Supplementary Data

For Age and Ageing paper: *Common eye diseases in older adults of Southern Germany: Results from the KORA-Age Study.* Graw, J et al.

**Appendix 1. Methodology.**

**Material**

**Eye disorders**

As part of a standardized face-to-face interview the participants were asked for the presence of eye disorders (cataracts, glaucoma, and/or other eye diseases including AMD or dry eyes); the interview is based upon the KORA-Eye questionnaire [9]. Briefly, the ophthalmologists were asked to validate and specify the self-reported diagnosis based upon their records with respect to cornea disorders (including dry eyes), cataracts (with and without surgery; nuclear, cortical, posterior and/or subcapsular cataracts as subtypes), glaucoma (defined by increased inner-ocular pressure, visual field anomaly, optic nerve excavation or as open- or closed-angle glaucoma), and retinal disorders (including wet and dry AMD). For details of the questionnaire sent to the ophthalmologists for validation and specification refer to supplementary data, Appendix 2. For further calculations, only cases validated by an ophthalmologist have been considered. As “controls” we took those participants having said “no eye disease” without statement of an ophthalmologist, or whose ophthalmologist said “no eye disease. As “missings” we considered those participants, whose statements having an eye disease was not confirmed because the ophthalmologists did not reply to our invitation.

**Assessment of co-variables**

Besides age (in years) and sex, other co-variables (gathered in a structured face-to-face interview, in a questionnaire or in direct laboratory investigations) were included in the analysis.

As co-morbidities, we included diabetes (self reported plus medication taken), lung diseases (self reported asthma, emphysema, COPD without medication taken), and hypertension (actually measured ≥140/90 mmHg according to ISH-WHO 1999 [29], or corresponding medication taken given that the subjects are aware of having hypertension). The participants were asked to bring all medications along with them, which they had taken during the past 7 days preceding the interview. The medication data were registered (IDOM software [30]) and categorized according to the Anatomical Therapeutical Chemical (ATC) classification index. Composite variables were defined for local or systemic use of corticoids, thyroid hormones, insulin and oral antidiabetics, antihypertensive drugs, diuretics, beta-blocking agents, angiotensin-II-enzyme antagonists, angiotensin antagonists, and drugs used in airway diseases. Medication parameters were included into the statistics, if it was taken at least by 2.5% of participants in one of the sex-stratified subgroups. Life-style factors like body mass index (BMI), smoking status and alcohol consumption were also considered. To test any influence of physiological parameters on eye diseases, we included also some laboratory parameters (blood count, enzyme activities, metabolites and ion concentrations).

**Statistics**

Regression analyses for each of the four major disease outcomes (dry eye, AMD, glaucoma and cataract) were conducted and risk factors were identified by a group-wise selection procedure based on three groups of variables: co-morbidities (diabetes, lung disease and hypertension), general medication (n=14), life-style factors together with data from laboratory investigations (n= 21). For each disease an overall regression model was found by selecting a subgroup of risk factors with a P-value less than 0.05 or the risk factor with the strongest standardized effect based on separate multiple logistic regressions for each variable group. The analyses were stratified by sex and adjusted for age. The odds ratios were calculated per unit change and reported with corresponding 95% confidence intervals and P-values of the finally selected models. The analyses were done by the SAS procedure GLIMMIX (SAS Institute Inc, Cary, USA).

**Analysis of Single Nucleotide Polymorphisms (SNPs)**

SNPs were analyzed of the coding region of genes, which have been shown previously to be involved in cataracts [9]. Similarly, SNPs were investigated for genes involved in Glaucoma and AMD (Suppl. Table 1). Genotyping of genomic DNA was performed as described previously [31, 32]. Genotype, allele frequencies and violations of Hardy-Weinberg-Equilibrium are computed routinely for each SNP using the statistical computing environment R (version 2.15.3) [33] with the *genetics* package and *SNPassoc* package.

