Impacts and interactions of PDGFRB, MMP-3, TIMP-2, and RNF213 polymorphisms on the risk of Moyamoya disease in Han Chinese human subjects

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Polymorphisms of PDGFRB, MMP-3, TIMP-2, RNF213, TGFβ1, Raport and eNOS genes have been associated with Moyamoya disease (MMD) separately in studies, but their interactions on MMD have never been evaluated in one study. This study enrolled 96 MMD patients and 96 controls to evaluate the contributions and interactions of these polymorphisms on MMD in Chinese Hans. After genotyping, five polymorphisms loci were deemed suitable for analysis, rs3828610 in PDGFRB, rs3025058 in MMP-3, rs112735431 in TIMP-2, rs112735431 and rs148731719 in RNF213. Interactions of different loci on MMD were evaluated by multifactor dimensionality reduction (MDR) method. Signiﬁcantly higher frequencies of A allele and G/A genotype of rs112735431 in RNF213 were observed in MMD patients compared with controls (P = 0.011; P = 0.018, respectively). In the dominant model, G/A genotype of rs112735431 was associated with increased risk of MMD (P = 0.018). A higher frequency of G allele and C/G genotype of rs148731719 in RNF213 gene in patient than control group (P < 0.001; P < 0.01, respectively) was also detected. No signiﬁcant interaction between MMD and other three loci (P > 0.05) was detected. MDR analysis failed to detect any signiﬁcant interaction among these ﬁve loci in the occurrence of MMD (P > 0.05), but the combination of three loci (rs112735431 in RNF213, rs3828610 in PDGFRB, rs3025058 in MMP-3) could have the maximum testing accuracy (57.29%) and cross-validation consistency (10/10). The results indicated that RNF213 rs112735431 and rs148731719 may exert a signiﬁcant inﬂuence on MMD occurrence. Compared with this overwhelming effect, the inﬂuences of PDGFRB, MMP-3, and TIMP-2 on MMD may be unremarkable in Chinese Hans. There may be no prominent interaction among these ﬁve gene polymorphisms on the occurrence of MMD.

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1. Introduction

Moyamoya disease (MMD) is a progressive cerebrovascular disorder involving occlusive lesions of the bilateral internal carotid arteries (ICA) and their proximal branches, and is characterized by the formation of abnormal vascular network in the base of the brain (Kuroda and Houkin, 2008; Liu et al., 2011b; Ni et al., 2011; Scott and Smith, 2009).

The etiology of the disease is still unclear, but mounting evidence on ethnic predisposition and familial aggregation indicated that genetic factors may play a pivotal role in the pathogenesis of MMD (Roder et al., 2010a). Previous studies related MMD to several genetic changes, such as polymorphisms in platelet-derived growth factor receptor beta (PDGFRB) (Roder et al., 2010b), matrix metalloproteinase-3 (MMP-3) (Li et al., 2010), tissue inhibitors of metalloproteinase-2 (TIMP-2) (Kang et al., 2006), ring ﬁnger 213 (RNF213) (Kamada et al., 2011; Liu et al., 2011a, W. Liu et al., 2012; Miyatake et al., 2012a, 2012b; Miyawaki et al., 2012; Wu et al., 2012), transforming growth factor beta 1 (TGFβ1) (C. Liu et al., 2012), Raport (Liu et al., 2010), and endothelial nitric oxide synthase (eNOS) (Park et al., 2011).

Although various susceptibility genes of MMD obtained from different studies, some results cannot be replicated in validation studies of other populations. These conﬂicting results could be attributed to the following reasons, firstly, MMD is a complex disease with genetic heterogeneity absent from a classic pattern of inheritance.
The investigated SNPs might not have a causative effect on the pathogenesis of MMD, but rather they might only be related to the development or manifestation of the disease. Thus, single gene locus cannot sufficiently elucidate their genetic susceptibility, and its function may be dependent on the synergy of other genetic variations. Secondly, since the experiment is underpowered, even if a positive result based on crossing a threshold of statistical significance obtained, the study result will not reflect the fact owing to the inflated observation effect. When the same effects were regarded as observation indicator, the postosterity will not be able to replicate the result not representing the fact as previously stated reasons (Ioannidis, 2008). Previously, the impacts and interaction of these gene polymorphisms on the occurrence of MMD have never been compared and evaluated in a single study. We, therefore, hypothesize that polymorphisms of PDGFRB, MMP-3, TIMP-2, RNF213, TGFβ1, Raptor, and eNOS genes or some of them might contribute jointly in the development of MMD. We adopted a novel computational method, multifactor dimensionality reduction (MDR) to explore the gene–gene interactions in a case–control sample from Chinese Hans.

