Descriptively Probabilistic Relationship Between Mutated Primary Structure of Coagulation Factor IX and Clinical Severity of Hemophilia B

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ABSTRACT

Hemophilia B is a recessive bleeding disorder resulting from mutations in the coagulation factor IX gene. As this disease is characterized by clinical and molecular heterogeneity, the building of relationship between its genotype and phenotype would be great helpful for better diagnosis, prognosis and treatment. We use a descriptively probabilistic method, cross-impact analysis, to couple the changed primary structure of mutant human coagulation factor IX with the severity of hemophilia B with the help of the amino-acid distribution probability as a quantitative measure for mutation. Then we use the Bayesian equation to calculate the probability that the severity of hemophilia can be defined under a mutation. A patient has larger than 0.5 chance of being defined as severity of hemophilia B when a new mutation is found in coagulation factor IX. In this way, we take the first step towards further modeling of genotype-phenotype relationship in human coagulation factor IX.

INTRODUCTION

The coagulation factor IX precursor contains coagulation factor IXa light chain and heavy chain. After activation of coagulation factor IX to factor IXa, this enzyme interacts with the active cofactor form of factor VIII, to form a complex on membrane surfaces. This complex converts factor X to factor Xa [1]. Thus, the coagulation factor IX is one of critical components of the blood coagulation pathways, and its deficiency causes hemophilia B [2].

Hemophilia B is a recessive bleeding disorder that results from mutations in the coagulation factor IX gene on the X chromosome [3-5]. It occurs in one of 30 000 live male births in all populations [6, 7]. Major acute and chronic complications are often secondary to recurrent bleeding [8]. The unpredictable, recurrent, spontaneous bleedings mainly appear in soft tissues and/or major joints. Recurrent bleeding in large joints usually leads to crippling arthropathies in a majority of severely affected patients.

The clinical severity of hemophilia B corresponds to the level of circulating coagulation factor IX. Severe hemophilia occurs in less than 1% of coagulation factor IX activity. With moderate hemophilia, 1 –

5% of coagulation factor IX activity, there is infrequent, spontaneous bleeding. The presence of at least 5% of coagulation factor IX seems to protect those with mild hemophilia against spontaneous bleeding [6, 8]. Each individual case of hemophilia is characterized by a series of unique parameters, emphasizing the variability and heterogeneity of this disease. These parameters include the mode of initial presentation, the baseline level of the clotting factor, and the presence or absence of a relevant family history [9].

Although the affected males are born to carrier females, up to 50% of cases appear de novo as a result of new mutations [10-12]. Approximately 1 000 unique mutations causing hemophilia B have been reported in humans [13-21]. Approximately 3% of hemophilia B patients have major deletions in the coagulation factor IX gene, half of which are complete [22].

As hemophilia B is characterized by clinical and molecular heterogeneity [23], it is important to find a way to connect the mutations and their clinical outcomes together, by which we could approach to predicting a possibly clinical outcome when a mutation is found. For clinical manifestation, it is easy to consider its appearance/non-appearance as an event with two options, but it is hard to present a mutation, which can occur at different position with different amino acid at coagulation factor IX, as an event with limited choices. Without limited choices, it means that the coagulation factor IX needs to be represented as a number, then any mutation would leads this number to change. In other words, we need to convert a 20-letter symbolized protein sequence into a numeric sequence in order to reach this above aim. Actually, there are currently several ways in doing so, for simplest example, we can use the physicochemical property of amino acid to replace each amino acid in a protein to get the numeric sequence, however the physicochemical property of amino acid is not subject to mutation.

Since 1999, our group has developed three approaches to doing this conversion (for reviews, see [24-26]), and our approaches are more suitable to study the mutations. In this study, we use our approach to building a descriptively probabilistic relationship between mutated primary structure of coagulation factor IX and clinical severity of hemophilia B.

