Hemizygous F8 p.G201E mutation identified in a Chinese family with haemophilia A

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Abstract

Background: Haemophilia A (HA), inherited via an X-linked recessive pattern, is the most common severe lifelong bleeding disorder caused by mutations in the coagulation factor VIII gene (F8). It has significant socio-economic effects due to its long course of disease and high cost of care. These impacts argue for a more accurate genetic diagnosis in an increasingly complex clinical environment.

Methods: A three-generation Han-Chinese family with mild HA was recruited in the study. Exome sequencing was performed in the index case to detect potential disease-causing mutations, and Sanger sequencing was applied to verify the mutation in the family.

Results: A hemizygous c.602G > A variant in the F8 gene, leading to a single amino acid substitution at codon 201 from glycine to glutamic acid (p.G201E) within the factor VIII (FVIII) A1 domain, was identified in the HA family. This mutation detected in the proband was found in his affected sibling, while it was absent in the unaffected family member and the two hundred ethnically-matched controls. The mutation affects an evolutionary conserved residue, which may impact the tertiary structure of FVIII.

Conclusion: The study findings should provide for more dependable and precise genetic counseling which may assist in perfecting family management.

Keywords: Chinese; F8, FVIII; Genetic; Haemophilia A; Mutation

1. INTRODUCTION

Haemophilia A (HA, OMIM 306700), inherited in an X-linked recessive pattern, is the most common severe lifelong bleeding disorder, affecting 1 in 5000 male live births.1-3 It arises from mutations in the coagulation factor VIII gene (F8). HA was diagnosed on the basis of clinical manifestations and laboratory findings. Residual plasma factor VIII coagulant activity (FVIII:C) tests rank HA as severe (<1% FVIII:C), moderate (1-5% FVIII:C) or mild (5-40% FVIII:C).4 HA associates with prolonged activated partial thromboplastin time (APTT), but normal bleeding time, prothrombin time (PT) and platelet count.5 HA clinical hallmarks are intra-articular bleeding, intra-muscular bleeding and long-term potentially mortal post-trauma or post-operative hemorrhaging.5,6 Severe HA patients usually have 20-30 episodes of spontaneous or excessive bleeding in joint or muscle each year, though mild HA patients may occur excess bleeding simply after trauma or surgery.2

More than 2884 F8 mutations have been recorded in the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php). These mutations result in varied phenotypes including FVIII:C and FVIII antigen concentration in different families.4,7 Intron 22 (IVS22) and intron 1 (IVS1) inversions are responsible for 40-50% and 5% of all severe HA patients, respectively.6,9 The remaining severe, moderate and mild patients are induced by other different F8 mutations.7,8 The most serious FVIII replacement therapy complication is inhibitors (antibodies and anticoagulants) development which occurs in 20-35% of all severe HA cases, while mild HA cases rarely generate inhibitors.7

There are between 65,000-130,000 HA patients in China’s 1.3 billion population. FVIII replacement products supplies do not meet demand, and the budget for care is limited.1,10 F8 mutation detection is valuable for family genetic counseling and as a forecaster of inhibitor development.1,10 This study identified a hemizygous F8 c.602G > A (p.G201E) mutation in a Han-Chinese family with mild HA.

2. METHODS

2.1. Subjects

A three-generation Han-Chinese family with HA referred to us for diagnosis at The Third Xiangya Hospital, Central South University, China was recruited for the study (Fig. 1A). Clinical data and peripheral blood specimens were obtained from three family members, comprised of two HA patients (II:3 and II:5) and one unaffected individual (III:1). Two hundred unrelated ethnically-matched subjects (male/female, 100/100; mean age 45.3 ± 4.5 years) with no hemorrhagic disease were enrolled as controls. All participants executed the written informed consent. The study was performed in accordance with Declaration
Fig. 1 Pedigree of the family with mild HA and sequencing analysis of F8 c.602G > A (p.G201E) mutation. (A) Pedigree figure. The arrow indicates the proband. (B) Patient (II:3) with the hemizygous F8 c.602A allele. (C) Family member (III:1) with F8 c.602G allele. (D) Conservation analysis of the FVIII p.Gly201 amino acid residue. (E) Cartoon model of the FVIII protein structure (residues 20-744) by PyMOL 1.6.x based on the SWISS-MODEL: the glycine and the mutated glutamic acid at position 201 are shown as ball-and-stick model. HA, haemophilia A. F8, the coagulation factor VIII gene. FVIII, factor VIII.
of Helsinki and approved by the Institutional Review Board of The Third Xiangya Hospital, Central South University, China.

