



Clinical Implications

- We report the occurrence of a hereditary angioedema type 1 associated with developmental anomalies in a man with unaffected parents and/or relatives resulting from an unusual *de novo* pericentromeric rearrangement of chromosome 11 (11p11-12q12.2) sapping the complete *SERPING1* gene.

The proband is the youngest son of healthy, non-consanguineous Belgian parents. His 2 older brothers are normal. Pregnancy and delivery were normal. Birth weight was 2870 g, length was 48 cm, and head circumference was 31.7 cm. Clinical examination in the neonatal period revealed several dysmorphic features: microcephaly, antimongoloid eye position, epicanthal folds, broad nasal bridge, unilateral cryptorchidism, blind ending sacral fistula, and brachytelephalangy. Cardiological examination revealed a perimembranous ventricular septal defect. Brain computed tomography (CT) and magnetic resonance imaging scans at 4 months and 5 years of age were normal. Metabolic screening and ophthalmological examinations could not reveal anomalies. Biometric evaluation during infancy showed a linear growth of weight and height on the third percentile, whereas head circumference lagged behind with development below the third percentile. Psychomotor evaluations showed mild retardation with pronounced speech retardation. At 5.2 years, the IQ was 83.

The karyotypes performed on cultured fibroblasts and phytohemagglutinin (PHA)-stimulated lymphocytes suspected an interstitial deletion of the long arm of chromosome 11 (Figure E1, A, available in this article's Online Repository at www.jaci-inpractice.org). The single nucleotide polymorphism (SNP) array revealed a 9.3 Mb interstitial deletion of chromosome 11 including at least a small sequence of the short arm and a larger region of the long arm. However, SNP array resolution in the pericentromeric region is low as probes are absent from the highly repetitive alpha satellite DNA (Figure E1, C). Fluorescence *in situ* hybridization (FISH) experiments confirmed the 11q12 deletion but showed that the pericentromeric regions (which is not covered by probes of the SNP array) were retained on chromosome 11 (Figure E1, B). The combination of the

3 genomic tools highlighted a complex rearrangement of the pericentromeric region of chromosome 11 with 2 deletions separated by a normally represented centromere (Figure 1). Parental karyotypes and SNP arrays did not detect this chromosome 11 rearrangement.

Begin 2017, at age 24, he presented with an acute episode of vomiting and right fossa pain, mimicking appendicitis. Laparoscopy revealed a large amount of serous fluid in the abdomen. Furthermore, parts of small bowel and surrounding meso seemed inflamed. The appendix showed no signs of inflammation or perforation, but appendectomy was performed. Histopathological examination of the ascites revealed multiple neutrophils, compatible with an acute inflammatory exudate. Cultures remained sterile. A CT scan of the abdomen (Figure 2) showed concentric small bowel wall thickening, mainly ileum, with submucosal edema, prominent mesenteric vessels, and diffuse ascites. Luminal fluid accumulation is also present in some of the affected small bowel loops. Some segments of the colon were also affected, to a lesser degree. There were no signs of mechanical obstruction or perforation. Stool cultures were repeatedly negative. He was empirically treated with metronidazole and ciprofloxacin. An underlying inflammatory bowel disease (IBD) was suspected, but neither colonoscopy nor magnetic resonance enterography could confirm the diagnosis. He responded well to antibiotics.

In the next 6 months, he was readmitted twice with recurrent abdominal pain, diarrhea, and vomiting, lasting 2 to 5 days. Based on the clinical phenotype with recurrent episodes of enteritis and peritonitis, and taking into account that the above-described deletion on chromosome 11 includes the *SERPING1* locus (11q12.1), a hereditary angioedema (HAE) type 1 resulting from a quantitative C1-esterase inhibitor deficiency was suspected. History revealed the absence of angioedema at other localizations and to occur without urticaria or pruritus.

Table E1 (available in this article's Online Repository at www.jaci-inpractice.org) summarizes the complement profile in the patient, parents, and brothers. The results confirm the diagnosis of type 1 HAE in the patient. Because of repetitive bouts of pronounced and invalidating visceral angioedema, a long-term prophylaxis with plasma-derived C1 esterase inhibitor concentrate (Cinryze 1000 IU intravenously on a 3-day interval) was initiated. Since the start of this treatment he became asymptomatic.

Apart from the documentation of pulmonary infections during infancy, the patient's history was negative for recurrent and/or complicated infections, autoimmunity, and lymphoproliferation. Because the chromosome deletion included the *MS4A1* gene that is associated with common variable immunodeficiency (CVID), we assessed the IgG (and subclasses), A, M levels, and B memory cells. The normal results excluded the diagnosis of CVID.

