Biallelic *CXCR2* loss-of-function mutations define a distinct congenital neutropenia entity

Viviana Marin-Esteban,[1] Jenny Youn,[2] Blandine Beaupain,[3,4] Agnieszka Jaracz-Ros,[1] Vincent Barlogis,[5] Odile Fenneteau,[6] Thierry Leblanc,[7] Florence Bellanger,[8] Philippe Pellet,[8] Julien Buratti,[8] Hélène Lapillonne,[9] Françoise Bachelerie,[1] Jean Donadieu[2,3,4] and Christine Bellanné-Chantelot[4,8,10]

[1]Université Paris-Saclay, Inserm UMR996, Inflammation, Microbiome and Immunosurveillance, Clamart; [2]Sorbonne Université, Service d'Hémato-oncologie Pédiatrique, Assistance Publique–Hopitaux de Paris (AP-HP), Hôpital Trousseau, Paris; [3]Registre Français des Neutropénies Congénitales, Hôpital Trousseau, Paris; [4]Centre de Référence des Neutropénies Chroniques, AP-HP, Hôpital Trousseau, Paris; [5]CHU Marseille, Hôpital La Timone, Service d'Hémato-oncologie Pédiatrique, Assistance Publique–Hôpitaux de Marseille, Marseille; [6]AP-HP, Laboratoire d'Hématologie, Hôpital Robert-Debré, Paris; [7]AP-HP, Hôpital Robert-Debré, Service d'Hématologie Pédiatrique, Paris; [8]Sorbonne Université, Département de Génétique Médicale, AP-HP, Hôpital Pitié–Salpêtrière, Paris; [9]Sorbonne Université, CRSA–Unité INSERM, AP-HP, Hôpital Trousseau, Paris and [10]Inserm U1287, Villejuif, France

Correspondence:
CHRISTINE BELLANNÉ-CHANTELOT
christine.bellanne-chantelot@aphp.fr
VIVIANA MARIN-ESTEBAN
viviana.marin-esteban@universite-paris-saclay.fr

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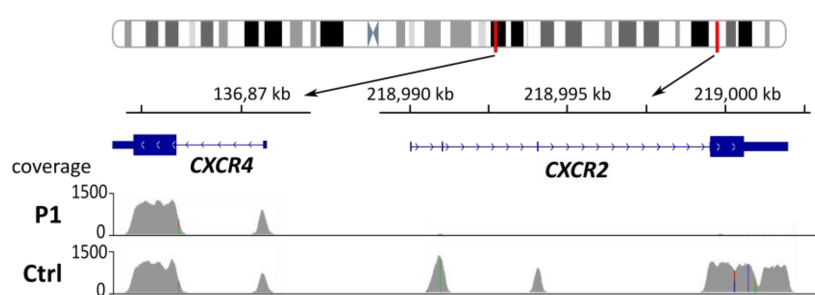
A

P1
*CXCR2*del/
*CXCR2*del



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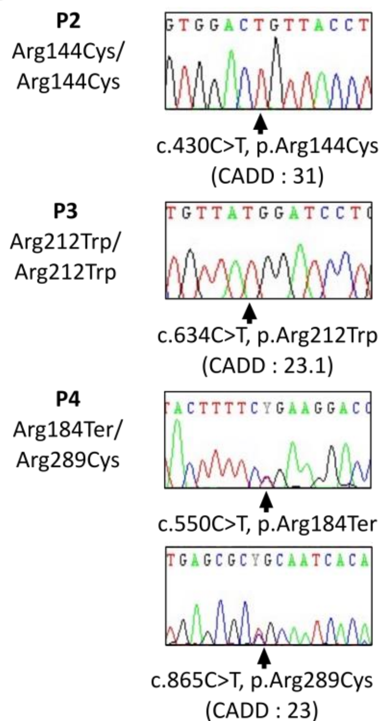