For the calculation of the detection of a relative risk with at least 80% power, we distinguish between different MAFs of the SNPs: for those SNPs with MAF 2.7% or greater, we have at least 80% power to detect a relative risk for AMD of 3.4 and 11.4 for heterozygous and homozygous SNP-carriers, respectively; for those SNPs with MAF > 19.5% we have at least 80% power to detect a relative risk of 2.1 and 4.6 and SNPs with MAF 40% or greater, we have at least 80% power to detect a relative risk of 2.1 and 4.3.

For those SNPs with MAF 3.7% or greater, we have at least 80% power to detect a relative risk for glaucoma of 2.7 and 7.4 for heterozygous and homozygous SNP-carriers, respectively; for those SNPs with MAF > 20.2% we have at least 80% power to detect a relative risk of 2.3 and 5.3 and those SNPs with MAF 40% or greater, we have at least 80% power to detect a relative risk of 2 and 3.8.

To explore genetic effects on age-related formation of eye diseases, we fitted a logistic regression for each SNP (additive model) adjusted for age and sex. To adjust for multiple testing, P-values were calculated controlling for false discovery rate [34]. SNPs were selected, if the adjusted false discovery rate was less than 0.05.

**References**

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**Appendix 2: Opthalmologists.**

**Specification of ocular diseases**

The patient suffers from „dry eye“ yes  no

There is/was a **cornea disease** yes  no

**If yes:** beginning of the disease (year) |\_\_|\_\_|\_\_|\_\_| surgery performed? yes  no

The cornea disease is/was characterized as

left right

yes no yes no

cornea dystrophy

Sicca syndrome

Keratopathia metaherpatica

[miscellaneous](http://dict.leo.org/ende/index_de.html#/search=miscellaneous&searchLoc=0&resultOrder=basic&multiwordShowSingle=on)

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There is/was a **cataract** yes  no

**If yes:** beginning of the disease (year) |\_\_|\_\_|\_\_|\_\_| surgery performed? yes  no

The cataract is/was characterized as

Left Right

yes no yes no

anterior c.

ncklear c.

total c.

cortical c.

posteriorc c.

subcapsular c.

[miscellaneous](http://dict.leo.org/ende/index_de.html#/search=miscellaneous&searchLoc=0&resultOrder=basic&multiwordShowSingle=on):

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b. w.

There is/was a **glaucom:** yes  no

**If yes:** beginning of the disease (year) |\_\_|\_\_|\_\_|\_\_| surgery performed? yes  no

The glaucoma is/was characterized as

Left Right

yes no yes no

increased inner ocular pressure

inner ocular pressure (mm Hg) |\_\_|\_\_| |\_\_|\_\_|

visual field anomalie

open-angle g.

closed-angle g.

optic nerve excavation

[miscellaneous](http://dict.leo.org/ende/index_de.html#/search=miscellaneous&searchLoc=0&resultOrder=basic&multiwordShowSingle=on):

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There is/was a **retinal disease** yes  no

**If yes:** beginning of the disease (year) |\_\_|\_\_|\_\_|\_\_| surgery performed? yes  no

The retinal disease is/was characterized as

Left Right

yes no yes no

retinitis pigmentosa

macular degeneration (AMD)

macular gliosis

macular edema

tumour

vessel anomalies

retinoschisis

thrombosis of the retina

[miscellaneous](http://dict.leo.org/ende/index_de.html#/search=miscellaneous&searchLoc=0&resultOrder=basic&multiwordShowSingle=on):

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[miscellaneous](http://dict.leo.org/ende/index_de.html#/search=miscellaneous&searchLoc=0&resultOrder=basic&multiwordShowSingle=on):

Unfortunately, there is no detailed specification possible

**Appendix 3 – Supplementary Tables and figures**

Suppl. Table 1:

**Genes tested for effects on age-related eye disease, grouped for cataract, glaucoma and AMD**

**Cataracts Glaucoma AMD**

*CRYAA (rs872331) MYOC (rs2234926) ABCA1 (rs2066714; rs2230808)*

*CRYBA1 (rs1047790) WDR36 (rs11241095) ARMS2 (rs10490924; 2736911)*

*CRYBA4 (rs5761637) CETP (rs5880)*

*CRYBB2 (rs16986560; rs8140949) CFH (rs1061170)*

*CRYBB3 (rs17670506; rs9608378) CX3CR1* (rs11715522)

