COMMENTARY

Congenital factor XIII deficiency: A commentary on ‘Homozygous intronic mutation leading to inefficient transcription combined with a novel frame-shift mutation in *F13A1* gene causes FXIII deficiency’

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The article entitled ‘Homozygous intronic mutation leading to inefficient transcription combined with a novel frameshift mutation in *F13A1* gene causes coagulation factor XIII (FXIII) deficiency’ by Wang *et al.*¹ is interesting. FXIII, which belongs to a family of transglutaminases, is a plasma glycoprotein that has an important role in the stabilization of fibrin clot in the final stage of blood coagulation. FXIII circulates as a heterotetramer, composed of two A (FXIII A) and two B (FXIII B) subunits in plasma. FXIII A possesses catalytic activity, and this catalytic subunit is carried and protected by the FXIII B subunit. Congenital FXIII deficiency is a rare autosomal recessive bleeding disorder affecting one in 1–5 million individuals. People with FXIII deficiency show severe bleeding tendency. The earliest and most characteristic clinical symptom is neonatal umbilical cord bleeding. Other typical findings are impaired wound healing and a high risk of miscarriage. The gene of the FXIII A subunit (*F13A1*) maps to the short arm of chromosome 6 (p24–25) and spans about 176.75 kb of genomic DNA. It consists of 15 exons, encoding a mature protein of 732 amino acids. Direct sequence analysis of *F13A1* is important in patients with congenital deficiencies and abnormalities of the FXIII A subunit. To date, 111 unique mutations (53 missenses, 19 deletions, 15 splicings, 10 insertions, 10 nonsenses, three gross deletions and

one promoter) have been reported in the FXIII deficiency, and these mutations are described in a database of mutations causing rare bleeding disorders by the International Society on Thrombosis and Haemostasis (Figure 1).

(<http://www.isth.org/default/index.cfm/publications/registries-databases/>)

These molecular defects do not appear to be clustered in specific regions and are scattered throughout *F13A1*. Nevertheless, the majority of missense mutations are located in the catalytic core domain, and no missense mutations have been reported so far in the activation peptide. On the other hand, a splice site mutation in intron 5 (IVS5-1G>A) seems to be the most common mutation, because it has already been reported in unrelated families from seven different countries.

The article concerned a Chinese patient with congenital FXIII deficiency that was identified by two novel different types of deletion (c.602_605del AAAG) and intronic mutation (IVS1+12C>A), using single-stranded conformation polymorphism analysis and direct sequencing.¹ The authors found a 4-bp deletion in the patient after electrophoresis of the exon-5 PCR fragment revealed two separate bands. Their subcloning and sequencing revealed a deletion of AAAG at nucleotides 602–605 (c.602_605del AAAG) or AAGA at nucleotides 603–606 in the GAGAAAGAAAGAGAAGAG region, leading to a predicted truncated peptide of 204 amino acid residues. To date, 22 deletions, including three gross deletions, have been described in the *F13A1* mutation database,

and there are four reports in the database of deletion in exon 5 that is the same as that reported in this article. Duan *et al.*² reported three unrelated Chinese patients with FXIII deficiency and found three novel mutations, including (c.599del AA). Peyvandi *et al.*³ reported a phenotype–genotype characterization of 10-unrelated Iranian patients and identified a novel small deletion (c.604del A). Similarly, Schroeder *et al.*⁴ described six novel mutations including (c.607del G). Although the nucleotide sequences of exon 5 in *F13A1* are not strict tandem repeat sequences, the sequence may be predisposed to deletion. In addition, Otaki *et al.*⁵ identified a gross deletion that is exactly 5984 bp long that includes the entire exon 5.

Another intronic mutation (IVS1+12C>A) is of particular interest. There is only one report of a 20-bp deletion in the promoter region,⁶ and no intronic mutation in *F13A1* has been reported in FXIII deficiency so far. The first intron of the various kinds of genes in *Homo sapiens* has been previously suggested to have an expression-augmenting activity.⁷ Previous studies of other genes other than *F13A1* have shown that intronic sequences are involved in regulating gene expression. Rohrer and Conley⁸ identified two Sp1 binding sites of intron 1 of the gene for Bruton's tyrosin kinase. Shamsheer *et al.*⁹ identified a regulatory element in intron 1 of the human protein C gene (*PROC*). The article by Wang *et al.*¹ is the first report in *F13A1*, although there is a report in other genes like this. To determine the functional effect of the (IVS1+12C>A) mutation, the authors found that the first

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Figure 1 Schematic of factor XIII A subunit (FXIII A) gene (*F13A1*) indicating the location of mutations of congenital FXIII deficiency and FXIII A-polypeptide. These mutations are described by database of the mutation causing rare bleeding disorders in the International society on thrombosis and Haemostasis (ISTH). (<http://www.isth.org/default/index.cfm/publications/registries-databases/>) 'M' squares indicated missense mutation, 'N' squares indicated nonsense mutation, 'S' squares indicated splicing mutation, 'D' squares indicated deletion and 'I' squares indicated insertion. Three double-headed arrows show gross deletion.

951-bp of intron 1 in *F13A1* are involved in regulating the expression of *F13A1*, and that the IVS1+12A mutation results in its reduced expression. Electrophoretic mobility shift assay indicated that the IVS1+12A mutation causes reduced protein binding. An Sp1 site was predicted in the sequence containing IVS1+12C, which the IVS1+12A mutation eliminates. Co-transfection of a plasmid expressing Sp1 revealed that Sp1 is involved in regulating the expression of FXIII A, and that IVS1+12A leads to inefficient transcription. Whereas the nucleotide of IVS1+12 (NT-007592:g.6260808) is adenine (A) in

ENST0000026480, in fact, the position of this nucleotide is a single nucleotide polymorphism (SNP: rs2815822). The SNP population diversity ratio in Africans and Europeans is approximately 0.9:0.1 (C alleles:A alleles). In Asians, the ratio of minor A alleles is slightly less than 0.05. In *F13A1*, initiation codon (ATG) exists in exon 2, and exon 1 is not translated and does not synthesize peptide. The size of intron 1 between exon 1 and exon 2 is 1904bp. It is thought that the intronic mutation that the authors identified is equivalent to a promoter mutation in the 5'-flanking region.

In conclusion, the authors proved that the position of this mutation is a regulatory mechanism in relation to Sp1 binding, although this mutation was classified into polymorphism in a frequency. These results provide the first insight into a novel regulatory mechanism involving intron 1 in the *F13A1* gene.

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