Identification of biallelic germline variants of *SRP68* in a sporadic case with severe congenital neutropenia

Barbara Schmaltz-Panneau,[1,2] Anne Pagnier,[3] Séverine Clauin,[4] Julien Buratti,[3] Caroline Marty,[1,2] Odile Fenneteau,[5] Klaus Dieterich,[6] Blandine Beaupain,[7,8] Jean Donadieu,[7,8,9] Isabelle Plo[1,2] and Christine Bellanné-Chantelot[1,4,8]

[1]Gustave Roussy Cancer Center, INSERM U1287, Villejuif; [2]Paris Saclay University, U1287, Villejuif; [3]Department of Pediatric Hematology and Oncology, CHU Grenoble Alpes, Grenoble, GIN, Grenoble; [4]AP-HP, Pitié-Salpêtrière Hospital, DMU BioGeM, Department of Genetics, Sorbonne University, Paris; [5]AP-HP, Robert Debré Hospital, Laboratory of Hematology, University of Paris, Paris; [6]Department of Medical Genetics, Univsersity Grenoble Alpes, INSERM U1216, CHU Grenoble Alpes, GIN, Grenoble; [7]French Registry of Chronic Neutropenia, Trousseau Hospital, Paris; [8]Reference Center for Chronic Neutropenia, Paris and [9]AP-HP, Trousseau Hospital, Department of Pediatric Hematology and Oncology, Paris, France

Correspondence: CHRISTINE BELLANNE'-CHANTELOT - christine.bellanne-chantelot@aphp.fr

doi:10.3324/haematol.2020.247825

Identification of biallelic germline variants of *SRP68* in a sporadic case with severe congenital neutropenia

submitted by Barbara Schmaltz-Panneau et al

Supplementary Materials and Methods

Ethics

Clinical information and samples were collected with written informed consent from the parents according to the Declaration of Helsinki. The case was registered with informed consent from his parents in the French severe chronic neutropenia registry (CNIL certificate 97.075). All material (blood, primary fibroblasts, and DNA/RNA samples) were declared to French Health Authorities in compliance with current legislation.

Supplementary methods

Whole exome sequencing and variant filtering

The exome of the trio was sequenced by the Genotypic and sequencing facility of the Institut du Cerveau et de la Moelle Epinière (Hôpital Pitié-Salpêtrière, France). Exons were captured from fragmented genomic DNA samples using the SureSelect Human All Exon 50 Mb exome kit (Agilent) and 75 bp paired-end sequencing was carried out on an Illumina HiSeq2500 according to manufacturers' protocols. Bioinformatics analyses were done using in-house pipeline. Sequence reads were mapped to the human genome build (hg19/GRCh37) using bwa-mem 0.7.10. Raw data quality was assessed with FastQC v0.11.5 and mapping file quality was assessed with picard-tools 1.121. Every analyzed sample exceeds the standard 90% coverage at 20X threshold. Single-nucleotide variants and short insertions/deletions were called with GATK 3.8 and annotated with SnpEff 4.3r. Functional predictions scores from dbNSFP v3 were added: CADD, SIFT, Polyphen3, REVEL, M-CAP, dbscSNV, as well as gnomAD allele frequency and number of heterozygous/homozygous, OMIM gene inheritance, HGMD and ClinVar annotations. An in-house script allowed us to select variants of interest, sorted by encoded categories: de novo, X-linked homozygous, homozygous and compound heterozygous. Variants were filtered based on their impact on the gene (missense, nonsense, frameshift, splice site-altering variants).

Sanger sequencing of *SRP68*

SRP68 gene (NM_003136.3) sequencing was performed using standard procedures. Primers are described in Supplemental Table S2. Sequences were analyzed with Seqscape software v2.5 (Life technologies) and variants were annotated with Alamut software v2.7 (Interactive Biosoftware).

Real-time quantitative PCR analysis

PCR based on SYBR-Green I fluorescence was performed using an ABI 7900 Sequence Detection System (Life Technology). Primers were designed for exon 1 and 2 of *SRP68* gene, and PCR were carried out with the SYBR Green PCR Master Mix (ThermoFisher) using 300 nmol/l of each primer and 20 ng of DNA extracted from blood. We used as reference the β–globin gene (HBB) and as control amplicon the exon 5 of the glucokinase gene (GCK). The number of copies was determined using the $2^{-\Delta\Delta CT}$. Each point has been performed in triplicate and replicated.

Analysis of *SRP68* transcripts

Total RNA were extracted either from primary fibroblasts or from cultured $CD34^+$ cells obtained from peripheral blood of the patient or from leukapheresis samples after mobilization of donors for control samples. $CD34^+$ progenitors were isolated by a positive selection using an immunomagnetic cell sorting system (Miltenyi Biotec) and cultured in serum-free medium supplemented with SCF (25 ng/mL), IL-3 (10 ng/mL) and G-CSF (20 ng/mL) for 12 days. Selected populations were sorted for granulocytic markers at days 12. The cells were sorted on a Becton Dickinson (BD) Influx cell sorter using conjugated anti-CD33-FITC, anti-CD15-V450, anti-CD11b-PE monoclonal antibodies (BD)

Quantitative RT-PCRs were carried out on the ABI Prism GeneAmp 5700 (Perkin-Elmer) using the Power SYBR Green PCR Master Mix (Applied Biosystems) containing the specific primers (Supplementary Table S2). Expression levels of all genes were calculated relatively to *HPRT* and to *PPIA* housekeeping genes using qbase+ software.