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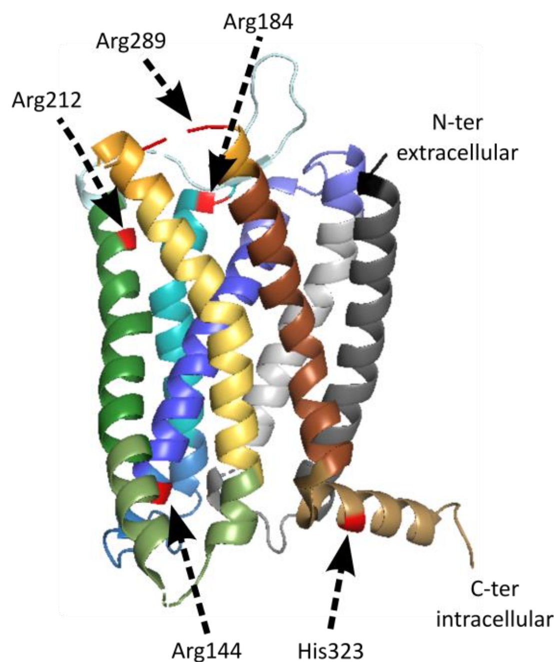
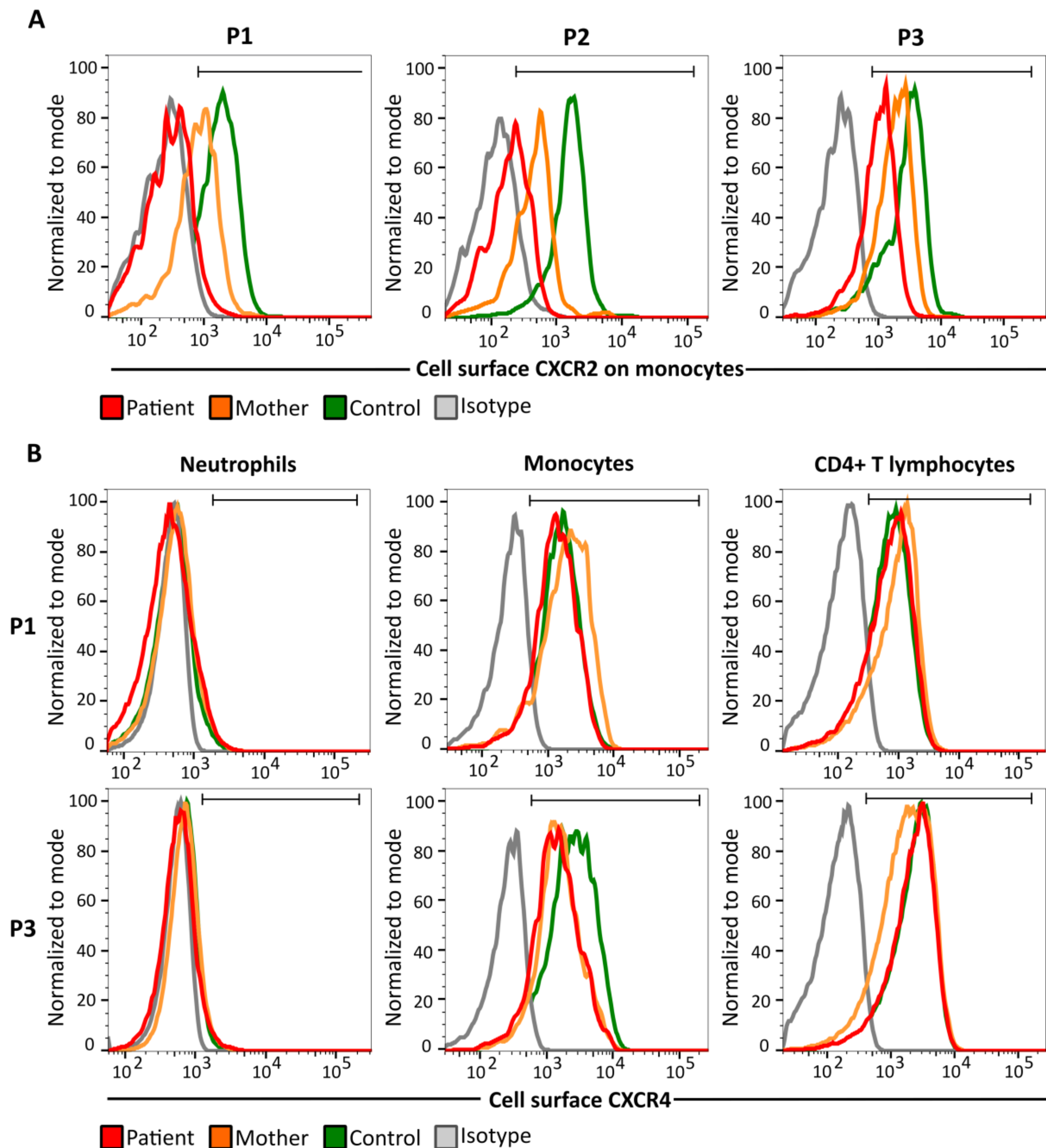
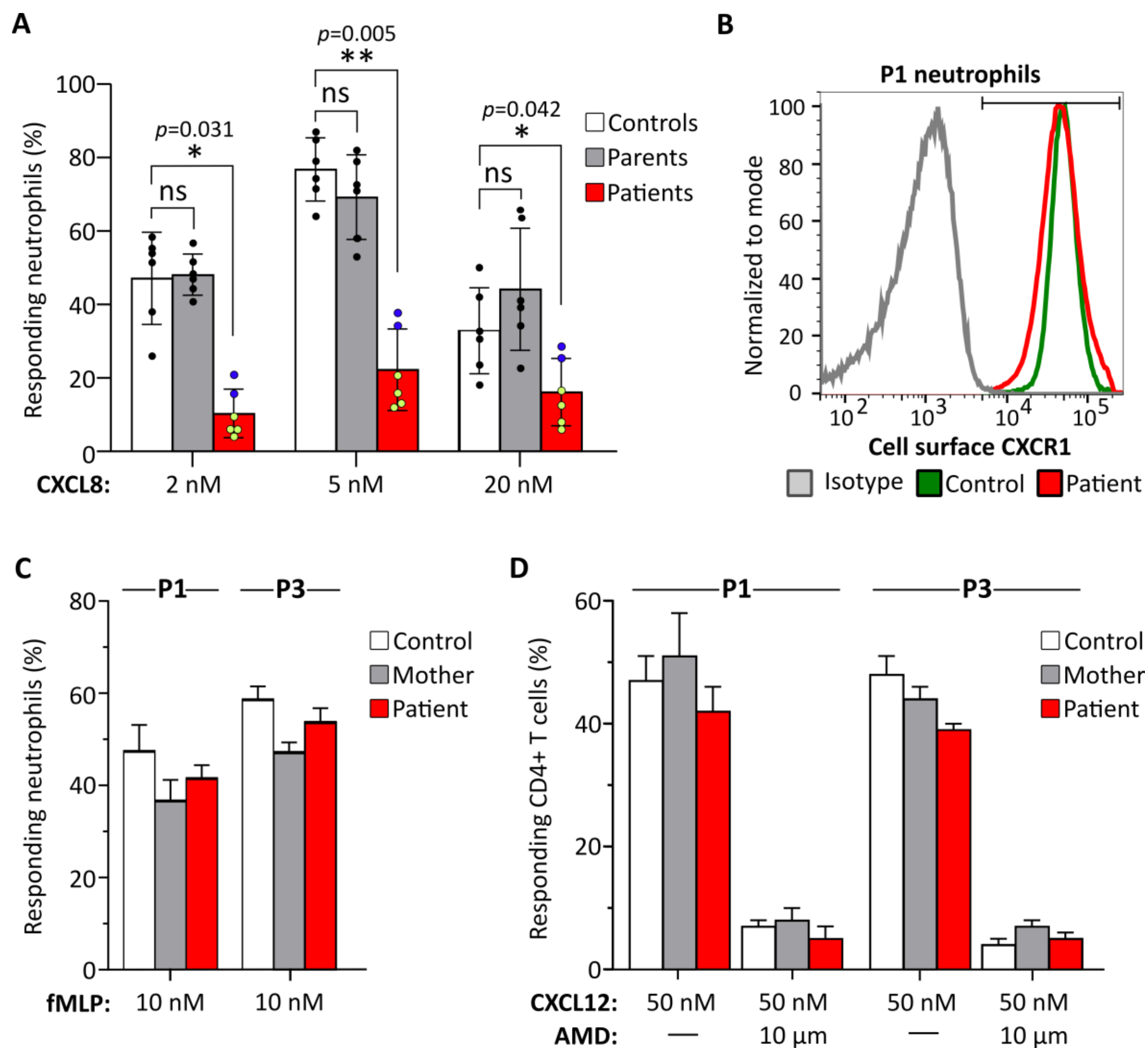