2. Material and methods

2.1. Subjects

A total of 96 consecutive unrelated patients with MMD from Nanjing Stroke Registry Program (NSRP) between April 2009 and December 2011 were recruited as the case group. All patients with MMD diagnosis were confirmed by digital subtraction angiography (DSA), magnetic resonance angiography (MRA), or computed tomography angiography (CTA). The inclusion criteria of MMD were in line with the guidelines revised in 1997 by the Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya disease) of the Ministry of Health and Welfare of Japan (Fukui, 1997). Patients with concomitant atherosclerosis, autoimmune disease, Down’s syndrome, sickle cell anemia, neurofibromatosis, irradiation to the head or neck, and those with unilateral intracranial carotid occlusion were excluded. All patients are sporadic individuals without family history of MMD. Ninety-six age- and gender-matched healthy individuals from the same demographic area were recruited as controls. To minimize the genetic heterogeneity, subjects were ethnically limited to Han Chinese. Written informed consent was obtained from each eligible participant prior to enrollment. The Institutional Review Board of Jinling Hospital approved the research protocol.

2.2. SNP selection

Fig. 1 is a flowchart delineating the process of SNP selection in this study. We initially searched PubMed and web of science databases to screen candidate gene loci responsible for MMD occurrence. Studies published before December 2011 were identified by extended computer-based searches using a combination of the following terms: (“Moyamoya disease” or “MMD”) and (“single nucleotide polymorphism” or “SNP”). The eligible studies have to fulfill the inclusion criteria: diagnostic criteria of MMD in studies were in accordance with the above-mentioned guidelines revised in 1997. Case reports, editorials and review articles were also excluded. Finally, eight gene polymorphism loci associated with MMD in previous papers were selected for genotyping, which included rs3828610 in PDGFRB, rs3025058 in MMP-3, rs8179090 in TIMP-2, rs112735431 and rs148731719 in RNF213, rs1800471 in TGFβ1, rs11220804 in Raptor, and eNOS 27 VNTRs. The too high melting temperature (Tm) as a result of the excessive GC sequence adjacent to the detection locus causes the ligation reaction interrupted, so the TGFβ1 gene was excluded. The eNOS 27 VNTRs was excluded because it is not suitable for the SNP genotyping method. After genotyping, Raptor was detected with only wild type, which was also excluded from data analysis.

Finally, five polymorphisms, rs3828610 in PDGFRB, rs3025058 in MMP-3, rs8179090 in TIMP-2, rs112735431 and rs148731719 in RNF213 were deemed suitable for the subsequent analysis.

2.3. Genotyping

Genomic DNA was extracted from anticoagulated venous blood by standard procedures using RelaxGene Blood DNA systems (TIANGEN Biotech (Beijing) Co., Ltd., Beijing) and diluted to working concentrations of 10 ng μl⁻¹ for genotyping and validation. Genotyping was performed by ligation detection reaction (LDR) method (Thomas et al., 2004; Yi et al., 2009), with technical support from Center for Human Genetics Research, Shanghai Genesky Biotech Co., Ltd. PCR primers were designed to amplify a fragment containing each variant. A detailed information concerning these SNPs was listed in Table 1. Technicians performing genotyping were blinded to case/control status of participants. The genotyping call rate for each SNP was over 95% in our study. A 10% random sample was selected to duplicate genotyping with complete concordance for quality control.

