

Supravalvular Aortic Stenosis Caused by a Familial Chromosome 7 Inversion Disrupting the *ELN* Gene Uncovered by Whole-Genome Sequencing

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Keywords

Elastin · *ELN* · Paracentric inversion · Supravalvular aortic stenosis · Whole-genome sequencing

Abstract

Apparently, balanced chromosomal rearrangements usually have no phenotypic consequences for the carrier. However, in some cases, they may be associated with an abnormal phenotype. We report herein the case of a 4-year-old boy presenting with clinically isolated supravalvular aortic stenosis (SVAS). No chromosomal imbalance was detected by array CGH. The karyotype showed a balanced paracentric chromosome 7 inversion. Breakpoint characterization using paired-end whole-genome sequencing (WGS) revealed an *ELN* gene disruption in intron 1, accounting for the phenotype. Family study showed that the inversion was inherited, with incomplete penetrance. To our knowledge, this is the first case of a disruption of the *ELN* gene characterized by WGS. It contributes to refine the genotype-phenotype correlation in *ELN* disruption. Although this disruption is a rare

etiology of SVAS, it cannot be detected by the diagnostic tests usually performed, such as array CGH or sequencing methods (Sanger, panel, or exome sequencing). With the future perspective of WGS as a diagnostic tool, it will be important to include a structural variation analysis in order to detect balanced rearrangements and gene disruption.

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Introduction

Supravalvular aortic stenosis (SVAS; MIM 185500), characterized by the congenital narrowing of the aortic lumen, is a rare cardiac anomaly with an incidence of approximately 1:25,000 births [Metcalf et al., 2000]. SVAS is the major feature of the elastin arteriopathy, related to haploinsufficiency of the *ELN* gene, located in 7q11.23, which encodes the elastin protein [Merla et al., 2012]. SVAS is a classical feature of Williams-Beuren syndrome (WBS; MIM 194050), due to a recurrent microdeletion in 7q11.23 encompassing the *ELN* gene [Pober, 2010]. This

syndrome is characterized by intellectual disability, hypercalcemia, impaired social interactions, facial dysmorphism, and elastin arteriopathy. SVAS may also be isolated and transmitted in an autosomal dominant manner with incomplete penetrance and variable expressivity. *ELN* Sanger sequencing identifies molecular alterations in approximately 50% of the cases [Metcalf et al., 2000]. Apparently, balanced chromosomal rearrangements usually have no phenotypic consequences for the carrier. However, in some cases, they may be associated with an abnormal phenotype due to a cryptic imbalance or a disturbance of gene expression at the breakpoints. Mapping of breakpoints using classical methods such as FISH or Southern blotting has scarcely been used in diagnostic settings. More recently, next-generation sequencing was found to be a rapid and efficient method to characterize the breakpoints at molecular level, providing information about their mechanisms and identifying disease-causing genes [Schluth-Bolard et al., 2013]. We report herein the case of a 4-year-old boy presenting with clinically isolated SVAS associated with an inherited paracentric chromosome 7 inversion. Characterization of this inversion by paired-end whole-genome sequencing (WGS) revealed *ELN* disruption.

