

Ethnic Heterogeneity of the Factor XIII Val34Leu Polymorphism

Flávia A. Attié-Castro¹, Marco A. Zago^{1,2}, João Lavinha³, J. Elion⁴, L. Rodriguez-Delfin¹, João F. Guerreiro⁵, Rendrik F. Franco²

From the ¹Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo and

²Blood Center of Ribeirão Preto, Brazil;

³Centro de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal;

⁴Laboratoire de Biochimie Génétique, INSERM U458, Paris, France;

⁵Laboratory of Human and Medical Genetics, Federal University of Pará, Belém, Brazil

Key words

Factor XIII, ethnic groups, thrombosis, risk factor

Summary

A polymorphism in the coagulation factor XIII gene (FXIII Val34Leu) has been recently described to confer protection for arterial and venous thrombosis and to predispose to intracerebral hemorrhage. At present it is known that FXIII Val34Leu is prevalent in Caucasians, but information upon its distribution in different ethnic groups is scarce. We investigated the prevalence of FXIII Val34Leu in 450 unrelated subjects of four ethnic groups: 97 Caucasians (Brazilians of European descent and Portuguese), 149 Blacks (Brazilians, and Africans from Cameroon, Zaire and Angola), 40 Asians (Japanese descendents) and 164 Amerindians from South America. PCR amplification of exon 2 of FXIII gene followed by *MseI* restriction-digestion was employed to define the genotypes. FXIII Val34Leu was detected in 44.3% of the Caucasians, in 28.9% of the Blacks, in 2.5% of the Asians and in 51.2% of the Amerindians. These data confirm that FXIII Val34Leu is highly prevalent in Caucasians and indicate that it is rarer in populations of African origin. The very high frequency among Amerindians indicates that FXIII Val34Leu is not absent among Asians, and since it has a very low prevalence in Japanese, a heterogeneity in its distribution in Asia may be inferred. Taken together, our data showed that FXIII Val34Leu exhibits a significant ethnic heterogeneity, a finding that is relevant for studies relating this polymorphism with thrombotic and bleeding disorders.

Introduction

Recently, a polymorphism in exon 2 of the coagulation factor XIII A-subunit gene (FXIII Val34Leu) was reported (1). This genetic variation results in increased transglutaminase activity (2) and, paradoxically, appears to be associated with decreased risk of myocardial infarction (3–5) and venous thromboembolism (6, 7) and with in-

creased risk of intracerebral hemorrhage (8). The putative anti-thrombotic effect of FXIII Val34Leu is not well understood, but the mutant allele seems to be linked to increased activation of FXIII and enhanced fibrin cross-linking (2, 9).

At present it is known that FXIII Val34Leu is prevalent in Caucasians, but information upon its occurrence in different ethnic groups is scarce. As FXIII Val34Leu may have a heterogeneous geographic and ethnic distribution, we investigated its prevalence in subjects of different ethnic backgrounds.

Subjects, Materials and Methods

Subjects

Blood samples were obtained after consent from 450 unrelated and apparently healthy adult individuals belonging to four ethnic groups: 97 Caucasians (49 Brazilians of predominantly European descent and 48 Portuguese); 149 Blacks, including 50 Brazilians and 99 Africans (34 from Cameroon, 33 from Zaire and 32 from Angola), 40 Asians, 112 Amerindians from Amazon and 52 Peruvian Indians. Subjects were selected in the context of population genetics studies, and denied any degree of racial admixture for at least three generations. Caucasian and Black Brazilians came from the town of Ribeirão Preto, State of São Paulo, Southeastern Brazil, and were blood donors recruited at the Regional Blood Center. Portuguese Caucasians came from Lisbon and participated as healthy controls in studies of genetic diseases carried out in the Human Genetics Center of Lisbon. African Blacks from Angola, Cameroon and Zaire were selected as controls for genetic population studies at local University Hospitals. The 40 Asians were first or second generation descendants of Japanese immigrants living in the state of São Paulo, Brazil. The Amerindians came from seven different tribes located in the Brazilian Amazon region (5 Wayampi, 22 Wayana-Apalai, 26 Kayapo, 21 Arara, 21 Yanomami, 7 Poturujara, 10 Katuena). The 52 Peruvians lived in the towns of Pasco (28 individuals) and Lima (24 individuals).

Methods

Genomic DNA was extracted from blood leukocytes by standard methods. Genotyping was performed by PCR amplification of exon 2 of the FXIII A-subunit gene followed by restriction digestion with *MseI*, as reported (7).

Statistical Analysis

Allele frequencies were calculated by counting genes from observed genotypes. Exact tests using a Markov chain were performed to assess Hardy-Weinberg equilibrium in each population and interpopulation differentiation (software ARLEQUIN).

