Coll. Antropol. **24** (2000) 2: 295–301
UDC 572:575.113.2
Original scientific paper

Anthropogenetical Analysis of Abnormal Human -globin Gene Cluster Arrangement on Chromosome 16*

D. Turčinov[1], R. Krishnamoorthy[2], B. Janićijević[1], I. Marković[1], M. Mustać, C. Lapoumeroulie[2], A. Chaventré[3] and P. Rudan[1]

[1] Institute for Anthropological Research, 10000 Zagreb, Croatia
[2] INSERM U 458, Hospital Robert Debre, Paris, France
[3] Laboratory for Anthropology and Genetic Demography, University P. Segalen, Bordeaux II, Bordeaux, France

A B S T R A C T

An earlier study of human globin gene polymorphism in two Adriatic islands of Olib and Silba showed an abnormal arrangement of -globin genes in two different individuals. The next step was to determine the degree of the kinship relationship between the two probands, one with a deleted and another with triplicated -globin gene on the island Silba, and to determine the stability of this disorder through generations. We reviewed the parish registers (Status Animarum) of the island of Silba, dating from the year 1527, and constructed family trees for the two probands. Restriction endonuclease mapping was performed to study the arrangement of the -globin genes in the offspring of our probands. A total of 183 ancestors completed the two family trees. The kinship relationship between them was established in the 5th, 6th, and 7th generation. The analysis of -globin genes in the offspring of our probands showed the triplicated -globin genes in two persons. We also found -globin gene triplication in other three relatives. We did not find any deleted -globin genes. We determined the kinship relationship between the two probands, one with deleted and the other with triplicated -globin genes. This finding enabled us to determine the stability of this gene disarrangement through generations. It also showed new possibilities in anthropogenetic research, by combining the analyses of parish registers with those of modern genetic methods, such as restriction endonuclease mapping.

Received for publication May 25, 1999.

* This research is funded by the Ministry of Science and Technology of the Republic of Croatia (project no. 01960101).

Introduction

The two island populations of Silba and Olib in the northeastern Adriatic (Croatian coast) (Fig 1) were holistically explored in the previous ten years. The problem and strategy of anthropological research was aimed at analyzing the biological and cultural (micro)evolution of the population, i.e. populational linguistic peculiarities, marital system, isonimy, morphology (anthropometry) and physiology (state of the cardiovascular and respiratory systems), dental status, dermatoglyphs of the digito-palmar complex, bone morphology (metacarpal bone dimensions by X ray), red blood cells markers, immunoglobulin allotypes, human globin gene polymorphism, polymorphism of esterases of *Escherichia coli,* HLA polimorphism strains and all results were compared between these two populations[1–15]. In 1989, Lapoumeroulie at al.[13] described an unusual arrangement of -globin gene on 16 chromosome in two different individuals from the island of Silba. They found that one individual (proband G.P., born in 1917) had single -globin gene on one chromosome as indicated by a 10.5 Kb Bam HI DNA fragment. The other individual (proband L.J., born in 1923) had three -globin genes on one chromosome as indicated by an 18 Kb Bam HI fragment. The authors observed that both the deleted and the triplicated -globin genes were found in the same island (population size in 1988 was 194 inhabitants) and had complementary geno-

