

Association of Complement Factor H (CFH) Y402H Gene Polymorphism and Serum CFH with Risk of Age Related Macular Degeneration in Egyptian Patients

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Abstract

Background: Age related Macular Degeneration (AMD) is stated as the most common cause of irreversible blindness among aging populations. It has been reported that both Complement Factor H (CFH) Y402H polymorphism and serum CFH concentration are linked to AMD.

Aim of Study: To determine whether Y402H polymorphism and serum level of CFH are associated with AMD in the Egyptian population.

Material and Methods: Eighty subjects were recruited; 40 AMD patients and 40 matched healthy individuals as control. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was performed for the analysis of CFH Y402H gene polymorphism. Serum CFH concentration was measured using Enzyme Linked-Immunosorbent Assay (ELISA).

Results: Susceptibility to AMD in Egyptian population was not associated with CFH Y402H polymorphism and the presence of C allele is not considered risky; Odds Ratio (OR): 0.545, 95% CI (0.291-1.022). No significant association between CFH genotypes and AMD. Serum CFH concentration showed no statistically significant difference between cases & controls ($p=0.706$). In controls, there was a statistically significant decrease in serum CFH level in CC genotype compared to both CT genotype ($p=0.038$) and TT genotype ($p=0.007$), while in AMD group, no statistically significant difference in serum CFH concentration between different genotypes.

Conclusion: CFH Y402H polymorphism does not confer risk of AMD in Egyptian patients. Further larger scaled studies are needed to confirm CFH role in AMD.

Key Words: Age related macular degeneration – Complement Factor H – Egyptian – Y402H.

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Introduction

GLOBALLY, it is estimated that Age-related Macular Degeneration (AMD) affect 196,000,000 people more than 50 years old [1]. It is considered the third worldwide leading cause of blindness [2]. This percentage is expected to increase with demographic changes and increasing life expectancy. Hence, AMD has become a significant public health concern and a major research focus [3]. AMD either presents as Geographic Atrophy (GA) of the Retinal Pigment Epithelium (RPE) or as Choroidal Neovascular (CNV); which might be complicated by RPE detachment, sub-retinal hemorrhage & scar formation. As for active CNV, different effective therapeutic options are available, while for GA no definite treatment protocol is specified & is still under trial [4]. Hence, till now no treatment is available for most AMD types, nor any effective means to stop AMD progression [5].

The complement system is an essential component of both innate and acquired immunity and is considered one of the most useful protagonists of both immune and inflammatory responses [6]. Complement Factor H (CFH) is one of the main regulators of the activation of the Alternative Pathway (AP) as it prevents the formation of the C3 convertase enzyme and promotes its dissociation. It also acts as a cofactor of the enzyme Factor I (FI) that mediates the proteolytic inactivation of C3b [7].

The glycoprotein chain of Factor H is single polypeptide combining 20 repetitive units of 60 amino acids named Short Consensus Repeats (SCR) [8]. The Tyr402His polymorphism lies in the SCR 7, among the positively charged amino acids in-

involved in binding heparin and C-Reactive Protein (CRP) with M protein [9].

In vitro functional studies revealed that the replacement of His by Tyr at position 402 changes the binding properties of SCR7 for different glycosaminoglycans and CRP [10,11] besides, it decreases its binding to retinal pigment epithelial cells [12,13]. Weismann and his team proved that CFH engages to a reactive decomposition product evolved from oxidative damage of the cell membrane lipids known as malondialdehyde (MDA). Also, the CFH variant p.Y402H found in AMD patients demonstrated a marked decrease in malondialdehyde binding compared to normal CFH. Their findings clarify the exact anti-inflammatory defensive role of CFH against oxidative damage and approve the hypothesis that dysfunctional CFH can lead to an abnormality in the outer retina function [14]. This defective regulation results in excessive release of different fragments such as C3a & C5a and the formation of the C5b9 complex; which are able to induce activation of endothelial cell and liberating growth factors as vascular endothelial growth factor or fibroblast growth factor [15].

