Prevalence of Factor V Leiden and G6PD 1311 Silent Mutations in Dalmatian Population

Vedrana Čikeš, Irina Abaza, Vjekoslav Krželj, Ivana Marinović Terzić, Robert Tafra, Anuška Trlaja, Eugenija Marušić and Janoš Terzić

Laboratory for Molecular Biology, University of Split, School of Medicine, Split, Croatia
Department of Paediatrics and Transfusion Medicine, University Hospital, Split, Croatia

Received for publication March 26, 2004; accepted July 7, 2004 (04/068).

Background. Factor V Leiden has been described as a common genetic risk factor for venous thromboembolism. The geographic distribution of this abnormality varies greatly, being high in Europe and almost absent in Asia and Africa. Particularly high prevalence is observed in some Mediterranean countries, which suggests the Mediterranean origin of this mutation. Similarly, prevalence of silent mutation 1311 of the G6PD gene seems to be higher among Mediterranean populations. Since the Dalmatian population (of south Croatia) geographically belongs to the Mediterranean populations we analyzed the prevalence of FV-Leiden and silent mutation 1311 in this region. Furthermore, because the coincidence of G6PD deficiency and venous thromboembolism was described earlier, we tested a possible association of FV-Leiden and G6PD deficiency.

Methods. One hundred sixty-eight healthy blood donors and 55 G6PD deficient individuals originating from the Dalmatian region were tested for the presence of FV-Leiden mutation and silent mutation 1311.

Results. Prevalence of FV-Leiden among blood donors was 2.4%, while among G6PD deficient individuals it was significantly higher, 11% (p = 0.011). Prevalence of silent mutation 1311 among blood donors and G6PD deficient individuals was 21 and 15%, respectively.

Conclusions. Observed allele frequencies among individuals originating from the Dalmatian region is similar to the neighboring European and Mediterranean populations. Interestingly, our results indicate the association of the FV-Leiden and G6PD deficiency and warrant further studies.

Key Words: Factor V Leiden, G6PD, Silent mutation 1311, Dalmatia.

Introduction

Factor V Leiden (FV-Leiden) is a point mutation in which adenine is substituted for guanine at nucleotide 1691 in the gene coding for coagulation factor V. This mutation impairs the proteolytic degradation of the factor V by protein C that makes it active for a long time (1). This abnormality of the hemostasis system is associated with an increased risk of venous thrombosis (2). The prevalence of the mutation varies among different populations, being high in Europeans and almost absent in the populations of Southeast Asia, Polynesia and Africa (3–4). The largest population study among Europeans revealed a mean frequency of 2.78% with a peak value of 12% in Cyprus with decreasing frequency from south to north and from west to east (5). This pattern suggests that FV-Leiden originated as a single event in the eastern Mediterranean and then spread throughout Europe (5).

A glucose-6-phosphate dehydrogenase (G6PD) deficiency is linked to the Mediterranean basin because it protects from
severe forms of malaria, the condition once very common in this region (6). Among many mutations that alter enzyme activity, G6PD-Mediterranean (C-T transition at position 563) is the most common in the Mediterranean basin. G6PD-Mediterranean mutation is always accompanied by a silent mutation at nucleotide 1311 in Europe but not in Asia, which suggests independent origin of this mutation in Europe and Asia (7). Interestingly, the European type of G6PD-Mediterranean mutation has been found in Nepal (8). Considering its diverse incidence in different populations, silent mutation 1311 could be used as a marker for population studies and, due to its location, in X chromosome inactivation studies (9).

The people of Dalmatia in southern Croatia geographically belong to Mediterranean populations. Thus, our aim was to determine the prevalence of FV-Leiden, as well as silent mutation 1311 in the Dalmatian population and compare it to that of neighboring populations, in order to test the hypothesis of Mediterranean origin of those mutations. For that purpose, we studied unselected healthy blood donors from the Dalmatian region.

Furthermore, because connection between G6PD deficiency and venous thrombosis has been indicated previously (10,11), we undertook a molecular analysis of the Leiden mutation among G6PD deficient individuals.

Patients and Methods

Patients

We studied two groups of samples, namely, 168 blood samples from healthy donors who had given blood at the Department of Transfusion, University Hospital, Split and 55 DNA samples of G6PD deficient individuals (belonging to 26 different families). The study was approved by the Ethics Committee on Human Research of University Hospital, Split, Croatia and informed consent was signed by all tested individuals.

