# The Effect of Genetic Variants Associated With Age-Related Macular Degeneration Varies With Age

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**PURPOSE.** The prevalence of age-related macular degeneration (AMD) increases dramatically with age. This large collaborative study investigates the effects of 51 late-AMD–associated genetic variants in different ages, focusing on individuals above the age of 90 years.

**METHODS.** The study included 27,996 individuals of the International AMD Genomics Consortium; 14,539 showed late AMD (51.9%) and 13,457 were controls (48.1%). Four age groups were compiled: 60 to 69 years, $n = 6514$, AMD = 2210 (33.9%); 70 to 79 years, $n = 12228$, AMD = 6217 (51.7%); 80 to 89 years, $n = 8285$, AMD = 5326 (64.3%); and $\geq$90 years, $n = 969$, AMD = 686 (70.8%). The effect sizes of 51 AMD-associated genetic variants were calculated for all age groups and were compared among the age groups.

**RESULTS.** Six variants were associated with late AMD in individuals $\geq$ 90 years of age ($P \leq 0.0006$). For rs10922109 and rs570618 (both in *CFH*), the minor allele (MA) was protective, and minor allele frequency (MAF) increased with age in cases and controls. For rs116503776 in *C2/CFB/SKIV2L*, the MA was protective, and MAF increased in cases. For rs3750846 in *ARMS2/HTRA1*, the MA increased risk, and MAF was lower in cases with increasing age. For rs6565597 in *NPLOC4/TSPAN10*, the MA increased risk. For rs5754227 in *SYN3/TIMP3*, the MA was protective, and there was no consistent variation in MAF with age. Variants in *CFH* and *ARMS2* showed lower effect sizes at greater age. Interaction analysis showed strong age-related effects for rs570618 ($P = 2.24 \times 10^{-7}$) and rs3750846 ($P = 0.001$). Total genetic risk was lower in individuals $\geq$ 90 years old (area under the curve [AUC], 0.795) than in those 70 to 79 years old (AUC, 0.831; $P = 0.03$)

**CONCLUSIONS.** Effect sizes and MAF of genetic risk factors for late AMD differed among the age groups. These results could guide future work on AMD risk assessment in older individuals.

Keywords: age-related macular degeneration, risk factors, genetic variants

Age-related macular degeneration (AMD) is a retinal disease and the main cause of severe vision impairment above the age of 50 in Western countries.[1] The early forms of AMD show drusen between Bruch's membrane and the retinal pigment epithelium. Late AMD is characterized by geographic atrophy of the outer retina and/or choroidal neovascularization with accumulation of fluid in or under the retina, resulting in vision loss. Multiple environmental and genetic factors are involved in the etiology of AMD.[2,3] Several AMD susceptibility loci have been identified.[4] Genome-wide association studies (GWASs) and large-scale resequencing initiatives have identified a large number of single nucleotide polymorphisms (SNPs) that increase

the risk for AMD.[5] The International Age-Related Macular Degeneration Genomics Consortium (IAMDGC) recently reported 34 independent AMD risk loci and 52 variants that were independently associated with late AMD.[6] Several variants increase the risk of late AMD, such as rs3750846 in *ARMS2/HTRA1*. In addition, variants have been found in genes involved in the complement system, such as *CFH*, *C2/CFB*, *C3*, and *CFI*, that either increase or decrease the risk of AMD. Other risk-inferring and risk-decreasing variants have been found in genes involved in, for example, collagen pathways (e.g., *COL15A1*, *COL8A1*, *LOXL2*, *COL4A3/COL4A4*, *MMP9*, *PCOLCE*), lipid pathways (e.g., *APOC2/APOE*, *CETP*, *LIPC*, *ABCA1*, *PLTP*), and extracellular

matrix pathways (e.g., *TNXB*, *VAV1*, *VEGFA*, *ACTG1*, *BCAR1*, *COL4A4*, *ITGA7*, *MYL2*, *VTN*).

