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

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Author's Contributions: VM-E, FB, JD and CB-C designed the study. VM-E performed and interpreted functional data. JY analyzed clinical data. BB collected biological and clinical data. AJ-R performed chemotaxis assays. VB and TL provided samples and clinical data. OF performed and reviewed bone marrow examinations. FB and PP performed molecular experiments and exome sequencing. JB performed exome annotation. HL performed cytological analysis. JD analyzed clinical data and performed statistical analysis. CB-C analyzed exome sequencing and performed variant interpretation; VM-E, FB, JD and CB-C analyzed the data and wrote the manuscript which was reviewed and edited by all authors.

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Data sharing statement. Technical information is available on request in order to assist other laboratories with characterization of CXCR2 variants.

Neutrophil homeostasis results from a balance between neutrophil production, release from the bone marrow (BM) and clearance from the circulation, where chemokines and their receptors play central roles.[1,2] Studies on mice demonstrated that CXCR4 and CXCR2 receptors antagonistically regulate BM neutrophil release.[2] While CXCR4 and its chemokine CXCL12, which is constitutively expressed in the BM, provide key signals for neutrophil retention, CXCR2 activation by the CXCL8 subfamily of chemokines promotes their release from BM.[1,2] Those events were shown in patients carrying heterozygous *CXCR4* gain-of-function mutations causing the rare autosomal dominant WHIM syndrome (WS), characterized by human papillomavirus (HPV)-induced warts, hypogammaglobulinemia, recurrent bacterial infections and myelokathexis reflecting an accumulation of senescent neutrophils in BM.[3] Profound neutropenia associated with myelokathexis was previously reported in two siblings carrying a homozygous truncating *CXCR2* loss-of-function mutation, supporting the importance of CXCR2 signaling in neutrophil mobilization.[4] Myelokathexis and recurrent severe infections[5] in that single pedigree led to its being included in the large series of WS and WS-like cases,[6] and it remains the only published case of CXCR2 deficiency. Herein, we report biallelic *CXCR2* mutations including one complete gene deletion in four patients with chronic neutropenia, harboring a wild-type (WT) *CXCR4* gene.

Patients were diagnosed during childhood with profound neutropenia in the context of recurrent gingivitis and oral ulcerations (Table 1). BM smears showed no major granulocytic maturation defect. Myelokathexis was present in only patient 1 (P1) affecting 35% of myeloid cells. The other hematological lineages, including lymphocyte subsets, were within their normal ranges. These four patients had high levels of circulating IgG and/or IgA at diagnosis that persisted throughout follow-up.

We investigated a possible genetic etiology using targeted-sequencing of genes known to be involved in inherited neutropenia and exome-sequencing. We excluded *CXCR4* mutations

and identified a homozygous *CXCR2*-gene deletion in P1, homozygous *CXCR2* missense mutations in P2 and P3, and compound heterozygous *CXCR2* mutations in P4 (Figure 1A and Online Supplementary Figure S1A and B). The *CXCR2* deletion was further confirmed by SNP-array analysis (data not shown) that revealed a homozygous 13.4-kb deletion in 2q35 (218,988,774_219,002,220) encompassing only *CXCR2*. To exclude other causal variants in P2, P3 and P4, who harbor missense *CXCR2* mutations, probands' and their parents' DNA were subjected to whole-exome-sequencing. Mean depth of exome coverage was 74X with 96% covered at least 20X. The *CXCR2* mutations were confirmed and no other potentially causative candidate variants were identified**.** The homozygous *CXCR2* genotypes of P1, P2, and P3 were consistent with the reported consanguinity of these pedigrees. Parents were heterozygous carriers and their blood-cell counts were within their normal ranges. The three *CXCR2* missense mutations (p.Arg144Cys, p.Arg212Trp and p.Arg289Cys) had been entered into the Genome Aggregation Database (gnomAD) with an allele frequency $<5\times10^{-5}$ but never as being homozygous. P2's mutation affects Arg144 that constitutes the critical DRY motif for G-protein activation.[7] P3's and P4's mutations affect Arg184, which is highly conserved between CXCR2 and CXCR1, and Arg212 and Arg289 that belong to domains cooperating with the CXCR2 N-terminal for the efficient docking of the CXCL8-chemokine ligand (Online Supplementary Figure S1C).[8]

We then examined cell-surface CXCR2 expression in neutrophils (Figure 1B), monocytes (Online Supplementary Figure S2A) and natural killer (NK) cells (data not shown) from P1, P2 and P3, their parents, and healthy control blood donors. As expected, CXCR2 was not expressed in the different cell populations derived from patient P1, who has a homozygous *CXCR2*-gene deletion. Her mother, who carries a heterozygous *CXCR2* deletion, had intermediate CXCR2 expression between P1 and control values. That mutant-dosage effect was also observed in carriers of *CXCR2* missense mutations, e.g., all pedigree-P2 blood cell

populations noted above and pedigree-P3 monocytes (Figure 1B, Online Supplementary Figure S2A). Whether the underlying mechanisms implicate altered turnover of the Arg144Cys mutant and, in a more cell-restricted fashion, the Arg212Trp mutant, remains to be investigated. As expected based on the patients' WT *CXCR4* genotypes , cell-surface CXCR4 expression was within the normal range for all tested blood cell populations as illustrated for P1 and P3 (Online Supplementary Figure S2B).

