Lack of association between SMOC2 polymorphism and age-related macular degeneration in Jordanian Arabs

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Age-related macular degeneration (AMD) is a late-onset disorder that affects the posterior part of the retina, the macula. The AMD is the most common cause of permanent elderly-blindness, and is a complex genetic disorder, in which several genetic, behavioral, and environmental factors can affect the disease onset. Characterizing risk factors for AMD is of great importance for improving our understanding of the disease's etiology, and for establishing preventive strategies. Genome-wide linkage analyses, as well as association analyses have identified several susceptibility loci that are likely to increase the preponderance to AMD.[1] In 2007, Radeke et al[2] found SMOC2 (SPARC-related modular calcium-binding protein), which is a matricellular protein that play a role in the regulation of interactions between cells and the extracellular matrix, to be differentially expressed in the macula of AMD patients.[2] This suggests that SMOC2 might affect the preponderance of AMD.

In this study, we aimed to investigate the contribution of SMOC2 single nucleotide polymorphism (SNP) rs13208776, to the risk of AMD in a set of Jordanian Arab population. Furthermore, we worked out the allele frequency of the SNP for the same population under study. The results we obtained give no indication for the association between SMOC2 SNP and the risk of AMD.

Study cases were recruited and diagnosed by an ophthalmologist from a large hospital, serving the northern part of Jordan (King Abdullah Hospital) from June 2008 until June 2011. Patients had exudative AMD in at least one eye with no other retinal disease, such as macular dystrophy, or diabetic retinopathy. A complete ophthalmologic examination was carried out for all patients including fundus examination, best corrected visual acuity measurements, and retinal photographs.

The patients underwent fluorescein angiography, and for some patients, optical coherence tomography and indocyanine green angiography were carried out. All 42 AMD patients completed a detailed questionnaire that included information regarding age, age of onset, gender, and family structure. All 92 controls were over 40 years, and reported no history of AMD. Informed consent was obtained from all participants in the study. This study was approved by the ethics Institutional Review Board of the Jordan University of Science and Technology.

Genomic DNA from patients and controls was extracted from peripheral blood using a genomic purification kit (Qiagen). The SMOC2 rs13208776 SNP was genotyped using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) assay. Detailed procedure and conditions were reported previously.[3]

Data were entered in Excel (Microsoft Corporation) for the calculation of allele and genotype frequencies. Hardy-Weinberg equilibrium was assessed by Chi square test. Allelic association *p*-values were determined using a Chi square test between cases and controls. Genotypic association *p*-values were determined by the Freeman-Halton extension of Fisher's exact test for a 2 × 3 contingency table, which evaluates the occurrence of all 3 genotypes as an array between the cases and controls. Logistic regression models are used to calculate odds ratio (OR), and 95% confidence interval (95% CI) for disease risk. A web-based calculator was used to compute *p*-values (OpenEpi: www.openepi.com). A $p<0.05$ was considered to be statistically significant for all tests. Power calculations were carried out by the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). Accordingly, we needed 31 cases to achieve 80% power to detect allelic association for SMOC2 SNP. Thus, our sample size had enough power to detect association.

We conducted DNA genotyping for 42 AMD patients, of whom 28.6% were diagnosed to have dry-AMD, 16.7% with wet AMD, 23.8% with bi-lateral dry and wet AMD, and 31.0% were unclassified. The gender of the cases was biased to males (73.5%), which might indicate that Jordanian men have higher AMD-risk than Jordanian women do.

The RFLP genotyping for the controls revealed a frequency of 8.7% for homozygote minor genotype (AA), and 44.6% for heterozygote genotype (GA).

Disclosure. This work was supported by a generous grant from the King Hussein Institute for Biotechnology and Cancer and from the Jordan University of Science and Technology, Irbid, Jordan.