Aging is a very important factor in the pathogenesis of AMD. The prevalence of AMD in individuals above the age of 90 years has been reported to be almost 60%, with almost 1/3 of the AMD cases (28.3%) being late AMD.[7] Especially in individuals of European ethnicity, the prevalence of the late forms of the disease increases rapidly after the age of 75 years.[8,9] Because of the high prevalence of AMD in older individuals, the increased aging of the population adds to the health care burden due to AMD. Risk factors may not be equal in all age groups; therefore, evaluation of the risk factors in the different age groups, especially very old individuals, could lead to improved late-AMD risk assessment and patient care. Previously, we demonstrated that the known genetic risk alleles rs1061170 in *CFH* and rs10490924 in *ARMS2* showed significantly lower effects on AMD development in individuals above the age of 90 years, whereas non-genetic factors maintained comparable effects with advanced age.[10] However, so far, most studies have included only small numbers of participants, particularly older individuals, and have evaluated only a limited number of genetic variants. Thus, our understanding of the genetic risk factors for the different age groups, especially those 90 years of age and older, is still very limited.

In this collaborative case–control study, we used the large IAMDGC genotyped cohort to investigate the effects of 51 known AMD-associated variants in 969 individuals 90 years of age and older compared to younger age groups. It is possible that age-specific genetic risk effects could be utilized to obtain more accurate disease risk assessments in older age groups.

## METHODS

### Study Population

Our analyses are based on individual participant data from 23 studies of the IAMDGC.[6] In our study, we included data for 27,996 individuals in the IAMDGC study who were between 60 and 102 years of age. We divided them into four age groups: 60 to 69 years ($n = 6514$), 70 to 79 years ($n = 12,228$), 80 to 89 years ($n = 8285$), and 90 years and older ($n = 969$). These individuals passed subject-level quality control as previously described.[6] Only unrelated individuals of European ancestry were included in the analysis.

Our study included healthy controls and late-AMD cases with documentation of the disease status. The AMD status of all included individuals was determined by fundus examination and/or fundus photography. Sixteen of the 23 included study sites additionally used fluorescein angiography and/or optical coherence tomography images for the grading of choroidal neovascularization. The individuals were recruited from studies based on ophthalmology clinics and population-based studies. Four studies recruited their controls from among spouses or friends. The late-AMD cases had geographic atrophy or choroidal neovascularization related to AMD. Controls had fewer than five drusen and no pigmentary abnormalities in either eye, and they had to be defined as "no AMD" based on the grading system of the study site. Due to only a very small number of early or intermediate AMD cases among the individuals above 90 years of age, those individuals with early or intermediate AMD were not included in this study. Further inclusion and exclusion criteria, as well as detailed information on ophthalmological

grading, quality control of genetic data, and imputation, are described elsewhere.[6]

The study followed the tenets of the Declaration of Helsinki and was approved by the ethics committee of the University Hospital in Cologne (Ethics Commission of Cologne University's Faculty of Medicine) and the local ethics review boards at the other participating sites. Informed written consent was obtained from each participant.

### Genotyping

Genotyping and imputation using the 1000 Genomes reference panel[16] were performed as described previously.[6]

The genotypes of the initial IAMDGC study are available from the Database of Genotypes and Phenotypes under accession phs001039.v1.p1, and GWAS summary statistics are available at http://amdgenetics.org/. Very rare variants with an allele frequency (AF) below 0.001 were excluded from the analysis. For this reason, the very rare variant rs121913059 (*CFH* p.Arg1210Cys) was excluded, and we analyzed the genotype dosages of 51 independently associated common and rare variants distributed across 34 loci (Supplementary Table S3; see also Ref. 6).

### Statistical Analysis

Genetic associations of the 51 genetic variants and AMD status were assessed as previously described by using allele dosages and by means of Firth bias-corrected likelihood-ratio tests as implemented in the Efficient and Parallelizable Association Container Toolbox (EPACTS) adjusted for PC1, PC2, and source of DNA.[6] Bonferroni correction was applied and $P < 0.001$ was considered statistically significant. Additionally, a sensitivity analysis was performed that included 885 individuals in each age group matched for sex, AMD status, source of DNA, and study cohort. Selection of the individuals for the sensitivity analysis was based on exact matches and performed using the nearest neighbor matching method of the R package MatchIt (R Foundation for Statistical Computing, Vienna, Austria).

