Short Communication

Fluorescence-Based Automated Fragment Analysis of Microsatellite Polymorphism within the Transmembrane Region of the *MIC-A* Gene

(*MIC-A* / STR / polymorphism / fragment analysis)

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Abstract. MHC class I chain-related genes (*MIC*) are located within the MHC class I region of chromosome 6. Sequence analysis of the *MIC-A* gene showed a trinucleotide repeat (GCT) microsatellite polymorphism within the transmembrane region. So far, six alleles of the exon 5 of the *MIC-A* gene, which consist of 4, 5, 6, 9 and 10 repetitions of GCT, or five repetitions of GCT with an additional nucleotide insertion (GGCT), have been identified. Recent works support the findings that *MIC-A* is associated with several autoimmune diseases. In our work we present a modification of a method used for microsatellite polymorphism detection within the transmembrane region of the *MIC-A* gene. It is the ALFexpress fluorescence-based automated fragment analysis. We also present the frequencies of *MIC-A* exon 5 alleles found in the Czech population. We have identified five alleles of the transmembrane region of *MIC-A*, which comprise 4, 5, 6 and 9 repetitions or five repetitions with an additional nucleotide insertion. The most frequent allele was A5.1 (59.3%) and the less frequent was the allele A5 (20.0%). No A7, A8 or A10 alleles were identified.

The human major histocompatibility complex (MHC) class I chain-related genes (*MIC*) are located within the MHC class I region of chromosome 6. Mapping studies have identified seven *MIC* genes (*MIC-A – MIC-G*), of which only *MIC-A* and *MIC-B* encode expressed transcripts (Bahram, 2000). The *MIC-A* and *MIC-B* genes are located telomeric to the *TNFA* gene between the B-associated transcript (BAT-1) and the *HLA-B* genes (Bahram and Spies, 1996). Both *MIC-A* and *MIC-B* genes contain long open reading frames encoding MHC class I molecules with three distinct extracellular domains (α1, α2 and α3), a transmembrane segment, and a cytoplasmic tail encoded by separate exons. Sequence analysis of the *MIC-A* gene showed a trinucleotide repeat (GCT) microsatellite polymorphism within the transmembrane region (Fodil et al., 1996; Ota et al., 1997). So far, six alleles of the exon 5 of the *MIC-A* gene, which consist of 4, 5, 6, 9 and 10 repetitions of GCT, or five repetitions of GCT with an additional nucleotide insertion (GGCT), have been identified (Ota et al., 1997; Perez-Rodriguez et al., 2000).

The polymorphism of the *MIC-A* gene and its location in the HLA region warrant studies aimed at identifying an association with the risk for autoimmune diseases. The *MIC-A* gene has already been found to confer genetic risk for Behçet's disease (Mizuki et al., 1997), ankylosing spondylitis (Yabuki et al., 1999) and autoimmune Addison's disease (Gambelunghe et al., 1999). Recent works also support the findings that *MIC-A* is associated with autoimmune diabetes mellitus (Gambelunghe et al., 2000; Gupta et al., 2003).

In this study, we introduce a modification of a method for *MIC-A* exon 5 genotyping and the frequencies of *MIC-A* exon 5 alleles evaluated in the Czech population.

Material and Methods

Analysed DNA samples were extracted from peripheral blood anticoagulated with EDTA using a QIAamp DNA Blood Kit (Qiagen GmbH, Hilden, Germany). All tested individuals had given their informed consent to DNA analysis. PCR amplification of the polymorphic transmembrane region encoded by exon 5 was carried out with primers forward Cy5-5'-GCTGGTGCTTCAGAGTCATTGGC-3' and reverse 5'-GGACCCTCTGCAGCTGATGTTTTC-3' (Generi Biotech, Hradec Králové, Czech Republic). The forward primer was labelled at the 5'-end with the fluorescent Cy-5 reagent. PCR was performed in a Biometra

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TGradient cycler in a final volume of 20 µl. The reaction mixture contained approximately 100 ng of DNA, 2.5 mM $MgCl_2$ (MBI Fermentas, St. Leon-Rot, Germany), 1 M betaine (Sigma-Aldrich, Dorset, UK), 200 µM of each dNTPs (MBI Fermentas), 2.0 µl of 10x PCR buffer (MBI Fermentas), 1 µM of each primer and 1 U of Taq polymerase (MBI Fermentas). An initial denaturation at 95˚C for 4 min was followed by 10 cycles of touchdown PCR – denaturation at 95˚C for 15 s, annealing from 64˚C to 55˚C (change of -1˚C per cycle) for 35 s, and extension at 72˚C for 50 s. The standard PCR protocol with 20 cycles of denaturation at 95˚C for 15 s, annealing at 54˚C for 35 s, and extension at 72˚C for 50 s succeeded touchdown PCR. A final extension at 72˚C for 5 min completed the reaction.

Before loading on polyacrylamide gel, amplified samples were mixed with stop buffer containing 99.5% formamide and denatured for 4 min at 96˚C. Approximately 10 fmol of the PCR product together with 10 fmol of each ALFexpress Sizer 100 and ALFexpress Sizer 150 (Amersham Biosciences, Buckinghamsire, UK) as internal standards were loaded on 8% polyacrylamide denaturing sequencing gel (HiResolution ReproGel, Amersham Biosciences). Electrophoretic separation of PCR products was performed in the ALFexpress standard plate under following conditions: voltage 1000 V, current 60 mA, power 50 W and gel temperature 55˚C. Signals were collected automatically every 2 s.