Patients and Methods

Clinical Reports

The index case is a 4-year-old boy (III:1, Fig. 1A) presenting with clinically isolated SVAS. He is the first born of a twin bichorial diamniotic pregnancy, an eutrophic premature at 32 weeks of amenorrhea. The neonatal period was uneventful. A rough aortic murmur 3/6 was found at 11 months of age, fully symptom free. The echocardiography showed an isolated SVAS measured at 5.7 mm with a maximal gradient at 118 mmHg and a mean gradient at 64 mmHg. The prestenotic aorta was measured at 15.8 mm for a poststenotic aorta at 13.8 mm. The left ventricle was not dilated, normokinetic, but hypertrophied, without mitral insufficiency. The aortic valve appeared asymmetric but tricuspid, without valvular stenosis. There was a micro aortic insufficiency 0.5/4 and a common origin of the innominate artery and the left primary carotid artery. The coronary arteries looked anatomically normal. A scan showed an SVAS of approximately 50% of the diameter without further anomalies of the thoracic aorta. He underwent a patch aortoplasty and plasty of the Valsalva sinuses at 32 months of age, without complications. Functional result was excellent. The last echocardiography, at 4 years of age, found no persistent SVAS, but an acceleration of the flow in the tubular aorta (2.6 m/s) with stable mild aortic insufficiency. Psychomotor development was normal, with regular schooling. Growth was normal as well, with a weight of 15 kg (−1 SD) and a height of 106 cm (average) at the last observation, 4 years of age. Blood pressure was normal. Serum calcium level was normal. There were no dysmorphic features suggestive

of WBS. The twin sister of the index case (III:2, Fig. 1A) is healthy. Both of the boy's parents (II:5 and II:4) have a heart murmur, with normal echocardiographic values as well as the maternal grandparents and 2 maternal aunts. Likewise among 3 paternal aunts, the eldest one (II:1) was monitored since infancy for an isolated symptom-free SVAS. The middle (II:2) is healthy, and the youngest (II:3) has a heart murmur, with normal echocardiographic values. The paternal grandmother (I:2) is healthy. The paternal grandfather (I:1) has a mitral valve prolapse in the context of a ballooned dystrophic mitral valve, with no left ventricle impact.

Materials and Methods

Array CGH was performed on extracted DNA from the lymphocytes of the index case with the QIAmp DNA Blood Midi kit (Qiagen, Vento, The Netherlands), with a custom 244K oligonucleotide microarray enriched at the 7q11.23 locus (SurePrint Custom-HD CGH Microarray, 1X244KK, AMADID 020793, Agilent Technologies, Santa Clara, CA, USA). The median resolution is about 200 kb, with a 440-bp resolution in 12.5 Mb of the WBS region (chr7:67558714_79837248, hg19). The array-CGH procedures were performed according to the manufacturer's instructions versus a control DNA. The 244K slides were scanned on an Agilent DNA Microarray Scanner, and images were extracted with Feature Extraction software (10.7.3.1). Data analysis was carried out with Agilent Cytogenomics (2.9.2.4). A copy number variation was validated if an abnormal $\log_2$ ratio was obtained for at least 3 contiguous oligonucleotides. The array-CGH results were analyzed with the UCSC (University of California, Santa Cruz, CA, USA) hg19 assembly.

Standard G- and R-banded blood karyotypes were determined by standard procedures.

WGS was performed on genomic libraries following the Illumina TruSeq protocol (Illumina, San Diego, CA, USA). Briefly, 3 µg of DNA were fragmented by sonication and purified to yield fragments of 350 bp. Paired-end adaptor oligonucleotides from Illumina were ligated on repaired A-tailed fragments, then purified, and indexed. DNA library was sequenced on an Illumina NextSeq 500 as paired-end 101-bp reads using the High Output (300 cycles) NextSeq500 kit. Image analysis and base calling were performed using Illumina Real Time Analysis Pipeline 2 with default parameters. The alignment on reference human genome hg19 was performed by BWA/GATK Whole Genome Sequencing workflow V1.6 from BaseSpace (Illumina). SVdetect21 V0.8b was then used for the rearrangement detection and Integrative Genomics Viewer Broad Institute V2.3 for their visualization. Polymerase chain reactions (PCR) were performed using primer pairs selected on each side of the breakpoint region delimited by WGS (primer sequences available from the authors upon request). Junction fragments were amplified using the Taq DNA Core kit 25 (MP Biomedicals, Solon, OH, USA) according to the following protocol: 120 ng DNA was mixed with 1.75 mM MgCl₂, 0.2 mM dNTP mix, 0.3 mM forward primer, 0.3 mM reverse primer in a final volume of 50 µL, and incubated with an initial denaturation for 10 min at 96°C, followed by 35 cycles of denaturation for 1 min at 96°C, hybridization for 1 min at 60°C, and elongation for 1 min at 72°C, with a final elongation phase for 10 min at 72°C. DNA from a negative control who was not a carrier of chromosomal rearrangement was amplified parallel to the patient. DNAs were also amplified with the primer pair for the *ATPIA3* gene (exons 7 and 8) as positive control. PCR products were verified on LabChip GX (PerkinElmer, Waltham,