Interaction analyses were performed with computing models that included two age groups, and the interaction term was estimated for each pair of age groups. Moreover, an overall interaction term of age and SNP was calculated that included all individuals in the analysis. Regression models were calculated for the different age groups and included all 51 genetic variants; the area under the receiver operating characteristic curve (AUC) was also computed. Differences between the AUCs were calculated using the R package pROC. Power calculations were performed along the lines of Cohen[17] using the R package pwr based on the odds ratios (ORs) for the overall population reported by Fritsche et al.[6]

## RESULTS

### Demographics

The dataset for individuals 90 years and older included 686 late-AMD cases (70.8%) and 283 controls without AMD (29.2%). The mean age of this study group was $92.08 \pm 2.25$ years (range, 90–102 years); 351 were male (36.2%), and 618 were female (63.8%). For comparison purposes, 27,027 individuals from other age groups were additionally included in

**TABLE 1.** Demographics of the Study Cohort

| Age Group | N | *n* (%) | | | |
|---|---|---|---|---|---|
| | | Female Sex | Male Sex | No AMD | Late AMD |
| 60–69 y | 6514 | 3753 (57.6) | 2761 (42.4) | 4304 (66.1) | 2210 (33.9) |
| 70–79 y | 12,228 | 6822 (55.8) | 5406 (44.2) | 5911 (48.3) | 6317 (51.7) |
| 80–89 y | 8285 | 4895 (59.1) | 3390 (40.9) | 2959 (35.7) | 5326 (64.3) |
| ≥90 y | 969 | 618 (63.8) | 351 (36.2) | 283 (29.2) | 686 (70.8) |

the study (Table 1). All samples belonged to the IAMDGC. Additional information regarding the included IAMGC study cohorts is provided in Supplementary Table S1.

## Association of Genetic Variants with AMD in Individuals 90 Years and Older

The associations with late AMD were determined for 51 AMD-associated variants present in the study cohort (Supplementary Table S2).

A total of six SNPs were significantly associated with AMD in the individuals 90 years and older after Bonferroni correction for 51 tests ($P < 0.001$): rs10922109 and rs570618 in the *CFH* locus, rs116503776 in the *C2/CFB/SKIV2L* locus, rs3750846 in the *ARMS2/HTRA1* locus, rs6565597 in the

*NPLOC4/TSPAN10* locus, and rs5754227 in the *SYN3/TIMP3* locus (Table 2).

For rs10922109 and rs570618, minor allele frequency (MAF) increased in both cases and controls with increasing age, but the increase was greater in cases than in controls. For *ARMS2* rs3750846, there were no age effects of MAF in the controls, but MAF in cases decreased with greater age. For *C2/CFB/SKIV2* rs116503776, there was no consistent relationship between MAF and age in the controls, but in cases an increasing frequency of MAF with age was observed. For *NPLOC4/TSPAN10*, there was no consistent change of MAF with age, as MAF dropped only in the controls ≥ 90 years of age. In *SYN3/TIMP3* rs5754227, no relationship between MAF with age in cases and controls could be detected (for details, see Table 2). Compared to the effects reported in the overall population (see also Supplementary Table S3),[6] smaller effects were detected in the nonagenarian and older group for rs10922109 and rs570618 in the *CFH* locus and for rs3750846 in the *ARMS2/HTRA1* locus (Table 2). Moreover, rs6565597 in *NPLOC4/TSPAN10* and rs5754227 in *SYN3/TIMP3* showed larger effects in the individuals 90 years and older, whereas rs116503776 in *C2/CFB/SKIV2L* showed similar effects (Table 2).

Power analysis of all 51 variants analyzed showed that the study design had greater than 80% power to detect the association of a third variant at the *CFH* locus, rs61818925 (Fig. 1); however, no association was detected for this partic-

