Article

SGCD a novel candidate gene for age-related macular degeneration

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Abstract: 1) Background: *CFH* and *HTRA1* genes are traditional markers of increased risk of AMD across populations. Recent findings suggest that additional genes, for instance, in the dystrophin-associated protein complex might be promising markers for AMD. 2) Methods: Here, we performed a case-control study to assess the effect of *SGCD* single nucleotide polymorphisms (SNPs), a member of this protein family, on AMD diagnosis and phenotype. We performed a case-control study of 134 cases with 134 unpaired controls. Cases were 60 years or older (CARMS grade 4-5, as assessed by experienced ophthalmologists following the AAO guidelines), without other retinal disease or history of vitreous-retinal surgery. Controls were outpatients aged 60 years or older, with no drusen or RPE changes on fundus exam and negative family history of AMD. We examined SNPs in the *SGCD* gene: rs931798, rs140617, rs140616, and rs970476 by sequencing and RT-PCR. Genotyping quality check and univariate analyses were performed with PLINK v.1.9. Furthermore, logistic regression models were done in SAS v.9.4 and haplotype configurations in R v.3.3.1. 3) Results: After adjusting for clinical covariates, the G/A genotype of the SGCD gene (rs931798) significantly increases the odds of being diagnosed with AMD in 81% (1.81, 95%CI 1.06-3.14, p=0.031), especially the geographic atrophy phenotype (1.82, 95%CI 1.03-3.21, p=0.038) compared to the G/G homozygous. Moreover, the GATT haplotype in this gene (rs931798, rs140617, rs140616, and rs970476) is associated with lower odds of AMD (Adjusted OR 0.13, 95%CI 0.02-0.91, p=0.041). 4) Conclusions: *SGCD* is a promising gene for AMD research. Further corroboration in other populations is warranted.

Keywords: *SGCD*, delta-sarcoglycan, candidate-gene approach, AMD.

Peer-reviewed version available at Genes 2018, 9, 467; doi:10.3390/genes9100467

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in individuals over 60 years in developed countries [1] and the third leading cause worldwide[2]. This condition accounts for ~14% of all legal blindness[1,3]. AMD is a complex, polygenic, and multifactorial disease characterized by progressive photoreceptor degeneration and cell death[4]. During the early stage of the disease, most patients have no noticeable decline of visual acuity[5] even though drusen deposits and pigmentary changes can be observed by fundoscopy[6]. Over a five-year period since the onset of the disease, up to 5% of asymptomatic patients will develop one or a combination of two advanced forms of AMD: (1) choroidal neovascularization (CNV, exudative, or wet AMD) or (2) geographic atrophy (GA) of the retinal pigment epithelium (nonexudative or dry AMD)[7]. The rate of progression between phenotypes is poorly understood. Hence the importance of studying risk factors (genetic and environmental) for these advanced forms.

Even though the AMD phenotype varies, both forms GA and CNV share several susceptibility genes including two major loci in complement factor H (*CFH*)[8] and in the age-related maculopathy susceptibility 2 (*ARMS2*)/HtrA serine peptidase 1 (*HTRA1*)[9]. Additionally, through genome-wide association (GWAS) and case-control studies, multiple genes in the complement pathway (*C2, CFB, C3, CFL, VNT*)[10], lipids and HDL cholesterol metabolism (*LIPC, CETP, APOE*)[11], oxidative stress, angiogenesis (*VEFGA*), and other candidate genes in several biological pathways (*TRL3, TRL4*) have been identified[12]. The importance of the identification of these genetic variants is that they might influence the onset, progression and possibly the response to treatment[7]. Currently, there are a total of 52 independently associated common and rare variants distributed across 34 loci. Since AMD is a multifactorial disease, many more loci might not be entirely elucidated yet[13]. Of importance, recently, an epistatic module describing the interaction of multiple loci for AMD, proposed the *SGCD* gene (an unexpected result) as a possible bridge between the aging process and the immune dysregulation in AMD[14]. Tang W. et.al evidenced *in silico* a significant interaction between *SGCD* and the *SCAPER* gene that correlated with the *MASP1* and *MASP2* genes which activate the complement pathway[14]. Additionally, Wan-Yu, Lin et al. in a secondary prioritized subset analysis of an AMD GWAS identified several *in silico* single nucleotide polymorphisms (SNPs) in the *SGCD* gene significantly associated with AMD[15]. Bioinformatics analyses so far have elucidated both findings, but no study has assessed these relationships in vivo.