In AMD patients, the homozygote minor genotype frequency is 9.5% which is higher than the controls, and for the heterozygote group are 35.7% which is lower than the controls (**Table 1**). In addition, the allelic frequency for the minor allele (A) was found to be similar in the controls (31.0%) and AMD patients (27.4%) (**Table 1**). Statistical analysis showed neither significant allelic (p=0.64), nor significant genotypic (p=0.62) differences between the AMD patients and controls (**Table 1**)

Our data suggest no association between the SMOC2 rs13208776 SNP and AMD in the Arab Jordanian population. This study determines the association between SMOC2 SNP and AMD. A previous study showed a significant decrease in the SMOC2 expression between the macula and extramacular region in AMD patients, suggesting that SMOC2 might be linked to AMD pathogenesis either directly or indirectly.[2] Furthermore, recently SMOC2 rs13208776 was reported to be associated with autoimmune generalized vitiligo[4] and AMD pathogenesis seems to have autoimmune basis,[1] which further supports the candidacy for SMOC2 in AMD etiology. Based on these facts, our aim was to examine the association of the SMOC2 rs13208776 polymorphism in an Arab Jordanian AMD patients compared to controls.

The SMOC2 SNP could play a dual role in AMD pathogenesis, as either a protective, or risk factor. The protective role could be explained by the fact that overexpressed SMOC2 synergize with VEGF and bFGF to stimulate DNA synthesis and angiogenesis. Thus, the down expression of SMOC2 might decrease the disease's severity by slowing down the choroidal neovascularization (CNV). On the other hand, as SMOC2 also play a role in calcium binding; due to the presence of 2 HF-hand calcium-binding domains, and as calcium transport affect cell adhesion it could be hypothesized that SMOC2 SNP impairs cell adhesion and attachment, thus contribute to the severe RPE detachment.

In this study on 42 AMD patients compared to 92 controls, we did not detect any association between the SMOC2 gene and AMD (p>0.05). If further studies in different populations, or different SNPs within SMOC2 reach the same conclusion, this may suggest that the reported microarray results, a decrease in SMOC2 expression,[2] was due to an indirect effect of other associated proteins. In this study, AMD patients were significantly older than the control patients. However, age in quartiles was not significantly associated with allelic frequencies and means of age did not differ according to the SNP genotypes (data not shown). Our study proved that SMOC2 does not play a role in the genetics of AMD, which would suggest that its differential expression in the macula of AMD patients might be a consequence of the disease, rather than causative to it. This is an important finding that suggests looking at other factors that contribute to regulating the expression of SMOC2, those other "unknown" factors might play a role in the etiology of AMD rather than SMOC2 itself.

In this study, we found an allele frequency of 31% for the SMOC2 SNP. According to the international HapMap project,[5] the allele frequency of our population is comparable to the allele frequency for the Utah population with western and southern European ancestry (34.1%), and Italy (28.2%). However, the SMOC2 SNP is almost absent in Asians from Japan, China, and in Nigeria with 0% allele frequency, it also has a low frequency in individuals from African ancestry in southwest USA with an allele frequency of 4.1%.[5]

The AMD is a multifactorial disease with many genes and environmental triggers involved. The ARMS2 and CFH are 2 major genes that are associated strongly with AMD with many replicating studies. On the other hand, other genes as well, have been reported such as: Apolipoprotein E, Complement component 3, Elastin, FB, scavenger receptor class B member 1 (SCARB1), vascular endothelial growth factor, and so forth. Given these facts and in this context, it maybe important as well to describe negative associations. One limitation of our study is the low sample number, even though our power calculation suggests enough power to detect association, more sample number would give more robust conclusions.

In conclusion, we could not detect an association between SMOC2 gene and AMD. This study tests the candidacy of SMOC2 gene to the etiology of AMD.

Table 1 - Genotype and allele frequency of rs13208776 of SMOC2 gene among age-related macular degeneration (AMD) patients and controls included in a study in King Abdullah Hospital, Irbid, Jordan.

Genotypes and alleles	AMD patients	Control	*P*-value	Odds ratio (95% CI)
GG	23 (54.8)	43 (46.7)	0.62	
GA	15 (35.7)	41 (44.6)		
AA	4 (9.5)	8 (8.7)		
G	61 (72.6)	127 (69.0)	0.64	0.84 (0.47-1.49)
A*	23 (27.4)	57 (31.0)		

CI - confidence interval; A* - minor allele of rs13208776

Our study was based on previous data linking differences between SMOC2 expressions in the macula of AMD patients. Further studies in a larger groups of patients or different study populations might be needed to confirm or negate a link between SMOC2 and AMD.

Acknowledgement. *We gratefully acknowledge all participants in this study. Likewise to Duaa Abuarqoub for helping out in the sample collection and technical help.*

Received 26th May 2012. Accepted 5th August 2012.

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