**TABLE 2.** Significantly Associated Variants in the 90 Years and Older Group in All Age Groups

| | | Chromosome | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 1 | 6 | 10 | 17 | 22 |
| | | Locus | | | | | |
| | | *CFH* | *CFH* | *C2/CFB/SKIV2L* | *ARMS2/HTRA1* | *NPLOC4/TSPAN10* | *SYN3/TIMP3* |
| | | Index Variant | | | | | |
| | | **rs10922109** | **rs570618** | **rs116503776** | **rs3750846** | **rs6565597** | **rs5754227** |
| | | Major/Minor Allele | | | | | |
| | | C/A | T/G | G/A | T/C | C/T | T/C |
| Overall population[6] | OR | 0.38 | 0.42 | 0.57 | 2.81 | 1.13 | 0.77 |
| | AF, cases | 0.233 | 0.580 | 0.090 | 0.436 | 0.400 | 0.109 |
| | AF, controls | 0.426 | 0.364 | 0.148 | 0.208 | 0.381 | 0.137 |
| | *P* | $9.6 \times 10^{-618}$ | $2.0 \times 10^{-590}$ | $1.2 \times 10^{-103}$ | $6.5 \times 10^{-735}$ | $1.5 \times 10^{-11}$ | $1.1 \times 10^{-24}$ |
| 60–69 y | OR | 0.35 | 0.35 | 0.53 | 3.08 | 1.17 | 0.77 |
| | AF, cases | 0.200 | 0.368 | 0.080 | 0.483 | 0.405 | 0.112 |
| | AF, controls | 0.416 | 0.625 | 0.143 | 0.217 | 0.372 | 0.141 |
| | *P* | $2.50 \times 10^{-138}$ | $4.09 \times 10^{-165}$ | $3.25 \times 10^{-26}$ | $9.36 \times 10^{-187}$ | $8.40 \times 10^{-05}$ | $3.79 \times 10^{-06}$ |
| 70–79 y | OR | 0.34 | 0.37 | 0.50 | 3.12 | 1.12 | 0.77 |
| | AF, cases | 0.203 | 0.392 | 0.083 | 0.461 | 0.399 | 0.107 |
