The Pro279Leu variant in the transcription factor MEF2A is associated with myocardial infarction


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Key words: myocardial infarction, risk factors, gene, MEF2A
Word count: 2634
Abstract.

Introduction: A myocyte enhancer factor 2A (MEF2A) mutation that segregated with coronary artery disease/myocardial infarction (CAD/MI) in a large family has been recently described. In addition, missense mutations in sporadic CAD patients were also reported. Together, these data suggested that mutations in exons 7 and 11 of MEF2A cause CAD/MI. However, this association was refuted by other study.

Methods: We analysed the genetic variation of exons 7 and 11 in a large cohort of Spanish MI patients and controls through SSCA and direct sequencing.

Results: A rare polymorphism, P279L, was detected both in patients and controls. Carriers of the 279 Leu allele would have a threefold risk of suffering MI compared to controls (p=0.009; OR=3.06, 95%CI=1.17-8.06). In addition, this allele was only found in controls < 50 years. Exon 11 showed a high degree of heterogeneity, caused by a polyglutamine (CAG)n polymorphism, but no significant differences in genotype or allelic frequencies were found.

Discussion: the 279 Leu allele should be a genetic risk factor for MI in our population. This effect could be a consequence of a reduce transcriptional activity on MEF2A with 279 Leu.
Introduction.

Myocyte enhancer factor 2 (MEF2) is a family of transcription factors composed of 4 members, MEF2A, MEF2B, MEF2C, and MEF2D. The MEF2 transcription factors bind to their cognate DNA sequence CAT(A/T)₄TAG/A present in the regulatory regions of several genes [1]. The promoter regions of several cardiac genes contain MEF2-binding sequences, and MEF2 would regulate inducible gene expression in muscle and endothelium of coronary arteries, among other cell-types [1-3].

Several acquired and inherited risk factors for coronary artery disease (CAD) and its most frequent complication, myocardial infarction (MI), have been described. CAD is caused by the development of atherosclerotic lesions in the walls of coronary arteries, and factors such as high cholesterol levels in blood, obesity, diabetes, and high blood pressure contribute to the origin and progression of atherosclerosis, are risk factors for CAD/MI [4]. A family history of CAD is also a significant risk factor for the disease, and in most of the patients the genetic susceptibility lies in common DNA-polymorphisms in candidate genes [4,5]. In addition, CAD segregates as a mendelian trait in several families. Some of the loci for these familial forms have been mapped to human chromosomes, but only recently a candidate gene has been identified [2,6]. MEF2A is located on chromosomal 15q26 region, which contains a susceptibility locus for familial CAD/MI, and a deletion in exon 11 of MEF2A that results in a seven amino acid deletion disrupting the transcriptional activation activity of MEF2A, has been identified as the causative mutation in a large CAD-MI family [2]. Subsequent analysis of the whole MEF2A gene in sporadic CAD patients identified a number of putative missense mutations in exon 7 in as many as 2% of CAD patients. These mutations would reduce the activation activity of MEF2A [7].

Although these initial studies supported the involvement of MEF2A-variants in the risk for CAD/MI, a recent report identified these variants in healthy individuals, thus raising the possibility that they were rare DNA-polymorphisms not directly related with the risk for CAD [8]. In order to clarify this issue, we searched for MEF2-variants in a large cohort of Spanish MI-patients and healthy controls, and compared the frequencies between both groups.
Methods.

Patients and controls. A total of 483 Spanish patients were analysed. These patients attended our Cardiology Departments between 1998 and 2004, and suffered a first episode of MI defined according to the WHO criteria [9]. Coronary angiograms were performed on all MI patients. According to the angiographic appearance, a vessel was regarded as diseased if it contained at least one stenosis involving >30% loss of lumen diameter.

The control group consisted of 1189 Spanish healthy individuals, who had not suffered episodes of cardiovascular disease. Data on clinical history were collected directly from controls by a normalised questionnaire. Ischaemic coronary, cerebral and peripheral vascular diseases were excluded on the basis of clinical history.

