Peripartum Iliac Arterial Aneurysm and Rupture in a Patient with Vascular Ehlers-Danlos Syndrome Diagnosed by Next-Generation Sequencing

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Summary

Vascular Ehlers-Danlos syndrome (vEDS), a genetic disorder caused by mutations in procollagen type III gene (COL3A1), may lead to fatal vascular complication during peripartum period because of the arterial fragility. We experienced a case of vEDS with peripartum life-threatening arterial rupture diagnosed by next-generation sequencing (NGS) and successfully treated the vascular complications. A 25-year-old female in pregnancy at 34 weeks had sudden and acute pain in the left lower abdomen. After successful delivery, her computed tomography scan showed a dissecting aneurysm of the left common iliac artery (CIA). Four days after delivery, she presented in hemorrhagic shock induced by arterial rupture in the CIA. Since her clinical presentations inferred vEDS even in the absence of familial history, we performed NGS-based genetic screening for inherited connective tissue disorders including vEDS with informed consent. Even though we started intensive medication, her iliac aneurysm was progressively enlarging within 3 weeks. After an urgent molecular diagnosis for vEDS (a splice-site mutation), cautious endovascular therapy for her CIA aneurysm was successfully performed. This is the first report for pretreatment molecular diagnosis of vEDS using NGS in an emergent situation of severe vascular complications.

Key words: Emergency, Genetic screening, Pregnancy

Case Report

A 25-year-old pregnant woman at 34 weeks of gestation developed acute left abdominal pain and was transferred to our hospital. Her pregnancy had been uneventful until this point. Her past medical history was notable for pneumothorax at the age of 9 years. Her mother and older brother had no vascular episodes or bruisingability (Figure 1). Her father’s clinical history was not available. Her younger brother with different paternity had bruisingability, an episode of a large hematoma in the thigh, and a vascular fragility during the surgery for a fracture at the left wrist (Figure 1).

On admission, she was 148 cm tall and weighed 55.8 kg. She had translucent skin and a large subcutaneous hematoma on the right forearm. Her blood pressure was 98/47 mmHg, and her pulse was 81 beats per minute. Laboratory blood count testing showed anemia (hematocrit 1180
32.1%, hemoglobin 10.6 g/dL, red blood cell count 396×10⁶/μL). Biochemical profiles were nearly normal considering the gestational period.

On the day of the admission, an emergent caesarean section was performed under lumbar anesthesia for severe fetal bradycardia, and a male baby was successfully delivered. A large hematoma was observed in her left peritoneal space. A computed tomography (CT) scan on day 3 of her hospitalization showed a dissecting aneurysm of the left common iliac artery (CIA) with a large peritoneal hematoma (Figures 2A, 2B). Because her symptoms had been improving, bed rest and careful observation were recommended after discussion with a vascular surgeon and cardiologists.

Four days after delivery, she developed a sudden abdominal pain and faintness just after a short walk to the bathroom. Her consciousness was soon recovered, but blood pressure decreased less than 80 mmHg. Emergent CT scan showed fresh bleeding in the right peritoneal space and very small ulcer-like projection in the right CIA, suggesting that another arterial dissection and/or arterial rupture in the right CIA resulted in hemorrhagic shock (Figure 2C). She was immediately transferred to the intensive care unit and stabilized with massive transfusion. Blood pressure control was started with calcium antagonist (nicardipine) and beta-blocker (celiprolol) as a standard treatment for CIA dissection and rupture. At this point, she was suspected to have vascular Ehlers-Danlos syndrome (vEDS) or other connective tissue disorders, although she had no familial history.4,5) We performed skin biopsy from her right arm to culture skin fibroblasts and assessed type III collagen measurement as previously described.6,7) However, this biochemical assay usually took a long time for the results. Then, we performed a next-generation sequencing (NGS)-based panel analysis in an urgent fashion.

Genomic DNA extracted from the peripheral blood of the patient was sequenced on an ion PGM (Thermo Fisher Scientific, Carlsbad, CA, USA) using an Ion AmpliSeq Custom Panel designed by Ion AmpliSeq Designer (https://ampliseq.com/browse.action) for 54 genes associ-
ated with hereditary connective tissue disorders, including COL3A1, encoding type III procollagen α1 and responsible for vEDS, as well as genes causative for other syndromes with various arterial fragilities (FBN1, TGFBR1, TGFBR2, and SMAD3) (Table). The sequence coverage of each gene is shown in the Table with a total coverage of 98.05%. The sequencing data were mapped to human genome hg19 using Torrent Suite software (Thermo Fisher Scientific), and single nucleotide variants and small insertions/deletions were detected from the mapped data using the Torrent Variant Caller plug-in. The variants detected were annotated using wANNOVAR (http://wannovar.wglab.org/), including validation with allele frequencies in public databases such as 1000 Genomes Project, Exome Aggregation Consortium (ExAC), NHLBI-ESP 6500 exomes and Complete Genomics 46 (CG46); dbSNP annotation, ClinVar annotation, and 15 prediction scores for non-synonymous variants such as SIFT, PolyPhen-2, and GERP++. We also referred to Human Genetic Variation Database (HGVD) (http://www.hgvd.genome.med.kyoto-u.ac.jp/) to exclude SNPs specific to Japanese.

