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MOLECULAR PATHOLOGY STUDY OF RARE BLEEDING DISORDERS (RBDS) IN INDIAN POPULATIONS

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ABSTRACT
Blood disorders that are rare can cause severe bleeding symptoms and are highly heterogeneous in their occurrence. In India, consanguinity is widespread and likely to increase the incidence of There are rare bleeding disorders in other countries that are autosomal recessive. We propose a comprehensive analysis of the mutation The nature and frequency of RBDs in Indian patients. The PubMed online search engine (www.pubmed.com) was used to locate research that the authors had published in Indian research on RBDs was located using PubMed search terms "rare bleeding disorders", "mutations", "India", “fibrinogen”, "afibrinogenemia", "factor II deficiency", "prothrombin" "factor VII deficiency", "factor V deficiency", "factor X deficiency", "factor XI deficiency", "com. The Human Gene Mutation Database (HGMD) has been referred to in order to compare Indian mutation frequencies with those of the world (www.hgmd.org). Molecular pathology of RBDs should be the focus of a network of Indian institutions. As a consequence, RBDs can be diagnosed at a lower cost and quicker as well as being useful for prenatal diagnosis of affected families during their first trimester.

Key words: Blood disorders, Indian mutation frequencies, Molecular pathology.

INTRODUCTION

Blood disorders such as hemophilia A (VIII deficiency) and hemophilia B (FIX deficiency) as well as Von Willbrand syndrome are the most common. The most rare bleeding disorders are fibrinogen deficiency, defective prothrombin (FII), defective factor V (FV), defective factor VIII alone (FV+VIII), defective factor VII (FVII), defective factor X (FX) and defective factor XI (FXI), as well as platelet. Blood disorders linked to the X chromosome are hemophilias, while RBDs are inherited recessively [1].

There may be severe bleeding diathesis in those who suffer from rare deficiencies of coagulation factors. It is possible for bleeding diathesis to be highly heterogeneous, even in the presence of severe deficiencies in coagulation factors. WFH reports that FXI and FVII deficiencies cause 37 and 23 percent of the total number of RBDs, respectively. FX and FXIII deficiencies are respectively 9% and 6%; FV deficiencies and fibrinogen disorders are each 10%. The most common bleeding disorders are reported to be a combination of FV+FVIII deficiencies (3%) and a FII defect (2%) [2].

There is a great deal of diversity within the Indian population. Different populations have largely separated and fixed their gene pools over centuries. There is still the practice of consanguineous marriage in some communities, with 6–40 percent of all marriages taking place under this arrangement [3]. Consequently, the bleeding disorders caused by autosomal recessive genes, which are uncommon in many parts of the globe, do not have as high an incidence in many regions of India.

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This vast country with approximately 1270 million people has yet to conduct a comprehensive cellular pathology study, so it is rational to try. The authors of this research describe molecular pathologies associated with different RBDs in different geographical areas, and compare them to internationally published literature on RBDs. As a result, the author compares the RBDs in India to those in various other countries.

METHODS AND MATERIALS

Procedure

Analyzing the biology of RBD from India is part of the review protocol. This study is comprehensive because it includes the entire literature on RBDs in India that has been published in English.

Eligibility criteria and Information source

A review of cases, reviews, and original papers on molecular pathology in RBDs in patients was used in the writing of this review.

Research

An Indian literature PubMed search was used to investigate the Molecular pathology of RBDs. As of August 2020, the Human Gene Mutation Database (HGMD) (www.hgmd.org) was consulted to determine whether Indian studies reported mutations that were more prevalent in global literature. "Rare bleeding disorders", "mutations", "India", "fibrinogen", "afibrinogenemia", "factor VII deficiency", "factor V deficiency", "factor X deficiency", "factor XI deficiency", "combined factor V and VIII deficiency", and "Glanzmanns thrombasthenia" were also used in many combinations for PubMed searches. The following key words produced 60 relevant articles.

Process of collecting data

Each RBD was compiled separately with the data from the master sheets. Wherever possible, the report style was adapted to conform to the HGVS nomenclature to keep the presentation uniform.

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These were the variables examined: defects, inheritances, bleeding, complications, laboratory anomalies, inherited disorders of fibrinogen, defective platelets, deficiencies of FII, FV, FV+FVIII, FVII, FX, FXI, FXIII, GT, BSS, and more.

