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# Combined immunodeficiency with severe inflammation and allergy caused by *ARPC1B* deficiency

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## CAPSULE SUMMARY:

In a boy born of consanguineous parents, pulmonary infections, vasculitis-like episodes and generalized eczema were noted. The persistent leukocytosis and mild bleeding tendency was accompanied by an actin polymerization defect in his blood cells. Proteomics analysis revealed absence of the Arp2/3 complex component ARPC1B, caused by a homozygous indel mutation in the *ARPC1B* gene (c.491\_495TCAAGdelCCTGCCIns). *Arpc1b* knock-out mice demonstrated clinical similarities with the patient. Together, these findings describe a novel combined immunodeficiency with recurrent infections, allergy and inflammation as the main clinical features of the *ARPC1B* gene defect.

## To the Editor,

One of the major protein complexes in actin polymerization and cellular motility is the Arp2/3 complex, consisting of seven polypeptides<sup>1</sup>. Two of the subunits are actin-related proteins of the Arp2 and Arp3 subfamilies. The remaining five regulatory subunits are referred to as ARPC1 (actin-related protein complex-1), ARPC2, ARPC3, ARPC4 and ARPC5. ARPC1 is present in two isoforms in humans, ARPC1A and 1B as a WD40 repeat-containing protein, encoded by different genes, whereas the other ARPC subunits do not contain common sequence motifs. *Arp2/3* gene deletions result in embryonic lethality in the mouse<sup>2</sup>. The genetic defects in the regulatory proteins for cytoskeletal rearrangements cause different syndromes, mostly dominated by blood and immune phenotypes<sup>3</sup>. The activities of nucleation-promoting factors for actin polymerization are mostly regulated by signal-transduction pathways, one of which involves the activation by the Rho-family GTPases CDC42 and RAC2, and the Wiskott-Aldrich Syndrome Protein (WASP) family as the intermediary Arp2/3 activator that can control actin assembly downstream of these small GTPases<sup>4,5</sup>.

Here we describe the first human genetic defect in a component of the Arp2/3 complex itself. The *ARPC1B* mutation results in a combined immunodeficiency with symptoms of immune dysregulation and a mild bleeding tendency, caused by defective actin polymerization in the immune cells, in a 7-year old male patient born as the first child of consanguineous, healthy Moroccan parents. He has one younger unaffected brother. The first weeks of his postnatal development were uneventful, and his umbilical cord detached spontaneously. At 2 months of age he was admitted because of gastric bleeding during a febrile period of clinical gastroenteritis. A gastroscopy showed profuse bleeding in the presence of normal coagulation and liver function tests in the presence of a striking leukocytosis (between 25.2-55.8x10<sup>6</sup>/ml), a normochromic anemia and a mild thrombocytopenia. The white blood cell (WBC) count slowly improved spontaneously during further follow-up, whereas the thrombocytopenia persisted (**Online Repository Table 1**). The mean platelet volume (MPV) was repeatedly found to be normal (mean of 8.8 fL, n=9 samples; normal range 7.4-11.7 fL). His second admission was due to an auricular infection (perichondritis by *S. aureus*), which was treated appropriately, but resulted in scarring of his right pinnacle (**Fig.1A**). At the age of 5 months, he presented again with a clinical picture of fever and purpuric lesions on his legs, arms (**Fig.1B/C**), and scrotum, and poor wound healing (data not shown). Skin biopsy showed a clear leukocytoclastic vasculitis with multiple microthrombi in the vascular lesions (**Fig.1D**). We measured autoantibodies against nuclear antigens (ANA, ENA), neutrophil cytoplasmic proteins (ANCA-IFT, MPO and PR3), platelet antigens, and lupus anti-coagulans (LAC), and found all tests to be negative (data not shown). He suffered from two similar episodes of vasculitis in the presence of a high number of eosinophils

(peaking at 3350 cells/ $\mu$ l at 25 months of age during an infection) that were treated with corticosteroids for 3 months each. His eosinophil counts remained within the normal range thereafter (**Online Repository Table 1**).