We evaluated the potential impact of *CXCR2* mutations on the CXCL8-driven chemotactic response of blood neutrophils derived from P1 and P3 pedigrees (Figure 1C). In transwell migration assays, healthy donors' neutrophils responded to CXCL8, yielding a typical bell-shaped dose-dependent chemotaxis-response curve. Blockade with the specific CXCR2 inhibitor SB265610 (SB) confirmed CXCR2 implication in the observed chemotaxis. Neutrophils from parents migrated similarly to controls despite lower cell-surface CXCR2 expression, supporting the reported dissociation between the expression level of chemokines-receptors and their functions.[9] In contrast, efficacy of the CXCL8-induced chemotaxis for P1-derived neutrophils was drastically reduced (up to 86%) for all tested CXCL8 concentrations. For P3-derived neutrophils, this response was more weakly lowered (up to 59%) indicating that the Arg212Trp CXCR2 mutation only partially abrogates CXCR2 function. This was further confirmed by the SB-mediated inhibition of the remaining Arg212Trp CXCR2-driven chemotaxis (Figure 1C). P3-derived neutrophils expressed similar levels of CXCR2 than control neutrophils (Figure 1B) and their remaining chemotactic responses toward CXCL8 were out of the range of the ones provided by control neutrophils (Online Supplementary Figure S3A), further supporting *CXCR2* loss-of-function phenotype. We extrapolated that this loss-of-function phenotype would be similarly conferred by P2's and P4's *CXCR2* missense mutations, affecting the protein's critical DRY domain[7] or N-terminal domain[8], respectively. CXCR1 could account for the remaining migration of P1's

neutrophils, which were not affected by the inhibitor SB.[10] Indeed, although CXCR1 and CXCR2 have closely linked actions, they differ notably in their signaling properties and chemokine-ligand spectra, with CXCR1 being engaged by CXCL5 and CXCL6 and having high affinity for CXCL8, while CXCR2 promiscuously binds to all seven CXCL8-family chemokines.[11] CXCR1 expression levels on P1 and control neutrophils were within the same range (Online Supplementary Figure S3B), thereby substantiating that hypothesis.

The patients described herein did not experience severe recurrent bacterial infections, suggesting that although CXCR2 actively participated in neutrophil recruitment into inflammatory tissues, this function was largely counterbalanced. Indeed, patients' neutrophils remained responsive to *N*-formylmethionine-leucyl-phenylalanine (fMLP) (Online Supplementary Figure S3C), indicating that they might be efficiently guided to inflammatory sites by chemoattractant signals, like fMLP and possibly others including the C5a complement factor, both abundantly generated in bacterial infection foci.[12] Likewise, CXCL12-driven migration was equivalent for $CD3^{+}CD4^{+}$ cells (Online Supplementary Figure S3D) and the other lymphocyte subpopulations (data not shown) from P1 and P3, their parents and controls. Those findings support the postulate of normal CXCR4 function in patients harboring *CXCR2* mutations acting as drivers of congenital neutropenias although it remains to be experimentally demonstrated.

Different clinical manifestations distinguish these four patients with *CXCR2* mutations from the 14 WS clinical spectrum enrolled in the French Severe Chronic Neutropenia Registry, as summarized in Table 2. Myelokathexis, a pathognomonic feature of WS,[6] was solely detected in P1, harboring the *CXCR2* gene deletion, thereby extending the description of the two previously reported cases with *CXCR2* loss-of-function mutations.[5] Its absence in the clinical pictures of P2, P3 and P4, together with the partial CXCR2-chemotaxis response retained by Arg212Trp, further suggests that their chronic neutropenia is not the only

consequence of a CXCR2-dependent mobilization defect; neutrophil homeostasis also seems to be impacted. That hypothesis is supported by the reported association of rare heterozygous *CXCR2* missense variants, including the one carried by P4, with low white blood-cell counts.[4]