| | AF, controls | 0.426 | 0.636 | 0.151 | 0.206 | 0.375 | 0.134 |
| | *P* | $6.56 \times 10^{-308}$ | $1.83 \times 10^{-310}$ | $1.31 \times 10^{-62}$ | $<1.00 \times 10^{-311}$ | $3.85 \times 10^{-05}$ | $6.56 \times 10^{-11}$ |
| 80–89 y | OR | 0.38 | 0.46 | 0.62 | 2.59 | 1.08 | 0.81 |
| | AF, cases | 0.238 | 0.454 | 0.095 | 0.398 | 0.397 | 0.111 |
| | AF, controls | 0.445 | 0.646 | 0.146 | 0.202 | 0.378 | 0.133 |
| | *P* | $1.10 \times 10^{-162}$ | $7.31 \times 10^{-123}$ | $1.47 \times 10^{-22}$ | $3.51 \times 10^{-148}$ | 0.04 | $1.78 \times 10^{-05}$ |
| ≥90 y | OR | 0.48 | 0.56 | 0.57 | 1.89 | 1.46 | 0.62 |
| | AF, cases | 0.284 | 0.509 | 0.114 | 0.346 | 0.423 | 0.111 |
| | AF, controls | 0.444 | 0.645 | 0.180 | 0.217 | 0.344 | 0.171 |
| | *P* | $9.16 \times 10^{-12}$ | $1.48 \times 10^{-08}$ | $8.93 \times 10^{-05}$ | $2.86 \times 10^{-08}$ | 0.0006 | 0.0006 |
| Variation of AF with increasing age | AF, cases | Increase | Increase | Increase | Decrease | No trend | No trend |
| | AF, controls | Increase | Increase | No trend | No trend | No trend | No trend |
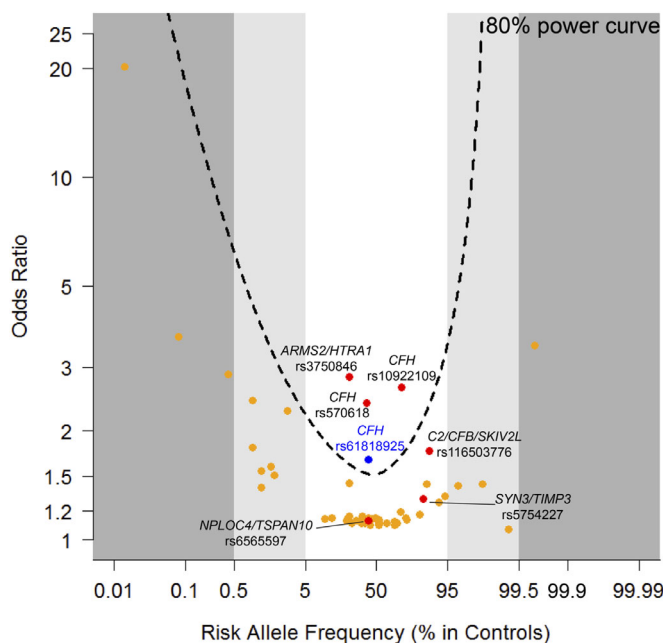
**FIGURE 1.** Power analysis of 51 variants in individuals 90 years of age and older. Significantly associated variants are marked in *red*; rs61818925 (marked in *blue*) did not show significant associations despite more than 80% power.
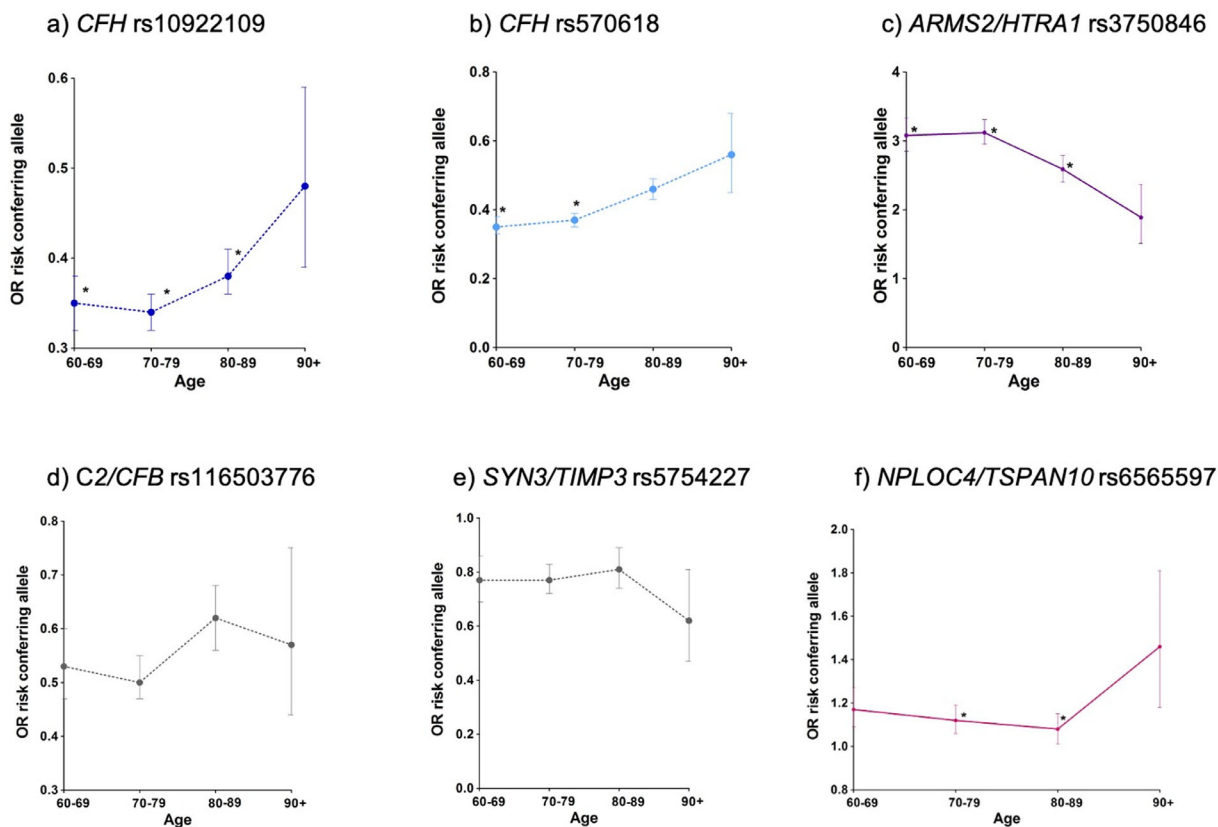