MATERIALS AND METHODS

Data

The human coagulation factor IX precursor with total 145 mutations is obtained from UniProtKB/Swiss-Prot entry [27]. Of them, 141 are missense mutations, 1 insertion and 3 deletions.

Amino-acid distribution probability before and after mutation

The position of amino acid in a protein can be associated with probability, computed using $\frac{r!}{(q_0!\times q_1!\times\ldots\times q_n!)}\times\frac{r!}{(r_1!\times r_2!\times\ldots\times r_n!)}\times n^{-r}$ [28], where r is the number of amino acids, n is the number of partitions, rn is the number of amino acids in the n-th partition, qn is the number of partitions with the same number of amino acids, and ! is the factorial function.

For example, there are fourteen glutamines (Q) in normal human coagulation factor IX, positioned at 2, 57, 90, 96, 143, 167, 185, 216, 219, 237, 241, 292, 370 and 408. According to the equation above, we can imagine the coagulation factor IX as 14 partitions with equal length, each contains 33 (461/14 = 32.93) amino acids because the coagulation factor IX is composed of 461 amino acids. Then, fourteen Qs have the distribution patterns as those in the second column in Table 1, whose amino-acid distribution probability is $r_1 = 1$, $r_2 = 1$, $r_3 = 2$, $r_4 = 0$, $r_5 = 1$, $r_6 = 2$, $r_7 = 2$, $r8 = 2$, $r_9 = 1$, $r_{10} = 0$, $r_{11} = 0$, $r_{12} = 1$, $r_{13} = 1$, $r_{14} = 0$, *and* $q_0 = 4$, $q_1 = 6$, $q_2 = 4$, $q_3 = 0$, $q_4 = 0$, $q_5 = 0$, $q_6 = 0$, $q_7 = 0$, $q_8 = 0$, $q_9 = 0$, $q_{10} = 0$, $q_{11} = 0$, $q_{12} = 0$, $q_{13} = 0$, $q_{14} = 0$, then

$$\frac{1!}{4!\times6!\times4!\times0!\times0!\times0!\times0!\times0!\times0!\times0!\times0!\times0!\times0!\times0!\times0!}\times\frac{1!}{1!\times1!\times2!\times0!\times1!\times2!\times2!\times2!\times1!\times0!\times0!\times1!\times1!\times0!}\times14^{-14}$$

$$=\frac{87178291200}{24\times720\times24\times1\times1\times1\times1\times1\times1\times1\times1\times1\times1\times1\times1}\times\frac{87178291200}{1\times1\times2\times1\times1\times2\times2\times2\times1\times1\times1\times1\times1\times1}$$

$$\times\frac{1}{11112006825558016}=0.1031$$

Table 1. *Glutamines and histidines and their probability before and after mutation at position 167 in factor IX*

Partition	Before mutation		After mutation	
	Glutamine (Q)	Histidine (H)	Glutamine (Q)	Histidine (H)
I	1	1	1	1
II	1	0	1	0
III	2	0	2	0
IV	0	0	1	1
V	1	0	0	0
VI	2	2	2	0
VII	2	4	3	3
VIII	2	1	0	3
IX	1	2	1	1
X	0	1	0	2
XI	0	-	1	0
XII	1	-	1	-
XIII	1	-	0	-
XIV	0	-	-	-
Probability	0.1031	0.0286	0.1544	0.0539

