β6 integrinosis: a new lethal autosomal recessive ITGB6 disorder leading to impaired conformational transitions of the αvβ6 integrin receptor

We read with interest the recent work by Schleier et al1 demonstrating consequences of impaired α4β7 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 (αvβ6). Specifically, a male dizygotic twin was delivered dystrophic (1715 g)
at 36 weeks of gestational age and developed intractable diarrhoea within the following 2 months, contrary to his twin brother. Severe systemic infection or parasitosis was ruled out, but subsequently low-serum IgG and severe neutropenia occurred due to consumption of neutrophils during the prolonged diarrhoea. Eventually, he developed cholestatic hepatopathy and thrombocytopenia and died of uncontrollable GI, dermal haemorrhages and hepatic failure at 7 months of age. Extensive diagnostics included biopsies of liver, muscle, bone marrow, small intestine, the exclusion of known congenital diarrhoea reasons and immunodeficiencies by leucocyte FACS, CD40L expression, WASP staining, et cetera with no results. Familial anamnesis revealed similar fatalities of a sister and further cousins from the patient’s known generation within their first year of life due to intractable diarrhoea (figure 1A; 5 fatalities/16 infants).

Using whole exome sequencing on both twins and parents we identified a single-nucleotide polymorphism (SNP) in the integrin beta-6-subunit-encoding gene (ITGB6G1312A|rs61737764) leading to a valine to methionine substitution (ITGB6V438M). The heterodimeric αβ6 receptor participates in mediating cell-cell and cell-extracellular matrix interactions. Further SNPs fitting to autosomal-recessive inheritance were improbable candidates due to lacking phenotype conformity (DSG4C156871) or relatively high population frequency (TTTC3G2771A). Next, we analysed the relevance of ITGB6V438M by structural simulation, cell-based interaction studies, immunohistochemistry and ITGB6 knockout in zebrafish. Anti-αβ6 monoclonal immunohistochemistry revealed diminished intestinal αβ6, which correlated with enriched LTBP1, possibly influencing TGF-β1 activation from its latent precursor (figure 1B). Evolutionary ITGB6V438 conservation within a hydrophilic motif in mammalian integrin β6 and human integrins β3, β5 and β6 emphasises its relevance (figure 2A). Comparative structure inspection on PDB ID 4UM8|ITGB6(wt) suggests that ITGB6V438M could affect the conformational transition between the inactive bent stage and the activated open conformation by establishing additional intramolecular hydrogen bonds (figure 2B–3). Possibly impairing proper αβ6 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 2D–5). We propose that improper conformational transition of αβ6 integrin receptors affects intestinal tissue integrity and barrier function explaining both diarrhoea and haemorrhages.
caused additional H bonds (green lines) bridging the hybrid domain and the N-exposed V438 localisation at the hybrid domain surface. (B3) Simulation of V438M substitution.

Figure 2  (A) Alignment: vertebrate β6 integrins and eight human β integrins. (B1) α,β6 headpiece subdomains participate in dimerisation. (B2) Magnified view demonstrating exposed V438 localisation at the β6 hybrid domain surface. (B3) Simulation of V438M substitution caused additional H bonds (green lines) bridging the hybrid domain and the N-terminal β6 domain. (C) F2H assay results. Top quartet: ITGB6(wt)-GFP (bait/green) enrichment at nuclear binding matrix. Colocalisation of ITGAV-βGF-(prey/red) indicated α,β6 interaction. Bottom quartet: Using ITGB6V438M-GFP no ITGAV-βGF 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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