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Association of hereditary angioedema type 1 with developmental anomalies due to a large and unusual *de novo* pericentromeric rearrangement of chromosome 11 spanning the entire C1 inhibitor gene (*SERPING1*).

Ebo Didier G, MD, PhD[1], Blaumeiser Bettina MD, PhD[2], Kooy R. Frank, PhD[2], Beckers Sigri, PhD[2], Van Gasse Athina L, MD[1], Saerens Michael, MD[3], Spinhoven Maarten, MD, PhD[4], Sabato Vito MD, PhD[1], Poirel Hélène A, MD, PhD[5]

[1] Department of Immunology-Allergology-Rheumatology, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp (Belgium) and, Antwerp University Hospital, Antwerp (Belgium)

[2] Department of Medical Genetics, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp (Belgium)

[3] Department of Gastroenterology, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp (Belgium)

[4] Department of Gastroenterology, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp (Belgium)

[5] Belgian Cancer Registry and GenHAP, Brussels (Belgium)

Correspondence:

D.G. Ebo, MD PhD

University of Antwerp

Faculty of Medicine and Health Sciences

Immunology -Allergology - Rheumatology

Campus Drie Eiken T5.95

Universiteitsplein 1

2610 Antwerpen

Belgium

Tel: ++ 32 (0) 3 2652595

Fax: ++ 32 (0) 3 2652655

immuno@uantwerpen.be

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Short title :

HAE and developmental anomalies due to a large pericentromeric rearrangement of chromosome 11

ORCID ID

Didier Ebo: 0000-0003-0672-7529
Athina Van Gasse: 0000-0003-1657-5135
Vito Sabato: 0000-0002-1321-314X

Summary

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

To the editor,

The proband is the youngest son of healthy, non-consanguineous Belgian parents. His two older brothers are normal. Pregnancy and delivery were normal. Birth weight was 2870 g, length 48 cm and head circumference 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 peri-membranous ventricular septal defect. Brain CT- and MRI-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, while head circumference lagged behind with development below the third percentile. Psycho-motor evaluations showed mild retardation with pronounced speech retardation. At 5.2 years, the IQ was 83.

The karyotypes performed on cultured fibroblasts and PHA-stimulated lymphocytes suspected an interstitial deletion of the long arm of chromosome 11 (Fig. E1A). 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. (Fig. E1C). FISH experiments confirmed the 11q12 deletion but showed that the pericentromeric region (which is not covered by probes of the SNP-array) were retained on chromosome 11 (Fig. E1B). 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 (Fig. 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 (Fig. 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 IBD was suspected, but colonoscopy nor MR-enterography could confirm the diagnosis. He responded well to antibiotics.

In the next six months he was readmitted twice with recurrent abdominal pain, diarrhoea and vomiting, lasting 2-5 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 absence of angioedema at other localisations and to occur without urticaria or pruritus.

Table 1 in the supplementary data 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® 1000IU intravenously on a three-day interval) was initiated. Since start of this treatment he became asymptomatic.

Apart from the documentation of pulmonary infections during infancy, patient's history was negative for recurrent/complicated infections, autoimmunity and lymphoproliferation. Since the chromosome deletion included the *MS4A1* gene that is associated with 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 than 500 different *SERPING1* alterations have been identified up to now, scattered over all exons of the gene (http://www.hgmd.cf.ac.uk/), mainly missense/nonsense substitutions (53%) and small indels (36%). The large gene rearrangements (11%) are mainly intragenic [1]. They are generated by nonhomologous recombination between *Alu* repeat sequences, due to their exceptionally high density in most introns of the gene [2]. Larger deletions encompassing the entire 17kb-long *SERPING1* gene are only reported in 7 patients [3-6]. Iwamoto *et al* [5] 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 al* [5] is higher than the mean age (about 8-12 years) associated with missense mutations. This confirms the poor genotype-phenotype correlation in type 1 HAE [1].

The deleted pericentromeric regions contains 67 genes, excluding 73 olfactory receptor (pseudo)genes (Supplementary Table 2). While *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 which 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 25-30% of HAE patients[7].

The centromere is an essential structure that is required for chromosome stability [8]. 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.

Legend of figures

Legend figure 1: Scheme of genomic rearrangement of chromosome 11 based on karyotypic, FISH and micro-array analyses.

The copy number estimated by SNP-array is in blue for 2 copies and in red for 1 copy, by FISH in yellow. The asterix pinpoint the SERPING1 locus, which is included in a large deleted segment.

The combination of the 3 different technics highlights a more complex rearrangement than expected by only one technic.

Legend figure 2: Coronal and axial CT 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 (asterix).

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Supplementary data

Supplementary Figure 1: Cytogenetic and molecular analyses

1A Karyotype : 46,XY,del(11)(q12q13)

The deleted chromosome 11 is highlighted by a red arrow.

1B FISH analysis with 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 (similar blue spots) on both chromosome 11 which are identified by the 11q25 control probe (green signals).

1C 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.

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, CA, USA).

CNV analysis was performed using CNV-Webstore [9].

Table 1 : complement profiles in the patient, parents and brothers

Table 2 : List of the deleted genes (excluding the olfactory receptor coding sequences)