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'EVIDENCE DRIVEN' CLINICAL SCENARIO

Clinical Exome Sequencing as a Novel Tool for Diagnosing Loeys-Dietz Syndrome Type 3

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CLINICAL VIGNETTE

A 35 year old pregnant woman noted blurred vision in her left eye. After delivering a healthy baby by Caesarean section, vaginal bleeding occurred from the uterine wound. Two days later she experienced chest pain with ST elevation in the precordial leads. A dissection of the left anterior descending coronary artery was stented (Fig. 1A–C). Visual symptoms recurred and a dissection of the left internal carotid artery was confirmed. "How should I manage this patient?"

Objective/Background: In rare genetic vascular syndromes the diagnosis may not be apparent from the phenotype, but might be important for proper management.
Methods: A previously healthy woman without dysmorphic features presented with pregnancy associated vascular dissections and aneurysms. Next generation clinical exome sequencing was performed.
Results: The differential diagnosis of spontaneous arterial dissection is outlined. The patient's diagnosis became evident after clinical exome sequencing detected a novel missense mutation in the evolutionary conserved region of *SMAD3*, confirming the diagnosis of Loeys-Dietz syndrome (LDS) type 3. A brief overview of the various types of LDS and their management is presented.
Conclusion: Clinical exome sequencing proved useful in diagnosing LDS type 3 where detailed vascular surveillance and timely intervention with a low threshold is recommended.

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DIFFERENTIAL DIAGNOSIS OF SPONTANEOUS ARTERIAL DISECTION

Postpartum status and multiparity are known risk factors for spontaneous coronary artery dissection, which is associated with a predisposing arterial disease in 80% of cases.[1] Systemic inflammatory conditions, such as the vasculitides, account for a small proportion of spontaneous coronary artery dissections, while most patients have non-inflammatory disease, such as fibromuscular dysplasia or one of the rare genetic syndromes (e.g., vascular type Ehlers-Danlos syndrome [EDS], Marfan syndrome, osteogenesis imperfecta, familial aortic dissection [also known as cystic medial necrosis], reticular fiber deficiency, homocystinuria, alpha-1 antitrypsin deficiency or others).[1] About 20% of spontaneous coronary artery dissections remain idiopathic.[1] The differential diagnosis of spontaneous carotid artery dissection is similar to that of coronary dissection with the addition of autosomal dominant polycystic kidney disease.[2]

Did the patient's history, physical examination, and laboratory tests offer any diagnostic clues? The patient's past medical history was unremarkable, and her family history