Table 2*. Computation on cross-impact analysis in Fig. 1*

P(2) = 57/141 = 0.4043
$P(\bar{2})$ = 1 – P(2) = 1 – 0.4043 = 0.5957 = 84/141
$P(1\mid\bar{2})$ = 51/84 = 0.6071
$P(\bar{1}\mid\bar{2})$ = 1 – $P(1\mid\bar{2})$ = 1 – 0.6071 = 0.3929 = 33/84
P(1|2) = 31/57 = 0.5439
$P(\bar{1}\mid 2)$ = 1 –P(1|2) = 1 – 0.5439 = 0.4561 = 26/57
$P(1\bar{2})$ = $P(1\mid\bar{2})$ × $P(\bar{2})$ = 51/84 × 84/141 = 0.3617 = 51/141
$P(\bar{1}\bar{2})$ = $P(\bar{1}\mid\bar{2})$ × $P(\bar{2})$ = 33/84 × 84/141 = 0.2340 = 33/141
P(12) = P(1|2) ×P(2) = 31/57 × 57/141 = 0.2199 = 31/141
$P(\bar{1}2)$ $P(\bar{1}\mid 2)$ P(2) = 26/57 × 57/141 = 0.1844 = 26/141

Any point mutation leads an amino acid to change to another one, which certainly changes the distribution pattern of both original and mutated amino acids, thus the amino-acid distribution probabilities will be different for both original and mutated amino acids in the normal and mutant coagulation factor IX.

For example, there is a mutation at position 167 changing Q to histidine (H), then we have 13 Qs after mutation (column 4, Table 1), that is,

$r_1 = 1, r_2 = 1, r_3 = 2, r_4 = 1, r_5 = 0, r_6 = 2, r_7 = 3, r_8 = 0, r_9 = 1, r_{10} = 0, r_{11} = 1, r_{12} = 1, r_{13} = 0$, *and* $q_0 = 4, q_1 = 6, q_2 = 2, q_3 = 1, q_4 = 0, q_5 = 0, q_6 = 0, q_7 = 0, q_8 = 0, q_9 = 0, q_{10} = 0, q_{11} = 0, q_{12} = 0, q_{13} = 0$, then

$$\frac{13!}{4!\,6!\,2!\,1!\,0!\,0!\,2!\,3!\,0!\,1!\,1!\,0!\,0!} \times \frac{13!}{1!\,1!\,2!\,1!\,0!\,2!\,6!\,0!\,1!\,1!\,1!\,1!\,0!} \times 13^{-13}$$

$$= \frac{6227020800}{24 \times 720 \times 2 \times 1 \times 1 \times 1 \times 1 \times 1 \times 1 \times 1 \times 1 \times 1 \times 1} \times \frac{6227020800}{1 \times 1 \times 2 \times 1 \times 1 \times 2 \times 6 \times 1 \times 1 \times 1 \times 1 \times 1}$$

$$\times \frac{1}{302875106592253} = 0.1544$$

For the mutated amino acid, there are 10 Hs in normal coagulation factor IX and 11 Hs in the mutant. Their distribution probabilities are 0.0286 and 0.0539 before and after mutation, so the mutation increases the distribution probability of H.

Because this mutation increases the distribution probability of both the original and mutated amino acids, its overall effect obviously brings about an increment of the distribution probability in the mutant coagulation factor IX, (0.1544 - 0.1031) + (0.0539 - 0.0286) = 0.0766. Actually we have used this approach in many our previous studies [29-47].

In this manner, we have different numbers for different mutations in coagulation factor IX and their documented clinical manifestation, and we therefore can build a quantitative relationship between changed primary structure of coagulation factor IX and clinical severity of hemophilia B.