Individuals included in this study were Caucasians from two Spanish regions (Asturias, Northern Spain, and Murcia, Southeastern Spain) and gave their informed consent to participate in the study, which was approved by Ethical Committee of Hospital Universitario Central de Asturias

Single-strand conformation analysis (SSCA) of MEF2A. DNA was obtained from patients and controls following a salting-out method [10]. Exons 7 and 11 of MEF2A were polymerase chain reaction (PCR) amplified with primers obtained from the MEF2A Ensembl Transcript (ID ENST 00000346108): GTTTGTGCCAAAGTATTTTAA and AAGAATGAAAGTTGAAGAAAGG (exon 7, annealing at 58°C), and CTGCAAGCCATCTGACC and CCATCCTCATCCGTTTACAG (exon 11, annealing at 58°C). Each reaction contained 100 ng of genomic DNA, 10 pmol of each primer, 2mM MgCl₂, and 2mM of each dNTP, in a final volume of 20 µl. After 32 PCR-cycles of 95°-30s, 58°C-40s, and 72°C-40 s, three µl of each reaction were mixed with 15 µl of deionized formamide and denatured at 95°C, and 10 µl were electrophoresed on 12% polyacrylamide gels (29:1 acrylamide: bisacrylamide; 30 cm length). Electrophoresis was for 18 hours at 6W and room temperature. Gels were silver stained to visualise the electrophoretic pattern of each sample.

The nucleotide sequences corresponding to all the different electrophoretic patterns identified through SSCA, were characterised by direct sequencing of purified PCR-fragments on an automated ABI310 system, using the PCR-primers and BigDye-chemistry.

Genotyping of the MEF2A (CAG)n polymorphism. The number of repeats of the polymorphic polyglutamine (CAG) tract in exon 11 of MEF2A was determined in each patient and control through PCR, followed by capillary electrophoresis in an automated system. Briefly, 50 ng of genomic DNA were amplified in a final volume of 15µl with primers: CTTGCAAGCCATCTGACC and CCGATCACTGCCATAGG (annealing at 58°C). The forward primer was 5'-end fluorescence labelled with FAM. After 30 PCR-cycles, five µl of each reaction were mixed with 50µl of formamide, heated at 95°C for 5 min, size-fractioned through capillary electrophoresis for 20 min in an ABI 310 Genetic Analyzer, and analysed with the GENESCAN software.

Statistical analysis. Allele and genotype frequencies in patients and controls were compared through a chi-square test. The χ² test was also used to determine if the observed genotype frequencies in patients and controls differed from those expected under the Hardy-Weinberg equilibrium. Odds ratios (OR) with 95% confidence intervals (CI) were obtained to calculate the relative risk of MI associated with the genotypes. The power (% chance of detecting) was also calculated. All statistical
analyses were performed with the SPSS statistical package (v.11.0) and the EpiInfo v 3.3 software.
Results.
We used SSCA and direct sequencing to analyse exon 7 of the MEF2 gene in 483 MI patients. We found a rare exon 7 SSCA-pattern in 11 patients (Figure 1). After sequencing, we found that these were heterozygous for c.1250C>T (nucleotide number according to Genbank accession NM005587), which results in a missense change (P279L). The same genotype was found in 9 of the 1189 controls. The frequency of 279Leu carriers was significantly higher in MI-patients compared to controls (2.3% vs. 0.8%; p=0.009; OR=3.06, 95%CI=1.17-8.06) (Table 2). The 279L allele was only found in controls < 50 years; (9/890 ; range:30-47 years) (Table2). The power of the study for this polymorphism at significance level of 0.05 was 70%.

Exon 11 showed a high degree of SSCA heterogeneity. We sequenced cases representative of all the observed SSCA-patterns, and we found that the variation was caused by a previously described polymorphic trinucleotide CAG repeat (dbSNP rs3138597) (11). In addition, two previously described amino acid silent SNPs, S417S and G451G (dbSNP rs3730059 and rs325400, respectively) and 2 length-variant alleles in an adjacent poly-proline repeat (5 and 4 prolines), were identified. The (CAG)n polymorphism was also analysed in 211 patients and 301 controls through a fluorescent capillary-automated electrophoresis. Frequencies for the different (CAG)n alleles did not differ between patients and controls (Table 3). In addition, the 21-bp deletion previously found in a large family with CAD/MI was not found among our MI patients and healthy controls.

Table 1. Clinical characteristics in the MI patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (N=483)</th>
<th>Controls (N=1189)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age±SD (years)</td>
<td>54±12</td>
<td>52±12</td>
</tr>
<tr>
<td>Smokers</td>
<td>406 (84%)</td>
<td>440 (37%)</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>188 (39%)</td>
<td>262 (22%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>58 (12%)</td>
<td>120 (10%)</td>
</tr>
<tr>
<td>Male</td>
<td>430 (89%)</td>
<td>951 (80%)</td>
</tr>
<tr>
<td>≥ 1 diseased vessel</td>
<td>459 (95%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Genotype frequencies for the P279L single nucleotide polymorphism.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total participants</th>
<th>Younger participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients N=483</td>
<td>Controls N=1189</td>
</tr>
<tr>
<td>279PL</td>
<td>11 (2.3%)</td>
<td>9 (0.8%)</td>
</tr>
<tr>
<td>279PP</td>
<td>472 (97.7%)</td>
<td>1180 (99.2%)</td>
</tr>
</tbody>
</table>

* p=0.009; OR=3.06, 95%CI=1.17-8.06
† p=0.01; OR=2.9; 95%CI=1.04-8. Younger participants: male < 55 years and female < 65 years.

