evaluation. Baseline mean and SD for thinnest corneal thickness, maximal corneal curvature ($K_{\text{max}}$), mean corneal curvature $K_{\text{m}}$ anterior, astigmatism, anterior chamber (AC) depth, and corneal volume at 7 mm were 482.1±36.8 μm, 52.3±3.7 diopters (D), 46.0±2.2 D, $-3.25±1.6$ D, 3.3±0.3 mm, and 23.5±1.6 mm$^3$, respectively.

Anterior and posterior corneal curvature ($K$ values) measurements were found to be repeatable (Table 1); for curvature measures the worst repeatability limits were for $K_{\text{max}}$ with a value of 1.97 D. Summary data in terms of KCN power deviation, AC depth, AC volume, and AC angle estimates were moderately repeatable, but were all greater than normative values. Front surface elevation maps were more repeatable than back surface elevation maps. Pachymetry estimates had good repeatability limits for pupil center, corneal apex, and thinnest corneal thickness, with most measures inside normal limits.

The reproducibility of the single image between the 2 observers was found to be generally good (Table 1). When the mean of 2 images was used, the limits of reproducibility improved by 20% on average. When the mean of 3 images was used, the limits on average did not improve further; however, certain parameters showed marked additional improvement (Table 1). $K$ values for anterior and posterior measures showed good reproducibility, with the maximum limit of reproducibility of <0.45 D. With a single image, $K_{\text{max}}$ had reproducibility limits well outside normal, but was similar to normal limits when the average of 3 images was used. Index of height decentration and center KCN index had tight reproducibility limits, remaining within normative limits, suggesting these are among the most reproducible parameters in KCN patients.

McAlinden et al determined the $r$ and $R$ values for 100 normal subjects using the same methodology as implemented herein. A sample size of 100 eyes gives 99% confidence limits at level that are within 13% of the true value, whereas a sample size of 32 gives confidence limits on estimates within 23%. McAlinden et al gave an estimate of 95% limit of repeatability in $K_{\text{max}}$ to be 0.8 D in normal subjects; therefore, the 99% CI around it is 0.7 to 0.9 D; herein, we have reported that $K_{\text{max}}$ has a repeatability of 1.97 D. Therefore, the 99% CI of this estimate is (1.5–2.4 D). Because the confidence limits do not overlap, it is clear that these are significantly different. In this way, we compared the repeatability and reproducibility limits of normal and KCN eyes which revealed that 36% and 44% (n = 13/36 and 16/36) of ocular parameters for KCN patients were wider than normative limits but that 28% and 36% (n = 11/36 and 13/36) of the parameters were narrower than normative limits. Reproducibility limits for the indices (KCN index, index of vertical asymmetry, and index of horizontal asymmetry) were notably wide for KCN eyes. However, repeatability limits for axis anteriorly and posteriorly were markedly narrower ($r < 24°$; Table 1) than in normal eyes ($r > 95°$; Table 1).

According to the guidelines of patient selection for CXL, a change of >1 D in the $K_{\text{max}}$ parameter is required, stating that a change of this magnitude is “3 times the standard deviation of measurement error.” This study has demonstrated that measurement error is far greater than previously reported, especially in the $K_{\text{max}}$ parameter. In the future, this difference may be partly compensated for within the device, by using the average of parameters derived from several images. However, currently every ophthalmologist who uses the Pentacam device to monitor KCN patients should be aware of the inconsistency in measurement error between KCN and healthy eyes, such that real change can be better distinguished from variability. Future studies should take into account these observations when interpreting progression of KCN and results following CXL.

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References

Genetic Testing in Persons with Age-Related Macular Degeneration and the Use of the AREDS Supplements: To Test or Not to Test?

