VPS33B mutations cause ARKID syndrome affecting Rab protein interaction, collagen modification and epidermal structure

Gruber R¹, Rogerson C², Windpassinger C³, Strohal R⁴, Schmuth M¹, Kroisel P³, Janecke AR⁵, Gissen P²

¹Department of Dermatology, Venereology and Allergology, Medical University of Innsbruck, Austria; ²MRC Laboratory for Molecular Cell Biology, University College London, UK; ³Institute of Human Genetics, Medical University of Graz, Austria; ⁴Department of Dermatology, Academic Teaching Hospital Feldkirch, Austria; ⁵Department of Pediatrics I, Medical University of Innsbruck, Austria

Aut.-Rec. Keratoderma Ichthyosis Deafness Syndrome (ARKID)

Patients presented with severe palmoplantar keratoderma, ichthyosis and sensorineural deafness (Fig. 1). Exome sequencing revealed biallelic mutations in *VPS33B*, encoding VPS33B, which interacts with Rab11a and Rab25 proteins and is involved in trafficking of the collagen modifying enzyme lysyl hydroxylase (LH3). Patients 1a and 1b showed the homozygous missense variant c.[390G>A;392G>A] (p.Gly131Glu), while patient 1c was compound heterozygous for p.Gly131Glu and the splice site mutation c.240-1G>C.

Fig. 1: (a-c) Clinical features of ARKID patients.

VPS33B p.Gly131Glu does not affect VPS33B-VIPAR interaction

Overexpressed wild-type VPS33B interacts with VIPAR (Fig. 2a). In contrast to *VPS33B* mutations causing arthrogryposis renal dysfunction and cholestasis (ARC) syndrome the p.Gly131Glu variant does not result in a reduced interaction with VIPAR (Fig. 2b,c), likely due to its localization (Fig. 2d).

Fig. 2: mIMCD3 cells; YFP/RFP, yellow/red fluorescent protein; IP, immuno-precipitation.

VPS33B p.Gly131Glu cannot rescue defective LH3 delivery

In *VPS33B*-deficient mIMCD3 cells LH3 is not delivered to intracellular collagen IV carriers, however this can be rescued by cotransfection of wildtype (wt) *VPS33B* and VIPAR constructs into *VPS33B*-deficient cells. Unlike the wt construct, p.Gly131Glu mutant *VPS33B* cotransfected with VIPAR could not rescue LH3 trafficking (Fig. 3a). There were deficient LH3-specific collagen lysine post-translational modifications in urine and fibroblasts of ARKID patients (Fig. 3b).

Fig. 3: (a) Quantification of LH3/collagen IV colocalization. (b,c) LC-MS-MS analysis for relative quantification of hydroxylysines (Lys-OH), galactosyl-hydroxylysines (Lys-O-Gal) and glucosylgalactosyl-hydroxylysines (Lys-O-GalGlc) from (b) urine and (c) skin fibroblasts.

VPS33B p.Gly131Glu affects interaction of the VPS33B-VIPAR complex with Rab11 family proteins

The *VPS33B*-VIPAR complex interacts with both Rab25, a small GTPase necessary for LH3 delivery to intracellular collagen, and Rab11a, which is essential for epidermal lamellar body biogenesis. Compared with wt, p.Gly131Glu mutant *VPS33B* had markedly reduced colocalization and coimmunoprecipitation with Rab25 (Fig. 4a-c) and Rab11a (Fig. 4d-f).

Fig. 4: (a-f) mIMCD3 cells; YFP/RFP, yellow/red fluorescent protein; IP, immuno-precipitation. (g,h) Hemagglutinin (HA) co-IP on lysates of HEK293 cells co-expressing HA-VPS33B or HA-VPS33B p.Gly131Glu with Myc-VIPAR and Rab25-GFP.

Epidermal structure of ARKID patients mirrors defects in tamoxifen-inducible VPS33B deficient Vps33b^{fl/fl}-ERT² mice

Both patients and murine models revealed an impaired basement membrane zone and epidermal barrier structure with less prominent hemidesmosomes, a reduced number of anchoring fibrils, insufficiently fixed collagen fibers to the fibrils and aberrant secretion of lamellar bodies (LBs) (Fig. 5).

Fig. 5: (a) Control and (b) ARKID palmar skin, H&E. (c-e) ARKID skin TEM (c) LBs (*) and (d) lipid bilayers (arrows) within corneocytes, (e) inhomogeneous LB secretion (double arrows) and aberrant LBs. (f, g) Dermo-epidermal junction in (f) control and (g) ARKID abdominal skin, anchoring fibrils (arrows). (h) wt and (i) Vps33b^{fl/fl}-ERT² dorsal epidermis, H&E. TEM of (j) wt and (k) Vps33b^{fl/fl}-ERT² epidermis: normal (white arrows) versus entombed bilayers (black arrows), aberrant LBs at the SG-SC interface (double arrows).