Several AMD susceptibility loci have been examined, including CFH gene on chromosome 1q32 [16]. The most studied SNP at this locus is rs1061170 (T1277C) which causes a Tyr402His amino acid substitution in CFH. Many studies investigated the association of CFH Y402H and AMD in different populations with contradictory findings [7,15,17,19]. The 402His allele frequency differs dramatically among different populations, which may lead to the recognized differences in AMD incidence along different ethnic groups [20]. Not surprisingly, since then it has been concluded and widely accepted that bad control of complement activation by this variant is a cause of pathogenesis of AMD [21]. However, the definite mechanism through which this CFH variant influence the risk of AMD stays mostly unknown.

Based on the recommendations of a meta-analysis study by Nonyane et al., who reported that data of populations from Africa, Middle East and South America are needed for better understanding of the relation between AMD genetic risk and diverse ethnicities [22], we attempted to investigate the influence of CFH Tyr402His polymorphism on the serum levels of CFH protein in patients with AMD and whether or not there is an association between this polymorphism and AMD in Egyptian patients.

Patients and Methods

This is a cross sectional study that took place in the period between March 2014 and July 2014. It included eighty subjects; forty patients diagnosed with AMD and forty age and sex matched healthy subjects assigned as the control group. All patients were recruited from Kasr El-Ainy & Dar El-Oyoum out-patient clinics. AMD patients above the age of fifty years who presented with visual loss in at least one eye were included in the study while all patients presenting with progressive degenerative myopia were excluded.

All patients were subjected to thorough history taking with special emphasis on cigarette smoking, hypertension and diabetes mellitus. All patients were subjected to a detailed ophthalmic examination and the type of AMD was determined clinically using slit-lamp biomicroscopy and by performing both Fundus Fluorescein Angiography (FFA) as well as Optical Coherence Tomography (OCT). The research followed the tenets of the Declaration of Helsinki. The study was approved by the local Research Ethics Committee, and all patients provided an informed consent.

Blood sampling: Five ml of venous blood samples were withdrawn from the recruited subjects; Two ml were placed into EDTA vacutainer tubes for DNA extraction and further CFH Y402H gene polymorphism analysis, while three ml were placed into plain vacutainer tubes for serum CFH level measurement.

Serum CFH concentration measurement:

Serum was separated 20 minutes after blood clotting by centrifugation (1500xg at 4°C for 15min). It was then transferred to a fresh polypropylene tube, divided into portions, aliquoted and stored at (20°C) until analyzed. Serum CFH was measured using Human Complement Factor H - ELISA KIT, catalog # HK342-01 (Hycult biotech, The Netherlands). Serum CFH concentrations were measured in sera of 22 AMD cases and 15 controls, randomly chosen.

Detection of CFH Y402H (rs1061170) gene polymorphism by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP):

Genomic DNA was extracted from peripheral blood leucocytes using the salting out technique based on the method of Trowsdale [23]. CFH Y402H polymorphism, with the substitution of T to C at nucleotide position 1277 in exon 9 was determined by PCR-RFLP after modifying Lau and his col-

leagues protocol of work. 18 Each Polymerase Chain Reaction (PCR) reaction was carried out with 50ng of genomic DNA, 25pmol of each primer, 12.5 μl Master Mix (Dream Taq™, Green PCR Master Mix) (Fermentas Life Sciences, Lithuania) in a total volume of 25 μl.

CFH Y402H genotyping was performed with the primer pair (forward 5'-TCA TTG TTA TGG TCC TTA GGA AA-3' and reverse 5'-TTA GAA AGA CAT GAA CATGCTAGG-3' (Bioren, USA) with initial denaturation at 95°C for 4 minutes, followed by 35 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 57°C and 1 minute extension at 72°C and a final 10-minute extension at 72°C using a PCR Thermal Cycler (ThermoHybaid, UK).