**FIGURE 2.** Odds ratios in the different age groups of the six variants showing significant associations in individuals 90 years of age and older. (**a**) rs10922109 (*CFH*), (**b**) rs570618 (*CFH*), (**c**) rs3750846 (*ARMS2/HTRA1*), (**d**) rs116503776 (*C2/CFB/SKIV2L*), (**e**) rs5754227 (*SYN3/TIMP3*), (**f**) rs6565597 (*NPLOC4/TSPAN10*). *P-value interaction with the 90 years and older group < 0.05. (For details, see Table 3.)

TABLE 3. Interaction of Genetic Effects Between the 90 Years and Older Group and Other Age Groups and Interaction with Age as a Continuous Variable of Significantly Associated Variants

| Locus | Variant | *P* Interaction with 90 Years and Older Group | | | *P* Interaction with Age As a Continuous Variable |
| | | 60–69 y | 70–79 y | 80–89 y | |
| --- | --- | --- | --- | --- | --- |
| *CFH* | rs10922109 | 0.006 | 0.002 | 0.04 | 0.05 |
| *CFH* | rs570618 | $4.68 \times 10^{-5}$ | 0.0001 | 0.07 | $2.24 \times 10^{-7}$ |
| *C2/CFB/SKIV2L* | rs116503776 | 0.51 | 0.32 | 0.67 | 0.04 |
| *ARMS2/HTRA1* | rs3750846 | 0.0002 | $6.57 \times 10^{-5}$ | 0.01 | 0.001 |
| *NPLOC4/TSPAN10* | rs6565597 | 0.06 | 0.02 | 0.01 | 0.89 |
| *SYN3/TIMP3* | rs5754227 | 0.13 | 0.12 | 0.07 | 0.93 |

ular variant in the 90 years and older group (Supplementary Table S2).

### Genetic Risk Effects on AMD in the 90 Years and Older Group Were Different Compared to the Other Age Groups
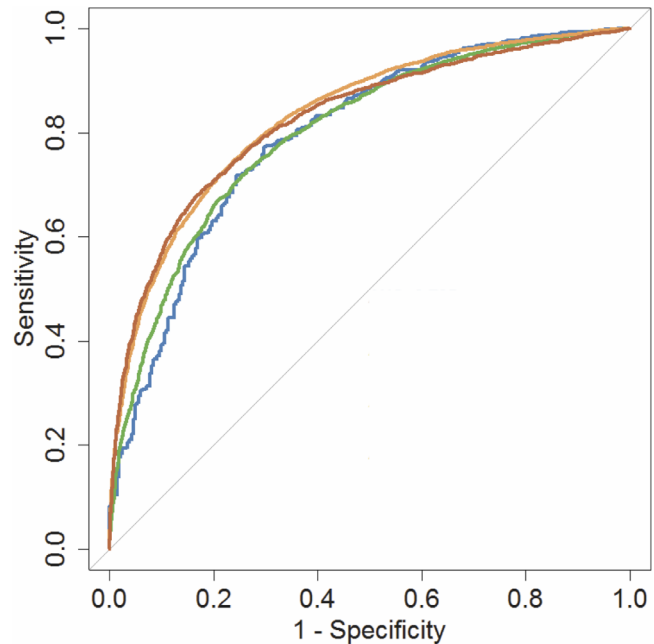
Associations with late AMD for the 51 genetic variants in the other age groups are presented in Supplementary Table S2. For rs10922109, rs570618 in the *CFH* locus, and rs3750846 in the *ARMS2/HTRA1* locus, the genetic effects decreased with increasing age (Figs. 2a–2c). For rs116503776 (*C2/CFB/SKIV2L*) and rs5754227 (*SYN3/TIMP3*), there were no significant differences in the effect sizes on late AMD in the different age groups (Figs. 2d, 2e). Interestingly, rs6565597 (*NPLOC4*/TSPAN10) showed larger effects in the 90 years and older group compared to two younger age groups (70–79 years and 80–89 years) (Fig. 2f). Details of the interactions in the 90 years and older group are displayed in Table 3.

Looking at the allele frequencies of cases and controls among age groups, variations could be detected for some of them with increasing age (Supplementary Table S2).