The controversy surrounding the use of genetic testing to guide the treatment of persons with age-related macular degeneration (AMD) continues. In 2001, the results of the Age-Related Eye Disease Study (AREDS), a placebo-controlled trial, demonstrated that oral supplementation with a combination of antioxidant vitamins and zinc reduced the risk of progression to late AMD by 25% in persons with intermediate AMD in ≥1 eye. Klein et al evaluated the influence of the genotypes complement factor H (CFH) (Y402H, rs1061170) and LOC387715/age-related maculopathy susceptibility 2 (ARMS2) (A69S, rs10490924) on the response to treatment with AREDS supplements (combination of antioxidants and zinc), zinc alone, or antioxidants alone in 876 AREDS participants who had available DNA and who were at high risk of developing advanced AMD. Although there was a possible interaction between CFH genotype and treatment, Klein et al concluded that the AREDS supplements were associated with a
Figure 1. Hazard ratios for the progression to late age-related macular degeneration (AMD) for each of the components of the Age-Related Eye Disease Study (AREDS) supplement: antioxidant, zinc, combination of antioxidant and zinc versus placebo in the analyses reported by Awh et al and the results of the replication of the analyses in the residual cohort of AREDS participants. These are stratified by the genotype treatment groups (GTG) defined by the Awh et al publication. A–C, The 4 genotype treatment groups. Note the differences seen in A and B, where a beneficial effect of the combination of antioxidant and zinc is greater in the analyses conducted in the residual cohort. The harmful effects of genotype treatment group 2 (B) in the Awh analyses were not replicated in the analyses conducted on the residual cohort. E, Participants are stratified by astrological signs, using outcomes for the basis of the subgrouping. Using the Awh cohort, we found a harmful effect of zinc in those born under the birth signs of Aries and Cancer. The analysis in the residual cohort demonstrated a beneficial effect with zinc, demonstrating the importance of replication in studies of genetic associations. ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H.
general reduction in the risk of developing late AMD in all genotype groups compared with placebo, and that neither antioxidant alone nor zinc alone was superior to the antioxidant and zinc combination in any of the genetic groups examined.

Awh et al. created a genetic test to evaluate CFH and ARMS2 genes, and performed retrospective analyses of AREDS subgroups (n = 989). They claimed that treatment with the AREDS supplements should be tailored according to the patient’s genotype, suggesting the need to genotype all patients taking the AREDS supplements. The AREDS investigators compared response to treatment in individuals with different genotype configurations in a larger group of AREDS participants (n = 1237) and failed to find significant differences in response to treatment with AREDS supplements. In this issue of Ophthalmology (see page 162), Awh et al have further refined their genetic subgroups based on outcome, and furthered their claim that AREDS supplements can be harmful to individuals with certain genotypes.

Are these findings by Awh et al true associations, or are they the result of chance, selection bias, or some other confounder? Our request for the identification codes of the AREDS participants in their analyses was turned down. Because the DNA and data used by Awh et al originated from our AREDS dataset, we have reconstructed their sample, which represents only a subset of AREDS participants for whom genetic information is available. Based on when and how the DNA were requested, we are confident of the identification codes for 893 (90%) of the 989 participants used in their analyses, which we verified by finding similar progression rates to late AMD and similar risk ratios for each of the supplements in each of their genetic risk groups.

We agree with Awh et al that the ultimate test of the validity of their study is a replication sample. Thus, it is fortuitous that Awh et al had access to only a portion of the AREDS patients with available DNA. We were able to assemble a validation cohort from the remaining patients (n = 526) and this cohort is referred here as the “residual cohort.” If the findings from the recent report by Awh et al are correct, the results of the analysis from this residual cohort will likely be in the same direction (either beneficial or harmful) and, on average, of the same magnitude as those published by Awh et al, validating their analysis. However, if the results by Awh et al were generated by selection bias and not true associations, the results would be different, likely regressing to the overall mean differences observed in the AREDS primary study results.