At the age of 4 years, he suffered from a period of prolonged bloody diarrhea from which *Salmonella typhimurium* was cultured due to a serious pan-colitis with neutrophil and eosinophil infiltration in the biopsies of his colon (**Fig.1E**). Recurrent pneumonias that responded to antibiotics have led to mild bronchiectasis. Apart from infections he developed serious eczema and showed anaphylaxis after ingestion of nuts. The immunoglobulin spectrum shows increased IgA and IgE (**Online Repository Table 1**).

Because of the early leukocytosis and initial bleeding tendency, we excluded leukocyte adhesion defects, including LAD-III<sup>6</sup>. The most eminent in-vitro findings consisted of the neutrophil defect in motility and directed movement (chemotaxis) due to an F-actin polymerization defect (**Fig.1F/G**); a result supported by confocal analysis (**Online Repository Fig.1A**), adhesion was unimpaired (data not shown). Enhanced azurophil granule release upon cell activation (**Online Repository Fig.2**) was noted by the release of proteolytic activity and the upregulation of CD63 as an integral membrane marker for azurophil granules, but normal activation of NADPH oxidase, phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli* (**Online Repository Fig.2**; data not shown). The initial bleeding tendency seemed to be associated with a very mild platelet dysfunction in a double-colored aggregation assay designed previously to determine platelet function under conditions of reduced platelet counts when standard aggregometry tests fail to be accurate<sup>7</sup> (**Online Repository Fig.3A**). Also, detection of GpIIb/IIIa integrin activation and upregulation of CD62P and CD63 from the granules were tested and compared with control platelets similarly activated (**Online Repository Fig.3B**). Although spreading of patient platelets was different from control platelets (**Online repository Fig.1B**) and CD62P and CD63 upregulation was slightly reduced, GpIIb/IIIa activation seemed intact. Clinically, the bleeding tendency is was not apparent anymore after these initial bleeding events during further follow-up in the presence of a mild thrombocytopenia.

Exome sequencing failed to pick up a mutation (because of the default 'variant caller' parameter settings in this complex genetic defect), but subsequent proteomics analysis of the platelets and neutrophils indicated a complete lack of ARPC1B (**Fig.2A-D**). ARPC1B is an essential hematopoietic component of the Arp2/3 complex for F-actin polymerization. The ARPC1B deficiency was caused by a homozygous complex frameshift mutation in the *ARPC1B* gene (c.491\_495TCAAGdelCCTGCCIns), as confirmed by additional targeted sequencing approaches with improved, customized 'variant caller' parameter settings for the detection of complex mutations (**Online Repository Fig.4**). The complex nature of the mutation in this family might be the consequence of a double strand break

(DSB) repaired by nonhomologous end joining (NHEJ), or by a microhomology-mediated end-joining mechanism (MMEJ)<sup>8,9</sup>. Proteomics analysis demonstrated the presence of the Arp2/3 complex in normal neutrophils, T cells and platelets to consist of ARPC1B, ARPC2, ARPC3, ARPC4, ARPC5 and ARPC5L, but absence of ARPC1A. Since ARPC1B was also expressed in tissue cells, the migration defect observed in neutrophils was expected to be present in primary fibroblasts as well. However, these cells showed normal migratory behavior and we may conclude that the non-hematopoietic expression of ARPC1A is apparently sufficient to rescue the fibroblasts from a detectable defect (**Online Repository Fig.5**). Expression of ARPC1A and ARPC1B were variably present in additional non-hematopoietic cell lines (HeLa, SKBR3 and HEK273) indicating redundancy of ARPC1 proteins, while only ARPC1B protein was detected in hematopoietic cell lines (Daudi, Ramos, Jurkat, NB4, U937), similar to the various findings with fibroblasts versus blood cells tested (data not shown). Together, these novel data on ARPC1 protein expression may well explain why this ARPC1B defect manifests primarily as an hematological and immunological disease.