Blood Samples and DNA Analysis

Blood samples were collected in Vacutainer tubes with Na-EDTA anticoagulant. DNA was isolated using the Perfect gDNA Blood Mini system (Eppendorf, Hamburg, Germany). DNA samples were assayed for FV-Leiden by polymerase chain reaction followed by MNII digestion (3). Silent mutation 1311 in the gene G6PD was detected as previously described (12). DNA fragments were separated on 2% agarose gels and visualized with ethidium bromide.

Statistical Analysis

The frequencies of mutations in two groups were compared by Pearson’s chi square test (13).

Results and Discussion

One hundred sixty-eight healthy blood donors (154 males) representing the Dalmatian population of southern Croatia were tested for FV-Leiden. Four individuals had FV-Leiden mutation with a prevalence of 2.4%. They were all males and heterozygous (Figure 1).

This prevalence is not different from that reported for the neighboring populations of northwestern Greece (3.3%), Italy (3.2%) and Austria (3.9), indicating European origin of Dalmatia’s inhabitants (14–16). This also supports the hypothesis of eastern Mediterranean origin of the FV-Leiden mutation and its later spreading throughout Europe, including Dalmatia.

Of 55 G6PD deficient individuals (28 males), 6 were positive for FV-Leiden, with a prevalence of 11%. There were four females and two males and they were all heterozygous. Observed allele frequency among G6PD deficient individuals is significantly higher than in healthy subjects ($p = 0.011$). Although G6PD deficient individuals came from 26 families, this higher frequency is not due to clustering of FV-Leiden mutation in certain families because only two heterozygous individuals were from the same family.

Deficiency of G6PD activity protects from severe forms of malaria, because the defective erythrocyte cannot support optimal growth of parasites within cells (6). Our finding that G6PD deficient individuals often have Leiden mutation suggests that the possession of both mutations may have additional protective roles against malaria. The protective mechanism of G6PD deficient erythrocytes is well understood (6), but a possible protective mechanism for Leiden mutation is speculative. We hypothesize that among individuals with Leiden mutation, who are more prone to coagulation events, plasmodium infested erythrocytes can more easily initiate the formation of a microthrombus, leading to the clearance of parasitic erythrocyte and protection from malaria. Another possibility explaining association of FV-Leiden and G6PD deficiency is that G6PD deficiency,
accompanied by FV-Leiden, was brought into the Dalmatian region from the eastern Mediterranean region where both mutations are more frequent-founder effect (16). Although this co-segregation of G6PD deficiency and Leiden mutation is hard to completely understand, this is an interesting observation and needs to be tested in other populations.

Allele frequency of silent mutation 1311 among tested blood donors was 21% and among G6PD deficient subjects (excluding 11 individuals with G6PD-Mediterranean mutation) was 16% (Figure 2). G6PD-Mediterranean mutation was excluded from calculation since it is known that all individuals with this mutation originating from the Mediterranean basin possess silent mutation 1311 (12,17).

Incidence of silent mutation 1311 among individuals with normal G6PD activity varies between 10 and 50%. In the Great Britain incidence of silent mutation among British is 24% while among Iranian subjects it was 44% (9). It seems that incidence of silent mutation is higher in the population of the Mediterranean basin than among populations of continental Europe (18–20). The Dalmatian population, as a part of the Mediterranean populations in contact with central European populations, with an incidence of 21% fit into this scheme, indicating mixed (Mediterranean/central European) origin of Dalmatian inhabitants. Also, due to low frequency of heterozygotes, this polymorphism is not a good marker for X chromosome inactivation studies in the Dalmatian population.

In conclusion, prevalence of Leiden mutation and silent mutation 1311 in the Dalmatian population (in the middle of prevalence spectra) supports the possibility of Mediterranean origin of these two mutations. Also, we report intriguing observation of association of Leiden mutation and G6PD deficiency that warrants further research.

Acknowledgment

This work was supported by the Croatian Ministry of Science and Technology grant No. 0216009 and by the Medical School in Split. Vedrana Cikes and Irina Abaza have contributed equally to the study.

References