To our knowledge, this is the first observation of visceral angioedema associated with developmental anomalies due to the largest and unusual pericentromeric rearrangement of chromosome 11 including the *SERPING1* gene.

Type 1 and 2 HAE are autosomal dominant diseases resulting from functional deficiency of the C1-inhibitor protein caused by alteration of the *SERPING1* gene. HAE is characterized by a high allelic heterogeneity, with almost each family carrying their own gene defect, and a poor genotype-phenotype correlation. More

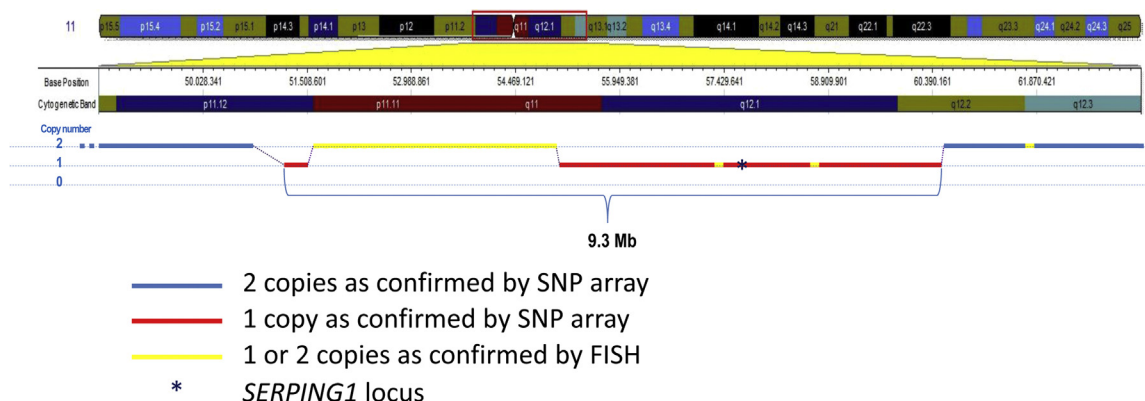


FIGURE 1. Scheme of genomic rearrangement of chromosome 11 based on karyotypic, FISH, and microarray analyses. The copy number estimated by the SNP array is in blue for 2 copies and in red for 1 copy, by FISH in yellow. The asterisk pinpoints the *SERPING1* locus, which is included in a large deleted segment. The combination of the 3 different techniques highlights a more complex rearrangement than expected by only 1 technic. *FISH*, Fluorescence *in situ* hybridization; *SNP*, single nucleotide polymorphism.



FIGURE 2. Coronal and axial computed tomography images of the abdomen during an acute episode of angioedema. Note the marked concentric small bowel wall thickening with submucosal edema (white arrow), prominent mesenteric vessels (black arrow), and ascites (asterisk).

than 500 different *SERPING1* alterations have been identified up to now, scattered over all exons of the gene,¹ mainly missense and/or nonsense substitutions (53%) and small indels (36%). The large gene rearrangements (11%) are mainly intragenic.² They are generated by nonhomologous recombination between *Alu* repeat sequences, due to their exceptionally high density in most introns of the gene.³ Larger deletions encompassing the entire 17-kb-long *SERPING1* gene are only reported in 7 patients.⁴⁻⁷ Iwamoto et al⁶ reported the largest deletion to date (650 kb) including 10 genes in a patient only affected by HAE.

The age of onset of the first symptoms of angioedema in our case and in that described by Iwamoto et al⁵ is higher than the mean age (approximately 8-12 years) associated with missense mutations. This confirms the poor genotype-phenotype correlation in type 1 HAE.²

The deleted pericentromeric regions contain 67 genes, excluding 73 olfactory receptor (pseudo)genes (Table E2, available in this article's Online Repository at www.jaci-inpractice.org). Although *de novo* deletions of this size and gene content are typically pathogenic, it is not possible to link genes apart from

SERPING1 to the clinical presentation of this patient. No comparable deletions and no known disease associations have been described that may explain the dysmorphic, psychomotor, and cardiac features. Normal complement profiles and genomic analyses in both parents confirmed that this deletion is *de novo*. *De novo* alterations of *SERPING1* are known to occur in 25% to 30% of patients with HAE.⁸

The centromere is an essential structure that is required for chromosome stability.⁹ FISH experiments demonstrated the persistence of the repetitive pericentromeric genomic sequences located within the interstitial deletion of chromosome 11, which are not covered by the SNP-array probes. These results suggest a more complex genomic rearrangement than a simple and large pericentromeric deletion, which by preserving the centromere maintains the stability of the abnormal chromosome 11.