When *Arpc1b*<sup>-/-</sup> mice (*Arpc1b*<sup>tm1a(EUCOMM)Wtsi</sup>) were generated and compared to the patient (**Online Repository Table 2**), no changes in whole blood cell counts were found. While serum total IgG, IgA and IgM levels were normal, total IgE level had increased at 16 weeks ( $0 \pm 0$  ng/ml in wild-type versus  $220.6 \pm 266.9$  ng/ml in *Arpc1b*<sup>-/-</sup> mice). In 75% of the *Arpc1b*-deficient mice mild inflammation of the blood vessels was observed, targeting the aorta and/or the mesenteric and pancreatic arteries (**Fig.2E**). Upon challenge with the attenuated *S. typhimurium* M525 and unlike their wild-type equivalents, *Arpc1b*<sup>-/-</sup> mice showed signs of salmonellosis by day 3 post-infection, with 5 out of 8 mice having to be sacrificed by day 5 (**Fig.2F**). In contrast, wild-type mice all survived the challenge.

The fact that ARPC1B is the only ARPC1 isoform in hematopoietic cells, whereas both ARPC1A and ARPC1B are present in tissue cells, suggests a differential use of Arp2/3 activities in tissue and blood cells. Severe food allergies, eczema, and autoimmunity are also observed in WASP- and WIP-deficient patients causing Wiskott-Aldrich(-like) syndrome<sup>3,5,10</sup>, but this was genetically excluded in our patient. These immunodeficiencies tend to have a more severe bleeding tendency and more pronounced thrombocytopenia.

We have identified a novel combined immunodeficiency with features of recurrent infections, allergic reactions, vasculitis and mild bleeding tendency. The biology as well as the supportive evidence from the mouse model suggests a direct causal relationship between the *ARPC1B* mutation and the clinical manifestations.

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**Key words:** Arp2/3 complex, actin polymerization, chemotaxis, NADPH oxidase, neutrophils, platelets, fibroblasts, proteomics, knock-out mouse

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**Authorship contribution:**

TWK diagnosed and is treating the patient; TWK conceptualized and wrote the paper; ATJT and MvH performed most of the neutrophil function tests, including F-actin, blotting and confocal experiments; IMdeC performed platelet studies; IvdB performed the fibroblast cultures and scratch assays; ABM and FvA were responsible for proteomics analysis; MdB and KvL performed genetic analyses; EC, GD, SC, RR and DA generated the mouse and performed the infection model; MJA interpreted the pathology; DR and TKvdB contributed by conceptualizing and commenting the manuscript.

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## LEGENDS

**Figure 1. Clinical, histological and in-vitro findings in the patient.** (A,B,C) remarkable clinical features of the affected index case, with skin manifestations appearing superficially, rather similar to vasculitic purpura-like lesions with poor wound repair under certain conditions. (D) Skin biopsy showing small-vessel vasculitis with leukocytoclasia and thrombosis (arrow), and (E) colon biopsy with severe active inflammation and crypt abscesses (arrows) (Hematoxylin and Eosin, 200x). (F) Defective chemotaxis over 3- $\mu$ m pore-size filters, and (G) F-actin polymerization in suspension after stimulation with C5a and fMLP (mean  $\pm$  SEM, n=7-9).

**Figure 2. ARPC1B deficiency and *Arpc1b*-knock-out mouse model.** (A,B,C) Mass spectrometry analysis showing the difference in protein level between the neutrophils of healthy controls and the patient (n =3; Volcano plot in C); ARPC1A is absent in proteomics analysis in control platelets and neutrophils (data not shown). (D) Absence of ARPC1B was confirmed by WB staining with anti-ARPC1B antibody of PMN and platelet lysates. Anti-GAPDH antibody was used as loading control (n=3). WASP was present by Western blotting (data not shown). (E) Vasculitis and renal glomerulonephritis in *Arpc1b*<sup>-/-</sup> mice with aortic vasculitis (200x; see arrows left panel), cardiac subendocardial vasculitis (200x; middle panel), and proliferative glomerulonephritis (400x; right panel). (F) Kaplan-Meier curve for survival following infection of *Arpc1b*<sup>-/-</sup> and wild-type mice with *S. typhimurium* M525.