Fragment sizes were determined automatically using the Fragment analyser v1.02 software (Amersham Biosciences) employing ALFexpress Sizer 50-500 (Amersham Biosciences) as an external size marker and ALFexpress Sizer 100 and 150 as internal standards.

Results and Discussion

Using the protocol described here we have been able to analyse the microsatellite polymorphism of the GCT repeat in the transmembrane region of the *MIC-A* gene in the Czech population. We have identified five alleles of exon 5 in 140 healthy individuals from the Czech population. The alleles comprise 4, 5, 6 and 9 repetitions of GCT, or five repetitions of GCT with an additional nucleotide insertion (GGCT). Their amplified sizes were, respectively, 123 bp, 126 bp, 129 bp, 139 bp, and 127 bp. The most frequent allele in the Czech population was the A5.1 allele with a frequency of 59.3%. The A5 allele had the lowest frequency (20.0%). We did not identify any A7, A8 and A10 alleles (alleles with 7, 8 or 10 repetitions of the GCT repeat). For all allele frequencies see Table 1. Frequencies of *MIC-A* exon 5 alleles found in the Czech population corresponded to those found in other Caucasian populations (Gupta et al., 2003).

Table 1. Allele frequencies (%) of MIC-A exon 5 in the Czech population

MIC-A ex. 5 allele	allele frequency (N=140)
A4	26.4 % (37/140)
A5	20.0 % (28/140)
A5.1	59.3 % (83/140)
A6	30.7 % (43/140)
A7	0.0 % (0/140)
A8	0.0 % (0/140)
A9	29.3 % (41/140)

In summary, the technology described here is a rapid, cost-effective, reproducible, and sensitive fluorescence-based fragment analysis method that can be used to generate *MIC-A* transmembrane region allele profiles in tested DNA samples. It is possible to obtain a *MIC-A* exon 5 allele profile within 3.5 h using the method described, so studies of several hundred samples could be completed quickly depending on the number of PCR thermocyclers and ALFexpress automated analysers available. Therefore, it could be used to comprehensively determine the diversity of *MIC-A* exon 5 alleles and haplotype frequencies in different ethnic groups and in case-control association studies of autoimmune and infectious diseases.

References

Bahram, S. (2000) MIC genes: from genetics to biology. *Adv. Immunol.* **76,** 1-60.

Bahram, S., Spies, T. (1996) The MIC gene family. *Res. Immunol.* **147,** 328-333

Fodil, N., Laloux, L., Wanner, V., Pellet, P., Hauptmann, G., Mizuki, N., Inoko, H., Spies, T., Theodorou, I., Bahram, S. (1996) Allelic repertoire of the human MHC class I MICA gene. *Immunogenetics* **44,** 351-357.

Gambelunghe, G., Falorni, A., Ghaderi, M., Laureti, S., Tortoioli, C., Santeusanio, F., Brunetti, P., Sanjeevi, C. B. (1999) Microsatellite polymorphism of the MHC class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. *J. Clin. Endocrinol. Metab.* **84,** 3701-3707.

Gambelunghe, G., Ghaderi, M., Cosentino, A., Falorni, A., Brunetti, P., Falorni, A., Sanjeevi, C. B. (2000) Association of MHC Class I chain-related A (MIC-A) gene polymorphism with Type I diabetes. *Diabetologia* **43,** 507-514.

Gupta, M., Nikitina-Zake, L., Zarghami, M., Landin-Olsson, M., Kockum, I., Lernmark, A., Sanjeevi, C. B. (2003) Association between the transmembrane region polymorphism of MHC class I chain related gene-A and type 1 diabetes mellitus in Sweden. *Hum. Immunol.* **64,** 553-561.

Mizuki, N., Ota, M., Kimura, M., Ohno, S., Ando, H., Katsuyama, Y., Yamazaki, M., Watanabe, K., Goto, K., Nakamura, S., Bahram, S., Inoko, H. (1997) Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behcet disease. *Proc. Natl. Acad. Sci. USA* **94,** 1298-1303.

Ota, M., Katsuyama, Y., Mizuki, N., Ando, H., Furihata, K., Ono, S., Pivetti-Pezzi, P., Tabbara, K. F., Palimeris, G. D., Nikbin, B., Davatchi, F., Chams, H., Geng, Z., Bahram, S.,

Inoko, H. (1997) Trinucleotide repeat polymorphism within exon 5 of the MICA gene (MHC class I chain-related gene A): allele frequency data in the nine population groups Japanese, Northern Han, Hui, Uygur, Kazakhstan, Iranian, Saudi Arabian, Greek and Italian. *Tissue Antigens* **49,** 448-454.

Perez-Rodriguez, M., Corell, A., Arguello, J. R., Cox, S. T., McWhinnie, A., Marsh, S. G., Madrigal, J. A. (2000) A new MICA allele with ten alanine residues in the exon 5 microsatellite. *Tissue Antigens* **55,** 162-165.

Yabuki, K., Ota, M., Goto, K., Kimura, T., Nomura, E., Ohno, S., Mizuki, N., Katsuyama, Y., Makysymowych, W. P., Bahram, S., Kimura, M., Inoko, H. (1999) Triplet repeat polymorphism in the MICA gene in HLA-B27 positive and negative caucasian patients with ankylosing spondylitis. *Hum. Immunol.* **60,** 83-86.