Elucidating the mechanisms underlying the relationship between the biallelic *CXCR2* mutations identified herein and neutropenia will require developing relevant experimental models. Alternative models to mice should be considered, in light of the lack of a murine CXCL8 homologue and the neutrophilia of mice lacking *Cxcr2*.[13,14] However, targeted *Cxcr2* invalidation in mouse neutrophils led to their retention in BM, reproducing a myelokathexis phenotype,[4] thereby suggesting a Cxcr2 role in the regulation of neutrophil biology and, intrinsically, in neutrophil trafficking. In contrast to patients with WS, who suffer from chronic lymphopenia, often associated with hypogammaglobulinemia,[6,15] patients with *CXCR2*-mutations experienced only transient episodes of lymphopenia and had elevated immunoglobulin levels, mostly IgG and IgA (Table 2). B-lymphocyte counts were normal, unlike mice invalidated for *Cxcr2* that exhibit B-cell expansion,[13] highlighting the limitation of mice to model of a CXCR2 deficiency. No HPV-induced warts, neoplasia or syndromic features, such as tetralogy of Fallot, observed in WS[15] were noted during patient follow-up. However, we could not exclude incomplete penetrance of these phenotypes, as reported in WS.[6,15]

In conclusion, CXCR2 deficiency seems to be a distinct molecular entity associated with congenital neutropenia with clinical severity and pathogenic mechanisms distinct from WS, thereby emphasizing the importance to determine *CXCR2* mutational status in patients with chronic neutropenia.

References

1. Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. Immunity. 2003;19(4):583-593.
2. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. J Clin Invest. 2010;120(7):2423-2431.
3. Hernandez PA, Gorlin RJ, Lukens JN, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet. 2003;34(1):70-74.
4. Auer PL, Teumer A, Schick U, et al. Rare and low-frequency coding variants in CXCR2 and other genes are associated with hematological traits. Nat Genet. 2014;46(6):629-634.
5. Bohinjec J. Myelokathexis: chronic neutropenia with hyperplastic bone marrow and hypersegmented neutrophils in two siblings. Blut. 1981;42(3):191-196.
6. Heusinkveld LE, Majumdar S, Gao J-L, McDermott DH, Murphy PM. WHIM Syndrome: from Pathogenesis Towards Personalized Medicine and Cure. J Clin Immunol. 2019;39(6):532-556.
7. Rovati GE, Capra V, Neubig RR. The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. Mol Pharmacol. 2007;71(4):959-964.
8. Liu K, Wu L, Yuan S, et al. Structural basis of CXC chemokine receptor 2 activation and signalling. Nature. 2020;585(7823):135-140.
9. Honczarenko M, Douglas RS, Mathias C, Lee B, Ratajczak MZ, Silberstein LE. SDF-1 responsiveness does not correlate with CXCR4 expression levels of developing human bone marrow B cells. Blood. 1999;94(9):2990-2998.
10. Richardson RM, Pridgen BC, Haribabu B, Ali H, Snyderman R. Differential cross-regulation of the human chemokine receptors CXCR1 and CXCR2. Evidence for time-dependent signal generation. J Biol Chem. 1998;273(37):23830-23836.
11. Nasser MW, Raghuwanshi SK, Grant DJ, Jala VR, Rajarathnam K, Richardson RM. Differential activation and regulation of CXCR1 and CXCR2 by CXCL8 monomer and dimer. J Immunol. 2009;183(5):3425-3432.
12. Petri B, Sanz MJ. Neutrophil chemotaxis. Cell Tissue Res. 2018;371:425-436.
13. Cacalano G, Lee J, Kikly K, et al. Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. Science. 1994;265(5172):682-684.
14. Broxmeyer HE, Cooper S, Cacalano G, Hague NL, Bailish E, Moore MW. Involvement of Interleukin (IL) 8 receptor in negative regulation of myeloid progenitor cells in vivo: evidence from mice lacking the murine IL-8 receptor homologue. J Exp Med. 1996;184(5):1825-1832.
15. Beaussant Cohen S, Fenneteau O, Plouvier E, et al. Description and outcome of a cohort of 8 patients with WHIM syndrome from the French Severe Chronic Neutropenia Registry. Orphanet J Rare Dis. 2012;7:71.

Table 1. Clinical profile of the four patients with biallelic *CXCR2* loss-of-function mutations