*CRYGB (rs796287) ERCC6 (rs2228529)*

*CRYGD (rs2305430) LIPC (rs6083; rs6078)*

*CRYGN (rs2075001) SERPING1 (rs4926)*

*EPHA2 (rs35903225)*

*GJA3 (rs11617415)*

*GJA8 (rs3766503)*

*LIM2 (rs8111243)*

*PITX3 (rs2281983)*

*SIX5 (rs2341097)*

Suppl. Table 2: **Validation of eye diseases in the questionnaire**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ocular disease | Participant: | Ophthalmologist: yes | Ophthalmologist: no | Validation\*  n (%) | Cases\*\*  (n) |
| Cataract | yes | 250 (95.4%) | 12 |  |  |
|  | no | 49 | 21 (30.0%) | 271 (81.6%) | 299 |
| Cornea/Dry eyes | yes | 23 (67.6%) | 11 |  |  |
|  | no | 106 | 188 (63.9%) | 211 (64.3%) | 129 |
| Glaucoma | yes | 45 (71.4%) | 18 |  |  |
|  | no | 27 | 246 (90.1%) | 291 (86.6%) | 72 |
| AMD | yes | 31 (81.6%) | 7 |  |  |
|  | no | 37 | 259 (87.5%) | 290 (86.8%) | 68 |

\*validation means that the treating ophthalmologist agrees with the statement of the participant (“yes/yes” or “no/no”).

\*\*As “case” is counted only, if the ophthalmologist says “yes”

Suppl. Table 3:

**Results for all co-variables tested in the KORA-Age2 Eye study**

1. Continuous parameters

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | men | | | women | | |
| Parameter | Number | Mean | SD | Number | Mean | SD |
| BMI | 411 | 27.98 | 3.95 | 400 | 28.11 | 4.56 |
| Alkaline phosphatase (µkat/l) | 404 | 1.17 | 0.43 | 397 | 1.19 | 0.35 |
| total cholesterol (mg/dl) | 404 | 197.40 | 41.87 | 397 | 219.20 | 40.09 |
| GGT – -glutamyl-transferase (µkat/l) | 404 | 0.98 | 1.26 | 397 | 0.69 | 1.35 |
| Glutamate-oxalate-transaminase (GOT. AST) | 404 | 0.33 | 0.15 | 397 | 0.30 | 0.14 |
| Glutamate-pyruvate-transaminase (GPT. ALT) | 404 | 0.36 | 0.18 | 397 | 0.31 | 0.15 |
| HDL - cholesterol (mg/dl) | 403 | 50.97 | 13.62 | 397 | 62.68 | 16.30 |
| Hemoglobin (g/l) | 402 | 135.30 | 14.49 | 395 | 127.30 | 10.84 |
| Uric acid (mg/dl) | 404 | 5.78 | 1.34 | 397 | 4.73 | 1.31 |
| Potassium (mmol/l) | 404 | 4.27 | 0.36 | 397 | 4.16 | 0.36 |
| Creatinine (mg/dl) | 403 | 1.17 | 0.40 | 397 | 0.95 | 0.26 |
| LDL - cholesterol (mg/dl) | 403 | 115.10 | 32.12 | 397 | 125.30 | 33.48 |
| MCH. mean corpuscular hemoglobin | 402 | 31.51 | 1.98 | 395 | 30.71 | 1.83 |
| MCV. mean erythrocyte volume (1/fl) | 402 | 91.53 | 5.21 | 395 | 89.67 | 4.70 |
| MPV - mean platelet volume (1/fl) | 399 | 8.63 | 1.04 | 392 | 8.62 | 0.99 |
| Sodium (mmol/l) | 404 | 139.60 | 2.62 | 397 | 139.60 | 2.80 |
| Thrombocytes (1/nl) | 402 | 197.30 | 65.02 | 395 | 225.10 | 54.75 |
| Erythrocytes (1/pl) | 402 | 4.31 | 0.49 | 395 | 4.15 | 0.36 |
| Leucocytes (1/nl) | 402 | 6.69 | 1.91 | 395 | 6.79 | 1.72 |