Individual studies and studies together are at risk for bias. The studies are all hospital-based, so symptomatically milder patients could have gone unnoticed unless a family study was conducted where one patient had severe symptoms.

Analysis of additional data

The present study is a descriptive-additional study, no additional analysis was required.

RESULTS

Fibrinogen deficiency

An extremely rare congenital bleeding disorder stemming from the mutations of three genes encoding fibrinogen chains (FGA, FGB, and FGG) affects 1 in 100,000 individuals [4], [5]. Molecular pathological analysis of 28 patients with fibrinogen deficiency has been reported in four and five reports [4, 5]. An exon 8 homozygous c.G8017A transition has been found in a study published in "Fibrinogen Mumbai.". At the C-terminus of the globular D domain, p.G434D results in a highly conserved amino acid residue. Based on the expression data [5], p.G434D impairs fibrinogen secretion, which results in severe hypofibrinogenemia. Four studies have examined the molecular pathology underlying fibrinogen deficiency among 27 patients [4].

In 27 patients, we found a total of 15 disease causing changes in the glycine-rich domains of fibrinogen alpha and beta (FGA, FGB, and FGG), including 8 frameshifts, 3 mutations affecting splice sites, 3 missense mutations, and 1 nonsense mutation. Three short-splice site mutations (There are 3 different elements in Asp296Fs*59, Thr466Fs*17, and Lys575Fs*74) were also identified in FGA, FGB, and FGG. The frameshift mutation p.Asp296Fs*59 in FGA and FGB, and the short-splice site mutation p.Lys185Fs*13 in FGG was observed in a third. These findings are noteworthy because they provide a first-line method for detecting mutations in this gene in Indian patients.

Prothrombin deficiency

Coagulation cascade protein thrombin is a serine protease which is produced by prothrombin (coagulation factor II). Prothrombin deficiency affects approximately one in every two million individuals, with diverse molecular causes. F7 gene mutations cause both bleeding and thrombosis. The Indian literature has reported six mutations, which result in prothrombin deficiency, so far. Among these two new mutations, one is the addition of p.Ala405Thr to the 'B' chain of *-thrombin, in other words A mutation in the F2 gene cannot prevent the production of prothrombin since severe malnutrition is lethal. The other mutation is the Mumbai mutation, which is a c.G269C mutation that causes the primary protein to be substituted with Cys90Ser.

Factor V deficiency

Bleeding symptoms are usually associated with congenital factor V (FV) deficiencies. There are more than 104 mutations known to be related to children with severe FV deficiency due to a mutation in the F5 gene. Among gene variants, exon 13 has been found to be the site of most mutations reported. In India, one report so far

describes mutations occurring in five unrelated individuals, with three novel deletions being found to have been identified two patients and a third patient with evidence for a deletion: g.50936–50937delAA, both present in two patients, as well as g.52162delC detected in another patient. This group of researchers found mutations only in exon 13. Prior to investigating exon 14 and 15, mutations in exon 13 should be screened. The results of the study are summarized below.

FVIII and FVV combined deficiencies

It occurs once in every 1,000,000 people with an autosomal recessive bleeding disorder. A mutation in either gene associated with these proteins can result in deficiencies of FV and FVIII. These genes represent either multiple coagulation factor deficiency 2 (MCFD2) or lectin mannose binding protein (LMAN1). It has been reported by two groups in Indian literature that 13 patients with combined FVIII and FVIV deficiencies have been molecularly characterized [9, 10]. The absence of mutations in either gene in one family suggests there is a third gene linked to the combined deficiency of FV and FVIII [10]. Four patients had one of these three abnormalities and MCFD2 (p.D122V), a 72-bp deletion in LMAN1 (c.813_822+62del72, p.K272fs) and a 35-bp deletion in MCFD2 (c.210_244del35). Mutation of the LMAN1 gene, which is a substitution from G to T in the second exon of the LMAN1 gene, was previously undiscovered [9].

An insufficient amount of Factor VII

In the coagulation cascade, FVII is produced in the liver using vitamin K.