2.4. MDR

To evaluate the gene–gene interactions in the occurrence of MMD, MDR was applied according to a previously reported process (http://www.epistasis.org/open-source-mdr-project.html). MDR is a non-parametric, genetic model-free method for identification and characterization of susceptibility genes for common complex multifactorial human diseases. By sorting the multilocus genotypes into two levels of high- or low-risk, this method collapses high-dimensional into a single dimensional multilocus-genotype variable. The procedure of cross-validation and permutation tests generates the prediction accuracy and empirical P-values, minimizing false-positive results thanks to the repeating tests. With 10-fold cross-validation, the dates are divided into 10 divisions equally, and 9/10 of the date as the training set is tested initially, then the remaining 1/10 as the testing set is checked. Testing balanced accuracy (TBA), testing accuracy (TA), the cross-validation consistency (CVC), and the statistical significance of the model are provided through MDR software analysis. The model was statistically significant when the P-value derived from the permutation test was less than 0.05. Then the one with maximized TA and CVC is considered to be the best model among all multilocus models.

2.5. Statistics

Statistical power was performed with the Power and Sample Size Program (Dupont and Plummer, 1990). Among these five gene SNPs, we calculated the power based on the lowest susceptibility minor allele frequency (MAF) to claim that our study group can reach higher power than this. Based on our sample size, the statistical power of the present study achieved 93.5% at a 5% significance level for the allele A of RNF213 rs112735431. Hardy–Weinberg equilibrium (HWE) for genotypes was tested by a goodness-of-fit test. Only SNPs consistent with HWE were further analyzed. The distribution of allele and genotype frequencies between patients and controls was tested by Pearson’s chi-square test or Fisher’s exact test as appropriate. The dominant genotype frequencies between patients and controls was tested with HWE were further analyzed. The distribution of allele and genotype frequencies between patients and controls was tested by chi-squared test or Fisher’s exact test as appropriate. The dominant genetic model was applied. The odds ratio (OR) and their 95% confidence intervals (CI) were estimated for the association of SNPs with MMD. Statistical analyses were conducted using SPSS software (version 18.0). The significant level was deemed as 0.05.

3. Results

The clinical and demographic characteristics of the participants are summarized in Table 2. Of the 96 MMD patients and 96 healthy controls, 50.0% patients and 52.1% controls were male. The mean age...
of MMD onset was 43.0 ± 13.7 (mean ± SD) and that of controls was 42.6 ± 9.4 years. In the case group, fifty patients were from Anhui Province; forty-two patients from Jiangsu province; two patients from Henan province; two patients from Hubei Province; while in the control group, forty, fifty-three, two and one, respectively. Cases and controls were matched well concerning gender, age and demographic area. Most MMD patients had an initial presentation of cerebral infarction (54.2%) or cerebral hemorrhage (42.7%). One patient (1.0%) presented with seizures, one (1.0%) with vertigo, and one (1.0%) with headache.

None of these tested SNPs demonstrated deviation from HWE at the significant level of 0.05. The genotype results are presented in Table 3. Among these SNPs, the frequencies of A allele and G/A genotype of rs112735431 in RNF213 gene were significantly higher in MMD patients than in control groups (5.21% vs. 0.52%, OR = 10.50, \( P = 0.011 \); 8.33% vs. 1.04%, OR = 8.74, \( P = 0.018 \), respectively). In the MMD group, the G allele and G/G genotype of rs148731719 in RNF213 gene had significantly higher frequency than in the control group (\( P < 0.001 \); \( P < 0.01 \), respectively). No significant differences were detected concerning allelic and genotypic distributions of
other three polymorphisms between patients and controls (all P > 0.05). Considering the rare variant of A/A genotype, we combined A/A with G/A to form a dominant model, in which rs112735431 was associated with a significant increased risk of MMD (GG vs. GA/AA, OR = 9.83, 95% CI 1.22–79.17, P = 0.018) and MMP risk (P > 0.05).

Considering the rare variant of A/A genotype, we combined A/A with G/A to form a dominant model, in which rs112735431 was associated with a significant increased risk of MMD (GG vs. GA/AA, OR = 9.83, 95% CI 1.22–79.17, P = 0.018) and MMP risk (P > 0.05).