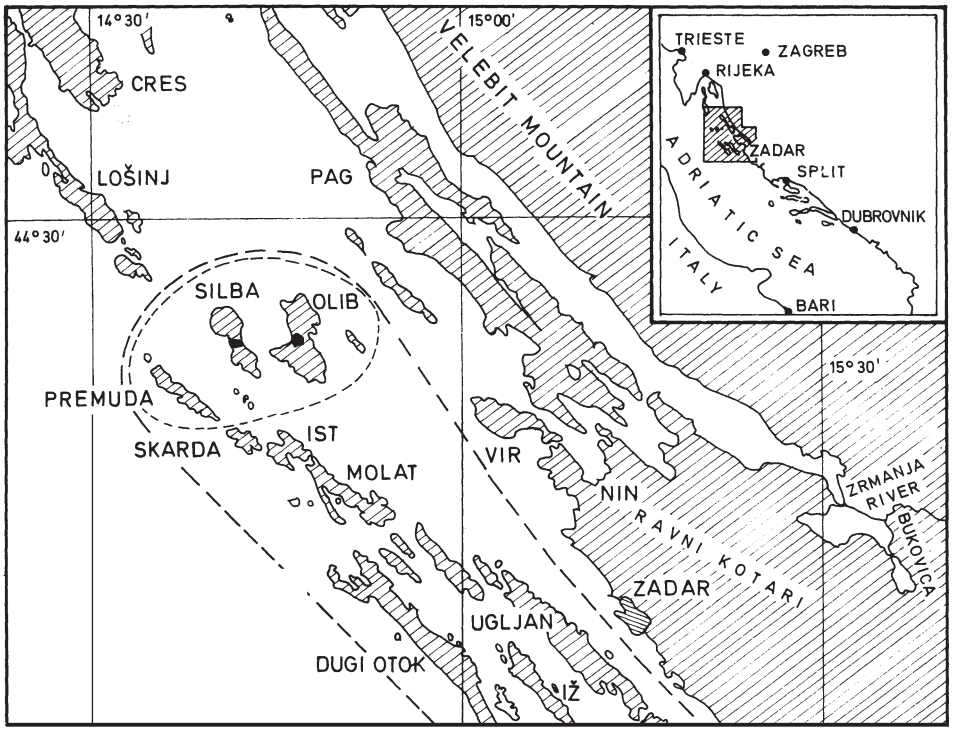

Fig. 1. Geographic position of the islands of Silba and Olib and location of the willages of »Silba« and »Olib« on the islands – geographic distance between the islands is 600 meters approximately.

types (– ^{3,7}/deletion versus $^{anti\ 3,7}$/) as if they were a product of a single and unique event of unequal crossing over between misarranged -globin genes on two different chromosomes.[13] As no kinship relationship between the two probands have been known since the second half of the last century, we carried out our investigation to establish this relationship by developing genealogies for these two subjects. The next step of our investigation was to explore -globin genes in their relatives and offspring in order to find out the stability of -globin gene disorders through generations.

Material and methods

The data utilized in this study were obtained from the parish registers (of baptism, marriage and death) of the island Silba. They were used for the reconstruction of the genealogies of the two subjects to uncover the details on their past kinship relationship. The earliest register dates from 1526 (Fig.2); the registers were written in the Croatian language and Glagolitic script afterwards in Latin script and Latin alfabet until the 19th century and kept in the Historical Archive in Zadar, Croatia. During the 19th century they were written in Italian and Latin script, and since the beginning of the 20th century they have been written in the Croatian language and Latin script and kept in the municipal office of the isand of Silba. On the basis of the data from the parish registers, we reconstructed the family trees for the two probands: G. P. born in 1917 with the deletion and L. J. born 1923. with the triplication of -globin genes to find out biological and kinship relationships linking them.

We studied 32 individuals from the island of Silba, including those who emigrated to the coastal towns of Zadar and Pula (in Croatia) and Trieste (in Italy). We employed the well known methodology for DNA analysis and rapid DNA extraction for in vitro enzymatic amplification as used by Lapoumeroulie et al.[13]

