In the presence of massive blur (Jackson Cross Cylinders), lens compensation relies more on chromatic cues

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Introduction

Several experiments, as shown below, have demonstrated that chicks can compensate for lenses in monochromatic light; these experiments have been interpreted as casting doubt on the role of longitudinal chromatic aberration as a cue to the sign of defocus. However, they show only that other visual cues exist.

![Graph showing change in refractive error over time with different lens conditions.](image)

![Graph showing change in eye length over time with different lens conditions.](image)

Purpose

Because other potential cues may depend on subtle spatial signals in the image, we attempted to reduce the efficacy of those cues by imposing astigmatic blur with strong Jackson Cross Cylinder lenses together with weak spherical defocus in hopes of magnifying the difference between lens compensation in white (as shown by McLean & Wallman, 2005) and monochromatic light.

Methods

Chicks wore lenses that presented astigmatic defocus (+5/–5 D crossed cylinders) combined with +3 D of spherical defocus over one eye and astigmatic defocus (+4/–4 D crossed cylinders) combined with –2 D over the other eye. Some chicks wore these lenses under white light, others under red monochromatic light. A third group of chicks wore lenses that imposed only spherical defocus of similar magnitude (+3 D and –3.5 D over the two eyes). We measured refractive error by Hardinger Refractometer and axial dimensions by high-frequency ultrasound; we present the data here as changes over the 3 days of lens-wear. In addition, choroidal responses were examined after 3 hours and 24 hours wear.

![Graph showing mean change in eye length over time with different lens conditions.](image)

Results

Refractive compensation despite astigmatic defocus was seen in white light, but not in red light. However, refractive compensation was seen with spherical defocus in monochromatic light.

Axial length compensation was seen in both astigmatic and spherical defocus conditions.

With astigmatic defocus, choroidal compensation was poorer in red than in white light. With spherical defocus, there was compensation in red light, but in green light, the choroidal responses were more transient, being evident at 3 and 24 hours (see below) but not at 3 days.

Conclusions

It appears that crossed cylinders attenuate lens compensation only under monochromatic light. We interpret this as evidence that, when other spatial cues are handicapped by massive blur, the use of chromatic cues to the sign of defocus are accentuated. Thus, these results imply that eyes use the signals provided by longitudinal chromatic aberration to discern the sign of defocus, but under normal circumstances, other cues are used as well.

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Poster created by Ashley Tang
Responses of different retinal areas to imposed defocus in chickens

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INTRODUCTION

- Recent experiments in monkeys suggest that defocus imposed in the periphery of the visual field can affect the development of foveal/central refractive errors.
- For designing spectacle lenses making use of this observation, it is important to know whether certain retinal areas are more responsive or whether changes in eye growth are just proportional to the defocused area.
- This question has previously been addressed in chicks by using spectacle lenses with central holes (4, 6 & 8 mm) (Schippert & Schaeffel, Vision Research 2006). These lenses induced changes in refraction in the periphery but scarcely in the center.

METHODS

- 54 chickens (Gallus domesticus), monocularly treated with:
  - ±7D full field lenses (6+8 chicks)
  - -7D hemi-field lenses (6 chicks)
  - “RRG” lenses (Rodenstock, Munich) (2 different power profiles, lens 1 and lens 2)
- Infrared photoretinoscopy at -45°, 0° and +45° eccentricity – NR, CR, TR
- A-scan ultrasonography
- “Image J” – self-written macro file to trace outlines of excised eyes

RESULTS

- Different RRG lenses are very differently effective in changing the central refraction. (see "key finding", above)
- Even after 5 days of treatment with RRG lenses that impose myopia in the periphery, there was little change in external eye shape - even though hyperopia could be induced. Obviously, the refraction changes were largely choroidal.

"RRG" lenses have been provided by the industrial partner of MyEuropia, Rodenstock, Munich, Germany

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Common polymorphisms in the COL11A1, PLOD and FBN1 genes are not associated with susceptibility to high myopia - Use of a DNA pooling approach

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INTRODUCTION
Myopia is a common eye disease in the world. This disorder is a significant public health and economic concern.1 High myopia, usually defined as ≥6.0 D or worse, is a leading cause of blindness. Since it is a predisposing factor to many ocular complications like retinal detachment, particularly in older adults. Myopia is a multifactorial disorder with contributing factors from genetics, environment and their interactions. Stickler syndrome type 2, Ehler-Danlos syndrome type 6 and Marfan syndrome are rare genetic diseases caused mutations in the COL11A1, PLOD and FBN1 genes, respectively.2 These syndromes have myopia as one of the consistent presenting features, and their causative genes are expressed in the sclera (and the vitreous for COL11A1). Therefore, we hypothesised that common polymorphisms in these genes may contribute to susceptibility to myopia.

AIM
The study aims to examine the possible association of high myopia and three candidate genes: COL11A1, PLOD and FBN1 genes. We used an efficient screening protocol based on accurate measurement of relative allele frequencies of DNA pools.

MATERIALS AND METHODS
SUBJECTS
Unrelated southern Chinese subjects were recruited for this study. Three hundred high myopes with spherical equivalent -8.0 D or worse for both eyes were included in case group and 300 individuals with emmetropia within ±1.00D were included in control group. Blood samples were collected and DNA was extracted.

