Association study between (TG) repeat polymorphism in PSMA6 gene and type II diabetes mellitus in Botnia

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Proteasome is a protein complex with multicatalytic proteinase activity. Type II or non-insulin-dependent diabetes mellitus (NIDDM) is multifactorial in origin with both genetic and environmental factors contributing to its development. Searching for susceptibility genes for type II diabetes mellitus, we analysed TG microsatellite polymorphism in the proteasomal PSMA6 (IOTA, PROS-27K) gene located on chromosome 14q13. Association study was performed in groups of 91 diabetes patients and 90 healthy controls from BOTNIA collection (Finland). The genotyping was made by PCR and fluorescent gel electrophoresis. Nine alleles were observed, varying in length from 13 to 25 TG repeats with 18 and 21 repeats being the most frequent in both diabetes patients and healthy individuals. Allele frequency of 22 repeats was twice higher in the diabetes group as compared to the control group. The difference was statistically significant ($P < 0.05$). This finding suggests that the region on chromosome 14q13 in general and PSMA6 (PROS-27K) gene itself could harbour the susceptibility site for type II diabetes mellitus.

Key words: non-insulin dependent diabetes mellitus (NIDDM), proteasome; PSMA6 (PROS-27K); TG repeat, intron, polymorphism

INTRODUCTION

Although the genetic origin of the non-insulin dependent diabetes mellitus (NIDDM) seems to be evident, elucidation of genes involved in the development of the multifacorial age-related form of the disease is still an unaccomplished task. Many genes coding for hormones, receptors and enzymes involved in carbohydrate and lipid metabolism were tested for possible mutations or allelic variants linked to NIDDM.

The ob gene, encoding for leptin, a hormone expressed in adipose tissue and regulating the feeling of hunger, seemed to be especially interesting, as mutation in its murine analogue leads to obesity and NIDDM. Although it is not the case in humans, as ob defects seem to be rare in human population [1], discovery of microsatellite sequences around the ob gene on human chromosome 7 triggered numerous studies of genome polymorphism around the gene locus. Probable linkage of certain variants of the locus structure is reported to be significant or suggestive with NIDDM [2–6]. Several genes located on chromosome 11 seemed to be perspective candidates for genetic determinants of NIDDM. Some allelic forms of the sulfonylurea receptor gene (SUR) seem to be more common in patients with NIDDM in Caucasian population, in North America and French population [7, 8], however, this is not the case in Japanese and Mexicans [9, 10]. Suggestive linkage was reported to cholecystokinin gene allelic also situated on chromosome 11 [11] and amplification patterns produced by microsatellites of this chromosome [12]. A positive correlation of A3 allele of fatty acid binding protein (FABP2) situated on chromosome 4 with NIDDM was found in several populations [13]. Special attention is paid to chromosome 20, harbouring several genes involved in carbohydrate and lipid metabolism. A positive correlation with NIDDM was found for several allelic variants of phosphoenol pyruvate kinase gene (PKC1) and am-

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plification patterns produced by several microsatellites situated on this chromosome [14]. The whole locus of chromosome 20 from adenosine deaminase gene to melanocortin receptor 3 gene on this chromosome is considered to be linked to body fat and insulin [15]. Several studies reveal linkage of NIDDM and obesity to mutation in the beta 3 adrenergic receptor gene [16, 17] and alpha2 adrenoreceptor alleles [18]. However, results of the most of above studies were not convincing and were hardly reproducible. A real breakthrough was achieved after a genome-wide screen for NIDDM genes that localised the susceptibility gene on chromosome 2. The diabetes susceptibility gene encodes calpain-10, an enzyme belonging to the calpain-like cysteine protease family. These enzymes perform extra-lysosomal protein catabolism in the cells [19]. This finding encouraged us to undertake a polymorphism linkage study to NIDDM of a gene encoding a protein involved in another extra-lysosomal protein breakdown pathway – the proteasomal core particle protein gene PSMA6 (PROS-27K, IOTA).

Proteasomes (prosomes, MCP-particles) are complexes composed of different combinations of proteins of MW 20 000 to 36 000 manifesting multicatalytic proteinase activity aimed at catabolism of ubiquitinated proteins. Proteasome core particle proteins can be classified into two superfamilies (alpha and beta) subdivided into 14 families, using the phylogenic approach [20]. Involvement of ubiquitin–proteasome mechanism in degradation of transcription factors, oncogene products, enzymes and muscle fibers determines their role in the pathogenesis of various diseases [21–23]. Thus, study of proteasome gene polymorphism seems to be promising for linkage studies with different diseases. Polymorphism of LMP2 and LMP7 proteasome protein genes encoded in the Major Histocompatibility Locus has already been studied in relation to insulin-dependent diabetes mellitus [24, 25]. In the present paper, we extend polymorphism studies on the PSMA6 gene in relation to NIDDM. The PSMA6 gene is one of the most evolutionary conservative representatives of the alpha-family. It is encoded by a single-copy gene on chromosome 14q13 [26]. Previously we have described the structure of the genomic domain of this gene. The gene domain spans over 30 Kbp, it is organized in 7 exons [27]. A TG dinucleotide repeat revealed in the intron 6 sequence seemed to be perspective from the point of view of the polymorphism studies [28]. In the present paper, we describe an association study of this repeat with NIDDM in the population of Western Botnia (Sweden).