The distribution among sex, AMD status, source of DNA, and cohort was variable among the age groups, but there were also differences in the size of these age groups. Therefore, an additional sensitivity analysis was performed in which the age groups were matched on these factors (*n* = 885 for all age groups). These analyses showed comparable results (Supplementary Table S4).

Receiver operating characteristic (ROC) curves including all 51 variants for all age groups are presented in Figure 3. The AUCs for the regression models (Table 4, Supplementary Table S5) were 0.825 for individuals 60 to 69 years of age, 0.831 for individuals 70 to 79 years of age, and 0.800 for individuals 80 to 89 years of age.

In the group of individuals 90 years and older, the AUC was the lowest: 0.795 (Table 4). The AUC for the individuals

TABLE 4. AUCs of Regression Models for the Different Age Groups Including All Genetic Variants

| Age Group | AUC | CI | *P* | | |
| | | | 70–79 y | 80–89 y | ≥90 y |
| --- | --- | --- | --- | --- | --- |
| 60–69 y | 0.825 | 0.814–0.836 | 0.31 | **0.001** | 0.08 |
| 70–79 y | 0.831 | 0.824–0.839 | — | $3.07 \times 10^{-7}$ | **0.03** |
| 80–89 y | 0.800 | 0.790–0.810 | — | — | 0.76 |
| ≥90 y | 0.795 | 0.763–0.826 | — | — | — |

Significant differences are marked in bold. CI, confidence interval.



FIGURE 3. ROC curves and AUCs of regression models of the 51 variants in the different age groups. *Red*, 60 to 69 years; *orange*, 70 to 79 years; *green*, 80 to 89 years; *blue*, 90 years and older. (For details, see Table 4.)

70 to 79 years old differed significantly from the 90+ group (*P* = 0.026) (Fig. 3, Table 4).

## DISCUSSION

In this study, we analyzed 51 genetic variants associated with late AMD in persons 90 to 102 years of age and compared the effect sizes to other age groups. In the oldest group, comprised of individuals 90 years and older, we detected six SNPs significantly associated with late AMD. The strongest associations were detected for rs10922109 and rs570618 located in the *CFH* locus and rs3750846 in the *ARMS2/HTRA1* locus. Previous reports have shown the importance of variants in *CFH* and *ARMS2* in the progression of drusen and progression into advanced AMD.[18–20] These very highly AMD-associated variants showed smaller effect sizes on late AMD in individuals over 90 years of age compared to the overall population and compared to younger age groups. For rs570618 in *CFH* and rs3750846 in *ARMS2/HTRA1*, we observed a strong interaction with age that supports this relationship. These results are in line

with previous reports describing lower effects sizes of other AMD-associated variants in *CFH* in older individuals.[10–12]

For some variants, allele frequencies in cases and/or controls increased or decreased with increasing age. For rs10922109 and rs570618 in *CFH*, the minor allele increased protection, especially in cases but also in controls. For rs3750846 in *ARMS2*, the minor allele was risk conferring. No age-related effects of MAF could be found in controls, but MAF decreased with age in cases. These findings may be explained by a survivorship effect, which has also been discussed by Adams et al.[11]

Minor alleles of rs5754227 in *SYN3/TIMP3* and rs116503776 in *C2/CFB/SKIV2L* are infrequent, which could have resulted in no clear or consistent change of MAF with age being detected, and there was not a clear association with age in the interaction models.

In our analysis, rs61818925 in *CFH* did not show significant associations with late AMD despite having sufficient power. Therefore, the protective effect of this variant on developing AMD might also be smaller in individuals above the age of 90 years. These findings point toward a smaller role for *CFH* and *ARMS2* in late AMD development at very advanced ages.

Previous studies have investigated the mortality risk resulting from AMD.[21–23] These reports describe associations of late AMD with mortality and therefore support the theory of the enrichment of non-risk, AMD-associated allele frequencies in a long-lived population. A large meta-analysis by McGuinness et al.[24] also concluded that late AMD is associated with increased rates of mortality of all causes, especially cardiovascular mortality. The authors therefore suggested the existence of shared pathways between late AMD and systemic diseases, which was also shown by Grassmann et al.[25]