Correspondence to: Rendrik F. Franco, MD, PhD, Laboratory of Haematology, Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, 14048-900 – Ribeirão Preto – SP-Brazil – Tel. +55166022223; Fax +55166331144; E-mail: rffranco@netsite.com.br

Table 1 Prevalence of FXIII Val34Leu genotypes in different ethnic groups

Ethnic group	Number of individuals	Heterozygous (Val/Leu)	Homozygous (Leu/Leu)	Leu 34 allele frequency
Caucasians	97	33 (34%)	10 (10.3%)	0.273
Brazilians	49	16	7	0.306
Portuguese	48	17	3	0.240
Blacks	149	36 (24.2%)	7 (4.7%)	0.168
Brazilians	50	12	1	0.140
from Zaire	33	10	3	0.243
from Cameroon	34	8	0	0.118
from Angola	32	6	3	0.188
Asians (Japanese)	40	1 (2.5%)	0 (0%)	0.013
Amerindians	164	72 (43.9%)	12 (7.3%)	0.293
Wayampi	5	1	1	0.300
Wayana-Apalai	22	12	1	0.318
Kayapo	26	8	1	0.192
Arara	21	9	1	0.262
Yanomami	21	11	4	0.452
Poturuja	7	3	0	0.214
Katueña	10	5	0	0.250
From Peru	52	23	4	0.298

Results

Table 1 shows the distribution of carrier and allele frequencies of FXIII Val34Leu in the ethnic groups examined. In Caucasians, FXIII Val34Leu was found in 43 individuals [33 heterozygotes (Val/Leu) and 10 homozygotes (Leu/Leu)], yielding a carrier frequency of 44.3% (allele frequency 0.273). Specific allele frequencies were 0.306 in Brazilians and 0.240 in Portuguese Whites. The mutant FXIII was found in 43 out of 149 Blacks (36 heterozygotes and 7 homozygotes) yielding a carrier frequency of 28.9% (allele frequency 0.168). Allele frequencies of 0.140 and 0.182 were observed in Brazilian and African Blacks, respectively. One heterozygote was observed among the 40 Asians (carrier frequency: 2.5%; allele frequency 0.013). Among the 112 Amazon Amerindians, 57 individuals carried FXIII Val34Leu (49 heterozygous, 8 homozygous), yielding a carrier frequency of 50.9% and an allele frequency of 0.290. In Peruvian Indians, 23 heterozygotes and 4 homozygotes were found (carrier frequency 51.9%; allele frequency 0.298). All the four major population samples were found to be in Hardy-Weinberg equilibrium (data not shown). The genotype distribution was clearly affected by ethnicity, as indicated by an exact population differentiation test (Table 2). The only pairwise comparison which did not reach the 95% confidence level was between Caucasians and Amerindians.

Discussion

We report on a significant ethnic heterogeneity linked to the FXIII Val34Leu polymorphism. Other polymorphisms causally related to vascular thrombosis are known to exhibit a significant ethnic hetero-

geneity (10, 11). Therefore, to determine whether FXIII Val34Leu frequency differs between ethnic groups is of relevance to adequately interpret the finding of the mutant allele in patient and control samples analyzed to investigate its role in vascular diseases.

We found that FXIII Val34Leu has a high prevalence in Brazilian and Portuguese Caucasians (allele frequencies 0.306 and 0.240, respectively). Previous studies reported allele frequencies ranging from 0.11 to 0.30 in Caucasians (12). Together, these data confirm that the Leu 34 allele has reached high frequencies in several Caucasian populations.

A recent report suggested that the Leu34 allele is rarer in Blacks, but the number of Blacks analyzed did not allow a definitive conclusion (7). Furthermore, the sample previously investigated was not selected in the context of a population genetics study. The data from the present study confirm and extend the previous findings by demonstrating a significantly lower prevalence of FXIII Val34Leu in Blacks as compared with Whites. The data also indicate that FXIII Val34Leu is an old mutation, which must have appeared in human evolution before the African/Nonafrican racial divergence.

Among Asians, only one individual was found to be heterozygote for FXIII Val34Leu. This finding agrees with previous studies suggesting that FXIII Val34Leu is absent or has a very low prevalence among Japanese (12). On the other hand, this polymorphism is highly prevalent in Amerindian populations, reaching allele frequencies not significantly different from those found among Caucasians (Table 2). The high prevalence in Amerindians cannot be explained by racial admixture, since the Amerindian tribes here investigated exhibit minimal degree of racial admixture. Thus, the finding of an allele frequency of 0.293 in Amerindians is an indication that FXIII Val34Leu may be present in other Asian populations, an observation that should encourage the search for a heterogeneous distribution of this polymorphism inside Asia.