The results of MDR for analyzing interactions of different gene polymorphisms in MMD risk were shown in Table 4. The single-locus model involving rs148731719 in RNF213 generated a testing accuracy (TA) of 50.52% and a cross-validation consistency (CVC) of 5/10, which is consistent with the results of previous direct genetic association analysis. Although both three- and five-locus models have the maximum CVC of 10/10, the former one has the highest testing accuracy. Hence, we considered that the best interaction model was the three-locus model (rs112735431 in RNF213, rs3828610 in PDGFRB, rs3025058 in MMP-3), with a highest testing accuracy of 57.29% and a perfect CVC of 79.17 (P = 0.018), or RNF213 was also associated with MMD (Kamada et al., 2011; Liu et al., 2011a; Miyatake et al., 2012a). RNF213 knockout zebrafish presented abnormal sprouting vessels in the head region (Liu et al., 2011a). The MMD patients carrying A/A genotype of RNF213 gene preferred to early age of onset, infarctions at initial symptoms and were inclined to involve posterior cerebral artery (Miyatake et al., 2012a; Wu et al., 2012). In this study only adult-onset MMD patients were included. In China, most of child patients are regularly sent to specialized children’s hospitals. Therefore, as a general hospital, we have very few cases of child-onset MMD, and patients in this cohort were relatively older than that reported in other studies. As a result, there is only one A/A homozygote in this cohort of adult MMD patients. Lack of this genotype prevented us from evaluating associations between A/A homozygote and MMD risk. In a study by Kamada et al. 2011, no pR8459K carriers were detected in 400 Caucasian healthy}

### Table 2

Summary of basic characteristics of cases and controls.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>48/48</td>
<td>50/46</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>43.0 ± 13.7</td>
<td>42.6 ± 9.4</td>
</tr>
<tr>
<td>Allele and genotype distribution frequencies of the four SNPs between cases and controls.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

We selected eight gene polymorphism loci associated with MMD risk from previous studies, due to genotyping failure and single-wild genotype, three genes TGFB1, eNOS and Raptor were excluded. Finally, five gene SNPs involving in similar pathological mechanisms of artery lesion were evaluated for their impacts and interactions on MMD risk. Single-locus analysis showed that rs112735431 and rs148731719 in RNF213 associated significantly with MMD. No significant gene–gene interaction on MMD risk was detected. RNF213 gene encoding a protein with a ring finger motif was considered to function as an E3 ubiquitin ligase which is involved in the cellular processes, DNA repair, and signal transduction pathways. Therefore, RNF213 gene has been related to multiple human diseases (Deshaies and Joazeiro, 2009). The region containing rs112735431 variant is a main transcript of RNF213. However, whether rs112735431 of RNF213 is capable of influencing ubiquitin ligase activity or changing the transcription and translation remains unclear. Recently, several genome-wide and locus–specific association studies as well as mutational analysis all identified that the single base substitution, rs112735431 (c.14576G>A) in exon 60 of RNF213 was associated with MMD (Kamada et al., 2011; Liu et al., 2011a; Miyatake et al., 2012a). RNF213 knockout zebrafish presented abnormal sprouting vessels in the head region (Liu et al., 2011a). The MMD patients carrying A/A genotype of RNF213 gene preferred to early age of onset, infarctions at initial symptoms and were inclined to involve posterior cerebral artery (Miyatake et al., 2012a; Wu et al., 2012). In this study only adult-onset MMD patients were included. In China, most of child patients are regularly sent to specialized children’s hospitals. Therefore, as a general hospital, we have very few cases of child-onset MMD, and patients in this cohort were relatively older than that reported in other studies. As a result, there is only one A/A homozygote in this cohort of adult MMD patients. Lack of this genotype prevented us from evaluating associations between A/A homozygote and MMD risk. In a study by Kamada et al. 2011, no pR8459K carriers were detected in 400 Caucasian healthy

