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Cutis laxa, exocrine pancreatic insufficiency and altered cellular metabolomics as additional symptoms in a new patient with ATP6AP1-CDG

Bianca Dimitrov[a,1], Nastassja Himmelreich[a,1], Agnes L. Hipgrave Ederveen[b], Christian Lüchtenborg[c], Jürgen G. Okun[a], Maximilian Breuer[a], Anna-Marlen Hutter[a], Matthias Carl[d,n], Luca Guglielmi[d,n], Andrea Hellwig[e], Kai Christian Thiemann[a], Markus Jost[a], Verena Peters[a], Christian Staufner[a], Georg F. Hoffmann[a], Annette Hackenberg[f], Nagarajan Paramasivam[g,h,o], Stefan Wiemann[i,j], Roland Eils[h,k,o], Matthias Schlesner[h,o], Sabine Strahl[l], Britta Brügger[c], Manfred Wuhrer[b], G. Christoph Korenke[m], Christian Thiel[a,*]

[a] Center for Child and Adolescent Medicine, Department I, Im Neuenheimer Feld 669, 69120 Heidelberg, Germany
[b] Leiden University Medical Center, Center for Proteomics and Metabolomics, Albinusdreef 2, 2333 ZA Leiden, Netherlands
[c] Heidelberg University Biochemistry Center (BZH), Im Neuenheimer Feld 328, 69120 Heidelberg, Germany
[d] Department of Cell and Molecular Biology, Medical Faculty Mannheim, Heidelberg University, 68167 Mannheim, Germany
[e] Department of Neurobiology, Interdisciplinary Center for Neurosciences, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany
[f] Division of Pediatric Neurology, University Children's Hospital Zürich, Steinwiesstrasse 75, 8032 Zürich, Switzerland
[g] Medical Faculty Heidelberg, Heidelberg University, 69120 Heidelberg, Germany
[h] Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[i] Genomics & Proteomics Core Facility, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, 69120 Heidelberg, Germany
[j] Division of Molecular Genome Analysis, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, 69120 Heidelberg, Germany
[k] Department for Bioinformatics and Functional Genomics, Institute for Pharmacy and Molecular Biotechnology (IPMB), BioQuant, Heidelberg University, 69120 Heidelberg, Germany
[l] Centre for Organismal Studies (COS), Glycobiology, Heidelberg University, Im Neuenheimer Feld 360, 69120 Heidelberg, Germany
[m] Klinikum Oldenburg, Zentrum für Kinder-und Jugendmedizin, Klinik für Neuropädiatrie u. angeborene Stoffwechselerkrankungen, Rahel-Straus-Straße 10, 26133 Oldenburg, Germany
[n] Laboratory of Translational Neurogenetics, Center for Integrative Biology, University of Trento, 39123 Trento, Italy
[o] Bioinformatics and Omics Data Analytics (B240), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

ABSTRACT

Congenital disorders of glycosylation (CDG) are genetic defects in the glycoconjugate biosynthesis. > 100 types of CDG are known, most of them cause multi-organ diseases. Here we describe a boy whose leading symptoms comprise cutis laxa, pancreatic insufficiency and hepatosplenomegaly. Whole exome sequencing identified the novel hemizygous mutation c.542 T > G (p.L181R) in the X-linked ATP6AP1, an accessory protein of the mammalian vacuolar H^+-ATPase, which led to a general N-glycosylation deficiency. Studies of serum *N*-glycans revealed reduction of complex sialylated and appearance of truncated diantennary structures. Proliferation of the patient's fibroblasts was significantly reduced and doubling time prolonged. Additionally, there were alterations in the fibroblasts' amino acid levels and the acylcarnitine composition. Especially, short-chain species were reduced, whereas several medium- to long-chain acylcarnitines (C14-OH to C18) were elevated. Investigation of the main lipid classes revealed that total cholesterol was significantly enriched in the patient's fibroblasts at the expense of phophatidylcholine and phosphatidylethanolamine. Within the minor lipid species, hexosylceramide was reduced, while its immediate precursor ceramide was increased. Since catalase activity and ACOX3 expression in peroxisomes were reduced, we assume an ATP6AP1-dependent impact on the β-oxidation of fatty acids. These results help to understand the complex clinical characteristics of this new patient.