On the basis of the known heterogeneous distribution of venous and arterial thrombosis in different ethnic groups and geographic regions, one might suggest a contribution of FXIII Val34Leu in determining this differential thrombotic risk. However, it should be pointed out that vascular thromboses are multifactorial disorders and as such the risk for the disease may vary in different human populations as a result of different combinations of genetic and environmental risk factors, Val34Leu being only one of them. Thus, caution is necessary before making firm statements upon the relation of FXIII Val34Leu with ethnic diversity of the occurrence of thrombosis. For instance, one might argue that the very low prevalence of the Leu34 allele in Japanese (in association with other risk factors for arterial disease) is in keeping with the elevated risk for stroke in this population, since Val34Leu seems to determine the risk for stroke (13). However, the same finding contrasts with the low prevalence of venous thrombosis in Japanese, most likely because other factors (for instance, virtual absence of factor Leiden and FII G20210A mutations) counterbalance the effect of the low frequency of Val34Leu in this population.

Table 2 Population pairwise differentiation test at the FXIII Val34Leu polymorphic locus: exact probability of nondifferentiation

Population	Africans	Amerindians	Asians (Japanese)
Amerindians	0.00000±0.0000		
Asians (Japanese)	0.00000±0.0000	0.00000±0.0000	
Caucasians	0.04092±0.0089	0.23250±0.0185 (NS)	0.00000±0.0000

NS, non-significant difference.

Result of the differentiation test between all populations: $P=0.00000±0.0000$.

In conclusion, the findings from the present study revealed a significant ethnic and geographic variation in the FXIII Val34Leu prevalence. The data here presented may be of value to design and interpret studies dealing with the role of FXIII Val34Leu in thrombotic and bleeding disorders.

Acknowledgements

F. A. Attié-Castro was a recipient of a FAPESP Grant (99/03215-9). This work was partly supported by FAPESP, FUNDHERP and Grant N. TS3*-CT93-0244 from the European Union. Authors are grateful to M.H. Tavella and L. Ferrão for excellent technical assistance.

References

- Mikkola H, Syrjälä M, Rasi V, Vahtera E, Hämäläinen E, Peltonen L, Palotie A: Deficiency in the A-subunit of coagulation factor XIII: two novel point mutations demonstrate different effects on transcript levels. *Blood* 1994; 84: 517-25.
- Anwar R, Gallivan L, Edmonds SD, Markham AF: Genotype/Phenotype correlations for coagulation Factor XIII: specific normal polymorphisms are associated with high or low factor FXIII specific activity. *Blood* 1999; 93: 897-905.
- Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ: Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost* 1998; 79: 8-13.
- McCormack LJ, Kain K, Catto AJ, Kohler HP, Stickland MH, Grant PJ: Prevalence of FXIII V34L in populations with different cardiovascular risk. *Thromb Haemost* 1998; 80: 523-4.
- Franco RF, Pazin-Filho AP, Tavella MH, Simões MV, Marin-Neto JA, Zago MA: Factor XIII Val34Leu and the risk of myocardial infarction. *Haematologica* 2000; 85: 67-71.
- Catto AJ, Kohler HP, Coore J, Mansfield MW, Stickland MH, Grant PJ: Association of a common polymorphism in the factor XIII gene with venous thrombosis. *Blood* 1999; 93: 906-8.
- Franco RF, Reitsma PH, Lourenço D, Maffei FH, Morelli V, Tavella MH, Araújo AG, Piccinato CE, Zago MA: Factor XIII Val34Leu is a genetic factor involved in the aetiology of venous thrombosis. *Thromb Haemost* 1999; 81: 676-9.
- Catto AJ, Kohler HP, Bannan S, Stickland M, Carter A, Grant PJ: Factor XIII Val34Leu. A novel association with primary intracerebral hemorrhage. *Stroke* 1998; 29: 813-16.
- Kohler HJ, Ariëns RAS, Whitaker P, Grant PJ: A common coding polymorphism in the FXIII A-subunit gene affects cross-linking activity. *Thromb Haemost* 1998; 80: 704.
- Franco RF, Santos SEB, Elion J, Tavella MH, Zago MA: Prevalence of the G20210A polymorphism in the 3'-untranslated region of the prothrombin gene in different populations. *Acta Haematologica* 1998; 100: 9-12.
- Pepe G, Rickards O, Vanegas OC, Brunelli T, Gori AM, Iusti B, Attanasio M, Prisc D, Gensini GF, Abbate R: Prevalence of factor V Leiden mutation in non-European populations. *Thromb Haemost* 1997; 77: 329-31.
- Kangsadalampai S, Board PG: The Val34Leu polymorphism in the A-subunit of coagulation factor XIII contributes to the large normal range in activity and demonstrates that the activation peptide plays a role catalytic activity. *Blood* 1998; 92: 2766-70.
- Elbaz A, Poirier O, Canaple S, Chédru F, Cambien F, Amarenco P: The association between the Val34Leu polymorphism in the factor XIII gene and brain infarction. *Blood* 2000; 95: 586-91.

Received January 31, 2000 Accepted after resubmission May 31, 2000