In conclusion, we report the largest *de novo* and complex pericentromeric rearrangement encompassing the entire *SERPING1* gene among others of type 1 HAE associated with developmental anomalies. This finding is important because a detailed analysis of the deleted genes allowed a faster diagnosis of HAE, and a relevant genetic counseling for the relatives, without needing to sequence the whole *SERPING1* gene. It also emphasizes the need to search for large rearrangements including the entire gene in case of type 1 or 2 HAE without *SERPING1* mutation.

^aDepartment of Immunology-Allergy-Rheumatology, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

^bDepartment of Medical Genetics, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

^cDepartment of Gastroenterology, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

^dBelgian Cancer Registry and GenHAP, Brussels, Belgium

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Corresponding author: Didier G. Ebo, MD, PhD, Department of Immunology-Allergy-Rheumatology, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Campus Drie Eiken T5. 95, Universiteitsplein 1, 2610 Antwerpen, Belgium. E-mail: immuno@uantwerpen.be.

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REFERENCES

1. Institute of Medical Genetics in Cardiff. The Human Gene Mutation Database. Available from: <http://www.hgmd.cf.ac.uk/>. Accessed October 29, 2018.
2. Germenis AE, Speletas M. Genetics of hereditary angioedema revisited. *Clin Rev Allergy Immunol* 2016;51:170-82.
3. Stoppa-Lyonnet D, Duponchel C, Meo T, Laurent J, Carter PE, Arala-Chaves M, et al. Recombinational biases in the rearranged C1-inhibitor genes of hereditary angioedema patients. *Am J Hum Genet* 1991;49:1055-62.
4. Duponchel C, Di Rocco C, Cicardi M, Tosi M. Rapid detection by fluorescent multiplex PCR of exon deletions and duplications in the C1 inhibitor gene of hereditary angioedema patients. *Hum Mutat* 2001;17:61-70.
5. Roche O, Blanch A, Duponchel C, Fontan G, Tosi M, Lopez-Trascasa M. Hereditary angioedema: the mutation spectrum of SERPING1/C1NH in a large Spanish cohort. *Hum Mutat* 2005;26:135-44.
6. Iwamoto K, Tanaka A, Kawai M, Ishii K, Mihara S, Hide M. A large heterozygous deletion including the entire C1 inhibitor gene in a sporadic case of hereditary angio-oedema. *Clin Exp Dermatol* 2012;37:20-3.
7. Loules G, Zamanakou M, Parsopoulou F, Vatsiou S, Psarros F, Csuka D, et al. Targeted next-generation sequencing for the molecular diagnosis of hereditary angioedema due to C1-inhibitor deficiency. *Gene* 2018;667:76-82.
8. Ebo DG, Van Gasse AL, Sabato V, Bartholomeus E, Reyniers E, Vanbellingen JF, et al. Hereditary angioedema in 2 sisters due to paternal gonadal mosaicism. *J Allergy Clin Immunol Pract* 2018;6:277-279.e1.
9. Fukagawa T, Earnshaw WC. The centromere: chromatin foundation for the kinetochore machinery. *Dev Cell* 2014;30:496-508.