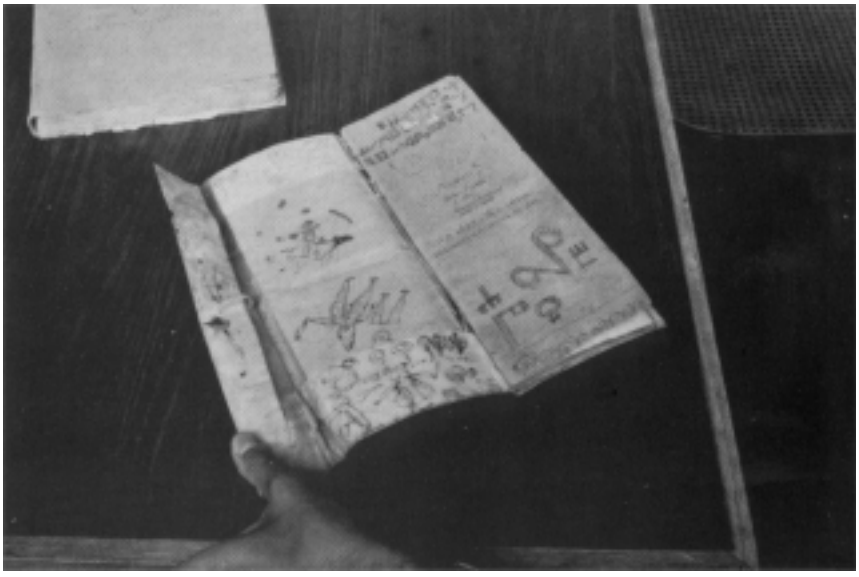

Fig. 2. The original parish register from the year 1526 (Historical Archive, Zadar).

The venous blood was drawn on EDTA and kept frozen until DNA extraction by the phenol and chloroform procedure. DNA was then digested by enzyme Bam HI and enzyme Bgl II (Boehringer Mannheim, Mannheim, FRG), to define the presence of three (– /) four (/) or five (/) globin genes. The digested samples were then fractionated by overnight electrophoresis on 0.8% agarose gel.

A solution of 1.5 M sodium chloride and 1.15 M sodium citrate was used for capillary transfer on Gene Screen Plus membrane (New England Nuclear, Boston, MA, USA). An -globin gene probe was performed for hybridization in sealed bags.

The amount of 200 L blood was spun down in a centrifuge, and suspended in 200 L of water and boiled to lyse the cells and nuclei. The supernatant fluid was taken for amplification by PCR using Hybaid intelligent heating block.