Characteristic	Patient (Registry ID)				
	P1 (8364)	P2 (6487)	P3 (6902)	P4 (8497)	Reference range
Clinical profile					
Age at diagnosis (years)	2.9	1.9	1.8	1.2	
Oral lesions	Yes	Yes	Yes	Yes	
Severe infections[a] (age)	No	1 cellulitis (13 months)	1 pneumonitis (22 months)	No	
Prophylactic treatment	No	Cotrimoxazole	Cotrimoxazole	No	
G-CSF therapy (dose, period)	Yes (2 µg/kg, 2 years)	No	Yes (5 µg/kg, 1 month)	No	
Age at last follow-up (years)	36	22.5	10	6.5	
Hematological values at diagnosis					
Neutrophils (10^9/L)	**0.56**	**0.6**	**0.18**	**0.6**	1.5-8.0
Monocytes (10^9/L)	0.31	0.46	0.52	0.46	0.1-1.0
Lymphocytes (10^9/L)	2.0	6.3	6.2	4.6	1.5-6.5
Hemoglobin level (g/dL)	12.4	12.2	11.8	10	11.5-15.5
Platelets (10^9/L)	359	371	368	225	150-400
Hematological values during follow-up					
Blood counts (No.)	18	24	23	7	
Neutrophils (10^9/L)	**0.57** (0.28-1.8)	**0.45** (0.1-0.85)	**0.54** (0.1-10.8[b])	**0.35** (0.3-1)	1.5-8.0
Monocytes (10^9/L)	0.29 (0.02-0.51)	0.43 (0.16-0.68)	0.53 (0.19-1.3)	0.52 (0.39-0.7)	0.1-1.0
Lymphocytes (10^9/L)	1.66 (1-2.7)	2.03 (1-6.3)	4.0 (0.97-10.8)	4.58 (1.9-4.8)	1.5-6.5
Hemoglobin (g/dL)	12.6 (11.5 -13.3)	11.5 (9.9-13.2)	12.2 (10.4-13.1)	10.7 (9.6-12.7)	11.5-15.5
Platelets (10^9/L)	357 (257-439)	277 (215-572)	324 (167-544)	523 (225-670)	150-400
Differential bone-marrow count					
Myeloblasts	4%	0%	1%	2%	0.3-4%
Promyelocytes & myelocytes	15%	5%	13%	2%	12-25%
Metamyelocytes & mature neutrophils	43%	29%	38%	37%	33-48%
Myelokathexis	35%	0%	0%	0%	
Immunoglobulin levels (g/L)					
IgG	***15.1*** *(14.2-20.5)*	***16.7*** *(14.3-17.3)*	*10.9 (10.2-11.8)*	***14.8***	5.98-11.1
IgM	0.91 (0.81-1.12)	**1.77** (1.51-2.0)	***2.41*** *(2.12-2.53)*	***1.63***	0.56-1.59
IgA	***2.98*** *(1.73-3.06)*	***3.75*** *(3.14-3.96)*	***1.75*** *(1.69-2.56)*	***3.35***	0.49-1.53
Lymphocyte subsets					
Subset determinations (N)	5	10	3	1[c]	
$CD3^+CD4^+$ T cells (10^9/L)	0.544 (0.333-0.700)	0.677 (0.611-0.853)	0.920 (0.766-1.075)	**1.456**	0.53-1.3
$CD3^+CD8^+$ T cells (10^9/L)	**0.288** (0.170-0.429)	0.501 (0.432-0.629)	0.611 (0.498-0.725)	**1.295**	0.33-0.92

$CD19^+$B cells (10^9/L)	0.157 (0.121-0.177)	0.295 (0.240-0.365)	0.468 (0.344-0.591)	***0.769***	0.11-0.57
$CD3^-CD16^+CD56^+$ NK cells (10^9/L)	0.088 (0.078-0.129)	0.171 (0.134-0.209	0.153 (0.134-0.172)	***0.778***	0.07-0.48

Results are expressed as medians (range), unless stated otherwise. **Bold type** indicates lower values and ***bold italics*** indicate higher values compared to the reference range. [a]Intravenous antibiotic-treated infections. [b]At the time of a fever of unknown origin (likely viral), the absolute neutrophil count increased spontaneously. [c]Evaluated at age of 4 years. *NK* natural killer