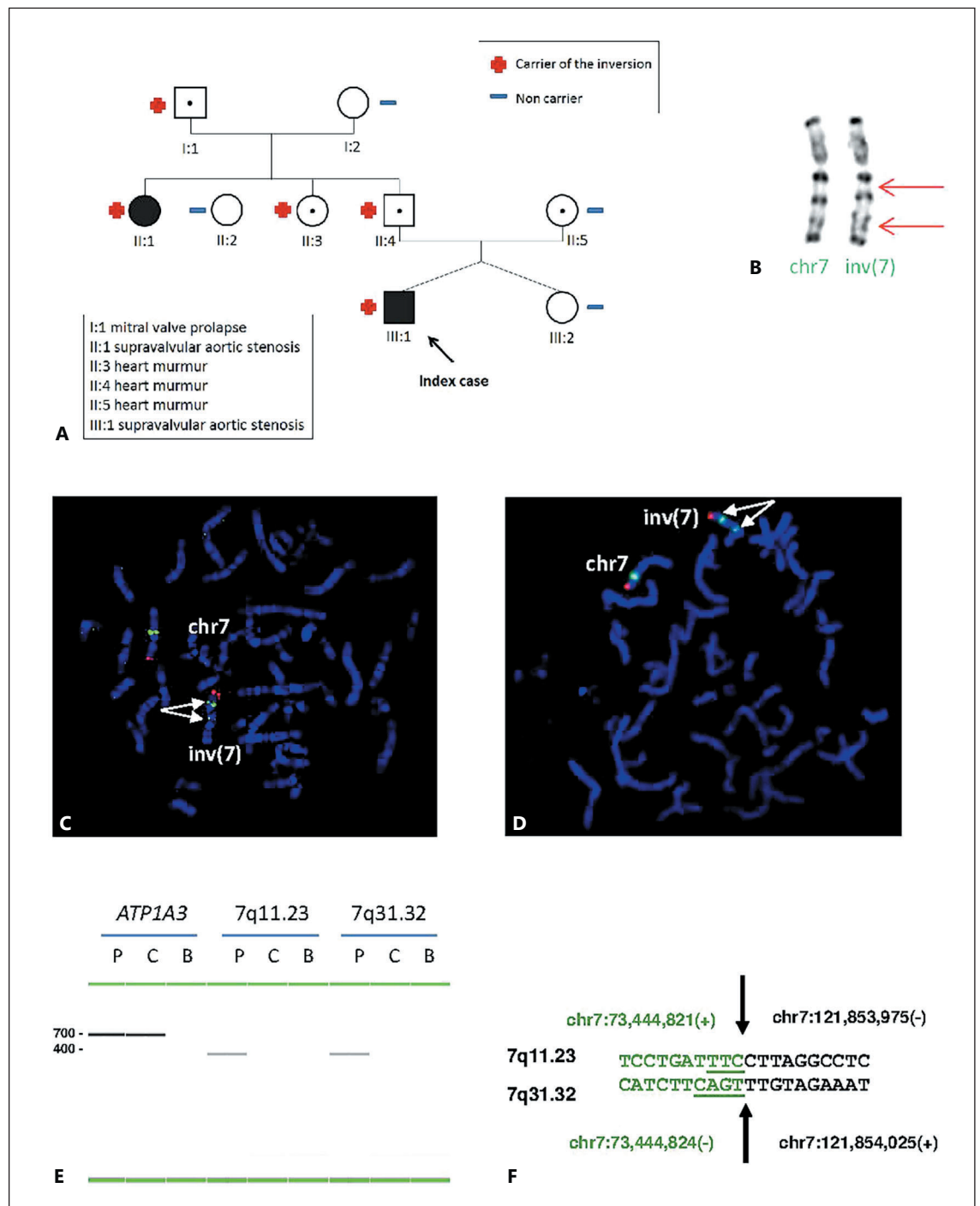


Fig. 1. **A** Family pedigree. **B** R-banded chromosome 7 showing a paracentric inversion *inv(7)(q11.2;q31)* (arrows). **C** FISH using RP11-644H24 probe (7q11.23) in green (FITC) and 7qter control probe in red showing a split green signal on derivative chromosome 7 (arrows). **D** FISH using RP11-560I19 BAC (7q31.32) in green (FITC) and 7qter control probe in red showing a split green signal on derivative chromosome 7 (arrows). **E** Amplification of junction fragments by PCR and migration on a 2% agarose gel. P, patient; C, control; B, blank; *ATP1A3*, positive control; 7q11.23,

proximal junction fragment; 7q31.32, distal junction fragment. **F** Sanger sequencing determining the breakpoints at bp level: sequence of proximal junction fragment (7q11.23) and sequence of distal junction fragment (7q31.32). The 7q11.23 sequence is in green, 7q31.32 is in black. Genomic coordinates are given according to hg19 and strand orientation is indicated (+ or -). Regions of microhomology are underlined. Note that there is a loss of 2 bp at the 7q11.23 breakpoint and of 49 bp at the 7q31.32 breakpoint.

Table 1. Cases of *ELN* disruption in the literature

	Curran et al., 1993	von Dadleszen et al., 2000	Duba et al., 2002	Malčić et al., 2008	Present case
Rearrangement	t(6;7)(p21.1;q11.23)	t(6;7)(q27;q11.23)	t(7;16)(q11.23;q13)	t(7;14)(q11.23;p12)	inv(7)(q11.23q31.32)
Inheritance	Familial	De novo	Familial	Familial	Familial
Affected individuals	5	1	5	3	5
Clinical features	SVAS, hoarse voice, facial dysmorphism	SVAS, hydrops fetalis	Hoarse voice, WBS	SVAS, left ventricular intracavitary stenosis, peripheral pulmonary artery stenosis	SVAS, heart murmur, mitral valve prolapse
Characterization	Southern blot	FISH	FISH	FISH	WGS
<i>ELN</i> disruption	Exon 28	Unspecified	Intron 5	Unspecified	Intron 1