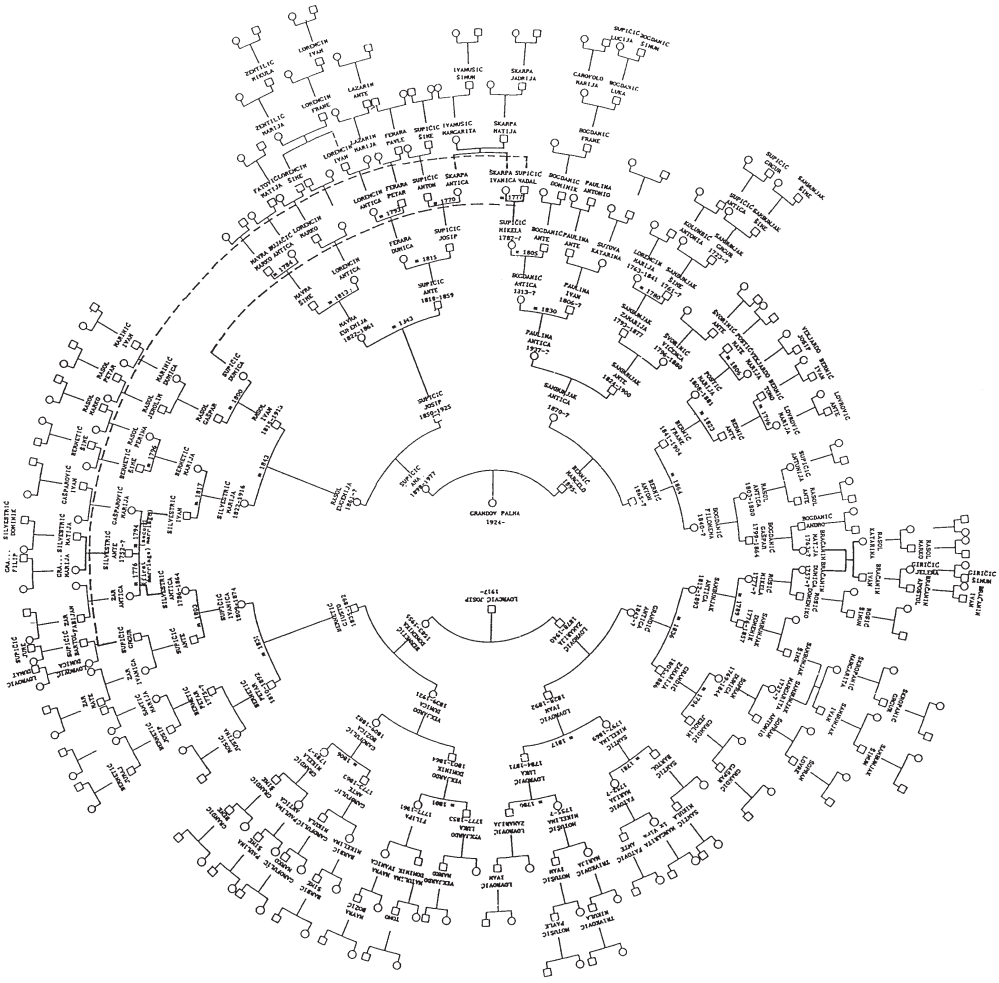

Fig. 3. The two family trees which includes a total of 183 ancestors of our two probands

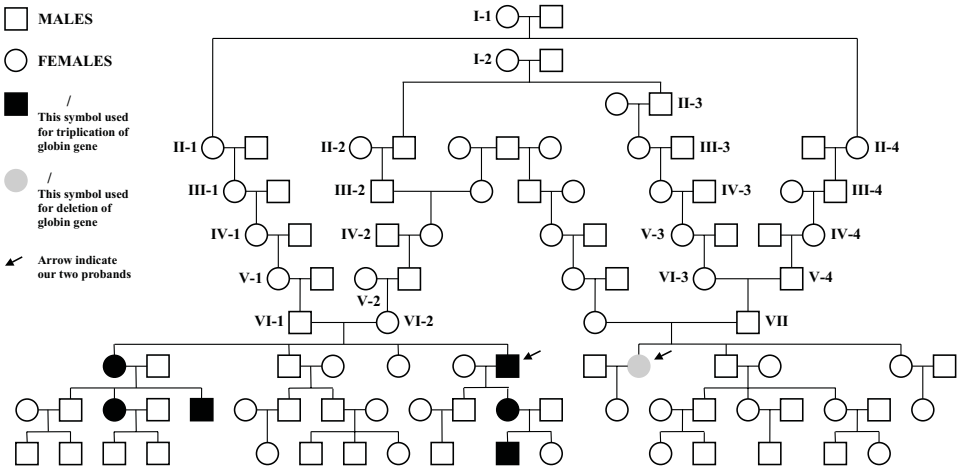

Fig. 4. The two family trees of G. P. and L. J. of two probands (arrow) and the relationship between the son, the daughter, three grandchildren and 15 other relatives of the proband with the triplicated (/) -globin genes, and the daughter and 11 other relatives of the proband with the deleted (– /) -globin gene.

Results

The reconstructed genealogies of G. P. and L. J. till the end 17-century (Fig. 3) include a total of 183 ancestors for the two family trees. The kinship relationship between them was established in the 5th, 6th, and 7th generation. Major events in the history of these two subjects are illustrated by the information on two marriages of S. A., who represents a common ancestor to both subjects. The first marriage with Z. A. occurred in 1776 and the second one with G. M. in 1794 (Fig. 3). Other common ancestors to both subjects include B. I. born in 1763, married to R. K., born in 1757, and S. N. married in 1777 to Š. I. (Fig. 3).

Restriction endonuclease mapping was used to study the arrangement of -globin genes in 32 individuals, offspring and relatives of the two subjects with misarranged -globin genes. We analyzed a son, a daughter, three grandchildren and 15 other relatives of the subject with triplicated (/) -globin genes. His (proband L.J.) daughter, grandchildren, and sister with her daughter and son all had triplicated (/) -globin genes (Fig. 4). We found that 15 other relatives had the normal -globin gene arrangement (/).

We analyzed a daughter and 11 relatives of the subject with deleted (– /) -globin gene (proband G.P.). All of them had the normal -globin gene arrangement (/) (Fig.4). All of the examinees had normal medical anamnesis concerning their physical and mental state. The same thing was found for the ancestors of both subjects as obtained by personal communication.