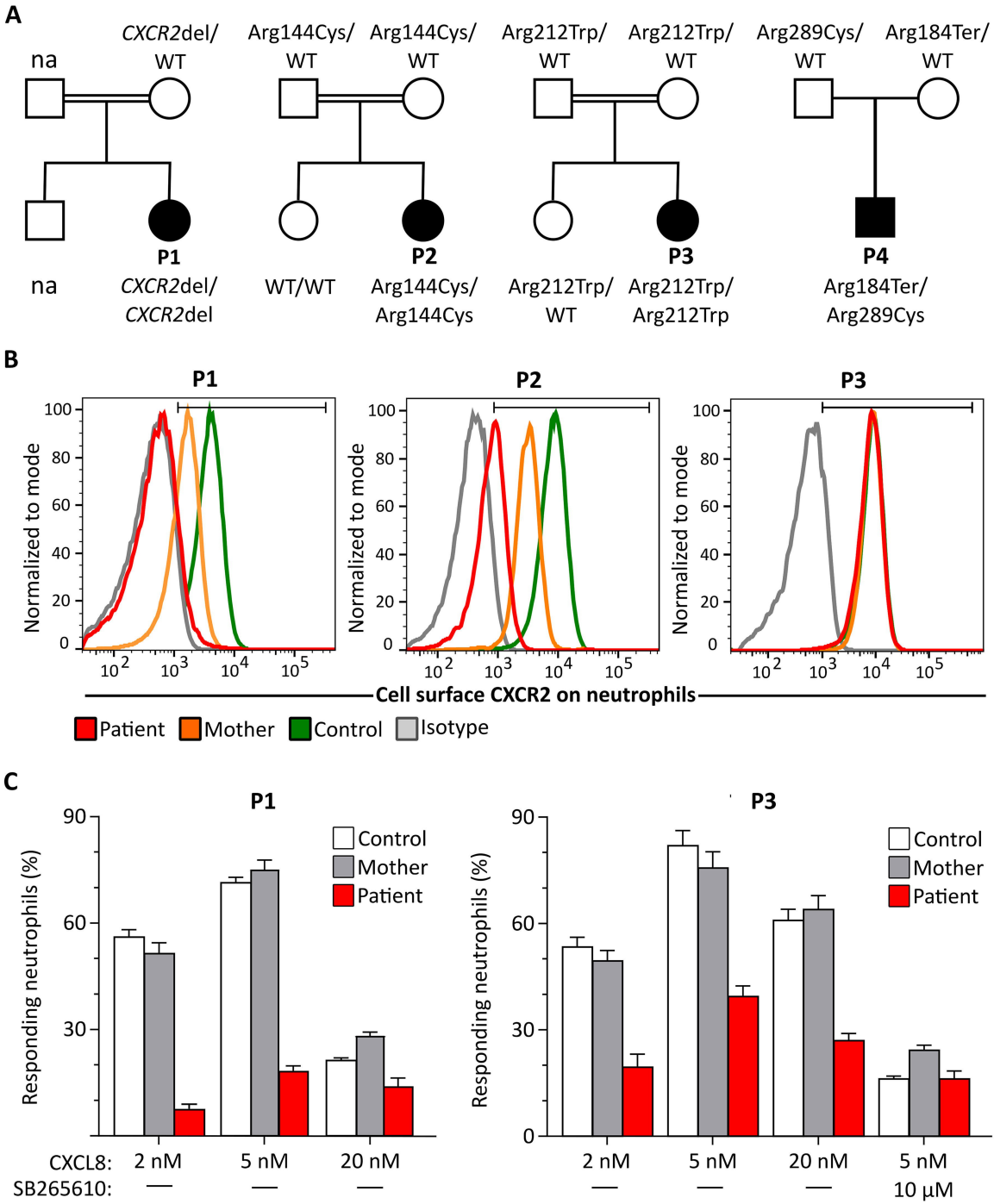
Table 2. Comparison of the clinical characteristics of 14 patients with *CXCR4* gain-of-function mutations and 4 patients with *CXCR2* loss-of-function mutations enrolled in the French Severe Congenital Neutropenia Registry.

Characteristic	Patients with *CXCR4* mutations	Patients with *CXCR2* mutations	Relevant differences
Number of patients	14	4	
Age at diagnosis (years)	4.9 (0.1-33)	1.8 (1.2-2.9)	
Age at last visit (years)	31.9 (8.9-77)	16.3 (7.2-36.5)	
Oral lesions	3/14	4/4	
Severe infections	10/14	2/4	
Warts	8/14	0/4	
Hematological values (all available CBCs)			
Neutrophils (10^9/L)	**0.221** (0.13-1.4)	**0.496** (0.18-0.57)	
Monocytes (10^9/L)	0.156 (0.06-0.44)	0.477 (0.29-0.54)	**
Lymphocytes (10^9/L)	**0.577** (0.16-1.9)	3.2 (1.6-4.5)	**
Hemoglobin (g/dL)	12.1 (8.6-13.8)	11.8 (10.7-12.6)	
Platelets (10^9/L)	220 (169-479)	338 (277-523)	*
Myelokathexis	14/14	1/4	*
Bone-marrow myeloid/erythroid ratio	3.5	3	
G-CSF treatment	3/14, poor responses	2/4, good responses	
Immunoglobulin levels (g/L)			
IgG	*8 (4.2-15)*	***16.2** (10.2-20.5)*	**
IgM	*0.64 (0.24-1.7)*	***1.63*** (0.81-2.53)	**
IgA	*0.86 (0.1-2.5)*	***3.33** (1.69-3.96)*	**
Lymphocyte subsets			
$CD3^+CD4^+$ T cells (10^9/L)	**0.37** (0.17-0.51)	0.80 (0.54-1.46)	**
$CD3^+CD8^+$ T cells (10^9/L)	**0.09** (0.04-0.10)	0.50 (0.29-1.29)	**
$CD19^+$ B cells (10^9/L)	**0.02** (0.01-0.05)	0.38 (0.12-0.77)	**
$CD3^-CD16^+CD56^+$ NK cells (10^9/L)	0.12 (0.06-0.16)	0.15 (0.08-0.78)	**
Solid tumors	8/14	0/4	
Tetralogy of Fallot	5/14	0/4	
Deaths	3[a]	0/4	

Results are expressed as medians (range), unless stated otherwise. **Bold type** indicates lower values and ***bold italics*** indicate higher values compared to the reference range. [a]Two deaths occurred between 30 and 40 years of age from vulvar cancer or atypical mycobacteria with liver failure, and one 77-year-old died of pneumonitis. Owing to the very low number of patients to be compared, the most relevant differences are indicated as *(P<0.01) or **(P<0.001).