Longevity studies describe the phenomenon of genetic differences between younger and older populations, explained by an enrichment of "longevity genes" in very old individuals.[13–15] Interestingly, a recent genome-wide association study for parent lifespan did not detect genome-wide significant associations with longevity or highly AMD-associated variants in *CFH* or *ARMS2*.[26] The study did detect variant rs429358 in *APOE* as a factor for survival with an increasing lifespan for carriers of the T-allele, a finding that was also reported by Pilling et al.[27] in 2017. In our study, *APOE* variant rs429358 showed significant associations in individuals 60 to 89 years of age with the C-allele as a protective factor with similar ORs. In contrast, in individuals older than 90 years of age, the effect was smaller and did not reach significance (Supplementary Tables S2). Although these findings may be explained by the smaller size of the oldest group, in our cohort the MAF was lower in very old individuals without AMD compared to other age groups (Supplementary Tables S2). Therefore, *APOE* may represent an example of a variant that is associated with both longevity and AMD. At higher ages, there are fewer carriers of the C-allele, possibly due to increased mortality.

Interestingly, in our cohort, rs6565597 in *NPLOC4/TSPAN10* showed a significant association with AMD in the 90 years and older age group, despite limited statistical power. In our study, the effect size for *NPLOC4/TSPAN10* was higher in the 90 years and older group than in the overall population and was specifically higher than in the individuals 70 to 79 and 80 to 89 years of age, indicating that this variant may be a specific risk factor for late AMD in the elderly. This was also seen in the sensitivity analysis, in which an association of this variant was detected only in the oldest age group. On the other hand, the frequency of the risk-increasing minor allele dropped only in the controls who were 90 years and older; there were no consistent changes in the late-AMD cases and no interaction with age when analyzed as a continuous variable, thus making it difficult to reach a clear conclusion regarding rs6565597 in *NPLOC4/TSPAN10*.

Regression models of all 51 SNPs showed the lowest discriminative power in the group of individuals 90 years and older and a similar AUC for the individuals 80 to 89 years of age. A significantly higher discriminative value was detected in the individuals 70 to 79 years of age. Therefore, we propose that the combined genetic influence of these 51 variants on AMD development decreases in individuals above the age of 80 years. As for rs570618 in *CFH* and rs3750846 in *ARMS2/HTRA1*, the strongest associations with age were found in the interaction analysis; these two variants likely are strong factors for lower AUCs in the two oldest groups. In older persons, it is plausible that primary age by itself and other non-genetic environmental factors such as smoking, alcohol use, physical activity, or sunlight exposure may become more relevant for disease risk.

Our results suggest that, in younger AMD patients, a high genetic risk, especially regarding the common variants in *CFH* and *ARMS2*, induces and accelerates the disease at earlier ages. Additionally, rare, highly penetrant variants will provoke disease development at younger age. In older individuals, aging processes in the retina and the subretinal structures may influence disease progression despite the presence of fewer genetic risk factors.

The results presented here are based on a large case–control cohort with a large number of individuals above the age of 90 years. Because it is very difficult to include very old individuals in clinical studies (especially controls without AMD), there are large differences in the sizes of the age groups that have been investigated. However, the results of our sensitivity analysis with matched group sizes show comparable results and suggest that no bias was introduced due to heterogeneity in the number of individuals, sex, AMD status, source of DNA, and cohort among the age groups. The multicenter case–control design with variable methodology does not allow the investigation of longitudinal or epidemiological parameters and limits reaching conclusions on real association trends with age. Furthermore, our analysis was restricted to 51 already known AMD-associated variants, and non-genetic factors were not included, further limiting interpretation. Future prospective studies using extended datasets and including both genetic and environmental factors are needed to identify additional AMD-associated risk factors in the nonagenarian and older population and to better draw conclusions on true survival effects.

In summary, in our study, risk alleles in *ARMS2* and *CFH* showed significantly smaller risk effects on AMD development in very old individuals. The influence of 51 known genetic risk factors on late AMD was less in the oldest individuals compared to persons between 70 and 79 years of age. Moreover, we have described for the first time, to the best of our knowledge, the genetic variant rs6565597 in *NPLOC4/TSPAN10*, which shows larger effects in individuals 90 years and older; therefore, rs6565597 may be a more specific risk factor for older persons. If these results are confirmed in other studies, they may have a significant

impact on AMD risk assessment in an increasingly aging population.

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