A total of 339 variants were detected and annotated through wANNOVAR. A pathogenic or likely pathogenic variant was not detectable in exonic regions or within 2 bp away from exon/intron boundaries of the genes. We then searched using the IGV visualization whether variant was present in intronic regions of a candidate gene COL3A1 and found a heterozygous splicing variant in intron 13 of the gene (NM_000090:c.951+5G > C) with the coverage as 305. The variant was confirmed through Sanger sequencing, performed on an ABI3130xl Genetic Analyzer using a BigDye Direct Cycle Sequencing Kit (Thermo Fisher Scientific). A different nucleotide change at the same position was reported previously (NM_000090:c.951+5G > A), suggesting in-frame exon 13 skipping (p.G300_A317del).9

In spite of intensive medication to control her blood pressure, her left CIA aneurysm progressively enlarged over the subsequent 3 weeks from her delivery (Figures 3A, 3B). An open vascular surgery to prevent rupture of the iliac arterial aneurysm was tried but abandoned because of severe tissue fragility. Two weeks after the hemorrhagic shock, we obtained the result of the NGS-based panel genetic screening with a molecular diagnosis of vEDS. Because open vascular surgery was extremely high risk for vEDS, an endovascular therapy for her CIA aneurysm was scheduled after transfer to Shinshu University, which had several experience to treat vascular complication of vEDS. In Shinshu University, a percutaneous covered stent implantation in the left CIA was successfully employed with general anesthesia (Figure 3C). The patient started to have celiprolol treatment, which was previously shown as specifically effective for vEDS,9 and was discharged home 1 week after the endovascular therapy without any additional complications.

Five months after the admission, we obtained the results of the biochemical assay for collagen production (Figure 4A). In cultured skin fibroblasts of the patient, type III collagen production was markedly decreased relative to normal control (22% of normal control). Furthermore, cDNA-based validation of the variant was performed. Total RNA was extracted form skin cultured fibroblasts of the patient, and cDNA was synthesized. Sanger sequencing using primers amplifying from exon 12 to exon 14 demonstrated skipping of exon 13 (Figure 4B). The variant was detected in her younger brother with bruisability and possible vascular fragility. It was not detected in her mother or older brother, both with no symptoms (Figure 1).

### Discussion

The variant detected in the current patient (c.951+5G > C), urgently identified through NGS-based genetic screening, was concluded to be pathogenic based on a segregation analysis of the family, a cDNA-based Sanger sequencing, and a biochemical analysis. Furthermore, considering that an unaffected mother without the variant had two affected children with the variant, a germline mosaicism is suggested in the family.

vEDS is an autosomal dominant connective tissue disorder with an estimated prevalence of 1 in 50,000-

### Table. A Gene List in the Present Custom Gene Panel

| Syndrome | Gene |
|----------|------|
| Ehlers-Danlos syndrome | COL5A1, COL5A2, TNXB, COL3A1, FBLN5, EFEMP2, ATP6V0A2, ELN, GORAB, PYCR1, LTBP4, RIN2, SLC2A10, PTDSS1 |
| Marfan syndrome | FBN1 |
| Loey-Dietz syndrome | TGFBR1, TGFBR2, SMAD3, TGFBR2 |
| Arterial tortuosity syndrome | SLC2A10 |
| Familial thoracic aortic aneurysms and aortic dissections | MYH11, ACTA2, MYLK |
| Beals syndrome | FBN2 |
| Occipital horn syndrome | ATP7A |
| Shprintzen-Goldberg syndrome | SKI |
| PLOD-related periventricular nodular heterotopia/Otoperipatalodigital syndrome | FLNA |
| Camurati-Engelmann disease | TGFBR1 |
| Osteogenesis imperfecta | PLS3, CRTAP, LEPRE1, PPIB, BMP1, FKB10, SERPINH1, SERPINF1, WNT1, SP7, TMEM38B, IFITM5, CREB3L1, PLOD2 |
| Cutis laxa | ALDH18A1, FBLN5, EFEMP2, ATP6V0A2, ELN, GORAB, PYCR1, LTBP4, RIN2, SLC2A10, PTDSS1 |

