RESEARCH ARTICLE

JP-3 gene polymorphism in a healthy population of Serbia and Montenegro

M. KECKAREVIĆ, D. SAVIĆ and S. ROMAC*

Faculty of Biology, University of Belgrade, Studentski trg 16, P.O. Box 52, Belgrade 11000, Serbia and Montenegro

Abstract

Expansions of CTG repeats in *JP-3* gene are associated with a phenotype similar to Huntington disease. These expansions are the cause of Huntington disease like-2 (HDL-2) phenotype. CTG repeats in *JP-3* gene are polymorphic in healthy population. Analyses of CTG repeat polymorphism of *JP-3* gene in various healthy populations could help in estimating the population at risk for developing HDL-2. CTG repeat polymorphism of *JP-3* gene was analysed in healthy population of Serbia and Montenegro. Study included 198 unrelated subjects. Analyses of *JP-3* locus were performed using PCR and sequencing. Six different *JP-3* alleles were obtained and they were in the range of 11 to 18 CTG repeats showing a bimodal distribution, with peaks at 14 and 16. Results show that the distribution of *JP-3* alleles in population of Serbia and Montenegro is consistent with distributions in other analysed populations. The absence of alleles with more then 18 CTG repeats suggests that HDL-2 is very rare in the populations of Serbia and Montenegro.

[Keckarević M., Savić D. and Romac S. 2005 *JP-3* gene polymorphism in healthy population of Serbia and Montenegro. *J. Genet.* **84**, 69–71]

Introduction

Junctophilin (JP) subtypes: JP-1, JP-2 and JP-3 are members of a recently described membrane protein family exclusively present in excitable cells (Takeshima *et al*. 2000). JPs take part in enabling communication between plasma membrane of an excitable cell and intracellular ion channels (Nishi *et al*. 2000). In general, JPs consist of a C-terminal domain spanning the membrane of endoplasmatic reticulum (ER), which in excitable cells serves as an intracellular calcium (Ca^{2+}) store, and a long cytoplasmatic domain interacting with plasma membrane (Nishi *et al*. 2000). JP-1 is predominantly expressed in sceletal muscles, JP-2 is expressed in both sceletal and cardiac muscles, while JP-3 is expressed specifically in brain (Takeshima *et al*. 2000; Nishi *et al*. 2000).

JP-3 gene is located on 16q23.4 and consists of 6 exons, including alternatively spliced 2A and 2B exons. Exon 2A contains CTG repeats and multiple splice acceptor sites leading to different reading frames. Depending on the splice acceptor position CTG repeats are in 3′ untranslated region or in the frame to encode polyalanine or polyleucine (Holmes *et al*. 2001). The number of CTG repeats in exon 2A of *JP-3* gene range from 8 to 28 repeats (Stevanin *et al*. 2002). Expansions of CTG repeats in *JP-3* gene, with more then 40 repeats, are associated with a phenotype named Huntington disease like-2 (HDL-2). Patients with clinical diagnosis of Huntington disease, but lacking mutations in HD gene, should be considered as possible HDL-2 patients. Analyses, so far, have shown that HDL-2 is very rare, accounting for less then 3% of all HDL patients (Stevanin *et al*. 2003).

Analyses of CTG repeat polymorphism of *JP-3* gene in various healthy populations could help in estimating population's risks for developing HDL-2, moreover, those data could be very useful in evolutionary, demographic and forensic studies.

*For correspondence. E-mail: stanka@bf.bio.bg.ac.yu.

Keywords. junctophilin-3; polymorphism; HD-like phenotype; DNA analyses.

Material and methods

A total of 198 unrelated (96 female and 102 male) subjects of Serbian and Montenegrian origin participated in this study. Blood samples were taken from all individuals after informed consent and genomic DNA was extracted using phenol-chloroform extraction method (Sambrook *et al*. 1989). Polymerase chain reaction with primers L237-1 and L237-2 (Holmes *et al*. 2001) was employed to amplify the region of *JP-3* gene that contains CTG repeats. The PCRs were performed in a 10 µl reaction volume, containing 100–200 ng genomic DNA, 50 mM KCl, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 20 ng of each primer, 200 µM dNTPs, 6% Glycerol, 0.5 µg/µl BSA and 0.3 U/µl *Taq* DNA polymerase (*Fermentas*, Germany). PCR running conditions were as follows: initial denaturation for 3 min at 96°C, followed by 5 cycles of 1 min denaturation at 94°C, 1 min annealing at 58°C and 1 min extension at 72°C and 25 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min extension at 72°C. After 10 min of final extension at 72°C and 30 min at room temperature, PCR products were kept at 4°C until further analysis. Denatured PCR products were run on 6% polyacrylamide sequencing gels, and alleles were visualized by silver staining (Bassam *et al*. 1991). Samples were genotyped using the sequencing ladder as a standard.

Results

In the sample of 396 chromosomes from subjects of Serbian and Montenegrian origin, we found 6 different alleles, ranging in size from 11 to 18 CTG repeats. Distribution of allelic frequencies was bimodal, with peaks at 14 (49.49%) and 16 (44.79%) repeats (as shown in figure 1). Population was in Hardy Weinberg equilibrium for this locus, and estimated heterozygosity was found to be 62%.