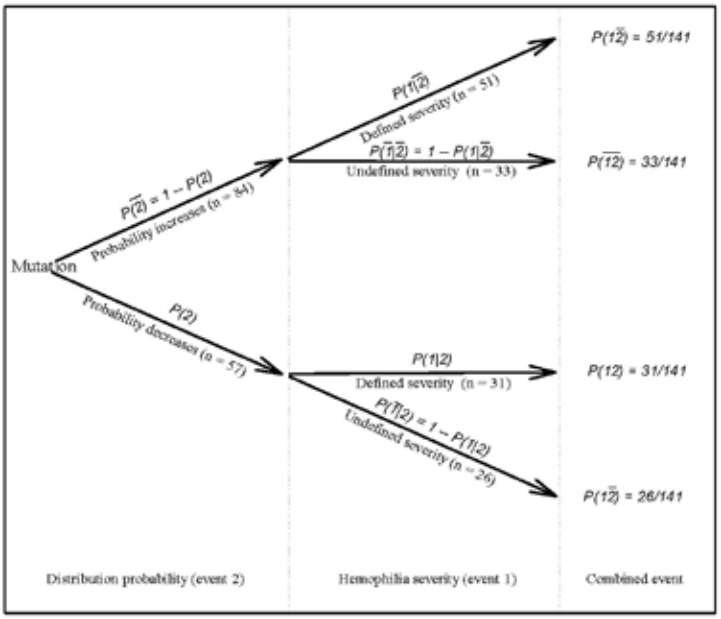
RESULTS AND DISCUSSION

Currently, 141 mutations are documented with hemophilia B, among which 82 are defined as severity. Thus, we can use the cross-impact analysis to build a quantitative relationship between the increase/decrease of distribution probability after mutations and the defined/undefined severity of hemophilia B, because the appearance/non-appearance is an event with two options, and the mutation effect on coagulation factor IX is also an event with two options as increased or decreased amino-acid distribution probability, while the cross-impact analysis is particularly suited for these [38, 48-53].

Figure 1 shows the cross-impact relationship between coagulation factor IX mutations and their hemophilia severity. At the level of amino-acid distribution probability, P(2) and $P(\overline{2})$ are the decreased and increased probabilities induced by mutations, and 57 and 84 mutations result in the distribution probability decreased and increased, respectively. At the level of hemophilia severity: (i)is the $P(\overline{1}|\overline{2})$ impact probability (conditional probability) that the hemophilia severity is defined under the condition of increased distribution probability, and 51 mutations have such an effect. (ii)is the impact probability that the hemophilia severity is not defined under the condition of increased distribution probability, and 33 mutations work in such a manner. (iii) P(1|2) is the impact probability that the hemophilia severity is defined under the condition of decreased distribution probability, and 31 mutations play such a role. (iv) $P(\overline{1}|2)$ is the impact probability that the hemophilia severity is not defined under the condition of decreased distribution probability, and 26 mutations fall into this category. At the level of combined events, we can see the combined results of mutations and disease severity.

Table 2 lists the computed probabilities with respect to Fig. 1,

Fig. 1*. Cross-impact relationship among coagulation factor IX mutations, hemophilia severity, and combined results.*



from which several interesting points can be found. (i) As $P(\bar{2})$ is larger than P(2), a mutation has a large chance of increasing the distribution probability in mutant coagulation factor IX. (ii) As is $P(\bar{1}|\bar{2})$ larger than $P(1|\bar{2})$, a mutation that increases the distribution probability has six tenths chance of being defined as the severity of hemophilia. (iii) As P(1|2) is slightly larger than $P(\bar{1}|2)$, a mutation that decreases the distribution probability has more than a half a chance of being defined as the severity of hemophilia.

From these probabilities, we can use the Bayes' law [54], $P(1|2)=P(2|1)\frac{P(1)}{P(2)}$, to determine the probability that the hemophilia severity defined under a mutation, which is P(1) in this equation. As P(2) and P (1|2) can be found in cross-impact analysis, while P (2|1) is the probability that the distribution probability decreases under the condition of hemophilia severity defined.

As P(1|2) = 31/57 = 0.5439 (Table 2), and P(2|1) = 31/(51 + 31) = 0.3780, $P(1)=\frac{P(1|2)}{P(2|1)}P(2)=\frac{0.5439\times 0.4043}{0.3780}=0.5817$ namely, the patient has larger than 0.5 chance of being defined severity of hemophilia B when a new mutation is found in coagulation factor IX.

In some sense, this study is somewhat similar to the currently popular analysis, genome wide association, the difference is that the single-nucleotide polymorphism is analyzed in genome wide association, while our association is a step ahead, because we have the probability that the occurrence of disease when a single-nucleotide polymorphism is found at protein level.

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