Table 3. Allele distribution of the (CAG)n repeat in the MI patients and controls populations

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184 (31%)</td>
<td>97 (16%)</td>
</tr>
<tr>
<td></td>
<td>316 (52%)</td>
<td>216 (51%)</td>
</tr>
<tr>
<td>Patients</td>
<td>136 (32%)</td>
<td>68 (16%)</td>
</tr>
</tbody>
</table>

* Number de (CAG)n repeats: 9, 10, 11 repeats; < 9: alleles with 6,7 or 8 repeats; >11: alleles with 12 or 13 repeats.
Discussion.
MEF2A belongs to a family of four closely related evolutionary conserved transcriptions factors (MEF2A to D). The role of MEF2 proteins in cardiovascular physiology has been established from animal models. Most MEF2A knockout mice die in the first week of life, and the few mutants that survive and reach the adulthood are also susceptible to sudden cardiac death. Heterozygous MEF2A +/- mice exhibits a normal phenotype and the cause of the sudden death in homozygous MEF2A -/- mice remains unknown [12]. Recently, Wang et al. described a MEF2A-mutation that segregated with CAD/MI in a large family with several affected members [2]. The mutation was a 7 amino acid deletion in exon 11, in a region of the protein required for nuclear localization. Because this mutant protein is sequestered into the cytoplasm and acts in a dominant negative manner, its final effect could be a downregulation of genes that contain the MEF2A-binding site in their promoters. MEF2A is expressed in endothelial and smooth muscle cells of coronary arteries, and MEF2A-mutations could disrupt the growth or differentiation of these cells, increasing the risk of developing CAD among mutation-carriers. In addition to this deletion in exon 11, a recent report described three putative missense mutations in exon 7 of MEF2A in 2% of sporadic CAD patients [7]. These rare nucleotide changes were absent in healthy controls, were clustered within or close to the major transcriptional activation domain of MEF2A, and significantly reduced the transcriptional activation activity of MEF2A, acting through a loss-of-function mechanism [7]. Together, these data suggested that mutations in the MEF2A gene were involved in the risk of CAD and MI, either familial or sporadic. In a recent report, Weng et al found some of these gene variants among healthy controls, challenging their role in the risk of CAD/MI and suggesting that MEF2A mutations were not a cause of familial or sporadic CAD [8]. However these authors did not exclude the presence of CAD in control group. To clarify this issue, we performed a screening of exons 7 and 11 in a large cohort of MI-patients and healthy controls from Spain. The only variant identified among patients was P279L but this was also found in controls. However, carriers of 279 Leu were significantly more frequency in patients, suggesting that this is a rare polymorphism associated with an increased risk for MI in our population. According to a previous report, 249 Leu significantly reduced the MEF2A transcription activity [7]. The (CAG)n repeat polymorphism in exon 11 was highly variable. Polyglutamine expansions are associated with several diseases [13]. However, no expansion of the MEF2A-polyQ repeat in our patients was observed. The association between P279L and MI was based on the comparison between 483 MI-patients and 1189 controls. Interesting, all the controls who were 289 Leu carriers were <50 years. The lack of 279Leu among elderly controls could reflect the predisposition to develop CAD/MI conferred by this MEF2A-allele. However, because we did not perform coronary angiography to confirm the lack of diseased vessels among the controls, our study could underestimate the risk conferred by 279Leu if this allele was associated with an increased risk to develop atherosclerotic lesions.
In conclusion, we found a significantly higher frequency of 279 Leu among MI patients. This allele could be a genetic risk factor for MI in our population.
Acknowledgements.
This work was supported by grants from Spanish Fondo de Investigaciones Sanitarias (FIS 03/05) and Red Temática de Centros de Genética Molecular (FIS C03/07).
References.
Figure 1. SSCA of exon 7 showing the distinct electrophoretic patterns for 279Pro homozygotes (Lanes 1, 3, 4, 8) and P279L heterozygotes (lanes 2, 6, 7, 9). Lane 5 is the electrophoretic pattern for a control carrier of a single nucleotide deletion in the flanking intron region (delC+14780/In6; nucleotide number according to ENST 00000346108).
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