ROLE OF MICA REPEAT POLYMORPHISM IN THE MANIFESTATION OF TYPE 1 DIABETES MELLITUS IN BENGALI INDIAN PATIENTS

Oindrila RAHA¹,², B.N.SARKAR¹, P.VEERRAJU², G. SUDHAKAR², P.RAYCHAUDHURI³, Soma MUKHOPADHYAY⁴, V.R.RAO⁵.

¹ Anthropological Survey of India, Kolkata, West Bengal, India.
² Department of Human Genetics, Andhra University, Visakhapatnam-530003, India.
³ Endocrinology Department, Calcutta Medical College & Hospital, Kolkata, India.
⁴ Netaji Subhash Chandra Bose Cancer Research Institute, Kolkata, India.
⁵ Department of Anthropology, University of Delhi, Delhi, India.


Background. The major histocompatibility complex class I chain-related gene A (MICA) (OMIM-600169) is a polymorphic gene in the HLA region expressed mainly by epithelial cells. The MICA protein encoded by the allele influences the activation of NK cells, which modify β-cells destruction and has been found to be involved in susceptibility of T1DM.

Objective. The aim of this study was to find the association of MICA alleles with T1DM among eastern Indian population.

Subjects and methods. Study was conducted in 134 eastern Indian patients and with 137 healthy controls for the possible role of MICA gene in T1DM pathogenesis.

Results. The MICA*A5 microsatellite allele, showed significantly higher frequencies in patients than controls (p=0.003, OR=1.746, CI=1.206-2.528). MICA A*6 was found to be protective in our study (p=<0.01, OR=0.406, CI=0.268-0.616).

Key words: MICA, T1DM, Bengali, India.

INTRODUCTION

Type 1 diabetes mellitus (T1DM; Online Mendelian Inheritance in Man-OMIM 222100) is an autoimmune disease characterized by destruction of the insulin-producing β-cells of islets of Langerhan’s of pancreas, leading to absolute insulin deficiency resulting in chronic
hyperglycemia (CONCANNON et al. 2005; ERLICH et al. 2008). T1DM is a polygenic disorder. T1DM has its highest incidence among adolescents, with a large number of presenting with diabetes in early puberty (Urban 2006).

The major histocompatibility complex class I chain-related gene A (MICA) (OMIM-600169) is a polymorphic gene in the HLA region expressed mainly by certain epithelial cells, keratinocytes, endothelial cells, fibroblasts, and monocytes. The MICA genes, members of the MIC [Major Histocompatibility Complex Class I chain-related] family are spread across the 2-Mb MHC Class I region (http://www.ncbi.nlm.nih.gov/gv/mhc/main.cgi?cmd=init). The MICA gene is the nearest neighbor to HLA-B (only 46 kb centromeric). The MIC-A and MIC-B genes contain long open-reading frames encoding for MHC class I molecules with three distinct extracellular domains (α1, 2, and 3), a transmembrane segment, and a cytoplasmic tail, each encoded by a separate exon (SANJEEVI et al. 2002). The expression of MICA gene is induced by heat shock and is broadly recognized by a subset of γδ T-cell that exists predominantly in the intestinal and other epithelia (KAWABATA et al. 2000). The broad recognition of MICA and MICB implies an unusual mode of interaction with the Natural Killer Cells (NK) and γδ T-cell. MICA has 5 exons and 91 alleles which encodes 71 proteins (http://www.ebi.ac.uk/imgt/hla/stats.html).

The microsatellite polymorphism consists of repetitions of GCT/AGC. (GCT/AGC)n encodes polyalanine, and the number of alanine (Ala) residues differs with the number of triplet repeats. Five alleles of exon 5 of the MICA gene, each consisting of 4, 5, 6 and 9 repetitions of GCT and five repetitions of GCT with an additional G insertion (GGCT), have been identified. These alleles have accordingly, been named A4, A5, A6, A9 and A5.1, and their sizes correspond to 179, 182, 185, 194 and 183 bp, respectively (Mehra et al. 2007). A5.1 allele contains five triplet repeats plus one additional nucleotide insertion (GGCT/AGCC), causing a frameshift mutation leading to a premature intradomain stop codon (TAA) in the transmembrane (TM) region. The MICA protein encoded by A5.1 allele influences the activation of NK cells, which modify β-cells destruction and thus involve in the age at onset of T1DM (PARK and EISENBARTH 2001). It has been observed that the MICA5.1 gene product expresses aberrantly at the apical surface of human intestinal epithelial cells instead of the basolateral surface, the site of putative interaction with intra-epithelial T and NK lymphocytes. Thus, MICA5.1-homozygous individuals may have altered immunological surveillance by T and NK cells (SUEMIZU et al. 2002).

