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CONCISE COMMUNICATION

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ATP6V0A2-related cutis laxa in 10 novel patients: Focus on clinical variability and expansion of the phenotype

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Abstract

In *ATP6V0A2*-related cutis laxa, the skin phenotype varies from a wrinkly skin to prominent cutis laxa and typically associates with skeletal and neurological manifestations. The phenotype remains incompletely characterized, especially in adult patients. Glycosylation defects and reduced acidification of secretory vesicles contribute to the pathogenesis, but the consequences at the clinical level remain to be determined. Moreover, the morphology of the elastic fibres has not been studied in *ATP6V0A2*-related cutis laxa, nor its relation with potential clinical risks. We report on the extreme variability in *ATP6V0A2*-related cutis laxa in 10 novel patients, expand the phenotype with emphysema and von Willebrand disease and hypothesize on the pathogenesis that might link both with deficiency of glycosylation and with elastic fibre anomalies. Our data will affect clinical management of patients with *ATP6V0A2*-related cutis laxa.

KEYWORDS

ATPV60A2-related cutis laxa, elastic fiber disarray, management, pulmonary emphysema, von Willebrand disease type 2

Beyens and Moreno-Artero equally contributed to this work.

Hadj-Rabia and Callewaert equally contributed to this work.

1 | BACKGROUND

ATP6V0A2 mutations have been associated with wrinkly skin syndrome (WSS, MIM 278250) and Debré-type cutis laxa (or autosomal recessive cutis laxa 2A, ARCL2A, MIM 219200). In wrinkly skin syndrome, patients show skin wrinkles mainly on the dorsum of the hands and feet, while patients with Debré-type cutis laxa manifest generalized and large redundant skin folds.[1] Both entities may associate with a characteristic facial appearance, a marfanoid habitus and variable lipodystrophy.[2–5] Neurological manifestations are reported in 78%[4] and may include microcephaly, cortical malformations resembling either lissencephaly or polymicrogyria ("cobblestone"-like brain malformations), seizures, cerebellar vermis hypoplasia and Dandy-Walker malformation.[2–6] Intellectual disability varies from mild impairment (as commonly observed in WSS) to a more severe mental retardation with anatomical brain defects (often present in Debré-type cutis laxa).[2–6] Skeletal findings include congenital hip dislocation, delayed closure of the fontanelles, scoliosis, joint laxity and soft tissue calcification.[3] Biochemically, most patients show a congenital defect of N- and O-glycosylation. *ATP6V0A2* encodes the alpha-2 subunit of the V-type H^+-ATPase. This vacuolar ATPase uses energy from ATP hydrolysis to pump hydrogen ions into vesicular compartments, resulting in the acidification of diverse cellular components mainly within the secretory pathway.[7,8]

2 | QUESTIONS ADDRESSED

The aim of this study was to evaluate the phenotype in ten novel patients with *ATP6V0A2*-related cutis laxa, with attention to the clinical variability and novel findings. As some of the features of *ATP6V0A2*-related cutis laxa point to elastic fibre dysfunction, we evaluated skin elastic fibres in skin electron microscopy. We formulate hypothesizes on pathophysiological links between novel clinical observations and elastic fibre morphology or glycosylation defects.

3 | EXPERIMENTAL DESIGN

We provide the clinical data of 10 patients (nine probands) with *ATP6V0A2*-related cutis laxa and extend the clinical spectrum to include variant von Willebrand disease and emphysema. Patients were evaluated by the referring physician by means of a checklist. Mutation analysis was performed using PCR-based next-generation sequencing on a MiSeq machine (Illumina, San Diego, CA, USA). We obtained glutaraldehyde 4%-embedded skin biopsies of two patients and describe the morphology of the elastic and collagens fibres.