SVAS, supraaortic stenosis; WBS, Williams-Beuren syndrome; WGS, whole-genome sequencing.

MA, USA). Following, specific products corresponding to the junction fragment were sequenced by Sanger method.

FISH was performed using bacterial artificial chromosome (BAC) clones spanning the breakpoint region as defined by the WGS results and selected through the UCSC genome browser. They were commercially available (RP11-644H24; chr7:73411275_73548266, hg19) (BlueFish, BlueGnome, Cambridge, UK) or FITC (fluorescein isothiocyanate) labeled by nick translation (RP11-560I19; chr7:121856401_121858491, hg19) and hybridized on metaphase spread with appropriate control probe (7qter) (Cytocell, Cambridge, UK).

Results

For the index case, array CGH with oligonucleotide enrichment at the 7q11.23 locus found no cryptic imbalance. Standard blood karyotyping revealed a balanced paracentric chromosome 7 inversion: 46,XY,inv(7)(q11.2q31) (Fig. 1B). In order to characterize the effect of this rearrangement on the patient's phenotype, a paired-end WGS was performed. It yielded a 10.9× physical coverage and made it possible to delineate the breakpoints in chromosome 7 in the 176-bp region at 7q11.23 and in the 193-bp region at 7q31.32. Breakpoints were consistent with cytogenetic findings and were verified by FISH (Fig. 1C, D). Sanger sequencing of junction fragments, amplified by PCR (Fig. 1E), identified the breakpoints at the hg19 positions chr7:73444821_73444824 at 7q11.23 and chr7:121853975_121854025 at 7q31.32. A 3-bp microhomology with a 2-bp loss was detected at the proximal breakpoint and a 4-bp microhomology with a 49-bp loss at the distal breakpoint (Fig. 1F). The rearrangement disrupted the *ELN* gene (intron 1) at 7q11.23. No pathogenic gene was involved at the