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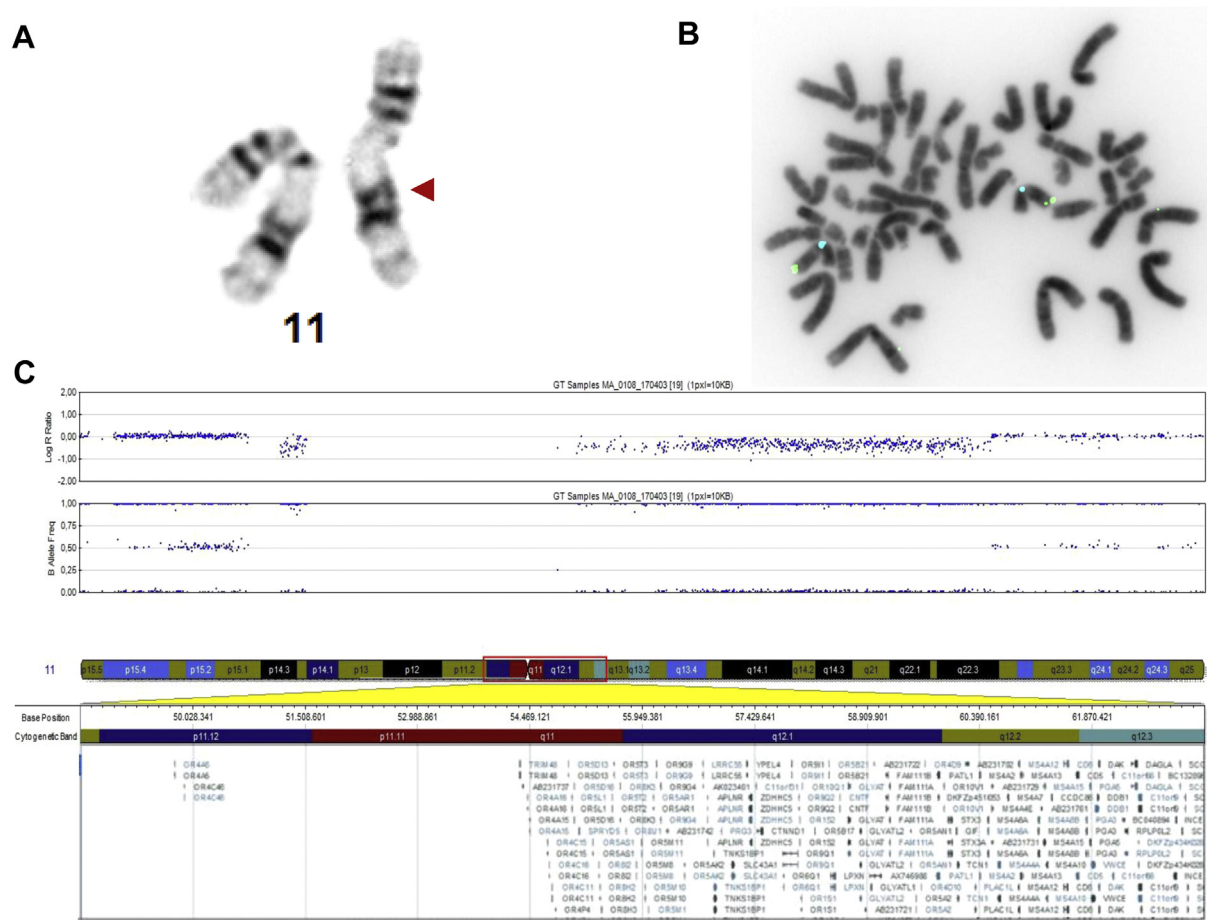


FIGURE E1. Cytogenetic and molecular analyses. **A**, Karyotype: 46,XY,del(11)(q12q13). The deleted chromosome 11 is highlighted by a red arrow. **B**, FISH analysis with the centromere 11 probe (Vysis CEP 11-D11Z1) and 11q25 probe (RP11-27H17). ish 11p11.11-q11(D11Z1x2),del(11)(q12.1)(RP11-872D17-,RP11-324G17-,RP11-691N7-),11q12.2(RP11-467L20x2). The centromere 11 probe hybridizes at the expected location (similar blue spots) on both chromosome 11 which are identified by the 11q25 control probe (green signals). **C**, SNP array: arr[hg19] 11p11.12q12.2(50766308x2,51191702-60531346x1,60547604x2). Top to bottom: LogR ratio, B-allele frequency, chromosomal position, and gene content. DNA was extracted from peripheral blood using standard methods. The SNP array was performed using an Infinium HumanCytoSNP-12 v2.1 BeadChip on an iScan System, following standard protocols as provided by the manufacturer (Illumina, San Diego, Calif). CNV analysis was performed using CNV-Webstore.^{E1} CNV, Copy number variation; FISH, fluorescence *in situ* hybridization; SNP, single nucleotide polymorphism.

TABLE E1. Complement profiles in the patient, parents, and brothers

	Patient	Father	Mother	Brother_1	Brother_2
C4 (0.10-0.40 g/L)	0.021	0.22	0.238	0.280	0.18
C1-INH function (69% to 127%)	15	104	96	127	119
C1-INH antigen (0.25-0.41 g/L)	0.04	—	—	—	—

TABLE E2. List of the deleted genes (excluding the olfactory receptor coding sequences)