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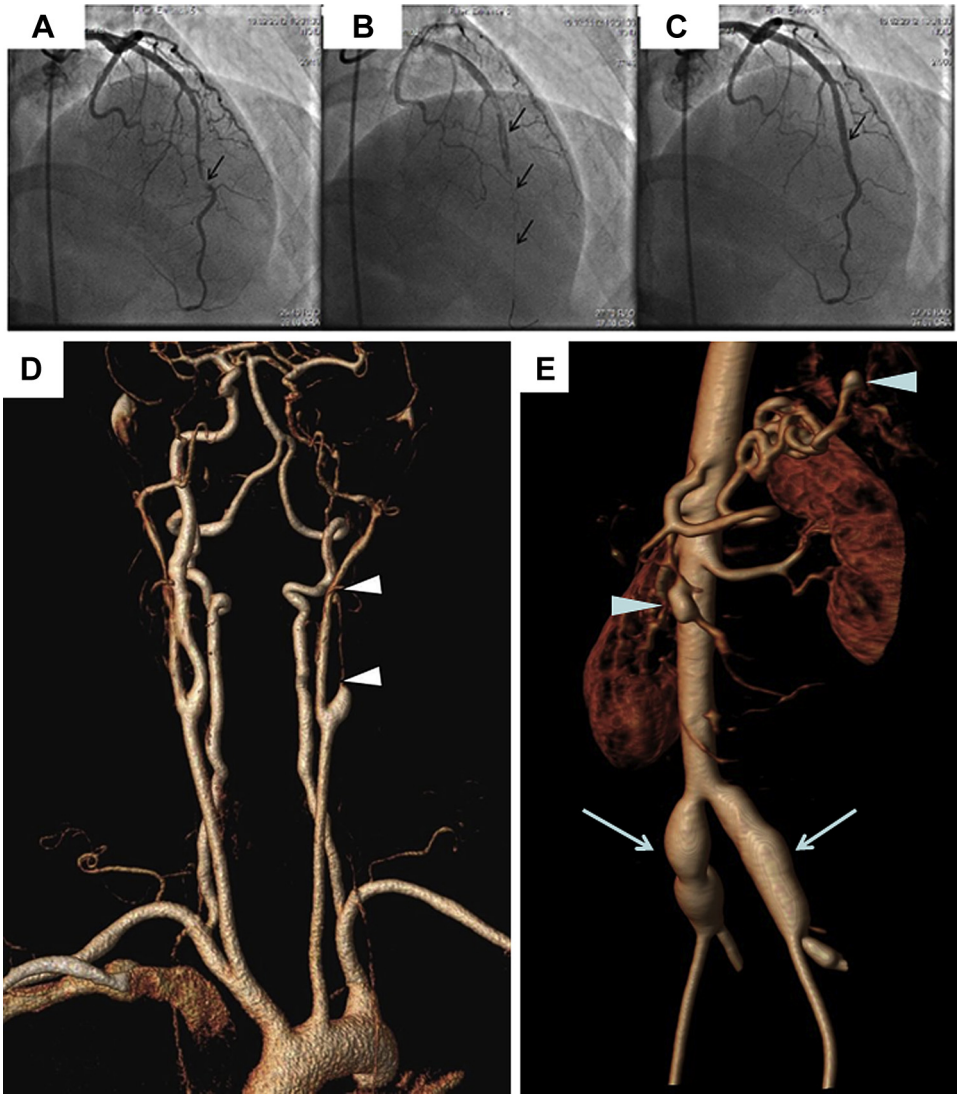

Figure 1. (A—C) Coronary angiography showing (A) a tight stenosis of the mid-left anterior descending coronary artery (LAD) (arrow); (B) dissection of the LAD progressing to total occlusion on passage of the guide wire (arrows); and (C) successful recanalization of the LAD after stenting (arrow). (D, E) Magnetic resonance angiograms with three dimensional reconstruction. (D) Long dissection of the left internal carotid artery (between arrowheads), (E) aneurysm of both common iliac arteries (arrows); aneurysm of the superior mesenteric artery (lower arrowhead), and of the splenic artery (upper arrowhead). Note the tortuosity of the splenic artery.

was negative for premature vascular disease. She was college educated, successfully employed, married, and had uncomplicated pregnancies in 2006 and 2009. On clinical examination, she was proportionally built, without dysmorphic features or neurological deficit, with a body height of 174 cm, body mass of 69 kg, and a body mass index of 22.8. There was no marked skin hyperelasticity or joint hypermobility. Serological screening tests for autoimmune diseases were negative and the inflammatory markers were normal, which argued against a systemic inflammatory condition such as large artery vasculitis.

The most common cause of spontaneous arterial dissection is fibromuscular dysplasia—a non-inflammatory, non-atherosclerotic disorder predominantly affecting women of childbearing age.[1,2] Fibromuscular dysplasia leads to arterial stenosis, occlusion, aneurysm, and/or dissection—most often in the renal and carotid arteries.[3] The etiology of fibromuscular dysplasia remains unknown, although a genetic predisposition with an autosomal inheritance has been described in some families.[3] Fibromuscular dysplasia has a characteristic angiographic appearance of a "string of beads" or, less commonly, circumferential or tubular stenosis,[3] none of which were present on this patient's angiogram (Fig. 1). Magnetic resonance angiography (MRA) showed normal aortic anatomy without dissection, but small aneurysms of both common iliac arteries were present, as well as a fusiform aneurysm of the superior mesenteric artery, which was the origin of the hepatic artery. The splenic artery was markedly tortuous and had a small aneurysm (Fig. 1E). Intracranially, a 3 mm aneurysm was found at the origin of the left anterior communicating artery.