Different MICA polymorphisms have shown various associations with autoimmune diabetes in different populations and have also varied with age at onset (BERZINA et al. 2002). Significant evidence is there for the association between MICA5.1 and adult-onset T1DM (TORN et al. 2004). It is observed that the frequency of A5.1 allele is highest in early-onset patients, intermediate in intermediate-onset patients and lowest in late-onset patients. A5.1 allele is strongly associated with HLA-B7 and Cw7, suggesting that MICA*A5.1-B7-Cw7 haplotype contains a gene responsible for age-at-onset. A4 allele was associated with a susceptible haplotype, DR4-DQB1*0401, and A6 allele is associated with a protective haplotype, DR2-DQB1*0601, suggesting that the association of MICA with T1DM Linkage Disequilibrium (LD) with Class II haplotypes (GAMBELUNGHE et al. 2001).

The Swedish study showed that the frequency of MICA*A5 was not associated with age in the healthy control group; neither it was significantly associated with the presence of any of the major islet autoantibodies. This analysis revealed the independent associations of
MICA*A5 and class II gene polymorphism with childhood/young-onset T1DM (age at onset before 25 yr). The interaction between the MICA gene and the HLA class II polymorphism was revealed by the increase in the presence of both MICA*A5 and HLA-DRB1*03-DQA1*0501-DQB1*0201 and/or DRB1*04-DQA1*0301-DQB1*0302 in young-onset T1DM (Gambelunghe et al. 2001). MICA*A5 was significantly associated with T1DM in the age group of 1–25 years at diagnosis in European Caucasians (Gambelunghe et al. 2001). In Indians also MICA*A5 allele is associated with T1DM (Sanjeevi et al. 2002). It has been observed that patients who are young and below the age of 18 years show higher association with MICA*A5 allele (Sanjeevi et al. 2002; Kawabata et al. 2000). The contrary reports are also available, like the study from Italy which shows negatively associated with age at the clinical onset of diabetes (Gambelunghe et al. 2000).

Meta-analysis also confirmed that the MICA*A5 variant was significantly associated with an increased risk for T1DM, while MICA*A6 was significantly associated with a decreased risk (Sanjeevi et al. 2002). Depending upon the risked allele is present, MICA*A6 confers either susceptibility or protection. For example, MICA alleles when present with T1DM associated high-risk MHC Class II haplotypes (DQ2-DR17), reveal that MICA*A6 was associated with an increased risk for T1DM. In contrast, MICA*A6 reduced the risk from the HLA-DQ8-DR4 T1DM-risk haplotype. MICA*A9 showed increased risk for T1DM when associated with DQ8-DR4 haplotypes and also MICA*6 and MICA*9 are less frequently transmitted to affected offspring (Alizadeh et al. 2007; Field et al. 2008).

Bengali ethnic population is a mixed breed of population broadly of Dravidians, Mongols and Aryans. There is some amount of admixture of aboriginals like Mundari and Santhals (Mazumdar 1998). Thus, it may be stated that Bengali as a community is not too homogeneous (Raha 1975). In the present study, we examine whether the results of association of certain MICA alleles with T1DM observed in Western populations can be substantiated in our population. MICA profiles of T1DM patients are available from various parts of India, though they are almost all based on single hospital data (Sanjeevi et al. 2002; Mehra et al. 2007; Das et al. 2002; Das et al. 2002; Datwa et al. 2002; Mehra et al. 2001; Mehra et al. 2002; Tandon et al. 2002) and none of them are from a population. In the present study in order to capture the allelic variation of MICA more comprehensively, we designed a sequencing-based typing (SBT) method that yields the genotype information for MICA gene for exon 5. The aim of this study was to find the association of MICA alleles with T1DM among eastern Indian population.

MATERIALS AND METHODS

Materials

We collected intravenous blood samples (10 ml) from 134 T1DM cases and 137 controls, recruited from six different hospitals – Calcutta Heart and Research Clinic, Kolkata; Endocrinology Department, Calcutta Medical College and Hospital, Kolkata; Endocrinology Department, SSKM Hospital, Kolkata; Netaji Subhash Chandra Bose Cancer Research Institute, Kolkata; Rabindranath Research Institute of Cardiac Sciences, Kolkata; School of Tropical Medicine, Kolkata. This research was approved by the Institutional Review Board of the Anthropological Survey of India as well as by the ethical committee of the respective hospitals.

The present samples comprised of T1DM patients and age, sex and ethnic matched controls. Patients with the age of onset of T1DM below 39 years and presenting with or without
acute ketosis with absolute insulin dependence as evidenced by a deficient C-peptide secretion i.e., a C-peptide value less than 0.5 (0.6-3.2) ng/ml., and a duration of T1DM of at least one year, were included. The mean age of patients were 22.80±9.36 and of controls were 37.02±15.61. Patients of at least one year duration were selected to exclude acute or “honeymoon” phases. Any participant with an obvious secondary cause like steroid therapy or fibrocalculous pancreatic disease, chronic infection or co-morbid illness was also excluded.