The F7 gene produces heterogeneous mutations (*140), leading to inheritance of blood coagulation (one in 500,000). Those suffering from FVII deficiency are affected by various symptoms. An unremarkable relationship between bleeding severity and FVII activity has been found. Twenty-one mutations and six polymorphisms have been identified in various studies conducted in India in patients with FVII deficiency [11]-[13]. The patient suffered from two mutations, p. Asp302Asn and p. Arg408His in exon 8 of the F7 gene. One of the two His408Arg substitutions within F7's exon 8 corresponds to the catalytic domain of the enzyme. The other Asp302 residue is completely conserved.

A total of 18 missense mutations, two nonsense mutations, and one frameshift mutation, among them 10 novel mutations, were reported in this study. Exon 8 of the F7 gene had mutations that caused the disease in 15 patients; exon 6 of the gene caused the disease in 7 patients, and six of the mutations were within the catalytic serine protease domain. A haplotypic study revealed there was a common founder between two variants in two patients: a premature termination codon was found in the propeptide region p.Leu55fs and a premature termination codon was found in the catalytic domain p.Gln287*. The successful prenatal diagnosis of a single affected child can be made by cordocentesis in postpartum, which is followed by an evaluation of FVII and other coagulation factors [13].

The F7 gene functional polymorphisms are also implicated in phenotyping of children who have severe hemophilia. A severe phenotype was linked to the F7353Q allele. Patients with a severe phenotype were found to have significantly higher levels of genotypes Gln/Gln and Arg/Arg (P = 0.045) than those with a milder phenotype [14].

Factor X deficiency

It is estimated that one in a hundred thousand people worldwide suffer from a bleeding disorder caused by FX deficiency. There are 21 exons and seven introns in the F10 gene, which lies on the chromosome 13 long arm [15]. At least 103 variants of the F10 gene have been identified, including deletions, nonsense and frameshift mutations (http://www.hgmd.org/). It has been found that missense mutations are most common in Gla domain exon 2 and in Exons 7 and 8 (the catalytic domain). The Indian groups have described and reported mutations in 22 Indian and one Nepalese patient suffering from FX deficiency. Five polymorphisms and 17 mutations have been reported, among them one frameshift, one nonsense, and 15 missenses. A total of 13 new mutations, along with 1 new polymorphism, have been identified [15, 16]. Nine out of the ten patients had a mutation in exon 8; in three of them, the mutation resulted in undetectable activity and a positive phenotype as a result of cross-reactive material. A c.C793T polymorphism was detected in nine out of twenty individuals in two independent study groups (p.Thr264Thr). During the second trimester of pregnancy, cord blood samples were taken from a family with a child diagnosed with FX deficiency [15].

Deficiency in Factor XI

Factor XI is present as zymogen in plasma. Two polypeptide chains with disulfide bonds form this homodimer. As a result of factor XIIa cleaving the internal peptide bond, factor XIa becomes activated, transforming into factor IX. There are approximately 1 in 100,000 to 1 in 1 million individuals in the world with FXI deficiency. Hemophilia C or Rosenthal syndrome is the medical term. Although FXI deficiency has been diagnosed in a few Indian patients [18], [19], only one research report has assessed the molecular profile of FXI deficiency in two patients and in a person with FIX deficiency and FXI deficiency combined [17]. The F11 gene from India has only been reported to have 3 mutations. A patient with p.Phe349Val mutation (representing the catalytic domain of FIX) in F9 and a homozygous p.Gly460Arg mutation in F11 were recently tested for coagulation mutations. The p.Gly460Arg mutation was found in a second patient in heterozygous condition, as was a novel p.Val271Leu

variation affecting the apple-3 domain of FXI [17]. Physiological donor splice sites are abolished with this mutation, resulting in an abnormal FXI transcript. Patient three has a mutation in exon 10 p.Tyr351Ser, which corresponds to the apple-4 domain in FXI. They reported two polymorphisms, -138C in intron A and p.Gly379Gly in two patients as heterozygous or homozygous (Possible Treatments 1 and 2) [17].