MATERIALS AND METHODS

DNA sample collection. DNA samples were taken from 91 type II diabetes patients and 90 healthy controls from the BOTNIA collection [29].

Microsatellite analysis. The length of TG repeat was determined by polymerase chain reaction (PCR). Forward primer: 5'-CTAATTGACTTGCAGTTG-CTGG-3' labelled with FAM fluorescent dye (DNA Technology A/S, Aarhus, Denmark); reverse primer: 5'-AACTGCCTCACAGGGCTG-3'. The reaction mix (15 μl) contained: 0.33x NH_4 buffer; 2.5 mM $MgCl_2$; 0.04 mM dNTP; 0.17 mM F and R primer; 25 ng DNA; 0.33x TaqMix (content of TaqMix: 1x NH_4 buffer; 1U Taq). PCR conditions were 3 min. at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, followed by chain elongation (10 min) at 72 °C. PCR was performed on GeneAmp PCR System 9600, Perkin Elmer). Fragment length analyses were performed on ABI Prism® sequencer 310 Genetic Analyser (Perkin Elmer) following manufacturer's protocol. Fragments with known size were included in each lane as DNA size standards (Genescan® – 400 HD (Perkin Elmer)). The length of amplified DNA fragments was calculated with the Genotyper 2.5 computer program.

Calculation of TG microsatellite length. To find out the TG repeat number corresponding to PCR product size, we have sequenced microsatellites in several alleles of different length. Amplified DNA fragments for sequencing were isolated from electrophoresis gel (3% agarose gel, 1x TAE, 15 μl Et-Br, 75 min.) by DNA Extraction Kit #K0513 (Fermentas, Vilnius, Lithuania). Sequencing reactions were performed with an ABI Prism® Big Dye™ terminator cycle sequencing ready reaction kit (Roche Molecular Systems, Inc.) according to the manufacturer's protocol. Sequencing primer was 5'-CGAAGCACTCAGTATGGATTTG-3' (27 bp upstream the reverse PCR primer). Electrophoresis was performed on an ABI Prism® 310 Genetic Analyser (Perkin Elmer).

Statistical analysis. The allele frequency data on the diabetes and control groups were compared using the χ^2 criterion.

RESULTS

PCR fragment analysis revealed fragments of 171, 181, 183, 185, 187, 189, 191, 193 and 195 bp in length. Comparing the size of PCR products with sequencing results we estimated the actual number of TG motifs in each PCR product – $(TG)_{13}$, $(TG)_{18}$, $(TG)_{19}$, $(TG)_{20}$, $(TG)_{21}$, $(TG)_{22}$, $(TG)_{23}$, $(TG)_{24}$ and $(TG)_{25}$, respectively.

Figure shows the frequencies of microsatellite alleles in the NIDDM and control groups. The most abundant alleles were $(TG)_{18}$ (26.7% in control group, 23.6% in diabetes group), $(TG)_{21}$ (29.1% – control, 23% – diabetes) and $(TG)_{19}$ (24.4% – control, 19.8% – diabetes). Allele $(TG)_{22}$ was twice more frequent in the diabetes group (18.1%) than in the control group (9.6%). Statistical analysis confirmed the significance

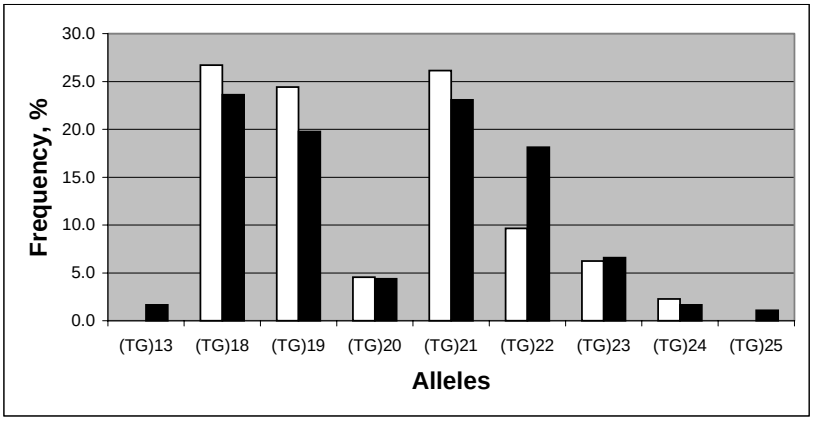

Figure. Frequencies of TG alleles in control group (white columns) and type II diabetes group (black columns)

of these differences ($P < 0.05$). Thus, allele $(TG)_{22}$ seems to be associated with type II diabetes.