As previously published, we genotyped CFH rs412852 and rs3766405, and ARMS2 c.372_815del443ins54 in our study cohort. Figure 1 demonstrates the results of the analyses of the subgroup of Awh et al and the residual cohort, stratified for each of the genotypic groupings suggested by Awh et al. Striking differences are displayed in the various genotypic groups of the genotypic groupings suggested by Awh et al. Striking subgroup of Awh et al and the residual cohort, stratiﬁed by Awh et al, whereas in the residual cohort the results showed a marked beneﬁcial treatment effect of the AREDS supplements and a smaller beneﬁcial effect by zinc, similar to that of the overall results of AREDS. For the group with 2 CFH risk alleles and no ARMS2 risk alleles (Fig 1A), the analysis of Awh revealed about a 3-fold increase in harmful effects for those assigned to either zinc or the AREDS supplements. However, the residual cohort analysis showed a beneﬁcial effect of AREDS supplements and a general regression to the mean, rather than in the direction of the analyses by Awh et al. The zinc group also regressed to the mean in the residual cohort. In the group with 0 or 1 CFH risk alleles and 1 or 2 ARMS2 risk alleles (Fig 1C), the results were similar in both studies. In those with 2 CFH risk alleles and 1 or 2 ARMS2 risk alleles (Fig 1D), whereas the Awh analyses barely showed a beneﬁcial treatment effect in any of the components, the residual cohort demonstrated the beneﬁcial effects of the AREDS supplements. In fact, for all 4 of the genotypic groups reported by Awh et al, the combination of antioxidants and zinc was found to be beneﬁcial and the treatment of choice in the residual cohort.

How do we explain the differences in these results? The major problem relates to how the genotypic subgroups are selected. Subgroup analyses are difﬁcult to interpret even when the subgroups are prespeciﬁed, but the subgroup deﬁnitions analyzed by Awh et al were guided by the same data used to evaluate their effectiveness. Thus, the genetic subgroups in the report from Awh et al were not prespeciﬁed, and were chosen from 9 different genetic subgroups they created based on CFH and ARMS2 genes. These 9 subgroups were then organized based on rates of progression to late AMD. Because of this built-in bias, statistical testing and P values as reported by Awh et al are difﬁcult to interpret. Finding signiﬁcant differences is almost a certainty when there are multiple groups (and hence multiple comparisons) and when subgroup selection is based on outcome. The fallacy of attributing associations to subgroups derived in this manner has been well-described. An example that is easily understood is to divide the population into subgroups by astrological sign. One can then select those with the most beneﬁcial treatment effects and those with the least beneﬁcial effects, easily demonstrating “statistical signiﬁcance” by comparing the 2 groups without accounting for multiple testing or for the lack of a prespeciﬁed comparison. Using the cohort of patients reported by Awh et al, those born under the sign of Aries or Cancer were “harmed” by treatment with zinc (Fig 1E). However, the residual cohort, which was not subjected to selection bias before analyses, showed no harmful effect of zinc for these astrological signs (Fig 1E).

These data strongly suggest and we believe that the conclusions from the report from Awh et al are not correct. The results could not be replicated with the analysis using the residual cohort. Because of the multiplicity of genetic subgroups, the large number of potential comparisons, and the lack of prespeciﬁed hypothesis, it is not unusual that initial associations with outcome cannot be replicated. Early reports of genetic inﬂuence on the response to anti-vascular endothelial growth factor therapy were promising suggesting a genetic association with response to intravitreal therapy but this too did not pass the crucial test of replication.

The combination of antioxidants and zinc, as found in both the AREDS and AREDS2 supplements, remains the only proven beneﬁcial formulation regardless of genotype, with no apparent indication for treatment with either antioxidants or zinc alone. Genetic testing is not recommended for initiating or determining the appropriateness of the AREDS formulation. One should not deprive patients of a therapy that has been proven to have signiﬁcant public health impact on the basis of a statistically ﬂawed, not replicated retrospective analysis of existing data.
A posterior staphyloma is a hallmark lesion of pathologic myopia. To date, retinal complications that develop on or around the edge of a myopic staphyloma have not been extensively reviewed. One reason for this paucity of data is that it is difficult to examine and photograph the edge of a wide staphyloma, except inferior staphylomas, by conventional 50° fundus photographs. However, this obstacle to quality imaging of the periphery has now been overcome by the relatively new Optos Optomap Panoramic 200A Imaging System (Optos, PLC, Dunfermline, Scotland).