Methods

DNA was isolated from the above samples following the standard protocol by phenol-chloroform method (SAMBROOK 2001). We have analyzed the exon-5 microsatellite repeat polymorphism of the MICA gene, by PCR primers flanking the TM region (Table 1). The primers were selected from previous work (GAMBELUNGHE et al. 2001; PARK et al. 2001). A total volume of 25µl was used for each PCR reaction. This included: 1.0µl of 10XPCRBuffer (containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl and 25mM MgCl2) (Roche Diagnostic, USA) 0.2µl of 10mM dNTPs (Roche Diagnostic, USA), 20pmol/µl of forward and reverse primers (Sigma), 1 unit of Taq polymerase (Roche Diagnostic, USA), and 100ng/µl of human DNA. The PCR carried out in an ABI Gene Amp PCR system 9700 were as follows: PCR conditions 96°C-10 minutes, 30 PCR cycles: 96°C- 30 seconds (denaturation) gradient specific temperature (56°C) - 30 seconds (annealing), 72°C- 45 seconds (extension), and two final cycles- 96º C for 10 min and 4º C for infinity. PAGE was used to examine the number of repeats in MICA microsatellite polymorphism. For PAGE, the vertical gel apparatus was used. The samples were electrophorosed in 6% polyacrylamide gel. 5 µl of the samples were mixed with 1/6th volume of gel loading dye. Electrophoresis was carried out at 60 volts/cm of the gel for 1.5 to 2.5 hours at room temperature depending on the size of the gel and the fragment size To visualize the DNA fragments, the gels were stained with ethidium bromide solution (0.5 µg/ml) for 30 minutes and then de-stained in water for 5 minutes. The PCR products were sequenced through automated DNA Analyzer (Applied Biosystems; Foster City, CA, USA).

Table 1. List of primers used in the present study to amplify genes

<table>
<thead>
<tr>
<th>Designation</th>
<th>Chromosome Location</th>
<th>Sequence</th>
<th>Annealing Temperature</th>
<th>Product Size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICA</td>
<td>6p21.3</td>
<td>F-5'</td>
<td>60º C</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCTTTTTTTTCAGGAAAGTGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCTTACCATCTCCAGAAACTGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(GAMBELUNGHE et al. 2001; PARK et al. 2001);

Statistical Analysis

All the HLA alleles at each locus were recoded as numbers to facilitate analysis with SPSS, v 17.0 for Windows statistical package (SPSS Inc., Chicago, IL). For each of the gene studied allele frequencies were calculated between T1DM cases and controls. Allele frequencies were estimated by gene counting method. Gene counting was carried out using SPSS. Odds
ratios were calculated for each gene using SPSS and recoding of the alleles was carried out in such a way that an allele with most similar frequency in both cases and controls was coded with the highest number to be used as the reference allele. Chi-square (χ²) analysis was carried out using SPSS. A p-value of < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

There are multiple conflicting reports in the literature about association of MICA gene and T1DM. The studies from Dutch, Italy, Korea, and North India show positive association of MICA alleles with T1DM (GAMBELUNGHE et al. 2001; GAMBELUNGHE et al. 2000; PARK et al. 2001); but at the same time, all the studies do not support an independent association between MICA variants and T1DM (SANJEEVI et al. 2002; ALIZADEH et al. 2007; KUMAR et al. 2012).

Some studies on trinucleotide STR (GCT/AGC)n in exon 5 of the MICA gene showed that allele A4, A5 or A5.1 predisposes to T1DM, and that MICA*A6 protects against T1DM. Whereas, another study found no association to any of the MICA alleles in T1DM(GAMBELUNGHE et al. 2007). These findings resulted in more controversy, with some investigators claiming that MICA has an HLA-independent effect on T1DM, whereas, others showed that the effect of MICA is confounded by LD between this locus and the HLA Class II DQ-DR haplotypes including DQB1*0201-DQA1*0501-DRB1*03 (DQ2DR17) and DQB1*0302-DQA1*0301-DRB1*04 (DQ8DR4) (KAWABATA et al. 2000; GAMBELUNGHE et al. 2001; ALIZADEH et al. 2007; PARK et al. 2001).

In our study we identified five alleles with four, five, six, and nine repeats of GCT (AGC for reverse primer) or five repeat of sequence GCT with one additional nucleotide insertion (GGCT). The alleles are A4, A5, A6, A9, and A5.1 respectively. We observed a significant positive association between A5 allele (OR=1.746, p=0.003) and T1DM. However, negative association was also observed between T1DM and the A6 allele (OR=0.406, CI=0.268-0.616, p=<0.01) in the present study (Table 1).