2.2. Exome capture
Genomic DNA, for all subjects, was extracted from peripheral blood samples via a phenol-chloroform extraction method.11 Exome sequencing, for the proband, was performed by the Novogene Bioinformatics Institute, Beijing, China. Paired-end DNA library construction and exome capture were performed using an Agilent SureSelect Human All Exon V6 Kit. Covaris S220 sonicator was used to break 0.6 μg of genomic DNA into fragments of 180-280 bp which were further subjected to end-repairing, A-tailing and adaptor ligation. After DNA quality assessment, the captured DNA library was paired-end sequenced (150 bp) with an Illumina HiSeq 2000 sequencing system (illumina Inc., USA).12,13 All operations were performed following the manufacturer protocols.

2.3. Variant analysis
Picard (http://sourceforge.net/projects/picard/), Genome Analysis Toolkit and SAMTools were applied to remove duplicates, obtain local alignment and do quality recalibration. An analysis-ready BAM alignment data was then acquired. The thresholds for calling single nucleotide polymorphisms (SNPs) comprised the alignment rate of sequencing reads ≥ 95% and the coverage of sequence depth ≥ 10×.14 ANNOVAR was used to annotate SNPs and insertions/deletions (indels).15 Common variants recorded in the public databases including SNPs build 151 (dbSNP151, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), 1000 Genomes Project (http://www.1000genomes.org/) and NHLBI Exome Sequencing Project (ESP) 6500 were filtered following the previous criteria.12,16 Variants remaining after this process were classified as ‘novel’. SNPs and indels located in exons or splicing sites were further analyzed. Sorting Intolerant from Tolerant (SIFT, http://sift.jcvi.org/) and Polymorphism Phenotyping version 2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/) were applied to predict non-synonymous SNPs function.

Locus-specific PCR amplification primers were designed referring to the human genomic sequences (accessed from UCSC database). Primer-BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to estimate primer specificity.17 Sanger sequencing, which relied on PCR products, was performed to verify putative disease-causing variants in the family and the two hundred ethnically-matched controls through ABI3500 sequencer (Applied Biosystems Inc., Foster City, CA, USA).17 Primer sequences for locus-specific PCR amplification and Sanger sequencing were listed: 5’-TCATCTACCTTCTATATAACAGC-3’ and 5’-AGACTGCTGATAACGCAAG-3’. NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to conduct multiple sequence alignment in diverse species. MutationTaster (http://www.mutationtaster.org/), Protein Variation Effect Analyzer (PROVEAN, http://provean.jcvi.org/index.php), SIFT and PolyPhen-2 were utilized to predict possible effects of mutation on protein function or structure.

3. RESULTS
3.1. Clinical findings
FVIII:C measurement was obtained via a one-stage clotting assay founded on the APTT.18 Two family members (II:3 and II:5) had mild HA with FVIII:C of 15.7% and 29.3% respectively. No FVIII inhibitors were detected in either patient. The proband (II:3) received recombinant human FVIII concentrate and regained a normal FVIII:C of 75.3%.7 Detailed clinical data was listed in Table 1.

3.2. Exome sequencing
A total of 35.65 million reads with an average read length of 100 bp were generated by exome sequencing, and 35.58 million reads (99.82%) were mapped to the human reference genome. Target region average sequencing depth was 51.95. The region was 97.7% covered by the target sequence at 10× or greater. A total of 125,316 SNPs, with 22,881 in the exon regions and 2475 in the splicing sites, were detected. A total of 14,507 indels, containing 613 in the exon regions and 371 in the splicing sites, were found.

3.3. Identification of pathogenic mutation
General known variants recorded in dbSNP151 with minor allele frequency (MAF) > 1%, 1000 Genomes Project with a frequency of > 0.5% and NHLBI ESP6500 were removed. SIFT (predicted values less than 0.05 are deleterious) and PolyPhen-2 (output close to 1.00 are probably damaging) software results predicted the possible pathogenic effects of nonsynonymous variants. Using screening criteria described above, a hemizygous c.602G > A (p.G201E) variant in F8 exon 5 was identified in the proband (II:3, Fig. 1B). No other potential pathogenic mutation for HA or diseasing-causing mutation in the von Willebrand factor gene (VWF) were reported to be responsible for von Willebrand disease, whose severe type 1 and type 2N share similar FVIII:C level with mild HA. The hemizygous F8 c.602G > A variant was confirmed by Sanger sequencing. The variant was found in his affected sibling (II:5). It was absent in the unaffected family member (III:1, Fig. 1C) and the two hundred ethnically-matched controls.
Glycine at position 201 (p.G201) is highly conserved in mammals (Fig. 1D). MutationTaster predicted that the F8 c.602G > A variant could be a disease-causing mutation with a score of 0.999. PROVEAN and SIFT program analyses of p.G201E variant support the deleterious function of this substitution with a score of -6.50 and 0, respectively, PolyPhen-2 software revealed this variant as a probably damaging mutation with a high score of 1.000 (sensitivity: 0.00; specificity: 1.00). According to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for variant interpretation, the mutation was classified as ‘pathogenic’.