The procedures used conform to the tenets of the Declaration of Helsinki, and their use was approved by the Ethics Committee of the Tokyo Medical and Dental University. We examined 777 eyes in 420 patients with pathologic myopia (spherical equivalent < -8.0 diopters or axial length ≥ 26.5 mm) were examined by wide-field fundus autofluorescence (AF) using Optos in the High Myopia Clinic at Tokyo Medical and Dental University. The mean age was 62.8±13.1 years, the mean refractive error was −13.5±4.9 diopters, and the mean axial length was 30.2±2.1 mm. Among the 777 eyes, 582 (74.9%) had a posterior staphyloma by stereoscopic ophthalmoscopy. Eyes with an inferior staphyloma owing to the tilted disc syndrome were excluded.

A linear or leaf-like lesion with an abnormal AF pattern that radiated from the staphyloma edge toward the peripheral fundus was detected in 45 of the 582 eyes with posterior staphyloma (7.7%, Fig 1; Figs 2 and 3, available at www.aaojournal.org), whereas they were not observed in any of the 195 highly myopic eyes without staphyloma. Twenty of the 45 eyes had >1 lesion per eye, and the average number of linear tracts per eye was 1.7±0.9 (range, 1–4). In total, 78 linear tracts were identified in the 45 eyes. The linear tracts were observed as an area of hyperpigmentation or hypopigmentation ophthalmoscopically; however, they were more obvious in AF images.

The tracts were most frequently observed to emanate from the supertemporal edge of the staphyloma (36/78 lesions; 46.1%) followed by those originating from the temporal edge (28/78 lesions; 35.9%), the nasal edge (10/78 lesions; 12.8%), and the inferotemporal edge (1/78 lesions; 1.3%). The tracts were oriented radially extending toward the periphery from the staphyloma edge in 44 of the 45 eyes.

The shape of the tracts varied according to their length. Short tracts had a triangular shape with their base at the staphyloma edge, whereas long tracts had linear or leaf-like shape. Long tracts seemed to be bent in the middle of the lesion and had a dog-earred appearance (Fig 3).

The AF findings of the linear tracts were divided into 3 distinct patterns: (1) Uniform hyper-AF, (2) granular hypo-AF surrounded by hyper-AF rim, or (3) confluent hypo-AF. In eyes with multiple lesions, the lesions had variable AF patterns.

Fluorescein angiograms of the linear tracts showed granular hyperfluorescence owing to window defect in the first and the second patterns, and hypofluorescence owing to choroidal filling delay in the third pattern. The optical coherence tomography images at the location of the tracts were obtained from 13 of the 45 eyes with tracts. These images showed a loss of outer retina and retinal pigment epithelium (RPE) in 70% of the lesions with the first AF pattern and in 100% of the lesions with the second or the third AF pattern. Serous retinal detachment overlying the tracts was found only in eyes with the first AF pattern (uniform hyper-AF). No eyes showed tissue staining suggestive of choroidal neovascularization at the staphyloma edge.

In 44 of 45 eyes (97.8%) with linear tracts, the AF features seen in the tracts were also observed at the staphyloma edge. Such AF abnormalities were found only in a limited part of the staphyloma edge where the tracts emanated. The optical coherence tomography images at the staphyloma edge were obtained in 10 of the 45 eyes, showing serous retinal detachment with subretinal precipitates in 5 eyes (50.0%) and the absence of outer retina and RPE in each of the 10 eyes.