7q31.32 breakpoint or in its neighborhoods. The balanced paracentric chromosome 7 inversion was inherited from the father and the paternal grandfather, and was shared with 2 paternal aunts including the SVAS-affected aunt (Fig. 1A).

Discussion and Conclusion

Only few cases of *ELN* disruption have been reported so far. They included 1 prenatal sporadic case [von Dadleszen et al., 2000] and 3 familial cases with multiple affected members [Curran et al., 1993; Duba et al., 2002; Malčić et al., 2008]. The phenotypic spectrum varied widely, even amongst members of the same family (Table 1) (SVAS and other cardiac anomalies, features belonging to the WBS spectrum, such as facial dysmorphism, hoarse voice [Curran et al., 1993; Duba et al., 2002], and intellectual disability [Duba et al., 2002]). It was proposed that elastin contribution to other connective tissues could explain the expanded phenotype as well as a long-range position effect for intellectual disability. However, all these previous cases were only characterized by FISH or Southern blotting, and no array CGH could exclude cryptic chromosomal imbalances. We report the first case of *ELN* disruption characterized by WGS, providing accurate genomic breakpoint positions and excluding any imbalances. Our family only presented with cardiac features. This supports the fact that precise characterization is necessary to improve the genotype-phenotype correlation in *ELN* arteriopathy.

WGS has proven to be a powerful tool in determining breakpoints at bp levels. It allows the assessment of gene disruption and the detection of associated cryptic imbalances. It may also contribute to elucidate breakpoint

mechanisms. In the current case, we observed micro-homologies and nucleotide deletions, suggesting micro-homology-mediated mechanisms such as MMBIR or FosTes [Ottaviani et al., 2014].

Over the past few years, there has been a tendency to replace karyotype and FISH analyses by array CGH in the first-line diagnosis of congenital malformations and intellectual disability, including WBS. Strategies for molecular diagnosis of *ELN* arteriopathy rely on Sanger sequencing to identify point mutations or small insertions/deletions and multiplex ligation probe amplification and real-time quantitative PCR to detect partial or complete exon deletions [Merla et al., 2012]. More recently, panel or exome sequencing have become alternative diagnostic approaches for mutation detection [Poninska et al., 2016; Seidelmann et al., 2017]. Other than FISH, neither of those techniques used in current diagnostic for elastin arteriopathy is able to detect balanced rearrangements susceptible to disrupt *ELN*. Although *ELN* gene disruption seems to be rarely involved, it should be kept in mind that a normal array CGH or a Sanger, panel, or exome sequencing result is not sufficient to rule out this hypothesis. With the future perspective of WGS as a diagnostic tool, it will be important to include a structural variation analysis in order to detect balanced rearrangements and gene disruptions that otherwise would be unnoticed.

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Acknowledgments

The authors wish to thank the patient and his family for their kind cooperation, all colleagues who provided help for this report, and the Hospices Civils de Lyon for their support.

Statement of Ethics

Informed consent was obtained from the family.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

P. Bouvagnet, M. Bakloul, S. Di Filippo, O. Metton, and J. Mitchell cared for the patient. L. Pons, A. Buisson, N. Chatron, A. Labalme, F. Diguët, P.-A. Rollat-Farnier, D. Sanlaville, and C. Schluth-Bolard performed the genetic analysis. L. Pons wrote the first draft of the manuscript, and all the other authors contributed to the manuscript final form.