The absence of dysmorphic features in the patient argued against Marfan syndrome or osteogenesis imperfecta, and the patient's normal cognitive function spoke against

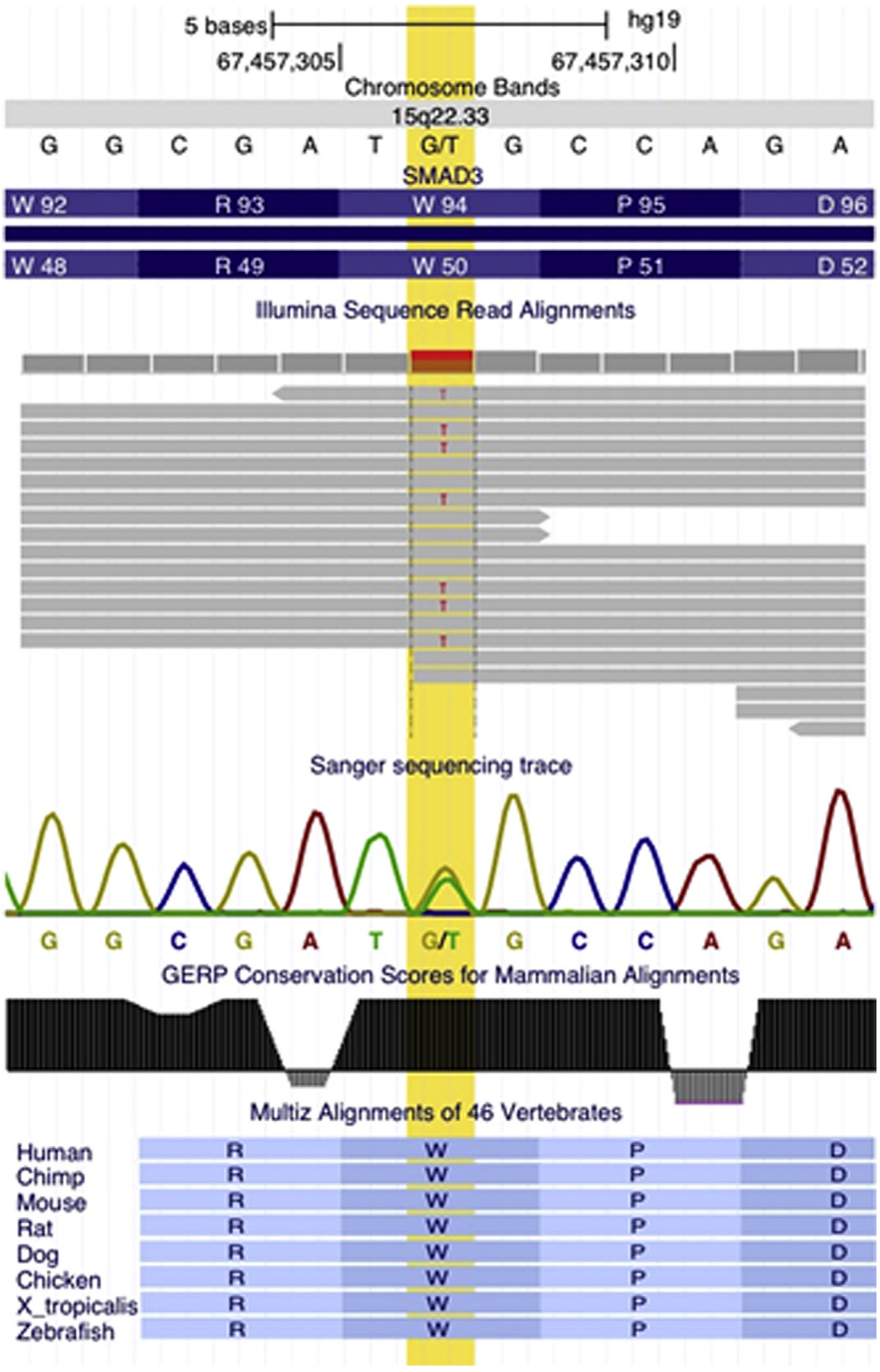

Figure 2. The patient's leukocyte DNA was subjected to Nextera fragmentation, and sequencing was performed using the TruSight Exome library preparation protocol (Illumina, San Diego, CA, USA), which included target enrichment for the coding exons of 2,761 genes related to inherited human diseases. The Illumina MiSeq sequencer was used in paired-end sequencing mode with 150 base pairs sequenced from each side of the fragments in the library. Over 93% of the sequences targeted in the capture set were covered at least 10 times. Primary data analyses, including de-multiplexing and fastq generation, were performed using Illumina MiSeq Reporter software. Secondary analyses were carried out using a custom pipeline, including the Burrows—Wheeler algorithm for reading alignment to hg19 human reference genome assembly, and variant calling using the Genome Analysis Toolkit (GATK) pipeline in accordance with GATK best practice recommendations (Broad Institute, Cambridge, MA, USA). Genetic variants were evaluated by ANNOVAR and snpEff algorithms, and the predicted pathogenicity was evaluated according to the SIFT, PolyPhen2, and MutationTaster algorithms. Information on the prevalence of variants in the general population was obtained from the 1000genomes, ESP6500, UK10K, and GoNL sequencing projects. The c.281G>T variant of *SMAD3* was detected in an evolutionary conserved region of *SMAD3* and confers the substitution of the evolutionary invariant amino acid Trp to Leu at position 94 in the transcript of *SMAD3*.