4 | RESULTS

All patients harbour biallelic premature truncation or splice site mutations in *ATP6V0A2*. This is in congruence with data from previous reports, where missense mutations represent only a minority of known mutations in *ATP6V0A2*, clearly indicating a common loss-of-function mechanism.[3,8,9] All patients presented with highly variable phenotypes (Table S1), but no clear genotype-phenotype correlations were observed, likely because all mutations are expected to result in loss of function. Therefore, our data can not the previous observation of a more severe phenotype in patients with loss-of-function mutations compared to missense mutations.[7] All patients had recognizable facial features with a long face, downslanting palpebral fissures, a convex nasal profile and generalized sagging skin (Figure 1), but showed highly variable neurological involvement ranging from moderate mental retardation with seizures, microcephaly and central nervous system abnormalities to normal development and macrocephaly. In our series, only four patients (40%) showed neurological manifestations with neuromotor delay; one had associated seizures, Dandy-Walker malformation and microcephaly. Corpus callosum hypo/aplasia, considered more reminiscent of *PYCR1*-related cutis laxa,[4] was present in one patient. Neurological involvement did not specifically correlate with the variable marfanoid skeletal features and lipodystrophy. Of note, in our patients, the evolution of the skeletal features seems much less severe than previously reported.[3] Two patients presented with cataract, an uncommon finding in ARCL2A in contrast to ARCL3 caused by *PYCR1* or *ALDH18A1* mutations.[4,10–12] Patient 6 had retinal detachment, unrelated to severe myopia. High bilateral and early myopia, one of the most frequent reported ocular abnormalities in *ATP6V0A2*-related cutis laxa,[4,5] was present in only one patient in our series (Patient 9).

Patients 1 and 2, sisters from a Moroccan consanguineous union, showed a prolonged bleeding time with easy bruising, frequent gingival bleedings and menorrhagia causing iron-deficient anaemia. Coagulation factor analysis in the older sibling revealed an increased von Willebrand factor (VWF) antigen (245%; normal: 50-150%), while ristocetin cofactor activity (VWF:Rco) was only 60% (normal: 58%-166%), leading to a decreased VWF activity/antigen ratio (0.24, normal ratio: 0.7-1.3) compatible with Type 2 von Willebrand disease. Multimer analysis (Figure S1) showed an unusual aspect with a large excess of unpolymerized VWF. Factor VIII was

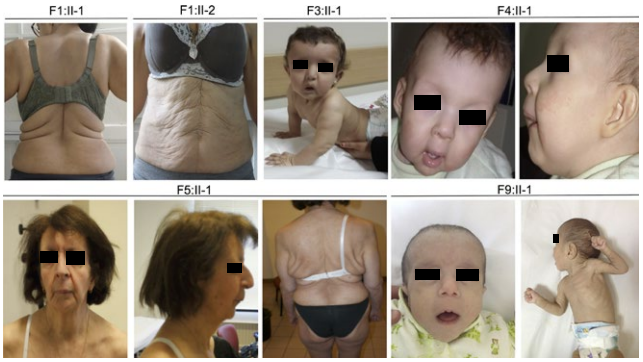

Figure 1. Clinical characteristics. Recognizable craniofacial and cutaneous features in 6 patients (the sisters F1:II-1 and F1:II-2, F3:II-1, F4:II-1, F5:II-1, F9:II-1). Note a long face, downslanting palpebral fissures, a convex nasal profile and generalized sagging skin

180% (normal: 60%-150%). Platelet count, activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen levels were within the normal range. Von Willebrand gene sequencing was normal. Interestingly, both sisters have the same biological profile with a decreased VWF activity/antigen ratio and excess of unpolymerized VWF. One report mentions Ehlers-Danlos–like skin lesions in *ATP6V0A2*-related cutis laxa. Although described as "pseudoecchymotic lesions" likely reflecting hemosiderin deposition, no coagulation analysis was performed.[13]

ATP6V0A2 deficiency causes V-type ATPase dysfunction with impaired retrograde vesicular trafficking in the Golgi complex and decreased acidification of the vesicles of the secretory pathway.[7,8] Von Willebrand factor (VWF) monomers are arranged into dimers in the endoplasmic reticulum. These dimers undergo further maturation in the Golgi apparatus with N-glycosylation in trans-Golgi and multimerization in post-Golgi vesicles.[14] The multimeric structure is essential to promote binding to platelet collagens and glycoprotein 1b, initiating adhesion, and platelet factor IIb/IIIa, inducing platelet aggregation and for binding to factor VIII, protecting the latter from rapid clearance.[15] While glycosylation anomalies may impair proper glycosylation and multimerization of VWF resulting in deceased functionality, we cannot exclude that extracellular matrix disarray could preclude proper interaction of VWF with the extracellular matrix, hence reducing its activity.[16] Indeed, VWD is not routinely observed in other CDG types, and electron microscopy shows both abnormal elastic and collagen fibres (Figure 2). Overall, elastic fibres are less abundant compared to controls. The microfibrillar structures are disorganized, and the deposited elastin shows a frayed and moth-eaten aspect with a network of fragmented elastin clumps rather than a smooth elastin core on the microfibrillar scaffold. However, the directionality of the elastin seems to be preserved. Collagen fibres show more variable diameters with wider interfibrillar spaces. The findings are similar but somewhat milder compared to cutis laxa due to mutations in other subunits of the v-ATPase, including ATP6V1E1 and ATP6V1A,[17] but are clearly different from cutis laxa not related to v-ATPase defects (unpublished data).