We also analyzed these samples for possible effect of MICA polymorphism on age of onset (Table 2). The result did not show any association with T1DM and age of onset and hence MICA gene has possibly no role for age of onset in the studied population.

<table>
<thead>
<tr>
<th>Ex 5 Microsatellite Alleles</th>
<th>Amplified Product bp</th>
<th>Cases (Total=268)</th>
<th>Controls (Total=274)</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>179</td>
<td>66</td>
<td>24.6</td>
<td>58</td>
<td>21.2</td>
<td>0.338</td>
</tr>
<tr>
<td>A5</td>
<td>182</td>
<td>98</td>
<td>36.6</td>
<td>68</td>
<td>24.8</td>
<td>0.003</td>
</tr>
<tr>
<td>A5.1</td>
<td>183</td>
<td>28</td>
<td>10.4</td>
<td>22</td>
<td>8.0</td>
<td>0.332</td>
</tr>
<tr>
<td>A6</td>
<td>185</td>
<td>42</td>
<td>15.7</td>
<td>86</td>
<td>31.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A9</td>
<td>194</td>
<td>34</td>
<td>12.7</td>
<td>40</td>
<td>14.6</td>
<td>0.517</td>
</tr>
</tbody>
</table>

CI=confidence interval
Most of the studies demonstrate a positive association between A5/A5.1 allele and T1DM. In European populations, the most common allele of the MICA-microsatellite is MICA*A5.1 (frequency 0.50), which contains five GCT repeats and a G insertion that causes a frame shift, leading to a premature stop codon and the truncation of the cytoplasmic tail. In Indian population, MICA*A5 is predominant among T1DM cases (Sanjeevi et al. 2002). The study from Cuttack showed that MICA*A5 allele is positively associated with T1DM (odds ratio(OR) =2.64, p < 0.05) (Sanjeevi et al. 2002). Further reports show association with age of onset (Sanjeevi et al. 2002). The recent study from North Indian population, shows the MICA*A5.1 allele with increased frequency in T1DM (29.6%, OR= 2.1, p =0.00017). However, it is also observed that it is in LD with HLA-B8-DR3-DQ2 haplotype (Kumar et al. 2012).

Significant associations between T1DM and MICA gene polymorphisms were observed in our Bengali-Indian study population. Our data showed a protective effect for the MICA*A6 allele. Also, the statistical significance is present for a positive association of the A5 allele with the T1DM. This is similar to the North Indian study. Our study and the North Indian study on HLA alleles highlights that T1DM patients have some common predisposing alleles such as DQA1*0201:DQB1*030101G, DQA1*050101:DQB1*030101G, and DQA1*0201:DQB1*030101G (Raha et al. 2013). Though, our study on Insulin VNTR gene did not show any predisposition with T1DM (Raha et al. 2011).

Eventually, our analysis of MICA Exon5 minisatellite brings forth the significance of the gene in the T1DM etiology. It was not only identifying the risk prone alleles but also to recognize the plausible protective alleles against T1DM. The observation from the present population indicates MICA*A6 (31.6%) frequency to be predominant, followed by MICA*A5 allele (25%) and MICA*A4 allele (21%).

Table 3: Allele frequencies of microsatellite polymorphism of MICA with respect to age-at-onset of type 1 diabetic patients

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Age at onset</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20 yrs (N=108)</td>
<td>≥20 yrs (N=30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>18</td>
<td>16.66</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>A5</td>
<td>47</td>
<td>43.52</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>A5.1</td>
<td>16</td>
<td>14.81</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>A6</td>
<td>18</td>
<td>16.66</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>A9</td>
<td>9</td>
<td>8.33</td>
<td>4</td>
<td>13.3</td>
</tr>
</tbody>
</table>

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O. RAHA et al: MICA REPEAT POLYMORPHISM IN TYPE 1 DIABETIS

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Oindrila RAHA1,2, B.N.SARKAR1, P.VEERRAJU2, G. SUDHAKAR2, P.RAYCHAUDHURI3, Soma MUKHOPADHYAY4, V.R.RAO5

1Antropološki pregled u Indiji, Kolkata-700016, Zapadni Bengal, Indija
2Odelenje humane medicine, Andhra Univerzitet, Visakhapatnam-530003, Indija
3Odelenje za endokrinologiju, Koledž medicine i bolnica. Kolkata, Indija
4Netaji Subhash Chandra Bose Institut za kancer,Delhi Univerzitet, 110007, Delhi, Indija
5Odelenje za antropologiju, Delhi Inivrzitet, 110007, Delhi, Indija

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