The *SGCD* gene located on chromosome 5q33.2-q33.3 encodes for δ-sarcoglycan (δ-SG), an integral membrane protein part of the sarcoglycan-sarcospan complex. δ-SG also is an essential component of the dystrophin-associated protein complex (DAPC). Sarcoglycans (SGs) as with other DAPCs were first described in muscular tissues, where they promote muscle membrane stability. Together with the dystrophin-glycoprotein complex, SGs also connect the extracellular matrix with the cytoskeleton and regulate signal transduction[16]. Molecular alterations in the *SGCD* gene have been linked to limb-girdle muscular dystrophy type 2 (LGMD2F) and dilated cardiomyopathy type 1L[17]. LGMD2F patients have visual disturbances not fully elucidated, such observational evidence, underscore a potential role of SGs in retinal function[18].

In the murine retina, SGs are partially dystrophin independent[19]. δ-SG is predominantly expressed in the outer retina, near the Müller glial cell fibers and the outer limiting membrane[20]. The correlation between the location of δ-SG expression and the higher metabolic regions of the retina might be indicative its function, this being the degradation of extracellular matrix through different enzymes; such as matrix metalloproteinases, cell cycle regulation, and DNA repair[14]. Some *SGCD* polymorphisms have also been implicated in drusen formation[14]. Furthermore, in a manuscript in preparation we have evidenced that the deficient Sgcd-null mice exhibit signs of retinal degeneration and frailty, highlighting the pivotal role of *SGCD* for the normal retinal function [21]. To date, there is limited or no information evaluating the relationship between *SGCD* polymorphisms, those reported

in silico, and the advanced forms of AMD. Hence, we aim to assess the effect of *SGCD* polymorphisms on AMD diagnosis and phenotype, using a candidate-gene approach, among Hispanics from Mexico.

2. Materials and Methods

We performed a hospital-based case-control study of 134 age-related macular degeneration cases with 134 unpaired controls. All participants were recruited at Institute of Ophthalmology "Conde de Valenciana" in Mexico City and agreed to participate by signing an informed consent. Our sample size was calculated taking 80% power to detect a significant difference in odds ≥ 1.50 considering the allelic frequency of our SNPs. 134 incident cases were assessed, and their status was assigned by experienced blinded ophthalmologists who stratified AMD phenotypes following the American Association Ophthalmology (AAO) clinical guidelines[22]. Controls were taken randomly from a pool at our clinic and were those without any evidence of advanced AMD at baseline but who could develop it. Our full case and control definition is displayed in Supplementary Table 1. We excluded cases with ophthalmological diseases that limited a thorough retinal examination by fundoscopy (e.g. cataracts) or those who withdraw their informed consent. To ascertain exposure, we recorded demographic characteristics, explicitly known risk factors for AMD, from electronic medical records such as age, gender, smoking history, and other clinical comorbidities (hypertension and diabetes). For our genotype data, exposed cases were those carriers of the heterozygous or less frequent homozygous alleles (See below, Statistical approach). All our assays were done in duplicate by blinded experienced laboratory technicians. We genotyped four SNPs in the *SGCD* gene significantly associated with AMD in bioinformatic analyses of Wan-Yu Lin et al.[15], each of these, was developed by two methods. First, for the SNP rs970476, we performed allelic discrimination assays with TaqMan probes on RT-PCR by duplicate. The rest (rs931798, rs140617, and rs140616) were close enough (less than 300 base pairs across) to be genotyped by automated sequencing (See below, DNA sequencing). The Institutional Review Boards approved this study at the Institute of Ophthalmology "Conde de Valenciana" (Clinical site) and Universidad Panamericana (Genotyping site). Our procedures and data collection followed the tenets of the Declaration of Helsinki, and all data was handled as directed by HIPAA.