Western-blot analysis

Protein expression was performed in primary fibroblasts using mouse anti-human SRP68 (Origene #TA351671) with anti-mouse β-Actin (Cell signaling #3700) as loading control.

Supplementary Table S1 : *SRP68* variants identified by whole exome sequencing

				Child			Mother			Father		
Position (hg19)	**Location**	**HGVS_cDNA**	**HGVS_protein**	**Reference[1] allele**	**Alternate allele**	**VAF**	**Reference allele**	**Alternate allele**	**VAF**	**Reference allele**	**Alternate allele**	**VAF**
74068513	Exon 1	c.60T>C	p.Gly20Gly	20	0	0,00	20	27	0,57	34	0	0
74068387	**Intron 1**	**c.184+2T>C**	**---**	**0**	**57**	**1,00**	**33**	**31**	**0,48**	**45**	**0**	**0**
74066447	Exon 2	c.251+12A>T	---	82	58	0,41	75	58	0,44	83	70	0,46
74056413	Exon 7	c.813G>A	p.Glu271Glu	56	51	0,48	44	26	0,37	59	34	0,37
74036573	Intron 14	c.1601-18T>A	---	57	45	0,44	59	42	0,42	107	0	0
74036468	Intron 15	c.1656+32G>A	---	107	0	0,00	55	37	0,40	113	0	0

[1.] read count of each allele, SRP68 accession number : NM_014260.3; VAF, variant allele frequency.

SupplementaryTable S2. Primers for Sanger sequencing, quantitative PCR and RT-PCR

Primer ID	Sequences 5' →3'
Design of primers for Sanger sequencing	
SRP68-ex1F	CACCTAGCCAGCCACAATTC
SRP68-ex1R	AGGTAAGGGCGAGAGAAACTG
Design of primers for real time-PCR	
SRP68-ex1Fq	GGATCGAAGGCAAACAAAGA
SRP68-ex1R	AGGTAAGGGCGAGAGAAACTG
SRP68-ex2Fq	TTTGTTTGGATTCTTGCTATGG
SRP68-ex2Rq	TGCTGCTGGGATTCCTTAATA
GCK-ex6Fq (control)	TGAAATGGATGTGGTGGCAAT
GCK-ex6Rq (control)	GATGGTCTTCGTAGTAGCAGGAGAT
HBB-354F (reference gene)	GTGCACCTGACTCCTGAGGAGA
HBB-455R (reference gene)	CCTTGATACCAACCTGCCCAG
Design of primers for RT-PCR	
SRP68-ex1Fcdna	GGCTGCTGAGAAGCAGGTC
SRP68-ex2Rq	TGCTGCTGGGATTCCTTAATA
Design of primers for quantitative RT-PCR	
SRP54-F	CCGCCACAAACAAGAAGACT
SRP54-R	TGGAGGCATCCATCACATAA
SRP68-F	CGGAGACTGTGCCCATTTCT
SRP68-R	TTACACTTGACCTCACCGGC
SRP72-F	TGCAGCTCAAAGCAATTGGG
SRP72-R	CCTTGGCCTATCAGTGCACA
ATF4-F	GTTCTCCAGCGACAAGGCTA
ATF4-R	ATCCTGCTTGCTGTTGTTGG
BAX-F	GTGCCTCAGGATGCGTCC
BAX-R	TCCAGTTCGTCCCCGATG
CHOP-F	AGAACCAGGAAACGGAAACAGA
CHOP-R	TCTCCTTCATGCGCTGCTTT
MDM2-F	ACCACCTCACAGATTCCAGCTT
MDM2-R	GCACCAACAGACTTTAATAACTTCAAA
NOXA1-F	GGACGTCCTGTGTGAAGTGGA
NOXA1-R	CACTTGGGGAAGATGCCGAT
P21-F	CGCTAATGGCGGGCTG
P21-R	CGGTGACAAAGTCGAAGTTCC
ULK1-F	TCATCTTCAGCCACGCTGT

ULK1-R	CACGGTGCTGGAACATCTC
XBP1 UNSPLICE-F	CAGCACTCAGACTACGTGCA
XBP1 UNSPLICE-R	ATCCATGGGGAGATGTTCTGG
XBP1 SPLICE-F	CTGAGTCCGAATCAGGTGCAG
XBP1 SPLICE-R	ATCCATGGGGAGATGTTCTGG
PPIA-F	GTCAACCCCACCGTGTTCTT
PPIA-R	CTGCTGTCTTTGGGACCTTGT
HPRT1_F	GGCAGTATAATCCAAAGATGGTCAA
HPRT1-R	TCAAATCCAACAAAGTCTGGCTTATAT
H2AFZ-F	GTGCGACGAAGGAGTAGGTG
H2AFZ-R	CTCTGCGGTGAGGTACTCCA