### Table 3

Allele and genotype distribution frequencies of the four SNPs between cases and controls.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Characteristics</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Odds ratio (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFRB</td>
<td>rs3828610</td>
<td>C</td>
<td>132 (68.75)</td>
<td>123 (64.06)</td>
<td>0.81 (0.53–1.24)</td>
<td>0.287</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
<td>60 (31.25)</td>
<td>69 (35.94)</td>
<td>1.00</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td></td>
<td>48 (50.00)</td>
<td>38 (39.58)</td>
<td>0.61 (0.33–1.11)</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td></td>
<td>36 (37.50)</td>
<td>47 (48.96)</td>
<td>1.06 (0.52–2.17)</td>
<td>0.816</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td></td>
<td>12 (12.50)</td>
<td>11 (11.46)</td>
<td>0.86 (0.27–3.16)</td>
<td>0.191</td>
</tr>
<tr>
<td>MMP3</td>
<td>rs3025058</td>
<td>6A</td>
<td>167 (86.98)</td>
<td>153 (79.69)</td>
<td>0.55 (0.29–1.03)</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5A</td>
<td>25 (13.02)</td>
<td>39 (20.31)</td>
<td>0.59 (0.34–1.02)</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6A/6A</td>
<td>73 (76.04)</td>
<td>61 (32.54)</td>
<td>1.00</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6A/5A</td>
<td>21 (21.88)</td>
<td>31 (32.29)</td>
<td>0.57 (0.30–1.08)</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5A/5A</td>
<td>2 (2.08)</td>
<td>4 (4.17)</td>
<td>0.42 (0.07–2.36)</td>
<td>0.416</td>
</tr>
<tr>
<td>TIMP2</td>
<td>rs8179090</td>
<td>G</td>
<td>160 (83.33)</td>
<td>161 (83.85)</td>
<td>1.05 (0.57–1.94)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>32 (16.67)</td>
<td>31 (16.15)</td>
<td>1.04 (0.61–1.78)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>69 (68.75)</td>
<td>67 (69.79)</td>
<td>1.00</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/C</td>
<td>28 (29.17)</td>
<td>27 (28.31)</td>
<td>0.95 (0.46–2.00)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/C</td>
<td>2 (2.08)</td>
<td>2 (2.08)</td>
<td>0.02 (0.15–4.82)</td>
<td>1.000</td>
</tr>
<tr>
<td>RNF213</td>
<td>rs112735431</td>
<td>G</td>
<td>182 (94.79)</td>
<td>191 (99.48)</td>
<td>10.50 (1.33–82.80)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>10 (5.21)</td>
<td>1 (0.52)</td>
<td>0.50 (0.01–2.35)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>87 (90.63)</td>
<td>95 (98.96)</td>
<td>1.00</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/A</td>
<td>8 (8.33)</td>
<td>1 (0.64)</td>
<td>8.97 (0.37–21.27)</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>1 (1.04)</td>
<td>0 (0)</td>
<td>NA</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dom</td>
<td>9.83 (12.22–79.17)</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>192 (100.00)</td>
<td>170 (88.54)</td>
<td>1.00</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>0 (0)</td>
<td>22 (11.46)</td>
<td>NA</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>96 (100.00)</td>
<td>85 (88.54)</td>
<td>1.00</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/A</td>
<td>0 (0)</td>
<td>11 (11.46)</td>
<td>NA</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dom</td>
<td>9.83 (12.22–79.17)</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dom: dominant model (homozygote frequent allele vs heterozygote + homozygote rare allele); NA: not applicable.
controls, and no mutation was identified in five Caucasian MMD patients after the full sequencing of RNF213. In a case–control study, Liu et al. [2011a] analyzed RNF213 in 50 Caucasian MMD patients and 384 Caucasian controls. No p.R4859K but four other variants of RNF213 was detected in four MMD patients. These results suggested that the genetic background of MMD in Caucasian is different from that in Asian populations. The reported low incidence of MMD in non-Asians may be a result of a low prevalence of RNF213 mutation. Given the significant difference on the RNF213 variance among ethnic populations, the relevance of RNF213 polymorphism detected in this study may not be generalized to other ethnic populations directly. Large cross-race studies are warranted to answer this question.