To allocate exposure in our cases and controls, we began by extracting genomic DNA from peripheral blood samples that were collected in EDTA tubes at baseline. We then immediately processed them using the PureGene DNA purification whole Blood Kit (QIAGEN, Germantown, MD, USA) following the manufacturers' specifications. DNA concentrations and purity were quantified using a Multiskan™ GO Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). To evaluate DNA integrity, we performed a 0.8% agarose (Thermo Fisher Scientific Inc., Wilmington, DE, USA) gel stained with GelRed (Biotium Corporate Headquarters Inc., Landing Pkwy, Fremont, CA, USA).

We genotyped the rs970476 of the *SGCD* gene with TaqMan SNP assay probes (Applied Biosystems, Foster City, CA, USA). We allocated a genotype to each case following conventional methods of melting curve analyses for real-time PCR with the PikoReal Real-Time PCR System (Thermo Fisher Scientific Inc., Wilmington, DE, USA). To ensure reproducibility and precision of our data; all assays were done in duplicate by blinded experienced laboratory technicians.

We mapped the three SNPs of our interest, those reported by Wan-Yu Lin et al.[15], in the second intron of *SGCD* gene (rs931798, rs140617, and rs140616) using the Ensembl genome browser 89 (http://www.ensembl.org/index.html). Since they are located less than 300 bases pairs (bp) apart, we designed the following primers with Primer3 v.0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) for automated sequencing: 5′ TGGCCCTTGGCTATCTCTTC 3′ and 5′ TCATGCTCATCCTAGGGTCCA 3′ under these parameters: 283 bp as length of theoretical fragment, 50 % of CG content, and alignment temperature of 54°C. These primers were synthesized at the Institute of Cellular Physiology (UNAM). For the PCR reaction, we used the AmpliTaq Gold DNA Polymerase (Applied

Biosystems, CA, USA) under an alignment temperature of 54°C using a Gradient Palm-Cycler™ (Corbett Life Science, Australia). To verify DNA amplification and integrity, we ran 2.0 % agarose gels (Thermo Fisher Scientific Inc., DE, USA) stained with GelRed (Biotium Corporate Headquarters Inc., CA, USA). Amplicons were compared to an O'RangeRuler 100 bp DNA Ladder (Thermo Fisher Scientific Inc., DE, USA). Our primers, per design, needed to be within 300 bp. To eliminate oligonucleotides and salts remnants, we purified the PCR products with DNA Clean & Concentrator-5 (Zymo Research, CA, USA). All samples after cleaning up were likewise verified in a 2.0 % agarose gel stained with GelRed and their concentration was measured using a MassRuler Expres LR reverse DNA ladder (Thermo Fisher Scientific Inc., DE, USA). Next, purified amplicons were marked with BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) and a PCR reaction was held in Gradient Palm-Cycler (Corbett Life Science, Australia) following manufacturers' specifications. The marked DNA was purified with the ZR DNA Sequencing Clean-up Kit (Zymo Research, CA, USA). 30 ng was used to perform a capillary electrophoresis and sequencing at 50°C with the ABI PRISM 310 Genetic Analyzer (Thermo Fisher Scientific Inc., DE, USA). Electropherograms were analyzed conventionally with FinchTV v. 1.4. (Geospiza, Inc., WA, USA; http://www.geospiza.com).

We began by describing the full sample and then performed stratified analyses by case/control status using Student's t-Test (continuous variables) and Chi-square tests (categorical variables) using SAS v.9.4.[23]. By this method, significant features at the 0.10-level were included in a logistic regression model (See below). For our genotyping data, we calculated allelic frequencies for the full sample and stratified by case/control status. We then corroborated if any of our single nucleotide polymorphisms significantly deviated from the Hardy-Weinberg equilibrium (HWE), especially in controls, using PLINK V. 1.9.[24]. We analyzed only those SNPs whose minor allele frequencies (MAF) were ≥ 0.01, HWE ≥ 0.05 among controls, and a genotyping call rate ≥ 95% by sequencing and RT-PCR. To decrease any information bias, we also performed all our genotyping assays by duplicate for both conventional sequencing methods and RT-PCR. Also, we estimated haplotypes, haplotype frequencies, and their unadjusted effect on AMD diagnosis using R version 3.3.1.[25]. Those haplotypes significant at the 0.10-level were included in a multivariate logistic regression model (See below).