Furthermore, Patient 1 presented with emphysema (forced expiratory volume in 1 second (FEV1), 47% of the expected value; and Tiffeneau index (FEV1/Forced Vital Capacity), 99%), despite the absence of clear environmental risk factors and her relatively young age. Major lung problems are typical in *ELN*-related ADCL, or *FBLN5*- and *LTBP4*-related ADCL,[18–20] but not in *ATP6V0A2*-related cutis laxa. However, most reported *ATP6V0A2*-related cutis laxa patients were young, and lung disease might not have been progressed to a clinically significant level. It may therefore be reasonable to suppose that elastic fibre anomalies predispose to pulmonary complications independent of the underlying mechanism and, if confirmed, should be monitored in all patients with *ATP6V0A2*-related cutis laxa. Within the same reasoning, a broad aortic root has been observed in a few patients with *ATP6V0A2* mutations,[13] and it might be advised to complement follow-up investigations with regular echocardiography.[5]

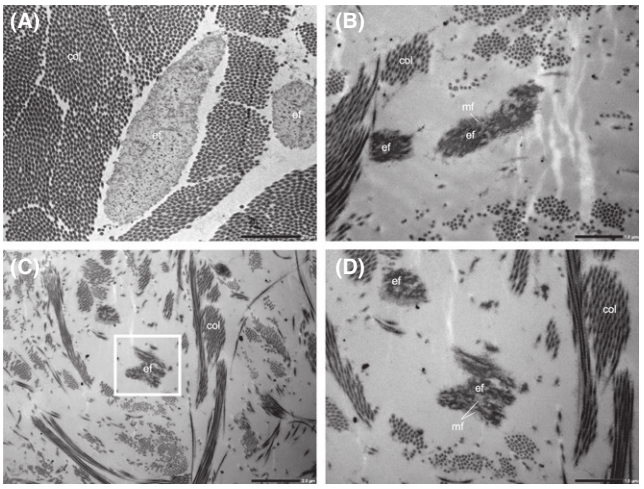

Figure 2. Electron microscopic findings in *ATP6V0A2*-related cutis laxa. A, Transmission electron microscopy of elastic fibres in a control skin biopsy. The elastic fibre consists of a dense elastic fibre core which is deposited onto a scaffold of microfibrils. Only a sparse mantle of microfibrils can be observed. **B-D**, Representative image of elastic fibres of Patient F1:II-1. In *ATP6V0A2*-related cutis laxa, we see extremely sparse elastic fibres with a frayed and moth-eaten aspect. D, A close-up image of the framed elastic fibre in C, ef = elastic fibre core; mf = microfibrils; col = collagen

5 | CONCLUSIONS

We further confirm the broad clinical spectrum of *ATP6V0A2* mutations with absent or minor neurological and skeletal features in a subset of patients, warranting caution when counselling about prognosis. Based upon this and previous observations, we propose to systematically evaluate the haemostatic profile in *ATP6V0A2*-related cutis laxa and complement follow-up with cardiopulmonary and ophthalmologic examinations.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

AB, AM-A, SH-R and BC interpreted the data and wrote the manuscript. CB, HC, AG, EYG, NK, PKVK, GO, AH, MV, AS and SH-R contributed clinical data and samples. MV and AS interpreted coagulation data. SS supervised the molecular analyses. BC designed and supervised the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. VWF multimer analysis. A, Citrated plasma samples were analysed on the Hydrasys 2 instrument (Sebia, Lisses, France) with a ready to use SDS agarose gel (Hydragel 5 von Willebrand multimers, Sebia), according to the manufacturer's instructions. In these conditions, multimers of high molecular weight have a slow migration and are present in the inferior part of the gel, giving a smear in normal plasma (C), whereas monomers migrate to the top of the gel. F: II-2: patient's plasma; C, control plasma. **B**, Densitometric analysis of electrophoretic gel image: the line in grey shows the distribution of multimers in normal plasma and in blue, the distribution of patient's multimers, showing a decrease in high molecular weight multimers and an accumulation of monomers, when compared to control

Table S1. Clinical and molecular data of the patients

Appendix S1. Supplementary methods

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