Abbreviations: CDG, Congenital Disorders of Glycosylation; CE, cholesterol ester; Chol, cholesterol; DAG, diacylglycerol; PC, phosphatidylcholine; -O, lipid containing ether/odd numbered fatty acyl; -P, plasmalogen; PE, phosphatidylethanolamine; PI, phosphatideylinositol; PS, phosphatiylserine; SM, sphingomyelin; TAG, triacylglycerol; Cer, ceramide; HexCer, hexosylceramide; PA, phosphatidic acids; PG, phosphatidylglycerols

* Corresponding author.
E-mail address: christian.thiel@med.uni-heidelberg.de (C. Thiel).

[1] Bianca Dimitrov and Nastassja Himmelreich contributed equally and are co-first authors.

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1. Introduction

The maintenance of pH homeostasis is essential for almost all cellular processes in an organism as structure, function and solubility of numerous proteins depend on a particular pH-value [1,2]. A variety of regulatory mechanisms control the pH, e.g. direct proton transport, indirect proton coupling to other ion transports or vacuolar H^+-ATPases (V-type H^+-ATPase, V-ATPase). The 1000 kDa V-ATPase is a multi-subunit complex which belongs to the family of V0V1-ATP synthases [3].

It is organized in the membranous V0 and the cytosolic V1 domain [4,5]. In mammals the V0 domain consists of nine subunits (a1, a2, a3, a4, d1, d2, c, c″ and e) while the V1 domain is composed of 13 subunits (A, B1, B2, C1, C2, D, E1, E2, F, G1, G2, G3 and H) [3]. Also accessory proteins are involved. The V-ATPase complex uses a V1-mediated and ATP-hydrolyzing rotor mechanism to transport protons across the cell membrane which then enter the V0 domain through a cytoplasmic hemichannel.

In recent years, a number of defects have been identified in different subunits of the V-ATPase in humans, as e.g. infantile malignant autosomal recessive osteopetrosis caused by mutations in *ATP6V0A3* (V0 subunit a3, also named TCIRG1) or the autosomal recessive renal tubular acidosis due to mutations in *ATP6V1B1* or *ATP6V0A4*, respectively [6,7,8,9]. Furthermore, mutations in the accessory subunit '(Pro)renin receptor' (M8-9 encoded by *ATP6AP2*) result in X-linked intellectual disability, epilepsy and parkinsonism [10,11]. Moreover, several defects of V-ATPase subunits influence the protein glycosylation machinery leading to distinct subtypes within the group of rare and heterogeneous metabolic diseases, called Congenital Disorders of Glycosylation (CDG) [12]. Deficiency of the V0 subunit α2 leads to ATP6V0A2-CDG [13], whereas ATP6V1A-CDG and ATP6V1E1-CDG are defects of the V1 subunits A and E1 [7]. These defects belong to CDG-II that affect the structure of N- and often also mucin-type O-glycans. In 2016 eleven male CDG-II patients with hemizygous mutations in the V-ATPase accessory subunit AC45 (ATP6AP1) were identified. The patients show mainly immunodeficiency (including hypogammaglobulinemia), hepatopathy and a spectrum of neurocognitive abnormalities [14].

Here we describe an ATP6AP1-CDG patient with a new hemizygous mutation and additional features such as rapidly improving cutis laxa, exocrine pancreatic insufficiency, treatable diarrhea as well as changes in the amino acid and lipid metabolism.

2. Material and methods

2.1. Patient material

The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty Heidelberg. Written informed consent was obtained from the parents of the patient for whole-exome sequencing. The parents also consented to molecular testing (Sanger sequencing) of their own ATP6AP1-status. Written informed consent was also obtained for use of photographs and clinical information.

2.2. Isoelectric focusing of serum transferrin

IEF of serum transferrin was performed as described by Niehues et al. [15].