PCR products (241bp) were digested by 2U of fast digest restriction enzyme Tsp509I (TasI) (Fermentas Life Sciences, Lithuania); the presence of T allele at nucleotide position 1277 created a recognition site for Tsp509I which led to digestion of the 241bp PCR product into restriction fragments of 60bp and 181bp. Digested products were visualized by electrophoresis on a 3% agarose gel stained with ethidium bromide and UV Transillumination.

Statistical analysis:

Results were analyzed using the SPSS computer software package, Version 15.0 (Chicago, IL, USA). Qualitative data was expressed as frequency and percentage. Quantitative data were presented as mean ± SD. Association between qualitative data was done by χ²-test. Risk estimate was calculated by Odds Ratio (OR). The p-value was considered significant at 0.05.

Results

The study included forty patients; 26 males (65%) and 14 females (35%) and forty healthy age and gender matched subjects with mean age: 64.03 ± (6.43) years. Exudative (wet) AMD was detected in 95% of patients (60% had active Choroidal Neovascular Membrane (CNVM) while 35% had a disciform scar) and 5% had atrophic (dry) type. There was either associated cataract or cataract surgery in 50% of the patients twenty three patients (57.5%) were smokers. Hypertension was found in 16 patients (40%) while 15 patients (37%) were diabetics.

CFH (Y402H) genotype distribution in AMD cases and controls (Table 1) showed no statistical significant association between the three genotypes and AMD (p=0.158). The risk estimation for the

(Y402H) SNP of CFH genotypes was not significant: (CC and CT) genotypes versus TT genotype (OR=0.393, 95% CI=0.149-1.038, p=0.056) and (T) allele versus (C) allele (OR=0.545, 95% CI=0.291-1.022, p=0.051), i.e. the presence of allele C is considered not risky.

Table (1): Genotype distribution & risk estimate of (Y402H) SNP of CFH genotypes & alleles.

CFH Y402H	Cases (n=40) N (%)	Controls (n=40) N (%)	Odds ratio	p-value
CC	9 (22.5)	13 (32.5)		0.158
TT	17 (42.5)	9 (22.5)		
CT	14 (35)	18 (45)		
CC & CT	23 (57.5)	31 (77.5)	0.393	0.056
TT	17 (42.5)	9 (22.5)	(0.149-1.038)	
C	32 (40)	44 (55)	0.545	0.051
T	48 (60)	36 (45)	(0.291-1.022)	

It was also found that there was no significant association between CFH genotypes and types of AMD (p=0.526) as shown in (Table 2).

Table (2): Association of CFH (Y402H) genotypes with AMD phenotype.

Type of AMD	CFH (Y402H) genotypes			p-value
	CC (n=9)	TT (n=17)	CT (n=14)	
Wet Type	7	13	11	0.526
Dry Type	2	4	0	

Table (3): Serum CFH level in the different CFH genotypes in the studied groups.

Serum CFH in the different CFH genotypes in AMD cases (n=22)			
CFH Y402H genotype	CC (n=7)	CT (n=8)	TT (n=7)
• Serum CFH level (ug/ml)	433.6±128	410±237.6	440.1±110
Serum CFH in the different CFH genotypes in control subjects (n=15)			
CFH Y402H genotype	CC (n=6)	CT (n=7)	TT (n=2)
• Serum CFH level (ug/ml)	329.2±87 ^a	488±143.5 ^b	665±162.7 ^b

Numbers carrying the same initials are not statistically significant.

Mean serum CFH level was 427.1 ± 164.8ug/ml in the studied cases (n=22) and 448.1 ± 164.4ug/ml in controls (n=15) with no statistically significant difference (p=0.706). Serum CFH concentrations in the different genotypes in cases and control groups are summarized in (Table 3). In the control group, there was a statistically significant decrease in serum CFH in CC genotype when compared with both CT genotype and TT genotype (p=0.038

and 0.007, respectively). In AMD group, serum CFH level was lower in CC genotype than in TT genotype, but this difference didn't reach statistical significance ($p=0.083$).