Loomis syndrome (Figure 1). In cultured skin fibroblasts of the patient, type III collagen production was markedly decreased relative to normal control (22% of normal control). Furthermore, cDNA-based validation of the variant was performed. Total RNA was extracted from skin cultured fibroblasts of the patient, and cDNA was synthesized. Sanger sequencing using primers amplifying from exon 12 to exon 14 demonstrated skipping of exon 13 (Figure 4B). The variant was detected in her younger brother with bruisability and possible vascular fragility. It was not detected in her mother or older brother, both with no symptoms (Figure 1).
The disorder is caused by mutations in COL3A1, resulting in qualitative and/or quantitative abnormalities of mature type III collagen protein. It is clinically characterized by thin, translucent skin; easy bruising; characteristic facial appearance in some individuals; and arterial, intestinal, and/or uterine fragility. Vascular complications include rupture, aneurysm, and/or dissection of major or minor arteries. Aortic and iliac artery ruptures often present catastrophically, with acute abdominal, back, or flank pain followed by cardiovascular collapse. Pregnancy for women with vEDS has an estimated 5.3% risk for death from peripartum arterial rupture or uterine rupture. In a series of surgical interventions for vascular lesions of vEDS, post-operative complications were highest when the diagnosis of vEDS was not known and when procedures were undertaken in an emergency setting. Shalhub, et al. stressed that molecular diagnosis was imperative to disease management: diagnosis confirmation, appropriate surveillance, and prophylactic interventions in an elective setting, which would be expected to improve surgical outcomes. However, a conventional method to diagnose vEDS is a biochemical assay for collagen type III protein and/or mRNA-based genetic screening using patients’ cultured skin fibroblasts, which are usually time-consuming, typically requiring several months such as this case. In the present patient, it was difficult to make a confirmatory diagnosis and a decision to undergo surgical intervention, including the selection of open or endovascular approach, only through clinical manifestations (aortic rupture and history of pneumothorax) in the absence of an affected family history. NGS-based panel genetic screening was crucial in the management of the patient through urgent molecular confirmatory diagnosis of vEDS in a non-invasive fashion without skin biopsy as well as appropriate differential diagnosis including several hereditary connective tissue disorders (such as Marfan syndrome and Loeys-Dietz syndrome) with clinical overlaps to vEDS.

**Conclusion**

This is the first report of pretreatment molecular diagnosis of vEDS using NGS in an emergent situation in a patient with severe vascular complications. NGS would be an irreplaceable piece in the management of patients with vEDS, enabling urgent, accurate, and non-invasive diagnosis expected to improve survival of those with vEDS through appropriate surveillance, prevention, and surgical intervention.
Figure 4. A: Biochemical assay for type III procollagen; fluorograms of sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis of collagen molecules obtained from the dermal fibroblast culture medium of the patient (P) and an age- and sex-matched normal volunteers (N1 and N2). Fibroblasts were cultured in medium containing [2,3-3H]-proline and 50 μg/mL l-ascorbic acid 2-phosphate for 24 h. After treatment of the collagen samples prepared from the culture medium with 0.1% pepsin, they were separated by SDS/5% polyacrylamide gel electrophoresis in the absence of reducing agent. Although the patient’s fibroblasts produced an equal amount of type I collagen to the normal control fibroblasts, production of type III collagen (α1 (III)) from the patient fibroblasts was markedly reduced. α1 (I), α2 (I), and α1 (III) 3: α1 chain of type I, α1 chain of type I, and trimer of α1 chain of type III collagen. B: cDNA-based Sanger sequencing; total RNA from the patient was extracted from skin fibroblast cells using a QiAamp RNA Blood Mini Kit and treated with an RNase-Free DNase Set (Qiagen, Valencia, CA, USA). Reverse transcribed was 500 ng of total RNA in 10 μL volume using PrimeScript RT reagent Kit (TaKaRa Bio, Shiga, Japan). Sanger sequencing was performed using a BigDye Direct Cycle Sequencing Kit (Thermo Fisher Scientific, Carlsbad, CA, USA) with M13-tailed PCR primers on an ABI3130xl Genetic Analyzer, according to the manufacturer’s instructions. Primers were designed using primer3 (http://bioinf.ut.ee/primer3/) as follows: COL3A1-RT-M13F: 5’-TGTAAAACGACGGCCAGTCCCTGCAGGTCCAACTTCAC-3’ and COL3A1-RT-M13R: 5’-CAGGAAACAGCTATGACCGGACACAGGCTTCGATGG-3’. The electropherograms showed skipping of exon 13 from exon 12 to exon 14, through the splice site variant “c.951 + 5G > C.”
Disclosures

Conflicts of interest: None.

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