2.3. Isoelectric focusing of apolipoprotein-CIII

IEF of ApoCIII was performed as described by Wopereis et al. [16].

2.4. Western blotting

Western blotting was performed as described by Lübbehusen et al. [17]. An HRP-coupled secondary antibody in a dilution of 1:10,000 in PBST was used for 1 h at RT. Visualization was performed by adding a chemiluminescence reagent (Pierce). Primary antibodies against ATP6AP1 (Sigma-Aldrich, rabbit anti-human, 1:500), HiF1α (ThermoFisher, mouse anti-human, 1:1000), ACOX3 (Atlas Antibodies, rabbit anti-human, 1:1000), BiP (Cell Signaling, rabbit anti-human, 1:1000) and LC3 (Novusbio, rabbit anti-human, 1:500) were used. Secondary antibodies used for Western blots were horseradish peroxidase (HRP)-conjugates (Dianova, goat anti-rabbit; Santa Cruz, goat anti-mouse).

2.5. Glycan analysis

N-glycans derived from patient and control fibroblasts were analyzed as described by Reiding et al. [18].

2.6. Whole exome sequencing (WES)

Trio-based WES (Agilent SureSelect target enrichment and Illumina HiSeq 2000 paired end sequencing) and analysis of the sequence data were performed at the German Cancer Research Center (DKFZ) at Heidelberg (Germany) using the previously described Heidelberg exome data analysis bioinformatics pipeline. Variants with a minor allele frequency (MAF) > 1% in the Exome Aggregation Consortium (ExAC) and 1000 genome phase III database were considered common alleles and removed from the candidate list. Local control samples were used to remove the recurrent technical artifacts and common alleles that were not seen in the above databases. Only non-synonymous exonic variants and variants ± 2 bases around the intron-exon junction, classified as splice-site variants, nonsense variants and indels were further considered [19]. Variants were assessed by 7 different variant effect prediction tools (SIFT, PolyPhen2, LRT, MutationTaster, MutationAssessor, FATHMM, and PROVEAN) from dbNSFP v2.0 and CADD scores. Sequence validation of the detected mutation was performed on cDNA and exon level by using Sanger sequencing (see below). The mutation was uploaded to ClinVar Submission Wizard (SUB2942359; www.ncbi.nlm.nih.gov/clinvar/).

2.7. Reverse transcriptase PCR

Using random hexamer primers (Invitrogen) and Maxima™ Reverse Transcriptase (Thermo Fisher) 500 ng of total RNA were reverse transcribed into cDNA. PCR was performed with 50 ng of cDNA as template using *ATP6AP1* specific primers *ATP6AP1*-Fw (5′-CCCAGTACATGACC TTATGGG-3′) and *ATP6AP1*-Rev (5′-GGTTAGTTCAAATAAGGCA CAG-3′) with *Pfu*-Turbo-Polymerase (Stratagene).

2.8. Quantitative real-time PCR

Quantitative real-time PCR was carried out by SensiFAST SYBR No-ROX-Mix (Bioline) with 50 ng of reverse transcribed RNA in a CFX Connect Real-Time System (BioRad) with the following parameters: step 1: 95 °C, 2 min; step 2: 95 °C, 15 s; step 3: 60 °C, 10 s; step 4: 72 °C, 10 s; step 5: 100 °C to 25 °C, 5 min; step 6: 4 °C, forever. Steps 2–4 were repeated 35-times. Primers used were ATP6AP1-qPCR-Fw (5′-GAGTG ACCGGGACTTGTGG-3′), *ATP6AP1*-qPCR-Rev (5′-CAGGAAGCACCAGT GAGGAG-3′), *RAB7A*-qPCR-Fw (5′-TGGGAGATTCTGGAGTCGGG-3′) and *RAB7A*-qPCR-Rev (5′-CACACCGAGAGACTGGAACC-3′). Expression levels were normalized to *Ras-related protein* (*RAB7A*).

2.9. Sanger sequencing

ATP6AP1-cDNA (RefSeq NM_001183.5) was analyzed on a 1% agarose gel and extracted with the QIAquick gel extraction kit (Qiagen).