Table (4) summarizes the comparison of serum CFH concentration in the AMD group stratified according to different risk factors. There was no detected statistically significant difference.

Table (4): Serum CFH level in AMD cases stratified according to different risk factors.

Risk factor associated with AMD cases (n=22)	Serum CFH (ug/ml)	p-value
<i>Gender:</i>		
Female (n=9)	420.6±199	0.881
Male (n=13)	431.7±145	
<i>Smoking:</i>		
Smoker (n=10)	462.2±201.8	0.375
Non-smoker (n=12)	397.9±128.4	
<i>History of cataract and cataract surgery:</i>		
With history (n=12)	434.3±195.6	0.829
Without history (n=10)	418.5±128.3	
<i>Hypertension:</i>		
Hypertensive (n=7)	462.9±178	0.463
Non-hypertensive (n=15)	404.5±166	
<i>Diabetes Mellitus:</i>		
Diabetic (n=9)	450±164.3	0.601
Non-diabetic (n=13)	1.3±170	

Discussion

The complement system has been proven to play a significant role in AMD pathogenesis. This has been further supported by identifying CFH as a major susceptibility locus for AMD. Multiple genetic variants in the CFH-CFHHR1-5 and CFB genomic regions have been characterized of conferring risk or protection to AMD development [24,25]. CFH Y402H polymorphism is not the single or the most significant regulator of complement proteins involved in AMD pathogenesis and its role may be outgrown by other studied SNPs [17], yet it has been unquestionably associated with AMD in patients from distinct ethnic backgrounds [26]. Nevertheless, this has not been widely studied in Egyptian population.

In the current study, there was no association between CFH Y402H polymorphism and AMD. Similar results were reported in Japanese population [27,28] as well as Northern Chinese patients [29]. On the other hand, Sodi and his colleagues revealed an association between CFHY402H genotype and atrophic AMD [30]. Also, studies from North America [18] Europe [31,32], Asia [33,34] and Brazil [7]

have found this polymorphism to be a risk factor for AMD development.

The genotype distribution in our studied AMD cases showed that the wild type TT was the most prevalent (42.5%) followed by CT (35%) then CC (22.5%) which is in concordance with those of Uka et al., [27]. The frequency of C allele; (0.4 in cases and 0.55 in controls), is comparable to reports in Caucasians [15]. Lower frequencies were reported in Japanese control 0.04 compared to 0.45 in Caucasians [28] as well as among Chinese population (0.1 in cases and 0.08 in controls) [29]. On the contrary, Sodi et al found that genotype C/C has higher prevalence in AMD group than in controls ($p<0.001$) and C allele in the AMD group was more frequent than in controls ($p<0.001$) [30].

Additionally, no statistically significant association between CFH genotype distribution and AMD phenotypes was found in the current study ($p=0.526$). Similar findings were reported in previous studies that recruited Austrians [35], Tunisians [36] and Italians patients [31].

Although the risky C allele frequency in the current study was comparable to that of European population, yet it didn't confer increased risk of AMD. On the contrary, the protective T allele was more in AMD cases (0.6) relative to controls (0.45). These results do not agree with those in the only published study on Egyptian patients. This could be due to small number in their study 26 AMD patients and 20 controls [37].

In their meta-analysis study, Nonyane et al., reported that the frequency of Y402H risk allele declared a positive association with the prevalence of late AMD across ethnicities except among those having an African descent. They added that although the allele frequencies in both African and European populations were similar yet the prevalence of late AMD among African descendants did not match [22]. The low prevalence of late AMD in African Americans is hypothesized to be due to the high frequencies of Y402H and delCHFR1 alleles [20]; the latter confer a protective effect in Europeans [38].