Unlike that reported in other studies, this study failed to detect any significant association between PDGFRR, MMP-3, or TIMP-2 gene variants and MMD occurrence. The possible reason is as follows, PDGFRR rs3828610 was reported to be associated in Caucasian patients with MMD, and these inconsistent results may be a result of differences in ethnicity involved. The incidence of MMD varied greatly throughout the world, the highest being in the Japanese population, which was about twice as high as in Nanjing, five times higher than in Hawaii and 20–40 times higher than in Taiwan and Iowa. In addition, 15% of MMD patients had a family history in Japan, while 1.5% in Nanjing (Kleinloog et al., 2012). We reviewed the family history of each patient enrolled in this study, and detected only one patient (1/97, 1.03%) with a family history of MMD in patient screening stage. Compared with sporadic MMD, familial MMD is rarer in this study. This result is in accordance with that of a large epidemiology study in Nanjing, in which a low familial MMD (1.48% of all MMD) was also observed (Miao et al., 2010). However the rate is lower than that reported in a recent study from Beijing, in which the familial proportion of MMD is 5.2% (Duan et al., 2012). We think there may exist some asymptomatic or mild familial MMD patients who neglected their diseases. But this kind of patients may be diagnosed by regular physical examination, which is more likely in regions with advanced economic development, such as Beijing. In the Japanese population, the proportion of familial MMD was reported as 12.1% in a national survey (Kuriyama et al., 2008). This observed discrepancy concerning familial MMD prevalence in different ethnic populations may be an integrated reflection of genetic and environmental differences. To increase the homogeneity of the enrolled subjects, we excluded this only one patient with a familial history of MMD, and limited the subjects to sporadic MMD patients. TIMP-2 rs8179090 polymorphism was found associated with familial MMD patients, while our patients were sporadic MMD patients. MMP-3 rs3025058 polymorphism was associated with MMD patients in a much larger cohort than our relatively small samples.

As a relatively novel method for analyzing genotype–phenotype association, MDR exhibited several advantages over traditional methods such as logistic regression. MDR can detect the high-order gene–gene interactions in relatively small samples with increased power, avoid the curse of dimensionality, reduce type I errors, and is not limited to a special genetic model. So this method is qualified for analyzing the relationship between five gene polymorphisms and risk of MMD.

No significant gene–gene interactive effects of these genes in MMD occurrence were detected in this study. This unexpected result may be explained by three possibilities. The first explanation lies in that the effect of the RNF213 gene on MMD may be predominant, and the interactive effects among genes may be overwhelmed by this main effect. A study including 72 Japanese MMD patients and 45 controls revealed that the polymorphism of rs112735431 in RNF213 could increase the risk for MMD with an OR of 190.8 (P = 1.2 × 10⁻⁴). (Kamada et al., 2011). In another larger sample (204 Japanese patients and 283 controls) study, the heterozygous variant significantly increases the risk with an OR of 236 (P < 0.001), and the homozygous variant with an OR of 259 (P < 0.001) (Miyatake et al., 2012a). The strong association of this polymorphism with MMD was confirmed in a large-scale study based on East Asian populations (251 cases and 707 controls) with an OR of 111.8 (P = 10⁻¹³) (Liu et al., 2011a). The second explanation is that these genes, although they result in similar pathological outcome (intra-mitima injury and artery occlusion), function via different pathophysiological pathways. Variations in PDGFRR gene may lead to decreased cellular reaction on VSMC, prolonged intimal cytokine exposure, and intimal thickening, all of which increase the possibility of arterial wall injury and risk of MMD (Minato et al., 2007). Variants in MMP-3 gene and its inhibitor TIMP-2 gene were associated with increased matrix protein deposition (Li et al., 2012) and decreased matrix protein degradation, both of which may cause angiostenosis in MMD (Fu et al., 2011). But for RNF213 gene, little is known concerning its mechanisms in MMD etiology. The third reason is that Chinese MMD patients have a relatively lower genotype frequency of rs112735431 of RNF213 than Japanese and Korean patients. Therefore, MMD in Chinese population has more complex genetic backgrounds compared to Japanese and Korean MMD. Due to a genotyping failure, some potential SNPs associated with MMD were not included in this study. Further large studies with extensive coverage of potential genes are warranted.

5. Conclusions

In conclusion, polymorphisms in rs112735431 and rs148731719 of RNF213 gene may associate with MMD, and influences of PDGFRR, MMP-3, and TIMP-2 polymorphisms on MMD occurrence may be unremarkable in the Chinese population. There may not exist interactive effects among polymorphisms of PDGFRR, MMP-3, TIMP-2 and RNF213 genes on the occurrence of MMD. For the first time, this study gave prominence to the importance of gene–gene interactions over single-locus analysis with regard to the genetic pathogenesis of MMD.

Conflict of interest statement

We have no conflicts of interest to disclose.

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