The data were also compared by gender and body mass index (BMI). Subjects with BMI <25 were included in normal weight group, and persons with BMI > = 25 formed the overweight and obese group. The number of people (*n*) in the subgroups was: male control – 36, male diabetes – 39, female control – 52, female diabetes – 52, normal weight control – 33, normal weight diabetes – 18, overweight control – 59, overweight diabetes – 73.

Comparing control and diabetes groups by gender, we did not reveal any significant differences in allele frequencies. No significant differences were found for the following subgroups: normal weight control / normal weight diabetes, overweight control / overweight diabetes, normal weight control / overweight control, normal weight diabetes / overweight diabetes. These results suggest that type II diabetes is not associated with a specific subgroup analysed above.

DISCUSSION

Our results indicate that the polymorphism of the intronic TG microsatellite of the PSMA6 gene is associated with non-insulin-dependent diabetes mellitus – allele of 22 TG repeats is twice more frequent in NIDDM patients. The allele is not associated with other traits such as gender or body mass index. The intronic microsatellites seem to be able to modify the level of gene expression by silencing or enhancing the transcription and splicing events [30, 31], thus the PSMA6 gene expression might be altered in the NIDDM patients. This might influence the efficiency of proteasome-mediated degradation of some transcription factors or enzymes involved in the mechanism of insulin susceptibility. The recently described alterations in the intronic sequences of the calpain 10 gene domain characteristic of NIDDM patients are coupled to decreased transcription of the gene [19]. Taken together, our data indicate a perspective for association studies of NIDDM and the proteasome gene polymorphism.

ACKNOWLEDGEMENTS

This work was supported by grants from the Latvian Council of Science and the Sigrid Juselius Foundation of Finland. We thank E. Grens for support and encouragement.

Received 11 October 2002

References

1. Carlsson et al. Obes Res 1997; 5(1): 30–5.
2. Duggirala et al. Am J Hum Genet 1996; 59(3): 694–703.
3. Lapsys et al. Obes Res 1997; 5(5): 430–3.
4. Shintani et al. Diabetologia 1996; 39(11): 1398–401.
5. Clement et al. Diabetes 1996; 45(5): 687–90.
6. Roth et al. Exp Clin Endocrinol and Diabetes 1997; 105(6): 341–4.
7. Inoue et al. Diabetes 1996; 45(6): 825–31.
8. Hani et al. Diabetes 1997; 46(4): 688–94.
9. Iwasaki et al. Diabetes 1996; 45(2): 267–9.
10. Stirling et al. Diabetes 1995; 44(8): 999–1001.
11. Vionnet et al. Diabetes1997; 46(6): 1062–8.
12. Elbein SC et al. Diabetes 1996; 45(3): 370–5.
13. Humphreys Diabetes 1994; 43(6): 800–4.
14. Maklecki et al. Diabetes 1997; 46(5): 876–81.
15. Lembertas et al. J Clin Invest 1997; 100(5): 1240–7.
16. Clement et al. N Engl J Med 1995; 333(6): 352–4.
17. Kadowaki et al. Biochem Biophys Res Commun 1995; 215(2): 55–60.
18. Oppert et al. Obes Res 1995; 3(3): 249–55.
19. Horikawa Y et al. Nature genetics 2000; 26(2): 163–75.
20. Scherrer K, Bey F Progr Nucl Acids Research and Mol Biol 1994; 49: 1–64.
21. Ciechanover A et al. BioEssays 2000; 22(5): 442–51.
22. Voges D et al. Ann Rev Biochem 1999; 68: 1015–68.
23. Desterro JMP et al. Cell Mol Life Sci 2001; 57(8–9): 1207–19.
24. Van Endert et al. Diabetes 1994; 43(1): 110–17.
25. Deng GY et al. Am J Hum Genet 1995; 56(2): 528–34.
26. Bey F et al. Mol Gen Genetics 1993; 237(1–2): 193–205.
27. Sjakste T et al. DNA Sequence 2001; 12(4): 261–5.
28. Sjakste T et al. DNA Sequence 2002; 13: 139–43.
29. Groop L et al. Diabetes 1996; 45(11): 1585–93.
30. Gabellini N Eur J Biochem 2001; 268(4): 1076–83.
31. Gebhardt F et al. J Biol Chem 1999; 274(19): 13176–80.