Discussion

The -globin gene cluster contains two duplicated units each spanning approximately 4 Kb.[16 17 22] The high degree of homology between these two units facilitates unequal crossing over events during meiosis resulting in a deleted (–) -globin gene on one chromosome and triplicated ( ) -globin genes on the other

chromosome.[18 19 20 21 22] A single 3' -globin locus may remain as a result of deletion of a DNA fragment about 4.0 Kb long containing the entire 5' -globin cluster.[19] Unequal crossing-over of a pair of structural loci produces in addition to one chromosome with a single locus, an opposite chromosome that bears three structural loci.[19] The deleted and triplicated -globin genes of our two probands have complementary genotypes.[13] The finding may suggest that the abnormal arrangement of -globin genes in two individuals occurred in the past in one occasion. The surveys suggests that such unique and unequal crossing-over occurred during meiosis in one of the three common ancestors, probably S. A. born in 1752, who had two marriages, as shown by the family tree (Fig. 3). There were approximately 194 inhabitants living on the island Silba in 1988.[1] These islanders lived in isolation for several generations with an absence of admixture with other population groups.[13] The restriction endonuclease mapping was used in 49 individuals (25% of the total population) of the island Silba.[13] The fact that the -globin rearrangement was found in only two individuals with complementary genotypes (– ^{3,7}/deletion versus $^{anti\ 3,7}$/) tends to suggest that it is a product of a single and unique event of unequal crossing-over between misaligned -globin genes on two different chromosomes.[13]

In this study we collected 32 blood samples from descendants and relatives of probands with the deleted and triplicated -globin genes as well. The restriction endonuclease mapping was used to confirm the stability of the two abnormal -globin genes through generations. The (anti 3.7/) triplication was found not only in the proband's daughter and one grand child, but even in his sister and her son and daughter (Fig. 4). This finding disclosed the fact that the triplicated form is transmitted through generations. Other relatives did not have abnormal arrangements of -globin genes. At the same time (– 3.7/) no deletion was found in the daughter or other relatives of the proband with the deleted -globin gene.

The two -globin genes on each chromosome are transcriptionally active and encode an identical protein product, deletion of a single gene or presence of an additional gene on one chromosome has been shown not to affect hematological parameters, especially in situations where globin gene cluster functions normally.[23] Three and five -globin genes encode a sufficient protein product that is in proportion with the production -globin gene. Only an imbalance in the relative amounts of and -globin chain synthesis represents an important factor in the pathophysiology of -thalassemia.[24] Probably this is why all examinees had normal medical anamnesis of their physical and mental state and the same applied to their ancestors as reported by personal communication. Further analysis of polymorphisms of the -globin gene cluster will anable to construct the haplotype map of critical individuals and will also allow to confirm the single event of recombination in this isolate.

REFERENCES

1. RUDAN, P., A. CHAVENTRE, Coll. Antropol. 13 (1989) 177. — 2. SUJOLDŽIĆ, A., Coll. Antropol. 13 (1989) 189. — 3. LAZAREVIĆ, A. S., Coll. Antropol. 13 (1989) 197. — 4. RUDAN, V., G. LEROTIĆ, S. M. ŠPOLJAR-VRŽINA, Coll. Antropol. 13 (1989) 207. — 5. SUJOLDŽIĆ, A., Coll. Antropol. 13 (1989) 223. — 6. SMOLEJ-NARANČIĆ, N., D. ŠIMIĆ, Coll. Antropol. 13 (1989) 229. — 7. SUŽNJEVIĆ. V., Coll. Antropol. 13 (1989) 241. — 8. PERINIĆ, J., Coll. Antropol. 13 (1989) 251. — 9. KUŠEC, V., Coll. Antropol. 13 (1989) 257. — 10. MILIČIĆ, J., Coll. Antropol. 13 (1989) 273. — 11. ARNAUD, J., N. BOROT, A. CHAVENTRE, B.