It should be noted that Egypt being central to the three continents Africa, Europe and Asia with its unique geographic location and due to the large-scale immigration as well as the fast pace of inter-continental transportation, its population is highly affected. Historically, Egypt has been ruled by the Greeks, Romans, Arabs, Turks, French and British who have also mixed with its people. Consequently,

that modern Egyptians represent a mosaic of all these legacies [39].

Few studies are available on studying the influence of CFH Y402H genotypes on serum CFH concentration and hence their association with risk of AMD. In the current study, there was no statistically significant difference in mean level of serum CFH between AMD cases and controls ($p=0.706$), which is in concordance with those reported by Esparza-Gordillo et al., [40] and Silva et al., [7]. In disagreement with our results, it was reported by Qureshi and Ambreen that serum CFH levels were significantly reduced in AMD patients compared to the controls ($p<0.0001$) [41]. In the AMD group, serum CFH concentration was lower in CC genotype than in TT genotype, yet the difference was not statistically significant ($p=0.083$). Similar results were obtained by Silva et al., who reported that no significant differences in serum CFH levels were observed in the groups with different CFH genotypes [7].

On the other hand, this study revealed a statistically significant decrease of serum CFH level in CC genotype compared to the CT genotype ($p=0.038$) and TT genotype ($p=0.007$) in controls. This is in contrast to results of Silva et al., who reported that the CFH Y402H polymorphism does not influence the plasma levels of CFH protein in the control group of normal individuals in Brazilian patients [7].

In the current study, serum CFH concentrations were not reduced in AMD cases associated with smoking, hypertension or diabetes mellitus in contrast to results reported by Esparza-Gordillo et al who observed that serum CFH was decreased in smokers [40].

In conclusion, this is the second study to be conducted in order to investigate the association between CFH Y402H polymorphism and AMD in Egyptian patients. it was found that there is absence of such an association with no relation between the genotypes and AMD phenotypes. The study additionally evaluated the influence of this polymorphism on the serum levels of CFH protein in the recruited subjects which showed no difference AMD cases and controls or with different CFH genotypes in AMD cases and it was not related to any of the risk factors. However, significant reduction in CFH levels was noted in CC genotype compared to other genotypes in controls. The relatively small sample size, though comparable to similar studies, remains one of the study limitations.

Replication of this study on a larger cohort is needed to confirm the above reported findings with further investigating of alleles frequencies. Future studies investigating the contribution of other genes related to the alternative complement pathway are highly recommended as haplotype and diplotype analysis seem to be of high importance.

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ارتباط الأشكال المتعددة لجين عامل الكومبلمنت (ك ف ه) "هاء" Y402H ومصل عامل الكومبلمنت "هاء" في حدوث مرض تحلل الشبكية الناتج عن الشيخوخة في المرضى المصريين

يعتبر مرض تحلل الشبكية الناتج عن الشيخوخة هو السبب الرئيسي للعمى الدائم في المسنين في جميع أنحاء العالم. العوامل المسببة لهذا المرض متعددة وتضم عوامل بيئية وعوامل جينية.

الهدف من هذه الدراسة هو دراسة إذا ما كان الأشكال المتعددة لجين عامل الكومبلمنت "هاء" Y402H ومستواه في الدم يزيد من احتمالية حدوث هذا المرض في عينة من المرضى المصريين

ولقد أشتملت الدراسة على ٨٠ شخصاً منهم ٤٠ مريض بمرض تحلل الشبكية الناتج عن الشيخوخة و٤٠ من الأفراد الأصحاء.

ولقد تم تحديد تعدد الأشكال لجين عامل الكومبلمنت "هاء" Y402H بواسطة التفاعل المتسلسل بالبوليميراز-رقلب.

وقد أسفرت نتائج الدراسة عن عدم وجود ارتفاع نو دلالة إحصائية في الأنماط الجينية في حالات المرضى عنها عن الأفراد الأصحاء.