Figure legend

Figure 1. Characterization of germline biallelic *CXCR2* mutations identified in four patients with chronic neutropenia. (A) Family pedigrees with identified homozygous (patients P1, P2, and P3) or compound heterozygous (P4) *CXCR2* mutations. Healthy parents were heterozygous carriers for the identified mutations. *WT* wild type. na, not available (B) Cell-surface CXCR2 immunostaining on neutrophils from P1, P2, and P3, one heterozygous carrier, and healthy donors. (C) Dose-dependent CXCL8-induced chemotaxis of neutrophils without or with SB265610 (SB), its specific CXCR2 inhibitor. Chemotaxis assays were run in duplicate, with whole blood samples (diluted 1:4 in RPMI with 1% human serum) using 12-mm–diameter transwell devises with 5-µm pores. For each assay including patient, parent and control, blood samples were collected concomitantly and equally treated. Samples were added in the upper chamber, CXCL8 in the lower chamber and SB in both chambers. Control wells without chamber were also added to determine the number and phenotype of total seeded cells. After 1 hour incubation, cells recovered in the lower chambers (responding cells) were counted and identified by flow cytometry. Results are expressed as percentage of responding neutrophils, calculated as [(Number of neutrophils recovered in the lower chamber with CXCL8) - (Number of neutrophils recovered in the lower chamber without CXCL8)] / (Number of total seeded neutrophils) x 100.


A
na
CXCR2del/ WT
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Arg144Cys/ WT
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Arg212Trp/ WT
Arg289Cys/ WT
Arg184Ter/ WT
P1
P2
P3
P4
na
CXCR2del/ CXCR2del
WT/WT
Arg144Cys/ Arg144Cys
Arg212Trp/ WT
Arg212Trp/ Arg212Trp
Arg184Ter/ Arg289Cys
B
P1
P2
P3
Normalized to mode
Cell surface CXCR2 on neutrophils
Patient
Mother
Control
Isotype
C
P1
P3
Responding neutrophils (%)
Control
Mother
Patient
CXCL8:
SB265610:
2 nM
5 nM
20 nM
10 µM