FXIII deficiency

Factor XIII, a plasma transglutaminase, is required for haemostasis and fibrinolysis. FXIII deficiency is an uncommon blood coagulation condition that is inherited as an autosomal recessive trait (1: 2,000,000). F13A is the most common gene implicated. Bleeding from umbilical stumps, persistent bleeding after an accident, menorrhagia, delayed wound healing, and spontaneous miscarriages are all signs of FXIII deficiency. In Indian investigations, the molecular pathology of 15 patients with FXIII deficiency was investigated. So far, 15 mutations have been discovered, including eight missense mutations, two duplications, one heterozygous deletion, one splice site mutation, and three nonsense mutations. We discovered a few polymorphisms, including one that was previously unknown. We discovered four mutations in exon 10 of F13A and three mutations in exon 6 of F13A. IVS1 polymorphism A246G was found in six unrelated cases [20], [21]. The heterozygosity frequencies of this polymorphism should be studied in order to provide afflicted families with prenatal diagnostics. Lists the mutations and polymorphisms that have been discovered.

Platelet function problems that are inherited

Glanzmann's Thrombasthenia is a kind of thrombasthenia found in Glanz (GT)

Glanzmanns thrombasthenia affects only one in every 200,000 people (GT). The platelet integrin *IIb*3 gene, which is encoded by ITGA2B and ITGB3, is deficient or absent in GT. Integrins serve as receptors for fibrinogen and von Willebrand factor, among other plasma glycoproteins. There are around 200 distinct mutations related with GT in the ITGA2B and ITGB3 genes. Deletions, point mutations, inversions, insertions, and splice site alterations are all examples [22].

A mutation study of 102 GT patients was undertaken based on Indian studies published in 2016, of which 75 had new mutations, 47 of which were novel. There were 32 missense mutations, 8 insertions, 3 nonsense mutations, 13 deletions, 1 insertion, 1 duplication, and 7 splice site changes among the 65 mutations found. A total of 5 new polymorphisms were discovered. According to one of the research [23]-[27], there have been five deletions and three insertions in exon 4 of ITGB3. Three polymorphisms, g.CIVS21(-7)G, c.T2621G in exon 26, and c.C3063T in exon 30, are in perfect linkage disequilibrium, confirming our prior findings. There are differences among Indian GT patients.

Bernard Soulier Syndrome is a condition that affects people (BSS)

This condition is caused by mutations in the GPIb*, GPIb*, and GP9 glycoprotein genes (GPIX). It's highly rare (1 in 1,000,000) and autosomal recessive, having autosomal recessive inheritance patterns. Platelet surface membrane expression of glycoprotein complexes is affected by genetic abnormalities in any gene. Thrombocytopenia, mucocutaneous haemorrhage, large platelets, and poor platelet adhesion are all symptoms of this condition. Only 42 mutations are known to cause BSS, according to the international literature (http://www.hgmd.org/). Two groups in India have reported characterisation of genetic alterations linked to 50 BSS cases, with 30 disease-causing variants found. Six nonsense, ten missense, three insertion, and eleven frameshift mutations were among the 25 new mutations. Indeed, both studies of BSS patients indicated that the p.Cys8Arg mutation was a prevalent disease-causing mutation in the GP9 gene. In ten patients, p.Arg42del was discovered to be a second frequent mutation in one of the investigations. There could have been a common founder among these patients from India's southern states. Screening for common mutations would make molecular diagnosis of BSS patients considerably easier in India's southern states.

Conclusion and Discussion

Several papers from throughout the world contain reports on the clinical and molecular pathophysiology of prevalent bleeding diseases. However, because unusual bleeding disorders are uncommon, knowledge of their molecular pathogenesis is scarce. Because of the rise in the number of patients presenting with RBDs, further research into their clinical and molecular pathologies is needed. By researching molecular variants and flaws, as well as their impact on coagulation proteins, we could get important insight into structure-function correlation.

The Indian studies and the HGMD have similar mutation types and percentage distributions. Although there was no statistically significant difference between the number of minor deletions detected in fibrinogen deficit patients and the number found in the international literature, fibrinogen deficiency patients had a substantially higher rate (53.33 percent). In India, all three mutations detected in patients with FV deficiency were tiny deletions, with small deletions accounting for 22.11 percent of FV deficiency patients. Two patients with FXIII deficiency (13.33 percent) had duplication mutations. GT was found to have 20% deletions, 12.3% insertions, and 1.53% duplications and indels, all of which have never been described in the literature. In patients with factor XI deficiency in India, three missense mutations were