homocystinuria. The negative family history was not in line with familial aortic dissection/cystic media necrosis. Alpha-1 antitrypsin deficiency was unlikely in the absence of lung or liver disease, and polycystic kidney disease was not present.

Older literature describes reticular fiber deficiency as an important cause of dissecting aneurysms of cerebral arteries.[4] Reticular fibers are composed mostly of type III collagen[5]; therefore, one could expect a significant overlap with vascular type EDS, which is caused mainly by mutations in *COL3A1*, which codes for type III collagen.[6] EDS is a group of genetic disorders of connective tissue characterized by skin hyperextensibility, joint hypermobility, and tissue fragility. The estimated frequency of EDS syndromes is 1 in 5,000, while that of vascular EDS is only about 1 in 100,000.[6] Vascular EDS is potentially life threatening, and differs from the classic and hypermobility forms in the increased risk of spontaneous vascular or visceral organ rupture, and the absence of large joint hyperextensibility.[6] Eighty percent of individuals with vascular EDS experience a major vascular event or rupture of an internal organ, for example gravid uterus, by 40 years of age.[6]

This patient had the clinical features of vascular EDS, but before being referred she had already undergone a skin biopsy, which did not show disorganized collagen structure. How could the suspected diagnosis of EDS be verified? Were there other diagnostic possibilities to be considered?

GENETIC TESTING: NEXT GENERATION CLINICAL EXOME SEQUENCING

In view of the high clinical suspicion of vascular type EDS, which was not corroborated by skin biopsy, genetic testing—next-generation clinical exome sequencing was opted for.

