$\beta 6$ integrinosis: a new lethal autosomal recessive ITGB6 disorder leading to impaired conformational transitions of the $\alpha_v\beta 6$ integrin receptor

We read with interest the recent work by Schleier *et al*¹ demonstrating consequences of impaired $\alpha 4\beta 7$ integrin-dependent gut homing of intestinal macrophages on wound healing, which fits well with own observations we have made in a case of congenital infantile intractable diarrhoea linked to impaired integrin receptors in intestinal epithelia ($\alpha_v\beta 6$). Specifically, a male dizygotic twin was delivered dystrophic (1715 g)

transition between the inactive bent stage and the activated open conformation by establishing additional intramolecular hydrogen bonds (figure 2B1–3),^{8–10} possibly impairing proper $\alpha_v\beta_6$ subunit interactions. To study the impact of ITGB6V438M on heterodimerisation we used fluorescent two-hybrid assays in hamster cells. Both subunits colocalised when ITGB6(wt)-GFP2 and ITGAV-RFP were cotransfected (figure 2C, top), but not when ITGB6V438M-GFP2 was cotransfected with ITGAV-RFP (figure 2C, bottom). Finally, ITGB6 morpholino injection led to altered tailfin epithelia recovery after standardised injuries in zebrafish embryos with significant delays in wound recovery when morpholinos were used at 0.3, 0.6 or 0.9 mM after 24 hours and increased mortality after 48 hours above 0.9 mM, supporting a role of ITGB6 in tissue integrity (figure 2D1–5). We propose that improper conformational transition of $\alpha_v\beta_6$ integrin receptors affects intestinal tissue integrity and barrier function explaining both diarrhoea and haemorrhages.

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Contributors ACJ, SW and RvdB collected the patient material. ACJ and JP performed the study design and coordinated the experiments. PW performed the exome sequencing and the *in silico* analysis. PW and VO performed the cell culture studies. DG performed the immunohistochemistry. TZ and SJ performed the zebra fish studies. ACJ and JP wrote the manuscript.

Figure 1 (A) Pedigree tree: patient (arrow) and known relatives. Red: verified ITGB6G1312A|rs61737764. (B) Immunohistochemistry/H&E stain on parallel target/control tissue sections using anti-human α_6 (6.2A1) or anti-human LTBP1 (Antibodies Online/ABIN1807165).

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Figure 2 (A) Alignment: vertebrate $\beta 6$ integrins and eight human β integrins. (B1) $\alpha_v \beta 6$ headpiece subdomains participate in dimerisation. (B2) Magnified view demonstrating exposed V438 localisation at the $\beta 6$ hybrid domain surface. (B3) Simulation of V438M substitution caused additional H bonds (green lines) bridging the hybrid domain and the N-terminal $\beta 6$ domain. (C) F2H assay results. Top quartet: ITGB6(wt)-GFP (bait/green) enrichment at nuclear GFP-binding matrix. Colocalisation of ITGAV-RFP (prey/red) indicated $\alpha_v \beta 6$ interaction. Bottom quartet: Using ITGB6V438M-GFP no ITGAV-RFP colocalisation was observed, suggesting impaired interaction. (D) Zebrafish tailfin wound healing after ITGB6 knockdown. (D1–3) Standardised injuries principle. (D4) Mortality after morpholino application. (D5) Delayed wound area recovery within 24 hours suggests impaired wound healing on ITGB6 knockdown.

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