1. Binary Factors

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | men | | | women | | |
| Parameter | Number total | Number observed | percentage | Number total | Number observed | percentage |
| Diabetes | 413 | 74 | 17.9% | 408 | 70 | 17.2% |
| Hypertension | 410 | 283 | 69.0% | 403 | 292 | 72.5% |
| Asthma/COPD/emphysema | 414 | 53 | 12.8% | 408 | 48 | 11.8% |
| Corticosteroide | 414 | 37 | 8.9% | 408 | 38 | 9.3% |
| Thyroid hormone | 412 | 42 | 10.2% | 408 | 132 | 32.4% |
| Drugs for diabetes | 414 | 59 | 14.3% | 408 | 53 | 13.0% |
| Antihypertensives | 414 | 295 | 71.3% | 408 | 285 | 69.9% |
| Beta-blockers | 412 | 160 | 38.8% | 407 | 175 | 43.0% |
| ACE-inhibitors | 412 | 149 | 36.2% | 407 | 127 | 31.2% |
| Angiotensin antagonists | 413 | 87 | 21.1% | 407 | 82 | 20.1% |
| Diuretics | 413 | 204 | 49.4% | 407 | 182 | 44.7% |
| Calcium antagonists | 411 | 86 | 20.9% | 408 | 93 | 22.8% |
| Other antihypertensives | 412 | 14 | 3.4% | 407 | 1 | 0.2% |
| Statines | 412 | 130 | 31.6% | 408 | 123 | 30.1% |
| Sympatomimetica | 414 | 22 | 5.3% | 408 | 17 | 4.2% |
| Glucocorticoides | 414 | 11 | 2.7% | 408 | 13 | 3.2% |
| Anticholinerics | 414 | 13 | 3.1% | 408 | 4 | 1.0% |
| No alcohol | 414 | 47 | 11.4% | 407 | 119 | 29.2% |
| actual smoker | 414 | 18 | 4.3% | 407 | 13 | 3.2% |

Suppl. Table 4:

**Results of the logistic regressions between AMD and analyzed SNPs (additive genetic model) adjusted for age and sex in KORA**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **gene** | **rs** | **Alleles\*** | **MAF NCBI** | **MAF KORA** | **OR** | **CI lower** | **CI upper** | **P val (corr)** |
| *ABCA1* | *rs2066714* | **A**/G | 0.133 | 0.117 | 1.13 | 0.61 | 2.07 | 0.822 |
| *ABCA1* | *rs2230808* | A/**G** | 0.199 | 0.256 | 1.00 | 0.64 | 1.57 | 0.998 |
| *ARMS2* | *rs10490924* | **G**/T | 0.199 | 0.195 | **2.28** | **1.48** | **3.51** | **0.005** |
| *ARMS2* | *rs2736911* | **C**/T | 0.208 | 0.158 | 0.47 | 0.23 | 0.95 | 0.261 |
| *CETP* | *rs5880* | C/**G** | 0.050 | 0.039 | 0.76 | 0.23 | 2.58 | 0.822 |
| *CFH* | *rs1061170* | C/**T** | 0.286 | 0.352 | **2.03** | **1.35** | **3.06** | **0.010** |
| *CRYAA* | *rs872331* | C/**T** | 0.438 | 0.408 | 0.92 | 0.61 | 1.40 | 0.822 |
| *CRYBA1* | *rs1047790* | **C**/T | 0.208 | 0.155 | 0.73 | 0.40 | 1.31 | 0.767 |
| *CRYBA4* | *rs5761637* | **C**/T | 0.183 | 0.156 | 1.29 | 0.79 | 2.11 | 0.767 |
| *CRYBB2* | *rs16986560* | **G**/T | 0.009 | 0.013 | 1.64 | 0.34 | 7.81 | 0.822 |
| *CRYBB2* | *rs8140949* | A/**G** | 0.208 | 0.250 | 0.88 | 0.55 | 1.41 | 0.822 |
| *CRYBB3* | *rs17670506* | A/**G** | 0.084 | 0.056 | 1.57 | 0.70 | 3.50 | 0.767 |
| *CRYBB3* | *rs9608378* | C/**G** | 0.308 | 0.392 | 1.12 | 0.75 | 1.67 | 0.822 |
| *CRYGB* | *rs796287* | **G**/T | 0.314 | 0.27 | 0.85 | 0.55 | 1.33 | 0.822 |
| *CRYGD* | *rs2305430* | **C**/T | 0.085 | 0.094 | 0.68 | 0.32 | 1.45 | 0.767 |
| *CRYGN* | *rs2075001* | **C**/T | 0.078 | 0.084 | 0.50 | 0.20 | 1.26 | 0.697 |
| *CX3CR1* | *rs11715522* | **A**/C | 0.353 | 0.387 | 0.56 | 0.36 | 0.87 | 0.096 |
| *EPHA2* | *rs35903225* | **C**/T | 0.018\*\* | 0.027 | 0.75 | 0.17 | 3.26 | 0.822 |
| *ERCC6* | *rs2228529* | **A**/G | 0.204 | 0.212 | 0.83 | 0.50 | 1.36 | 0.822 |
| *GJA3* | *rs11617415* | **C**/T | 0.225 | 0.213 | 0.92 | 0.56 | 1.51 | 0.822 |
| *GJA8* | *rs3766503* | A/**G** | 0.049 | 0.035 | 0.49 | 0.14 | 1.67 | 0.767 |
| *LIPC* | *rs6078* | A/**G** | 0.031 | 0.036 | 1.35 | 0.59 | 3.11 | 0.822 |
| *LIPC* | *rs6083* | A/**G** | 0.354 | 0.365# | 1.00 | 0.67 | 1.51 | 0.998 |
| *MYOC* | *rs2234926* | A/**G** | 0.158 | 0.133 | 0.67 | 0.34 | 1.31 | 0.767 |
| *PITX3* | *rs2281983* | C/**T** | 0.433 | 0.375 | 0.83 | 0.56 | 1.25 | 0.822 |
| *SERPING1* | *rs4926* | A/**G** | 0.200 | 0.271 | 1.09 | 0.71 | 1.67 | 0.822 |
| *SIX5* | *rs2341097* | **C**/T | 0.342 | 0.383 | 1.41 | 0.93 | 2.15 | 0.603 |
| *WDR36* | *rs11241095* | **A**/G | 0.301 | 0.309 | 0.92 | 0.60 | 1.42 | 0.822 |

\*major allele (NCBI) is given in **bold**;

#the major allele in KORA of the SNP rs6083 is A;

MAF: minor allele frequency; data according to dbSNP: for comparison, European data have been used (HapMap CEU with the largest chromosomal sample size);

\*\* ESP-cohort (no CEU data available);

E: exponent to the basis of 10;

OR: odds ratio; Cl lower: lower point of confidence interval; Cl upper: upper point of confidence interval;

P val (corr): p value corrected for false discovery rate during multiple testing.

**Legend to the supplementary figures:**

Suppl. Fig. 1: **KORA Populations**

The scheme demonstrates the relationship of the KORA-Age 2 study to the previous KORA cohorts. Originally, the surveys S1-S4 were formed, and a 7-years follow up of the S4 cohort (=F4, given in bold and boxed in dark blue) was first investigated to eye diseases (the age-group of 32-71 years had 2593 probands of the entire 3080 probands). The KORA-Age 1 cohort, however, consists of S1-S4 participants aged 65 years and older, and the KORA-Age2 cohort (given in bold and boxed in dark blue) reported here is a 3-years follow-up of an age- and gender-stratified sample from KORA-Age 1. Those cohorts, being not used for the evaluation of eye diseases, are given in grey and are boxed with light blue.

Suppl. Fig. 2: **Age-dependence of ocular diseases**

The percentages and their standard errors (error bar) of cataracts, glaucoma and AMD are given as percentage for the age groups in the population-based KORA-Age2 study; age is given in three 5-years blocks (and below 70 and above 86 years of age).

Supplementary Fig. 2: