Genetic Aspects of the Vascular Type of Ehlers-Danlos Syndrome (vEDS, EDSIV) in Japan

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Background  The vascular type of Ehlers-Danlos syndrome (vEDS, EDS type IV; MIM#130050) is an autosomal dominantly inherited disorder that results from mutations in the genes for type III procollagen (COL3A1). Affected individuals with vEDS are at risk of arterial rupture, aneurysm, and/or dissection; gastrointestinal perforation or rupture; and uterine rupture during pregnancy, which may lead to sudden death.

Methods and Results  Three unrelated Japanese individuals who exhibited symptoms of vEDS were analyzed. In order to identify mutations in the patients' RNA, one 3.8-kb reverse transcriptase polymerase chain reaction product containing the triple-helical domain of COL3A1 was prepared from cultured skin fibroblasts and then was sequenced directly. Three heterozygous mutations were identified; specifically, 2 novel missense base substitutions (Gly220Trp, Gly448Glu) in the (Gly-X-Y)n repeat of the triple-helical domain and a known splicing donor mutation of intron 20 (G+1, IVS20) of COL3A1. The genotype–phenotype correlations in Japanese vEDS individuals with COL3A1 mutations were also investigated.

Conclusion  There was no association between the type of complications in vEDS and the related COL3A1 mutation found. After the genetic diagnosis of COL3A1, the establishment of both a network among medical specialists, including clinical geneticists to perform genetic counseling, and long-term follow-up systems of vEDS may help to improve the management of vascular and visceral complications. (*Circ J 2007; 71: 261–265)

Key Words: Arterial rupture; Ehlers-Danlos syndrome; Familial aneurysms; Type III procollagen (COL3A1); Vascular type of Ehlers-Danlos syndrome (vEDS, EDS type IV)

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable connective disorders that cause symptoms of skin hyperextensibility, joint hypermobility, easy bruising, and tissue fragility. According to the latest revised nosology, 6 major types of EDS have so far been classified: classical, hypermobility, vascular, kyphoscoliosis, arthrochalasia, and dermatosparaxis! The vascular type of EDS (vEDS, EDS type IV; MIM#130050) is an autosomal dominantly inherited disorder caused by abnormal type III collagen resulting from heterogeneous mutations of the type III procollagen gene (COL3A1); vEDS differs from other types of EDS because individuals with vEDS are at risk from arterial rupture, aneurysm, and/or dissection; gastrointestinal perforation or rupture; and uterine rupture during pregnancy, which may lead to sudden death. A correct diagnosis of vEDS may prevent complications and influence the treatment of patients with unexpected bowel or arterial rupture, especially regarding surgery, and patients with a family history of similar events.

In the present study, we analyzed 3 unrelated Japanese individuals with clinical symptoms of vEDS. In order to identify mutations in the affected individuals, one 3.8-kb reverse transcriptase-polymerase chain reaction (RT-PCR) product containing the triple-helical domain of COL3A1 was prepared from cultured skin fibroblasts and then directly sequenced. This method is simple and easy for scanning mutations of COL3A1. Our results showed 3 heterozygous mutations in affected individuals; specifically 2 novel missense base substitutions (Gly220Trp, Gly448Glu) leading to the replacement of one Gly in the (Gly-X-Y)n repeat of triple-helical domain and a known splicing donor mutation of intron 20 (G+1, IVS20) of COL3A1. We also herein discuss the genotype–phenotype correlations in Japanese vEDS individuals with COL3A1 mutations.

Methods

Cell Culture

Dermal fibroblasts were obtained from explants of skin biopsy specimens from each affected individuals with vEDS, after appropriate informed consent had been given.

Mutation Identification

The COL3A1 mutation identification method we described previously4,5 was further modified to amplify 1 COL3A1 cDNA fragment. Total cellular RNA and genomic DNA were extracted from cultured dermal fibroblasts and complementary DNA was synthesized by priming with random hexamers as described previously. To cover the entire triple-helical coding sequence, long PCR amplification of a 3.8-kb fragment of COL3A1 cDNA was per-
formed using EX Taq Hot start version (TaKaRa, Kyoto, Japan). The forward primer ATGS1 was GGTGCTACTTGTTAGCACTGCTT (positions 36–55 of COL3A1 in Ref 6) and the reverse primer III-14 was CGGAATTACTCAGGACTAATGAGGCTTTC (positions 3,635–3,859 in Ref 6). The PCR fragments covering part of the N-propeptide and the entire sequence coding for the triple helix were screened for mutations by means of cycle sequencing and then were directly sequenced by the primer positions indicated in Fig 1. To confirm the identified mutations, the region of interest was amplified from genomic DNA and followed by direct sequencing. The sequences of primers were derived from previously published information on the intron–exon boundaries genomic sequence for COL3A1.7

Results

We screened for mutations of COL3A1 in 3 unrelated Japanese individuals clinically diagnosed as vEDS, using a one RT-PCR fragment direct sequencing method, and we identified 3 different mutations (Table 1). To confirm the mutations, we performed direct sequencing from genomic DNA and found identical mutations. The 3 mutations were heterozygous, including 2 novel missense base substitutions (Gly220Trp, Gly448Glu) leading to the replacement of one Gly in the (Gly-X-Y)n repeat of the collagen triple helix (Cases 1, 2) and a known splicing donor mutation of intron 20 (G+1, IVS20) of COL3A1 (Case 3) (Table 1). In this insertion mutation (Case 3), using the RT-PCR-direct sequencing method, the sequence could not be determined in progress (Fig 2A) as compared with the sequence that could be determined in a healthy control (Fig 2B). However, it could be read using another method; namely, sequencing based on PCR cloning of the RT-PCR product. The mutant allele mRNA contains 24 nucleotides from the intron 20 (IVS20) between exon 20 (Ex20) and exon 21 (Ex21) (Fig 2C). This insertion mutation caused 8 amino acids to be added to in-frame COL3A1 but did not have glycine. The mutation was a substitution of A for G+1 of intron 20 (G+1, IVS 20) based on the genomic DNA analysis (Fig 2D).

Table 1 Summary of Clinical Features of Patients With Vascular EDS With COL3A1 Mutations

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age at first complication (years)</th>
<th>Thin skin</th>
<th>Arterial dissection or rupture</th>
<th>Bowel rupture</th>
<th>Pneumothorax</th>
<th>Family history of sudden death</th>
<th>Defects in COL3A1</th>
<th>Reference</th>
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<tr>
<td>1</td>
<td>19</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>GGG GGG Ex18</td>
<td>Gly220Trp</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>GGA GAA Ex27</td>
<td>Gly448Glu</td>
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<td>25</td>
<td>+</td>
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<tr>
<td>4</td>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>+</td>
<td>GCC GAC Ex44</td>
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</tr>
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<td>5</td>
<td>43</td>
<td>+</td>
<td>+</td>
<td>+/–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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</tbody>
</table>

Washington University (220 cases) 23.5 ND 79% 8% ND 48% 48%
Mayo Clinic (31 cases) 28.5 77% 84% 16% 58% 58%

EDS, Ehlers-Danlos syndrome.
Fig 2. Sequencing pattern of splicing mutation used in the screening strategy. (A) Direct sequencing of RT-PCR product from Case 3’s fibroblasts could not be determined in progress. (B) Direct sequencing of the RT-PCR product in the wild-type allele could be determined in progress. (C) Sequencing of the mutated allele based on the PCR cloning of the RT-PCR product. The mutant allele mRNA contains 24 nucleotides from the intron 20 (IVS20) between exon 20 (Ex20) and exon 21 (Ex21). (D) Direct sequencing of amplified genomic DNA of Case 3. A was substituted for G+1 at the splicing-donor site of intron 20 of COL3A1 (G+1 IVS20), resulting in mutant mRNA being spliced in the next GT after 24 nucleotides of G+1 IVS20. RT-PCR, reverse transcriptase polymerase chain reaction.

Fig 3. Pedigree structure of affected individuals with vEDS (Cases 2, 4, 5) showing vEDS affected (solid symbols) and unaffected (open symbols) in 3 generations (generations I–III). *Affected individuals in whom the COL3A1 mutation was found. (□) Female; (●) male; slash through symbol = deceased (the deceased’s age and cause of death are shown below the symbol). vEDS, vascular type of Ehlers-Danlos syndrome.
Three of them (Cases 2, 3, 5) had 2 or more family members with clinical symptoms of vEDS. From the pedigrees of these cases (Fig 3), affected individuals appeared in every generation and almost had at least 1 affected parent. In the other 2 cases, there were no other individuals with notable clinical feature of vEDS in 3 generations.

Discussion

The vEDS is caused by a deficit in type III collagen, which is a major component of the cardiac and vascular extracellular matrix. Type III collagen is a homotrimer formed by an association of 3 alpha 1 (III) chains derived from 1 gene (COL3A1). The core of the molecule is a triple-helix region with an amino acid sequence characterized by (Glyglicine)-X-Y343 repeats. To ensure the proper assembly of the alpha monomers, the Gly-X-Y repeats must not contain skips, and the length of the triple helix must be the same for each alpha chain.

The molecular defects described previously in vEDS individuals include 2 types of mutations of COL3A1. Most (approximately two-thirds) of the base changes are substitution of other amino acids for glycine in the (Gly-X-Y)343 repeats in the triple helix region of COL3A1. A previous report suggested that any glycine replacement in the triple helix region of COL3A1 will cause disease and in the present study the 2 novel mutations (Cases 1 and 2) are recognized as substitutions for glycine residues in the triple-helix region. Most of the remaining mutations affect the splicing junctions of the triple helix exons of COL3A1. The mutation G+1, IVS20 of COL3A1 (Case 3) has been previously reported by 2 separate groups. In those reports both had family histories of vascular rupture, but included only easy bruising with no other characteristic features of vEDS, which differs from the severe phenotype of the present Case 3. This observation confirmed that the phenotype heterogeneity of vEDS identified in the same family or unrelated family members had the same COL3A1 mutation.

The diagnosis of vEDS is confirmed by a collagen protein analysis of fibroblasts and/or cDNA analysis of the COL3A1 by RT-PCR because COL3A1 is a large gene containing 52 exons. The standard biochemical assay for vEDS may produce false-negative results because detecting a change in the protein level in all affected individuals with mutations is more difficult than in a mutation analysis. A mutation analysis of COL3A1 is the best method available for the diagnosis of vEDS and should be performed in all affected individuals when there is suspicion of vEDS, despite negative findings in the collagen protein analysis.

We analyzed fibroblast RNA by RT-PCR-direct sequencing to diagnose vEDS. Based on previous reports of RT-PCR-direct sequencing of COL3A1, some overlapping PCR fragments were amplified. In the present study, one fragment was amplified using long PCR, and each allele could be examined (Fig 1). Even if a patient has a splicing mutation, allele specific fragments also could be cloned easily to detect mutations (Fig 2). This method can screen and identify both mutation types easily and simply; however, it requires a skin biopsy to obtain a fibroblast culture, rather than a blood sample, and EB-transformed cells did not express COL3A1 (data not shown).

The natural history of vEDS can be summarized according to the clinical complications, results of therapeutic intervention, and information regarding survival. The age at first complication was 20 years in 20% of the present patients and by 40 years in 80%. As shown in Table 1, all vEDS individuals in Japan experience some type of significant medical problem by 45 years of age. Of the complications (Table 1), the frequency of pneumothorax (4 of the 5 cases) was higher than that previously reported. No association was observed between the type of complication and any specific mutations, as has been previously reported.

A family history is important for the interpretation of vEDS, which has an autosomal dominant inheritance pattern, but approximately 50% of affected individuals who have a de novo disease-causing mutation and seem to be sporadic cases. In 2 previous studies, the frequency of positive family history including sudden death was 48% and 58%. Clinical features of Japanese vEDS were recognized in 1 or more family members in 3 of the 5 present cases (Table 1). In these 3 cases, the appearance of affected individuals in every generation indicated the autosomal dominant inheritance (Fig 3). The other 2 cases were sporadic and did not have affected family member in 3 generations. A dominant pattern is thus considered to be straightforward; namely, affected individuals have a 50% chance of passing the mutated gene to each child. Owing to the recent biotechnological advances in gene diagnosis, clinical geneticists should therefore be consulted in order for affected individuals to receive appropriate genetic counseling.

The prevalence of vEDS in Japan is unknown. In the United States, the incidence is estimated at 1 in every 5,000 to 20,000 live births. Therefore, vEDS is a candidate disorder for diseases associated with aortic aneurysm with multiple, early onset, or a family history and is a differential diagnosis for Marfan syndrome.

This is the first report to summarize the symptoms of vEDS/COL3A1 mutations in Japanese individuals with vEDS. Most physicians in Japan are not familiar with this disorder, which is a separate form of EDS and there is currently no specific treatment. Tissue friability is a factor of this disease, which makes surgery technically challenging, and there is an increased risk in conducting invasive diagnostic arteriography in these patients because of the vessel friability. Clinical awareness and timely diagnosis of vEDS is still inadequate, as the disease is often diagnosed only after life-threatening complications or death. Complications require hospitalization and consultation with surgeons, radiologists, obstetricians and clinical geneticists. To improve the likelihood of a good outcome, physicians must become aware of the existence of vEDS. Improved diagnosis and establishment of both a network between the medical specialists and of long-term follow-up systems may help physicians to better manage the vascular and visceral complications associated with this disease.

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References


