein from the vacuolar H+-ATPase complex (Golgi pH homeostasis).
- 19 patients from 11 families reported to date.

Clinical manifestations in ATP6AP1-CDG
- Mild to fatal hepatopathy
- Immunodeficiency with variable severity and recurrent infections
- Neurological manifestations
- Neonatal icterus
- Connective tissue manifestations (i.e. cutis laxa...)

Laboratory findings in ATP6AP1-CDG
- Low serum copper and/or ceruloplasmin
- Hypogammaglobulinemia (IgG/IgM/IgA)
- Hypertransaminasemia
- Leukopenia

CONCLUSIONS

- Our patient seems to present a milder form of ATP6AP1-CDG, probably due to the 30% of wild-type transcript left.

- Our study provides new insights into the molecular bases of ATP6AP1-CDG by reporting the first variant affecting splicing in this gene.

- Our study highlights the value of RNA-seq for the molecular diagnosis of CDG in patients with unidentified genetic alterations.

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