To ascertain the effect of baseline characteristics and genotypes on AMD diagnosis and phenotype (GA vs. CNV), we performed unadjusted and adjusted logistic regression models. We a priori screened for modes of inheritance using PLINK v. 1.9.[24]. All our inferential analyses assume a genotypic effect. Our modeling followed: $log(OR_{AMD}) \sim$ Clinical features + SNP $\begin{pmatrix} AA_{00} \\ Aa_{01} \\ aa_{10} \end{pmatrix} + \in$. We then selected those genotypes with a significant association at the 0.05-level and included them in a final multivariate logistic regression model. All these models followed standard regression assumptions (Data not shown). This model was also adjusted by those clinical features significantly associated with either case/control status (See above) and those significant characteristics in our unadjusted models. To bolster our approach, we included *SGCD* haplotypes in these models using PLINK v.1.9.[24]. We also screened for epistasis however we did not evidence any significant effect (Data not shown). All our modeling was done in PLINK v. 1.9.[24] and corroborated in SAS V.9.4.[26].

3. Results

3.1. Characteristics of the study population and genotyped data

We studied 268 individuals; half were cases diagnosed with AMD. Our sample description is detailed in Table 1. On average, our study population was 74.3 years of age (± 8.2). 60% or more were at least 70 years or older and female. Almost half had hypertension (HTN), and 27.4% type 2 diabetes mellitus (T2DM). Up to 80% of our sample was either former or current smokers. Among AMD cases, the most prevalent phenotype was geographic atrophy (GA) (69.7%), followed by choroidal

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198 neovascularization (CNV) (30.3%). In our sample, we did not observe any progressions between
199 phenotypes, i.e., GA to CNV. To evidence if any of these characteristics were significantly associated
200 with the case/control status, we performed stratified analyses portrayed in Supplementary Table 2.

201 **Table 1.** Description of the sample (n = 268).

Characteristic	N (%)*
Age (years), mean ± SD	74.3 ± 8.2
Age (years), n (%)	
[50 – 60)	7 (2.6)
[60 – 65)	23 (8.6)
[65 – 70)	46 (17.2)
[70 – 75)	60 (22.5)
[75 – 80)	59 (22.1)
≥ 80	72 (27.0)
Sex	
Male	97 (36.2)
Female	171 (63.8)
Type 2 diabetes, n (%)	71 (27.4)
Hypertension, n (%)	134 (51.0)
Smoking history, n (%)	
Never	202 (80.5)
Former or current	49 (19.5)
AMD phenotype, n (%)^	
Geographic atrophy (GA)	92 (69.7)
Neovascular (NV)	40 (30.3)

202 Full sample description. Means and standard deviations, along with sample size and column
203 percentages are shown. We excluded one individual because of age.

204 * Numbers may not sum to totals due to missing data, and column percentages may not sum to 100%
205 due to rounding.

206 ^ Among cases (n = 134).

207 † One subject was excluded because of being younger than 50 years old.

208

209 Age is a significant feature in our sample. Controls were significantly five years younger than
210 cases (mean difference of 4.8 years, $p < 0.0001$). Almost half of our cases were 75 years or older
211 compared to controls (70 years or older, $p < 0.0001$). There were no significant differences in sex,
212 baseline comorbidities (T2DM, HTN) or smoking history between cases and controls. Regarding our
213 genotype data, all four least frequent alleles in the *SGCD* gene had a minor allele frequency greater
214 than 0.01 (Table 2). Such alleles in our sample are concurrent with already reported single nucleotide
215 polymorphisms for the Hispanic population in Hap Map[27]. Moreover, to provide evidence of bias in
216 our genotyping, we stratified our results based on their case/control status. All our data followed the
217 Hardy-Weinberg expected distribution without any significant deviations. Furthermore, all our
218 experiments we had a genotyping call rate greater than 95%.

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Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 August 2018 doi:10.20944/preprints201808.0137.v1

Peer-reviewed version available at Genes 2018, 9, 467; doi:10.3390/genes9100467

Table 2. Minor allele frequencies and Hardy-Weinberg equilibrium (n = 268).