Although genetic testing is of little value in the acute setting, for example in a case of acute arterial dissection, the correct diagnosis may have implications for proper long-term patient management. Genetic testing used to be slow and expensive when large arrays of genes were sequenced serially according to Sanger, or insensitive when a small number of known gene variants was sought by comparative genomic hybridization.[7] Next-generation sequencing, based on massively parallel sequencing of clonally amplified DNA molecules, coupled with sufficient computational power and appropriate software for efficient data analysis, has brought genetic diagnostics into clinical practice at an affordable price.[7,8] An especially useful approach is clinical exome sequencing, that is concentrating on the roughly 1% of coding DNA in the human genome, where mutations are much more likely to have clinical consequences than in the remaining 99% of non-coding human DNA.[9] Genetic testing is recommended in all patients with suspected genetic aortic disease and their first degree relatives.[10,11] The patient did not have aortic disease in the strict sense, but had a high probability of a vascular disease syndrome with the potential to affect the aorta.

Clinical exome sequencing was performed, followed by bioinformatic analysis and interpretation of genes associated with inherited monogenic vascular diseases (*ACTA2*, *COL3A1*, *COL5A1*, *COL5A2*, *PLOD*, *EFEMP2*, *FBN1*, *FBN2*, *GATA5*, *MYH11*, *MYLK*, *NOTCH1*, *SLC2A10*, *SMAD3*, *TGFB2*, *TGFBR1*, *TGFBR2*). Sequencing data analysis showed the presence of a heterozygous missense variant in *SMAD3* (c.281 G>T, corresponding to an amino acid change Trp94Leu), located in the MH1 domain of the gene, linking the patient's phenotype to Loeys-Dietz syndrome (LDS) type 3. As it resides in a highly evolutionary conserved segment of *SMAD3*, which mediates the SMAD3 dependent regulation of the transforming growth factor (TGF)-β pathway, the c.281 G>T mutation was predicted as pathogenic in a consensus vote of all three pathogenicity prediction algorithms employed (Fig. 2). The mutation has not been reported previously in any individual in population studies, nor has it been tracked in the dbSNP v138 database of human variation (National Center for Biotechnology Information). Genetic testing of the patient's children showed the same mutation in the eldest daughter, while no abnormalities were found in *COL3A1*, *COL5A1*, *COL5A2*, or *PLOD1* in the patient or her children, thus refuting the original suspected diagnosis of the vascular type EDS and suggesting the diagnosis of LDS type *3.*

LDS

LDS is a rare, autosomal dominant genetic disorder associated with dysregulation of the TGF-β signaling pathway, which affects connective tissue in many parts of the body and represents a life threatening condition because of arterial aneurysms and dissections.[12–14] Most cases of LDS, especially type 1, are characterized by Marfan like skeletal features with additional craniosynostosis, hypertelorism, and bifid uvula or cleft palate.[12–14] In rare cases, especially LDS type 3, there may be no external dysmorphic features, which obscures the correct diagnosis.[14,15]

LDS was first described in 2005–06 as a consequence of mutations in *TGFBR1* and *TGFBR2*.[12,13] LDS type 1, caused by mutations in TGF-β receptor 1 (TGFBR1) mutations, accounts for about 75% of all cases of LDS and is characterized by aortic dilatation, aneurysms and dissection, pronounced arterial tortuosity, and by heart defects, hypertelorism, bifid uvula, cleft palate, craniosynostosis, and skeletal problems similar to that of Marfan syndrome (scoliosis, pectus excavatum or pectus carinatum, and elongated limbs with joint deformities).[12–14] LDS type 2, caused by TGF-β receptor 2 (TGFBR2) mutations, is less severe and is characterized by aortic dilatation, vascular tortuosity, and soft, translucent skin; skeletal abnormalities are usually absent.[14] Mutations of *SMAD3* cause LDS type 3,[14,15] which is characterized by aortic and arterial aneurysms and dissections, as well as by osteoarthritis of the knees, joints of the hands, and spine that usually becomes apparent in early to mid-adulthood.[14,15] Mutations in *TGFB2* cause LDS type 4, which manifests as aortic aneurysms, vascular tortuosity, and skeletal manifestations.[14] It has been proposed that a mutation in any of these four genes in combination with arterial aneurysm or dissection or family history of

documented LDS is sufficient for establishing the diagnosis of LDS.[14]