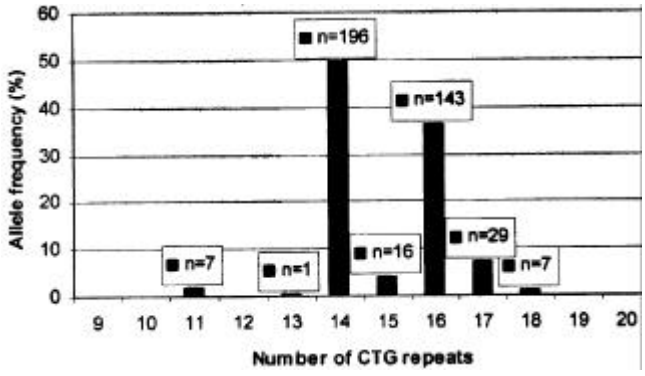

Figure 1. CTG repeat polymorphism in *JP-3* gene in population of Serbia & Montenegro (396 analysed chromosomes), *n* = number of chromosomes.

Discussion

About 20 patients of 6 pedigrees have been identified yet with HDL-2 associated CTG repeat expansions in *JP-3* gene, and all of them were of African, African-American and North African origin (Holmes *et al*. 2001; Stevanin *et al*. 2002). Results of Stevanin *et al*. suggest that the distribution of allelic frequencies of *JP-3* locus in the North African population (8–28 CTG) is broader compared to French population (9–18), exhibiting larger alleles that are not present in the French population.

If larger alleles undergo expansions (like in Huntington disease and Spinocerebellar Ataxias), it could be concluded that the North African population is at higher risk of developing HDL-2 then the French population. Population of Serbia and Montenegro shows very narrow distribution of *JP-3* alleles (11–18) without larger alleles, which implies that it is not to expect *JP-3* mutations among HDL patients of Serbia and Montenegro. Those expectations are in accordance with unpublished results of Keckarevic *et al*. showing that HDL patients have no expansion in *JP-3* gene, too. Bimodal distribution of allele frequencies with peaks at 14 and 16 CTG repeats is consistent with findings of Bauer *et al*. (2002), although there is a difference in distribution range of CTG repeat lengths between population of Serbia & Montenegro (11–18) and the German population they analysed (10–28), probably due to a different size of sampling (results of Bauer *et al*. (2002) are based on 1600 analysed patients). This difference implies that larger alleles are very rare in European white population, and that is unlikely that mutations in JP-3 gene are causative mutations for HDL in those populations. On the other side, in North African population, on 51 analysed subjects, distribution of CTG repeat alleles was 8–28. Wide range of exhibited alleles on such a small sample implies higher risk for developing HDL-2 in this population.

Narrow distribution of *JP-3* alleles in healthy French population and population of Serbia and Montenegro and low frequency of larger alleles in German population based on very extensive samples, compared to North African population, could be the result of a genetic drift or trans-acting factors in North African population responsible for variability of *JP-3* alleles and expansions associated with HDL-2.

Aknowledgements

This work was supported by Serbian Ministry of Sciences, grant # 1521.

References

Bassam B. J., Caetano-Annoles G. and Gresshoff P. M. 1991 Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem*. **196**, 80–83.

Bauer I., Gencik M., Laccone F., Peters H., Weber B. H., Feder E. H., Weirich H., Morris-Rosendahl D. J., Rolfs A., Gencikova A. *et al*. 2002 Trinucleotide repeat expansions in the junctophilin-3 gene are not found in Caucasian patients with a Huntington's disease-like phenotype. *Ann. Neurol.* **51**, 662.

Holmes S. E., O'Hearn E., Rosenblatt A., Callahan C., Hwang H. S., Ingersoll-Asworth R. G. *et al*. 2001 A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. *Nature Genet.* **29**, 377–378.

Nishi M., Mizushima A., Nakagawara K. and Takeshima H. 2000 Characterization of human junctophilin subtype genes. *Biochem. Biophys. Res. Commun.* **273**, 920–927.

Sambrook J., Fritsch E. F. and Maniatis T. 1989 Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor: CSHL.

Stevanin G., Camuzat A., Holmes S. E., Julien C., Sahloul R., Dode C. *et al*. 2002 CAG/CTG repeat expansions at the Huntington's disease-like 2 locus are rare in Huntington's disease patients. *Neurology* **58**, 965–967.

Stevanin G., Fujigasaki H., Lebre A. S., Camuzat A., Jeannequin C., Dode C., Takahashi J., San C., Bellance R., Brice A. and Durr A. 2003 Huntington's disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. *Brain* **126**, 1599–1603.

Takeshima H., Komazaki S., Nishi M., Iino M. and Kangawa K. 2000 Junctophilins: a novel family of junctional membrane complex proteins. *Mol. Cell* **6**, 11–22.

Received 8 March 2004; in revised form 9 December 2004