## ON-LINE REPOSITORY FIGURES

**Supplementary Figure 1: Actin localisation upon neutrophil activation. (A)** Polymerized F-actin was stained in control and patient PMNs. Polymerized actin staining on glass cover slide (red= actin, blue=nucleus). Control cells were activated by fMLP (upper panel) and compared with patient cells (lower panel) at the indicated time points. Upon activation with fMLP, the normal lamellipodia are not observed with the patient cells and instead the formation of filopodia are the major effect induced in the patient cells, which is in line with expected role of a dysfunctional Arp2/3 complex, resulting in lack of actin polymerization by branching. **(B)** Polymerized F-actin was stained in control and patient platelets activated by collagen and showed defective spreading behavior similar to what was observed with neutrophils. Representative for 3 independent experiments (see Online Methods)

**Supplementary Figure 2: Neutrophil function tests. (A)** Protease release of control and patient PMNs after stimulation with fMLP, PAF/fMLP, CytoB/fMLP, and TX-100 (maximal slope in RFU/min; mean  $\pm$  SEM, n=3). **(B)** CD63 expression on control and patient PMNs after stimulation with fMLP, PAF/fMLP and CytoB/fMLP (mean of two independent experiments) **(C)** NADPH-oxidase activity was quantified as H<sub>2</sub>O<sub>2</sub> release after stimulation with zymosan, serum-treated zymosan (STZ), PMA or PAF/fMLP (nmol H<sub>2</sub>O<sub>2</sub>/min/10<sup>6</sup> PMNs; mean  $\pm$  SEM, n=4). **(D)** Killing of *S. aureus* by control and patient PMNs. Killing was quantified by determining CFU after incubation with PMNs at different time points, t=0 was defined as 100% (mean  $\pm$  SEM, n=4). See Online Methods.

**Supplementary Figure 3: Mild defect in patient platelet aggregation and activation to multiple ligands. (A)** Platelet aggregation was determined as double-colored events (see Online Methods). PMA (100 ng/ml), TRAP (20  $\mu$ M) and background levels without stimulation are shown (mean  $\pm$  SEM, n=4; \* p<0.05). **(B)** Platelet expression of CD62P, CD63 and PAC-1 compared to CD61 upregulation (expressed as a ratio) upon activation by thrombin-related activation peptide TRAP (CD62P and CD63, platelet-rich plasma), or by collagen and PMA (PAC-1/CD61 ratio, washed platelets) (mean  $\pm$  SEM; n=3).

**Supplementary Figure 4. Identification of ARPC1B c.191\_195 TCAAGdelCCTGCCins mutation. (A)** Screenshot from the Integrative Genome Viewer (IGV from Broad Institute) shows the exon 5 sequence reads from the *ARPC1B* gene of the patient obtained with the Ion Torrent PGM system. All reads show the c.191\_195 TCAAGdelCCTGCCins mutation. **(B)** Electropherograms are shown from the Sanger sequencing results obtained with the exon-5 forward primer. The arrow indicates the start of the homozygous c.191\_195 TCAAGdelCCTGCCins mutation in the genomic DNA of the

patient in exon 5 of *ARPC1* gene (second row). The mother and father of the patient (third and fourth row) show a heterozygous pattern. The healthy control (upper row) shows the wild-type sequence.

**Supplementary Figure 5: Fibroblast migration and ARPC1A/ARPC1B expression.** Comparison of fibroblast migration in a scratch assay showed no difference between patient and control cells in response to PDGF and FCS. **(A)** Still images of patient and control cells after stimulation with 10% (v/v) FCS. **(B)** Quantification of the wound area after 0, 15, 30 and 45 hours after stimulation (mean  $\pm$  SD of 12 different areas). The wound area at t=0 was defined as 100%. **(C)** Western blot of patient and control fibroblast lysates (upper panel) and control fibroblasts and blood cell lysates (lower panel) with anti-ARPC1A antibody and actin as a loading control.