JANIĆIJEVIĆ, A. E. SAMMARTINO, A. SUJOLDŽIĆ, P. RUDAN, R. JAMMBOU, Coll. Antropol. 13 (1989) 281. — 12. DUGOUJON, J. M., N. BOROT, A. CHAVENTRE, P. RUDAN, Coll. Antropol. 13 (1989) 291. — 13. LAPOUMEROULIE, C., C. Y. LU, C. MURA, B. JANIĆIJEVIĆ, P. RUDAN, A. CHAVENTRE, J. ELION, R. KRISHNAMOORTHY, Coll. Antropol. 13 (1989) 299. —14. PICARD. B., Ph. GOULLET, A. SAMMARTINO, A. CHAVENTRE, Coll. Antropol. 13 (1989) 305. — 15. CAMBON-THOMSEN, A., E. SOMMER, A. SEVIN, A. CHAVENTRE, N. BOROT, E. OHAYON, P. RUDAN, Coll. Antropol. 13 (1989) 311. — 16. MICHELSON, A. M., S. H. ORKIN, J. Biol. Chem. 258 (1983) 15245. — 17. HESS, J. F., M. FOX, C. SCHMID, C. K. J. SHEN, Proc. Nat. Acad. Sci. USA. 80 (1983) 5970. — 18. ZIMMER, E. A., S. L. MARTIN, S. M. BEVERLEY, Y. W. KAN, A. C. WILSON, Proc. Natl. Acad. Sci. USA. 77 (1980) 2158. — 19. GOSSENS, M., A. M. DOZY, S. H. EMBURY, Z. ZACHARIADES, M. G. HADJIMINAS, G. STOMATOYANNOPOULOS, Y. W. KAN, Proc. Natl. Acad. Sci. USA. 77 (1980) 518. — 20. LIE INJO, L. E., A. R. HERRERA, Y. W. KAN, Nucleic. Acids. Research. 9 (1981) 3707. — 21. TRENT, R. J., D. R. HIGGS, J. B. CLEGG, D. J. WEATHERALL, Brit. J. Haematol. 49 (1981) 149. — 22. DODE, C., R. KRISHNAMOORTHY, J. LAMB, J. ROCHETTE, Brit. J. Haematol. 82 (1992) 105. — 23. LIEBHABER, S. A., F. E. CASH, S. K. BALLAS, J. Biol. Chem. 261 (1986) 15327. — 24. ORON, V., D. FILON, A. OPPENHEIM, D. RUND, Br. J. Hematol. 86 (1994) 377.

D. Turčinov

University Hospital for Infections Diseases, Mirogojska 8, 10000 Zagreb, Croatia

ANTROPOGENETSKA ANALIZA NEPRAVILNOG UREĐENJA KLASTERA -GLOBIN GENA NA 16. KROMOSOMU

S A Ž E T A K

Ranije istraživanje polimorfizma ljudskog globin gena na dva Jadranska otoka, Olibu i Silbi su pokazala neobično uređenje -globin gena u dvije različite osobe. Slijedeći korak je odrediti stupanj srodstva između dva probanda, jednog sa delecijom i drugog sa triplikacijom -globin gena na otoku Silbi, i odrediti stabilnost ovog oštećenja kroz generacije. Pregledali smo crkvene matične knjige (*Status Animarum)* otoka Silbe, datirane od 1527. godine, i izgradili porodična stabla za ova dva probanda. Obavljeno je mapiranje endonukleaza restrikcijom za istraživanje uređenja -globin gena potomaka naših probanda. Dva obiteljska stabla su upotpunjena sa ukupno 183 predaka. Dokazano je srodstvo između njih u 5, 6, i 7 generaciji. Analiza -globin gena u potomaka naših probanda je pokazala da triplicirani -globin gen u dvije osobe. Našli smo također triplikaciju -globin gena u tri ostala rođaka. Nismo našli nijednu deleciju -globin gena. Ovaj nalaz omogućuje nam zaključiti, stabilnost ovog poremećaja kroz generacije. Također ukazuje nove mogućnosti u antropogenetskom istraživanju, koristeći analizu crkvenih matičnih knjiga i modernih genetskih metoda, kao što je mapiranje endonukleaza restrikcijom.