MANAGEMENT OF PATIENTS WITH LDS

The recommended management of patients with LDS consists of vigilant vascular surveillance, blood pressure control, timely vascular surgical intervention, and orthopedic care and genetic counseling.[14]

To reduce hemodynamic stress on the vasculature, pharmacological beta blockade is regarded as the standard of care in all patients with syndromic aortic conditions.[14] Angiotensin receptor blockers are also recommended in LDS because they are expected to reduce the overactive TGF-β signaling pathway.[14] However, the data in favor of angiotensin receptor blockers are based on patients with Marfan syndrome in whom losartan reduced the rate of aortic root dilatation.[16,17] A recent randomized trial did not show a benefit of losartan over atenolol in children and young adults with Marfan syndrome.[18] Losartan is effective in "haploinsufficient" patients with Marfan syndrome with mutations of *FBN1*, where a decreased amount of normal fibrillin-1 is found in connective tissues but less so in "dominant negative" patients with Marfan syndrome where an abundance of mutant fibrillin-1 is present.[19] In spite of the lack of evidence based data in patients with LDS, the recommendation to prescribe an angiotensin receptor blocker was followed,[14] and losartan was added to the patient's medications with up-titration to 100 mg/day.

Patients with LDS require echocardiography at least at yearly intervals to assess the aortic root, ascending aorta, and heart valves.[14] Head to pelvis MRA or computed tomography angiography (CTA) should be performed at diagnosis, after 1 year, and thereafter according to the location, size, and progression rate of aneurysms, but at least every 2 years.[14] Type B aortic dissections should be followed aggressively for rapid aortic growth.[14] Patients with LDS should be advised to avoid contact or competitive sports, isometric exercises, and exercising to exhaustion.[14] As aortic dissections and aneurysm ruptures tend to occur in patients with LDS at smaller dimensions than in Marfan syndrome and other types of aortic disease, a lower threshold for vascular surgical intervention has been proposed.[10,11,14] The guidelines for patients with LDS syndrome suggest a surgical threshold in adults with an aortic root diameter of $>$4 cm or expansion at a rate of $>$0.5 cm/year, a personalized decision in the ascending aorta above a diameter of 4 cm, a threshold diameter of 4—5 cm in the descending thoracic aorta, and a threshold of 4.0—4.5 cm in the abdominal aorta, or an expansion rate of $>$1 cm/year at any location.[14] The European Society of Cardiology guidelines on aortic disease suggest intervening on the ascending aorta at diameters $\geq$ 4.2 cm,[10] while the American guidelines on thoracic aortic disease suggest considering surgical repair at a thoracic aortic diameter of $\geq$4.2 cm, determined by transesophageal echocardiogram, or $\geq$ 4.4 cm by CTA or MRA.[11] Although all these recommendations are based on expert opinion it is clear that monitoring of patients with LDS should be diligent, and a low threshold for vascular surgical intervention should be kept in mind. There is concern about using aortic stent grafts in patients with genetic aortic aneurysm syndromes for fear that the landing zones might easily be compromised by the underlying disease,[14] but stent grafts have been successfully used in aortic replacement where the landing zones were within existing synthetic grafts from previous operations.[20]

CONCLUSION

When a patient has a high probability of a genetic vascular syndrome, clinical exome sequencing is helpful in establishing the correct diagnosis which is a prerequisite for management. Clinical exome sequencing established the diagnosis of LDS type 3 in this patient, which prompted her caregivers to perform detailed vascular imaging, including screening for cerebral aneurysms, and keeping in mind the low threshold for vascular intervention.

CONFLICT OF INTEREST

None.

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