Supplementary Figure S1. Identification of germline biallelic *CXCR2* mutations in four patients with severe congenital neutropenia. (A) Visualization with Integrative Genomic Viewer (IGV) of the homozygous *CXCR2* whole-gene deletion identified in patient P1 by targeted sequencing. Comparison of *CXCR2* and *CXCR4* coverages for P1 and a healthy control DNA (Ctrl). (B) Sanger sequencing confirmation of the *CXCR2* mutations identified by targeted sequencing and exome-sequencing (KAPA HyperExome probes, Roche). Arrows indicate mutation positions. Mutation nomenclature is based on the reference sequence NM_001557.3. The CADD scores are indicated for missense mutations. (C) Ribbon representation of human CXCR2 (PDB code 6flfl) with the mapping of the four *CXCR2* mutations identified herein (Arg144Cys, Arg212Trp, Arg184Ter and Arg289Cys) and the His323fs mutation reported by Auer et al.[4]



Supplementary Figure S2. CXCR2 and CXCR4 expression levels. (A) Cell-surface CXCR2 immunostaining for monocytes from the patients P1, P2, and P3, one heterozygous carrier (mother), and a healthy donor (Control). Monocyte CXCR2 expressions, compared to the Control, were 52% lower and absent for P1's mother and P1, respectively; 87% and 71% lower for P2 and her mother, respectively; and 61% and 37% lower for P3 and her mother, respectively. (B) Cell-surface CXCR4 immunostaining on neutrophils, monocytes and $CD4^+$ T lymphocytes from P1 and P3.



Supplementary Figure S3. Chemotaxis responses and CXCR1-expression level. (A) Dose-dependent CXCL8-induced chemotaxis of neutrophils. Results from two separate assays with samples from patient P1 (yellow dots) and one assay with a sample from P3 (blue dots). Each sample from patients was simultaneously assessed with samples from one heterozygous carrier (parent) and one healthy control. Statistical analyses were computed, using the Friedman test followed by Dunn's multiple comparison test. *$0.005<p<0.05$; **$p\leq 0.005$; ns: not significant. (B) Cell-surface CXCR1 immunostaining of neutrophils from P1 and a healthy control. (C) *N*-formylmethionine-leucyl-phenylalanine (fMLP)-induced chemotaxis of neutrophils from Controls, P1 and P3 and their mothers. (D) CXCL12-induced chemotaxis of $CD4^+$ T lymphocytes from Controls, P1 and P3 and their mothers. AMD3100 (AMD) is a specific competitive CXCR4 antagonist. Chemotaxis assays were performed and analyzed as described in legend of Figure1. For lymphocyte chemotaxis assay, incubation time was 4 hours and cells were labelled for immunophenotyping before counting them by flow cytometry.