SNP	A1*	A2	Pooled (n = 268) MAF	Cases (n = 134) MAF	Controls (n = 134) MAF	p†
rs970476	T	G	0.455	0.459	0.452	0.601
rs931798	A	G	0.330	0.358	0.302	0.538
rs140617	G	A	0.164	0.160	0.168	0.766
rs140616	C	T	0.494	0.485	0.504	1.000

Genetic data summary with allele frequencies (A1, A2) of the full sample (Pooled), and stratified by case/control status. The least frequent allele in our sample (A1) is concurrent with single nucleotide polymorphisms in the Mexican population. Hardy-Weinberg equilibrium $\chi 2$ test is shown to evidence any significant deviations, especially in controls.

† P-value for HWE $\chi 2$ test evaluated at the 0.05 level.

* All least frequent alleles for our sample are single nucleotide polymorphisms for the Mexican population as reported in HapMap.

CHR – Chromosome, SNP – rs ID, A1 – least frequent allele in the sample, MAF – minor allele frequency, p – significance of HWE in controls.

3.2. Unadjusted effect of baseline characteristics and genotypes with AMD status and phenotypes

To test if any characteristic was significantly associated with AMD status, we modeled each feature (either baseline demographics, risk factors, or genotype data) using logistic regression. Our results are portrayed in Supplementary Table 3. We assumed for our genetic data a genotypic mode of inheritance since the rest of patterns were not significant in a screen done in PLINK v.1.9 (Data not shown)[24]. In our sample, the unadjusted effect of a unit-increase in age is 8% increased odds of AMD (1.08, 95%CI 1.05-1.12, $p < 0.001$). We fail to evidence any significant association with sex, comorbidities, and smoking history. But, the effect of a heterozygote G/A in the rs931798 SNP compared to the G/G ancestral homozygote is 74% increased odds of AMD (95%CI 1.04-2.90, $p = 0.034$). To complement our approach, we then stratified our analyses by AMD phenotype either GA or CNV. These results are displayed in Supplementary Table 4. Age is significantly associated with both phenotypes (Supplementary Table 4). For each case, a unit increase elevates the odds of being diagnosed with GA in 8% and CNV in 9% ($p<0.0001$ for both comparisons). Furthermore, the bivariate effect of the above G/A genotype seems to be associated with the geographic atrophy phenotype, as it increases 82% the odds of GA compared to the G/G genotype (95%CI 1.03-3.21, $p=0.038$).

3.3. Adjusted effect of baseline characteristics and genotypes with AMD status

To determine the independent effect of our significant predictors in the unadjusted analyses, we performed a multivariable logistic regression model of those features significantly associated with AMD or those with clinical significance. Our results are displayed in Table 3. After adjusting for age and sex, in our sample, the independent effect of the G/A genotype in the rs931798 SNP is 81% increased odds of AMD compared to the G/G ancestral genotype (95%CI 1.06-3.14, $p =0.031$). Also, a one-unit increase in age rises 8% the odds of AMD (95%CI 1.05-1.12, $p<0.0001$). To further corroborate the effects on AMD phenotype (See above), we additionally ran controlled stratified analyses portrayed in Table 4. The independent effect of the G/A genotype is seen in the geographic atrophy phenotype. Compared to the G/G genotype, the G/A heterozygous is associated with 81% increased odds of geographic atrophy holding constant sex and age (1.01-3.26, $p = 0.038$). Age is significantly associated with both GA and CNV. Holding constant all variables in the model, a unit-increase in age increased 8% the odds of GA and 8% the odds of CNV (Table 4).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 August 2018 doi:10.20944/preprints201808.0137.v1

Peer-reviewed version available at Genes 2018, 9, 467; doi:10.3390/genes9100467

Table 3. Multivariable logistic regression model of factors associated with AMD (n = 268).

Characteristic	Adjusted OR (95% CI)	p†
Age (years)	**1.08 (1.05, 1.12)**	**<0.0001**
Sex		
Female	1.00	—
Male	0.63 (0.37, 1.08)	0.096
rs931798		
G/G	1.00	—
G/A	**1.81 (1.06, 3.14)**	**0.031**
A/A	1.11 (0.45, 2.69)	0.822

Adjusted associations between baseline characteristics and AMD diagnosis (0–No, 1–Yes AMD). For genetic data, we assumed a genotypic mode of inheritance. Such models follow: $\log(OR_{AMD}) \sim$ Baseline characteristics $+$ SNP$\begin{pmatrix} AA_{00} \\ Aa_{01} \\ aa_{10} \end{pmatrix} + \in$ where: AA is the most frequent allele in our population, taken as reference. We considered statistically significant predictors of odds of disease those whose p-value < 0.05 holding constant all variables in the model.