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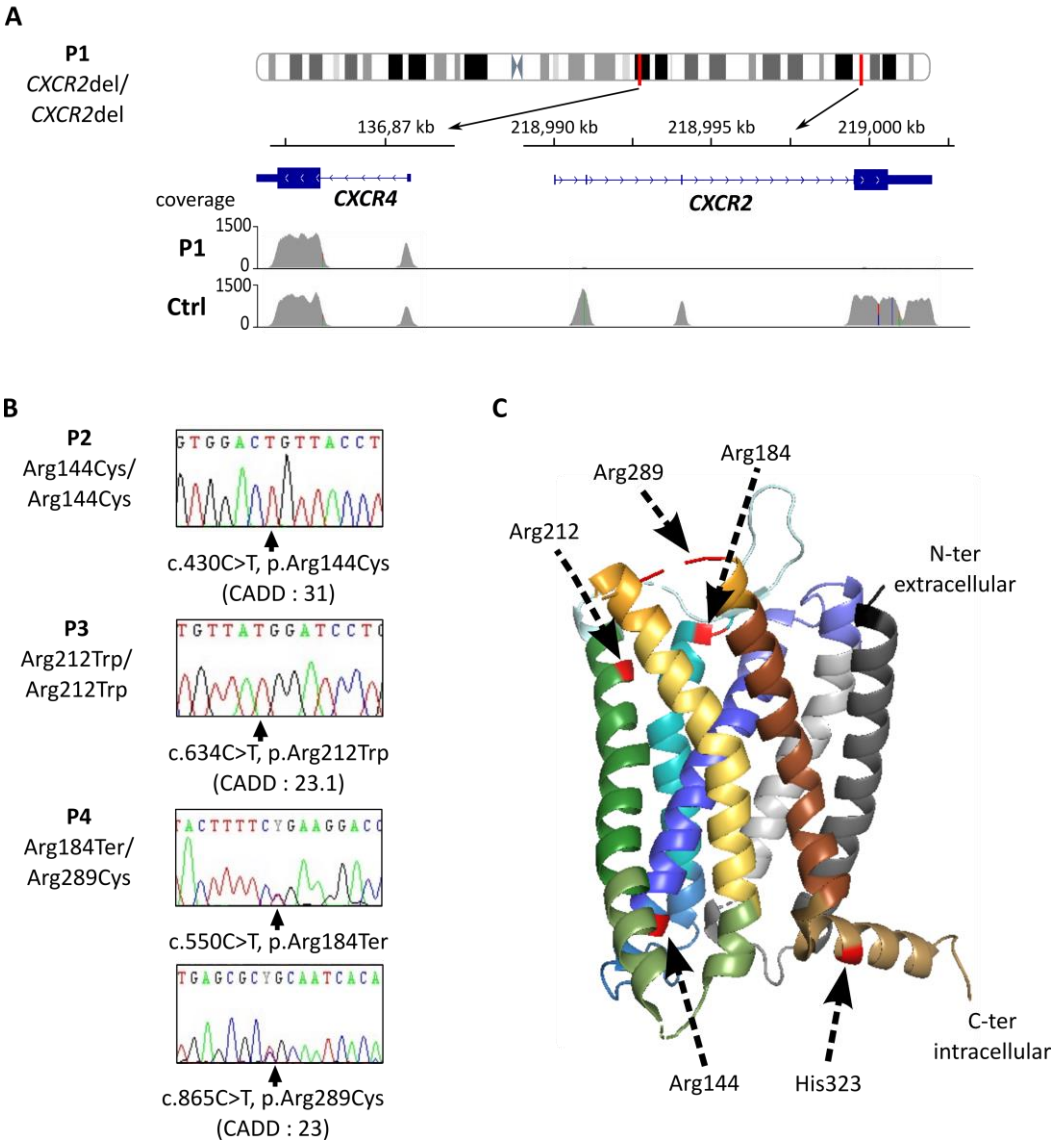
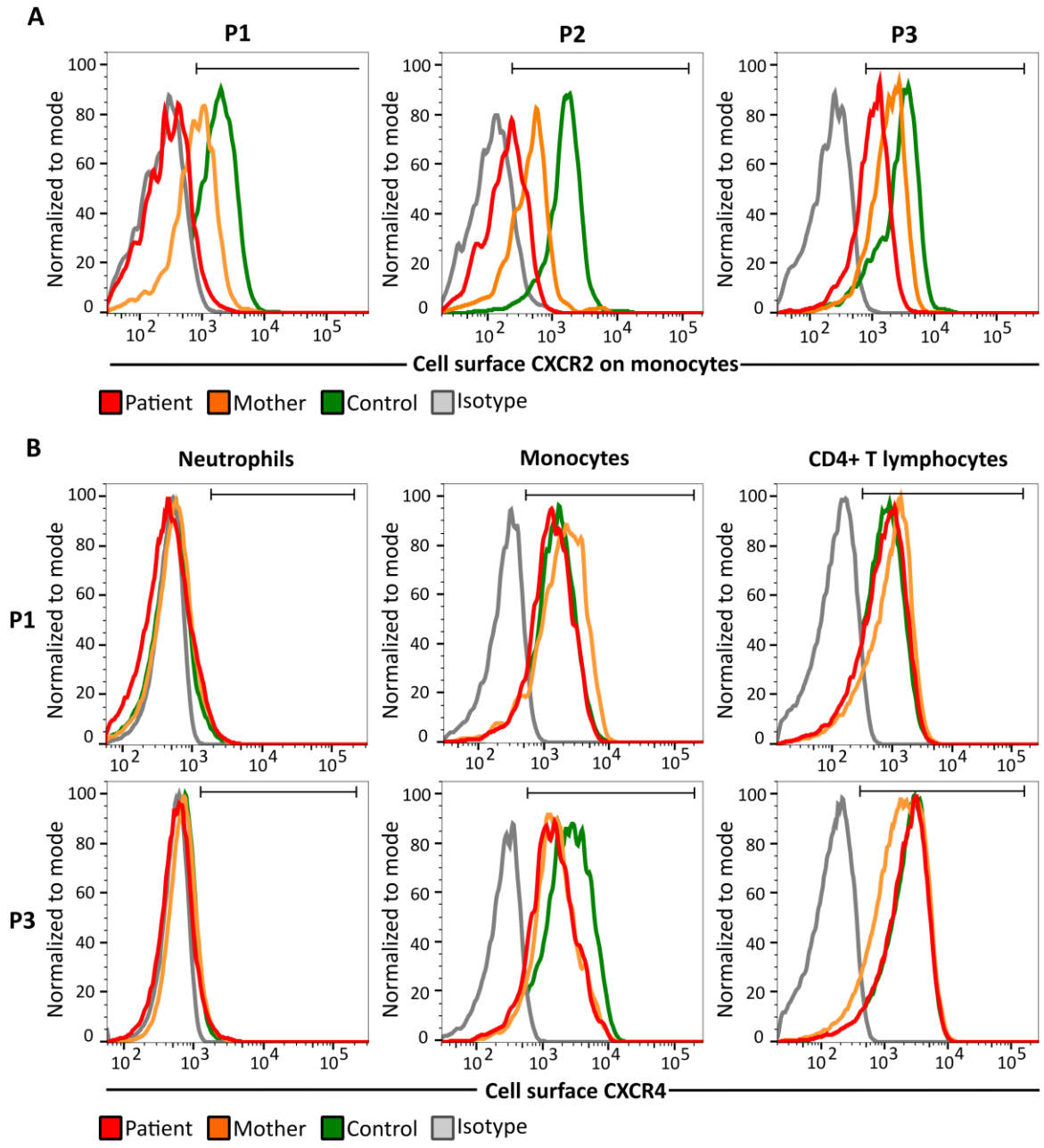
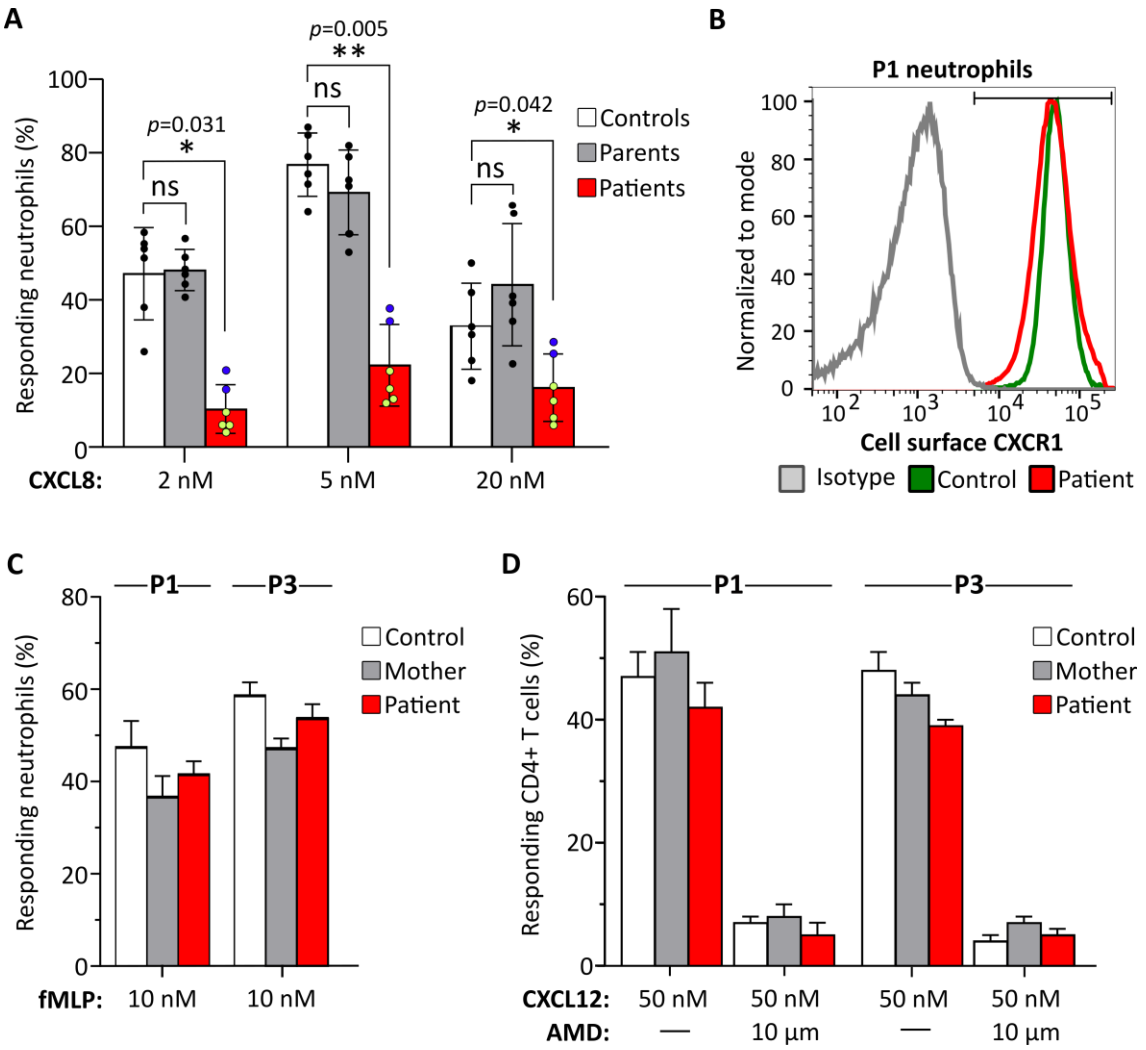
Viviana Marin-Esteban *et al*.



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. *$0.005<p<0.05$; **$p\leq0.005$; 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.