Gene symbol	Gene name	Reported in OMIM
APLNR	Apelin receptor	OMIM
BTBD18	BTB domain containing 18	—
C11orf31	Chromosome 11 open reading frame 31	OMIM
CLP1	Cleavage and polyadenylation factor I subunit 1	MORBID: Pontocerebellar hypoplasia, type 10#615803
CNTF	Ciliary neurotrophic factor	OMIM
CTNND1	Catenin delta 1	OMIM
DTX4	Deltex E3 ubiquitin ligase 4	OMIM
FAM111A	Family with sequence similarity 111 member A	MORBID: Gracile bone dysplasia #602361; Kenny-Caffey syndrome, type 2 #127000
FAM111B	Family with sequence similarity 111 member B	MORBID: Poikiloderma, hereditary fibrosing, with tendon contractures, myopathy, and pulmonary fibrosis #615704
GIF	Gastric intrinsic factor	MORBID: Intrinsic factor deficiency #261000
GLYAT	Glycine-N-acyltransferase	OMIM
GLYATL1	Glycine-N-acyltransferase-like 1	OMIM
GLYATL2	Glycine-N-acyltransferase-like 2	OMIM
LINC00301	Long intergenic nonprotein coding RNA 301	—
LOC101927120	Uncharacterized LOC101927120	—
LOC101927204	Uncharacterized LOC101927204	—
LOC283194	Uncharacterized LOC283194	—
LPXN	Leupaxin	OMIM
LRRC55	Leucine rich repeat containing 55	OMIM
MED19	Mediator complex subunit 19	OMIM
MIR130A	microRNA 130a	OMIM
MIR3162	microRNA 3162	—
MIR6128	microRNA 6128	—
MIR6503	microRNA 6503	—
MPEG1	Macrophage expressed 1	OMIM
MRPL16	Mitochondrial ribosomal protein L16	OMIM
MS4A1	Membrane spanning 4-domains A1	MORBID: Immunodeficiency, common variable, 5#613495
MS4A12	Membrane spanning 4-domains A12	OMIM
MS4A13	Membrane spanning 4-domains A13	—
MS4A14	Membrane spanning 4-domains A14	—
MS4A15	Membrane spanning 4-domains A15	—
MS4A18	Membrane spanning 4-domains A18	—
MS4A2	Membrane spanning 4-domains A2	MORBID: Atopy, susceptibility to (susceptibility factor)#147050
MS4A3	Membrane spanning 4-domains A3	OMIM
MS4A4A	Membrane spanning 4-domains A4A	OMIM
MS4A5	Membrane spanning 4-domains A5	OMIM
MS4A6A	Membrane spanning 4-domains A6A	OMIM
MS4A6E	Membrane spanning 4-domains A6E	OMIM
MS4A7	Membrane spanning 4-domains A7	OMIM
MS4A8	Membrane spanning 4-domains A8	OMIM
OOSP1	Oocyte secreted protein 1, pseudogene	—
OOSP2	Oocyte secreted protein 2	—
OSBP	Oxysterol binding protein	OMIM
P2RX3	Purinergic receptor P2X 3	OMIM
PATL1	PAT1 homolog 1, processing body mRNA decay factor	OMIM
PRG2	Proteoglycan 2, pro eosinophil major basic protein	OMIM

(continued)

TABLE E2. (Continued)

Gene symbol	Gene name	Reported in OMIM
PRG3	Proteoglycan 3, pro eosinophil major basic protein 2	OMIM
RTN4RL2	Reticulon 4 receptor-like 2	OMIM
SERPING1	Serpin family G member 1	MORBID: Angioedema, hereditary, types I and II#106100; Complement component 4, partial deficiency of #120790
SLC43A1	Solute carrier family 43 member 1	OMIM
SLC43A3	Solute carrier family 43 member 3	—
SMTNL1	Smoothelin-like 1	OMIM
SSRP1	Structure specific recognition protein 1	OMIM
STX3	Syntaxin 3	OMIM
TCN1	Transcobalamin 1	OMIM
TIMM10	Translocase of inner mitochondrial membrane 10 homolog (yeast)	OMIM
TMX2	Thioredoxin related transmembrane protein 2	OMIM
TMX2-CTNND1	TMX2-CTNND1 readthrough (NMD candidate)	—
TNKS1BP1	Tankyrase 1 binding protein 1	OMIM
TRIM48	Tripartite motif containing 48	—
TRIM51	Tripartite motif-containing 51	—
TRIM51HP	Tripartite motif-containing 51H, pseudogene	—
UBE2L6	Ubiquitin conjugating enzyme E2 L6	OMIM
YPEL4	Yippee like 4	OMIM
ZDHHC5	Zinc finger DHHC-type containing 5	OMIM
ZFP91	ZFP91 zinc finger protein	—
ZFP91-CNTF	ZFP91-CNTF readthrough (NMD candidate)	—

MORBID, Genetic disorder reported in OMIM; *OMIM*, Online Mendelian Inheritance in Man database.

REFERENCE

- E1. Vandeweyer G, Reyniers E, Wuyts W, Rooms L, Kooy RF. CNV-WebStore: online CNV analysis, storage and interpretation. *BMC Bioinformatics* 2011; 12:4.