† p-value for β significance adjusted for other covariates.

In bold significant predictors at the 0.05 level.

Table 4. Multinomial logistic regression model of factors associated with AMD phenotype (n = 268).

Characteristic*	Geographic atrophy OR (95% CI)	p†	Neovascular OR (95% CI)	p†
SNP2^				
G/G	1.00	—	1.00	—
G/A	**1.81 (1.01, 3.26)**	**0.047**	**1.40 (0.65, 3.01)**	**0.393**
A/A	1.24 (0.47, 3.24)	0.666	1.09 (0.31, 3.87)	0.890
Age(years)	**1.08 (1.04, 1.12)**	**<0.0001**	**1.09 (1.04, 1.15)**	**<0.0001**

Adjusted associations between baseline characteristics and AMD phenotype (either 1–GA, 0–else; or 1–NV, 0–else). For genetic data, we assumed a genotypic mode of inheritance. Such models follow: $\log(OR_{AMD}) \sim$ Baseline characteristics $+$ SNP$\begin{pmatrix} AA_{00} \\ Aa_{01} \\ aa_{10} \end{pmatrix} + \in$ where: AA is the most frequent allele in our population, taken as reference. We considered statistically significant predictors of odds of disease those whose p-value < 0.05 holding constant all variables in the model.

† p-value for β significance

NS: not significant at the 0.05 level.

*Controls or non-diseased phenotype are set as reference for all multinomial logistic regression models.

^We took the most common allele and set it as reference.

In bold significant predictors at the 0.05 level.

3.4. Haplotype analyses

To bolster our approach, we also ran haplotype analyses. The haplotype GATT (rs931798, rs140617, rs140616, and rs970476) (Supplementary Table 5) is associated with 86% decreased odds of being diagnosed with AMD (95%CI 0.02-0.93, p = 0.011). However, it is less frequent in our population. After adjusting for other covariates in a multivariate regression model, the independent

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effect of the GATT haplotype remained (Table 5). This haplotype significantly decreases the odds of being diagnosed with AMD in 87% (95%CI 0.02-0.91, p = 0.41). However, we were not able to produce stratified analyses by phenotype given our sample size.

Table 5. Multivariable logistic regression model of haplotypes and factors associated with AMD (n = 268).

Characteristic	Adjusted OR (95% CI)	p†
Age (years)	**1.09 (1.05, 1.13)**	**<0.0001**
Haplotype #1 (GATT)	**0.13 (0.02, 0.91)**	**0.041**

Adjusted associations between haplotype configurations of SNPs in the SGCD gene and AMD diagnosis by logistic regression.

† p-value for β significance

NS: not significant at the 0.05 level.

In bold significant predictors at the 0.05 level.

4. Discussion

The global burden of age-related macular degeneration on severe visual impairment and legal blindness is increasing. For North America, in 2015, around 15.9% of the regional blindness was directly attributable to AMD in those aged 50 years or older[3]. Moreover, there is a disproportionate prevalence of cases among high-income countries, especially those whose population is aging. Since this disease is multifactorial, there is great interest in characterizing molecular underpinnings and environmental exposures that may influence AMD phenotype, prognosis, progression, and response to treatment, towards decreasing AMD incidence[28]. Herein, we provide experimental evidence of a novel association between the *SGCD* gene and AMD diagnosis and phenotype.

δ-sarcoglycan is a structural protein that forms a complex with dystrophin among others[29]. In the murine retina, the gene product – δ-SG– is expressed diffusely but in abundance around the external (photoreceptors, ELM) and internal (ILM, optic nerve, ganglion cells) segments[19]. The significance or role of the retinal function or homeostasis is poorly understood. Key partners such as dystrophin-71 (Dp71), expressed in Müller glial cells, aid in regulating the extracellular milieu and potassium homeostasis[30]. Others such as the sarcoglycan complex promote intercellular junctions and thereby stability[19]. In a manuscript in preparation, we have also evidenced that Sgcd-null mice compared to a wild-type had increased retinal frailty, decreased layer thickness, and electroretinogram aberrances. These results strongly support the relevance of δ-sarcoglycan and its partners and encourage future characterization of this protein in the development of retinal impairment, a trait shared in many retinal diseases.