4. DISCUSSION

Since the F8 was first cloned in 1984, insights into the molecular bases of HA have made significant progress. This study found a hemizygous F8 c.602G > A (p.G201E) mutation in a three-generation Han-Chinese family suffering from mild HA. Sanger sequencing confirmed it co-segregated with disease in the family, and mutation prediction software supported it as a pathogenic mutation.

The F8 gene, located at the most distal band of the long arm of X-chromosome (Xq28), spans approximately 186 kilobases (kb) of genomic DNA and has 26 exons. It encodes a polypeptide of 2351 amino acids including a hydrophobic signal peptide of 19 residues and a mature protein of 2332 amino acids. The mature FVIII protein is a multidomain glycosylated heterodimer consisting of a heavy chain, comprising A1 (residues 1-336)-A2 (373-710)-B (741-1648) domains, and a light chain that includes A3 (1690-2019)-C1 (2020-2172)-C2 (2173-2332) domains. Three short acidic interdomain regions disperse in the A1 and A2 domains, the A2 and B domains, and the B and A3 domains, respectively, which were designated as a1 (337-372), a2 (711-740) and a3 (1649-1689). These acidic regions, located close to FVIII proteolytic cleavage sites and including a total of six highly conserved tyrosine residues, may play an important role in FVIII procoagulant activity. The B domain is an orphan domain with no known homology with other proteins. The C domains structurally associate with factor V (FV) C-terminals, and C2 domain is a necessary recognition region for molecules anchoring to procoagulant phospholipid surfaces. In plasma, FVIII binds with von Willebrand factor (vWF) by the A3 and C2 domains to generate a steady noncovalent complex which prevents degeneration. The F8 is activated through thrombin or factor Xa, which further separates from vWF and binds to negatively charged phospholipids. It contributes as a cofactor to activated factor IX (FIXa) in the tenase complex. Subsequently, factor X is activated and blood coagulation cascade continues.

F8 missense mutations are associated with a variety of impacts relying upon mutation type and position. Hydrophobic and hydrophilic amino acids locations in proteins are known to be different. They are generally determined by what the side chains are. The formers incline to locate into a pocket in the core of molecule, while the latters are more frequently on the protein surfaces. Mutations occurring between these two parts may affect protein tertiary structure. The hemizygous c.602G > A mutation leads to a single amino acid substitution at codon 201 (p.G201E) in the A1 domain of FVIII (Fig. 1E). Glycine tends to be a neutral, hydrophobic amino acid while its replacement, glutamic acid, is a negatively charged, hydrophilic amino acid. Located at the same position, another F8 mutation p.G201R was found in UK and Taiwan HA patients, respectively. Arginine is a positively charged, hydrophilic amino acid. These mutations may result in a denatured FVIII protein which may lose its procoagulant activity by disturbing the tertiary structure. The F8 c.602G > A (p.G201E) mutation was previously reported in a Lebanese severe HA case, suggesting that the nucleotide c.602G may be a mutational hotspot and originate in different ancestors. Similarly, both severe and mild HA was described in different patients with a same mutation, such as F8 c.409A > G (p.Y137A) mutation. Phenotypic differences in different patients may, for the most part, be caused by personal gene expression levels as moderated by genetic background or environmental factors.

In conclusion, a hemizygous F8 c.602G > A (p.G201E) mutation was identified as the genetic cause of mild HA in a Han-Chinese family. To our knowledge, this is the first report of p.G201E in East Asian population. The records of different mutations at codon 201 (p.G201R and p.G201W) in the literature will enhance our understanding of this important position of the FVIII protein and the pathogenic mechanisms of HA. These findings facilitate more dependable and exact genetic counselling and may be helpful in improving family management.

ACKNOWLEDGMENTS

We thank the participating members and investigators for their cooperation and efforts in collecting the genetic information, clinical data and DNA specimens. This work was supported by the National Natural Science Foundation of China (81670216, 81800219 and 81873686), National Science Foundation of Hunan Province (2016J2166 and 2018J2660), Scientific Research Project of Health and Family Planning Commission of Hunan Province, China (B20180729, B20180760 and B20180834), Grant for the Foster Key Subject of the Third Xiangya Hospital of Central South University (Clinical Laboratory Diagnostics), the New Xiangya Talent Project of the Third Xiangya Hospital of Central South University (20150301), China.

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