discovered. However, there is a case can be made that India lacks sufficient patients per RBD to ascertain their mutation distribution simply on numbers. The genetic composition of the Indian population differs from that of western populations. In India, marriage normally takes place inside the same community where a group of individuals resides. Inherited diseases such as RBDs and inherited platelet disorders should be more common in particular communities as a result of consanguineous marriages. In the literature, Indian patients had 71 percent BSS mutations, 27 percent GT mutations, 22 percent combined FV and FVIII mutations, 16.49 percent FX mutations, 1.33 percent FXI mutations, 15 percent FXIII mutations, 10 percent FII mutations, 8 percent FVII mutations, 7 percent fibrinogen disorders, and 3 percent FV deficiency mutations.

The presence of multiple novel mutations and polymorphisms is reported in this work, which adds to the database's value while also allowing for a better understanding of molecular diseases and the creation of better RBD diagnostic techniques. In 14 individuals with fibrinogen insufficiency, two defects (FGA: c.G364+1A, FGG: p.Lys185fs*13) shared a founder mutation; in two patients with FVII deficiency, two defects (p.Leu55fs and p.Gln287*) shared a founder mutation. Eight of the affected patients had p.Cys24Arg and ten had p.Arg42del, two prevalent BSS mutations. Seven of the nine patients have two common MCFD2 gene mutations (p.Glu71fs and c.G149+5A), indicating that this gene is linked to the F5F8D gene. Each of the five patients had a mutation in exon 4 of GPIIIa (ITGB3), two in GPIIb (ITGA2B), one in the promoter and one in exon 12, and four each in exon 4 and 12. Patients with FVII deficiency have mutations in these exons as well as the serine protease domain. As a result, screening these exons first and subsequently the remaining exons is an economical technique. Independent research groups have also discovered frequent mutations in patients with Factor X and FXIII deficiency [15], [16], [20], [21]. In practically all RBDs and platelet diseases, we discovered many novel polymorphisms. The heterozygosity distribution of these polymorphisms is intriguing, and it would be fascinating to investigate it more.

A network of institutions is needed to unravel the molecular diseases of RBDs. A national registry for RBD mutations and polymorphisms should be established to collect data from various investigations. Patients in India will benefit from faster and less expensive molecular pathology diagnostic testing, as well as prenatal diagnosis during the first trimester of pregnancy by screening for common mutations and gene domains where the majority of mutations are concentrated. Even in the lack of information regarding the molecular pathology of the index cases, families with FVII and FX deficits could acquire prenatal diagnosis in the second trimester [13], [15]. A first trimester prenatal diagnosis was successfully given to a GT family with one afflicted child using the linkage assessment technique [23].

The clinical signs of disorders like FVII deficiency may not necessarily correspond to the disorder's molecular pathophysiology [12]. A number of variants in the F7 gene have been associated to a higher prothrombotic proclivity. A670C transversion and repeat polymorphisms in intron 7 (seven or higher repeats) have been found to be independent risk factors for ischemic stroke in young adults by researchers. These polymorphisms could help patients with severe FVII deficiency since they can prevent them from bleeding symptoms. Many of the rarest coagulation factor illnesses have a prevalence of mutations in specific exons, according to the current study and other data from throughout the world. This exon preference has an impact on prenatal diagnosis, especially when mutations in the family are unknown.

The majority of RBDs do not have a significant mortality rate. Menorrhagia and severe mucosal bleeding put patients at danger. Many patient families relocate to locations where care is more readily available. The progeny may inherit the founder mutations from these migrations. However, fewer founder mutations were discovered in Indian patients with RBDs. There are few molecular research facilities for coagulation proteins in India, which could explain this anomaly. Alternatively, these patients may have died early due to insufficient health infrastructure in this country, or they may not have attended a haemostasis laboratory for further research due to the milder nature of some of these bleeding illnesses. Furthermore, these centres are spread throughout states that are vastly separated from one another. Individuals with RBDs may be ignored in distant areas of India due to the distances involved in diagnosis and treatment for many patients with little financial resources. Despite the fact that many new patients and mutations are being found in India, there is a growing need to enhance diagnostic and treatment facilities. In addition to being prohibitively expensive and unavailable to the majority of patients, current therapy options do not address the majority of rare factor deficits.

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