On top of these promising results in animal studies, others have evidenced an association of *SGCD* with AMD in silico. Tang et al. (2009) provided intriguing results of biomarkers in this gene interacting with the SCAPER gene[14]. Even further, in secondary prioritized subset analyses from already well-established AMD cohorts, Wan-Yu Lin (2010) identified several SNPs in silico who might increase the odds of AMD[15]. Here, we examined these markers in a case-control study done in Hispanics. The G/A genotype from rs931798 increased the odds for AMD, specifically of the geographic atrophy phenotype. Moreover, there might be an encouraging haplotype of all these biomarkers, but future studies warrant its replication. Currently, the specific function of this SNP is unknown. A plausible role might be in modulating complement levels in drusen[14]. In support of this hypothesis, are co-expression analyses that correlate both *SGCD* and *SCAPER* with *MASP1* (p-value, <1x10-5) and *MASP2* (p-value, <1x10-2) genes. Both are activators of the complement pathway[14]. Also, it may exert influence through the regulation of the aging process, as pieces of evidence show

that *SCAPER-SGCD* epistasis might be involved in cell cycle regulation and DNA repair. Moreover, all these four SNPs we have evaluated are in significant linkage disequilibrium (r2 > 0.8) with a neighboring region significantly associated with the expression of *FBLN1*[14]. Members of this family have been associated with AMD as they can act as cofactors for the matrix metalloprotease *ADAMTS1* and may play a role in proteoglycan degradation by *ADAMTS1* under inflammation[14]. Biomarkers in *SGCD* might also favor the development of AMD, especially of the geographic atrophy phenotype, in a similar pathway to *HTRA1* by regulating the degradation of extracellular matrix and facilitating access of other degradative matrix enzymes, such as matrix metalloproteinases to their substrates[14].

Our findings though promising are exploratory and limited to the Hispanic population. Despite our sample size, we were powered to detect significant differences in odds from 1.2 or higher. Future replication in other ethnicities is warranted to test the effect of such biomarkers on disease phenotype and progression. A new line of research should focus on dissecting the molecular pathway of *SGCD* in AMD, especially characterizing retinal alterations in patients with Limb-Girdle Muscle Dystrophies; caused by disruptions of this gene.

Author Contributions: Conceptualization, Francisco J. Estrada-Mena; Methodology, Andric C. Perez-Ortiz, Francisco J. Estrada-Mena, Alexandra Luna-Angulo, Juan Carlos Zenteno; Validation, Alexandra Luna-Angulo; Formal Analysis, Andric C. Perez-Ortiz, David Jimenez-Collado; Investigation, Alexandra Luna-Angulo, , Liliana Guadalupe Cortes-Ballinas, Andric C. Perez-Ortiz, Stefany Jacob-Kuttothara; Resources, Francisco J. Estrada-Mena, Juan Carlos Zenteno; Data Curation, Andric C. Perez-Ortiz; Writing-Original Draft Preparation, Francisco J. Estrada-Mena, Andric C. Perez-Ortiz, David Jimenez-Collado, Martha Janneth Peralta-Ildefonso, Bani Antonio-Aguirre; Writing-Review & Editing, Andric C. Perez-Ortiz, David Jimenez-Collado, Alvaro Rendon, Francisco J. Estrada-Mena, Liliana Guadalupe Cortes-Ballinas, Juan Carlos Zenteno; Visualization, Israel Ramírez, Francisco J. Estrada-Mena, Andric C. Perez-Ortiz; Supervision, Francisco J. Estrada-Mena, Alvaro Rendon, Israel Ramírez, Andric C. Perez-Ortiz; Project Administration, Francisco J. Estrada-Mena, Alexandra Luna-Angulo; Funding Acquisition, Francisco J. Estrada-Mena

Funding: This research was funded by Universidad Panamericana grant number through the grant "Fomento a la Investigación UP 2017", under project code UP-CI-2017-CS-MX-01.

Acknowledgments: We are grateful to Esmeralda Lira-Romero, MSc for providing technical support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; inal the writing of the manuscript, and in the decision to publish the results.

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