SELECTION OF SNPS
In total, 21 tag single nucleotide polymorphisms (SNPs) were selected from these 3 candidate genes for analysis by this DNA pooling strategy. Tag SNPs were selected from a region encompassing each gene locus and 3 kb upstream and 3 kb downstream of the gene, based on the HapMap data for Chinese subjects.

CONSTRUCTION OF DNA POOLS
All DNA samples were accurately quantified by PicoGreen. Equal amounts of DNA were mixed to create DNA pools (Figure 1). Six case pools and six control pools were prepared, each consisting 50 distinct individuals of the same disease status.

ESTIMATION OF RELATIVE ALLELE FREQUENCIES IN DNA POOLS
Relative allele frequencies in DNA pools were quantified by denatured high performance liquid chromatography (DHPLC) analysis of primer-extended products (peak heights) on the WAVE Fragment Analysis System (Transgenicom) (Figure 2). The estimation depends on a single PCR followed by a single primer extension and a single analysis in DHPLC. Each DNA pool was estimated in triplicates.

STATISTICAL ANALYSIS & FOLLOW-UP STUDY
Relative allele frequencies were compared between case pools and control pools by nested ANOVA.

A threshold P value of <0.10 was used as cut-offs for follow-up by individual genotyping. Follow-up individual genotyping study was performed for confirmation of significant result.

RESULTS
All SNPs tested gave P values greater than 0.10 and thus were not associated with high myopia, except for one SNP. The SNP (rs17127371) within the COL11A1 gene gave a P value of 0.0993.

Follow-up study by genotyping individual samples of the original sample set was performed for confirmation. Restriction fragment length polymorphism (RFLP) was applied. The results did not produce a significant difference between cases and controls.

DISCUSSION
• A rapid screening strategy based on DNA pooling and analysis of primer-extended products by DHPLC genotyping system was used. It greatly reduced in genotyping work.
• Thus, the strategy provides a great saving in time, expense and DNA samples that are beneficial for laboratory with limited resource.
• In order to explore and identify probable susceptibility genes for further investigation, a relatively less stringent threshold significant level (P value <0.10) was applied and no correction for multiple comparisons was made.
• It is understood that the effect of haplotypes of the SNPs would be lost in the DNA pooling strategy. This disadvantage would lead to the missing of significant effect of haplotypes of the SNPs when the SNPs individually showed no association with the disease.
• Nowadays, the cost-saving DNA pooling strategy is mainly applied in genome-wide association study (GWAS). It is also favourable to test many different selected potential candidate genes very quickly and conserve the efforts for testing promising susceptibility genes.
• Despite mutations of the COL11A1, PLOD and FBN1 genes being recognised to be associated with eye disorders showing high myopia as a clinical feature, the current study demonstrated that the common polymorphisms in the genes do not have a role in predisposition to myopia.
• This might indicate the different biological mechanisms between pathologic myopia and high-grade myopia.

CONCLUSION
Common polymorphisms in the COL11A1, PLOD and FBN1 genes are not associated with susceptibility to high myopia in Southern Chinese.

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REFERENCES
Association mapping of myopia susceptibility genes by a DNA pooling strategy: study of candidate genes expressed in scleral extracellular matrix

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INTRODUCTION
Myopia is the most common eye disorder worldwide and is a significant public health problem.1 High myopia (-6.0 D or worse) is associated with many ocular complications like glaucoma and retinal detachment. Myopia is a complex disease influenced by genetic and environmental factors, and their interactions. Human myopia develops mostly because of excessive axial eye size from accelerated eye growth. Sclera plays a significant role in the enlargement of the eyeball.2 It undergoes active remodeling when elongation of the eyeball is induced in animals. Many genes are expressed in the sclera, including genes encoding extracellular matrix proteins.3

AIM
This study aims to investigate the possible association of high myopia and candidate genes expressed in scleral extracellular matrix (ECM) by an efficient screen of DNA pools.4

MATERIALS AND METHODS

SUBJECTS
In total, 600 unrelated Han Chinese subjects were recruited in the study. They included 300 cases (high myopes: spherical equivalent -6.0 D or worse) and 300 controls (emmetropes: spherical equivalent ≤1.00 D). Blood samples were collected and DNA was extracted.

GENES AND SNPS SELECTION
Eight candidate genes expressed in sclera ECM were selected for study: COL1A2, COL10A1, ACAN, DCN, LUM, FMOD, KERA, and EPYC. In total, 78 tag single nucleotide polymorphisms (SNPs) were selected from these 8 genes for analysis by this DNA pooling strategy.

CONSTRUCTION OF DNA POOLS
The DNA samples were accurately quantified by PicoGreen measurement method. To construct DNA pools, equal amount of DNA solutions were mixed (Figure 1). Finally, 6 case pools and 6 control pools were constructed, each consisting 50 distinct individuals of the same disease status.

ESTIMATION OF RELATIVE ALLELE FREQUENCIES IN DNA POOLS
Relative allele frequencies in DNA pools were estimated based on analysis of primer-extended products (peak heights) by denatured high performance liquid chromatography (DHPLC) (Figure 2). The analysis included a single PCR followed by a single primer extension and a single analysis in DHPLC. Each DNA pool was estimated in triplicates.

RESULTS
All SNPs tested gave P values greater than 0.10 and thus were not associated with high myopia, except for 16 SNPs from two candidate genes (ACAN and COL1A2) (Table 1).
For the aggrecan (ACAN) gene, the P values were <0.05 for 3 SNPs, and 0.05-0.10 for 6 SNPs; 2 of these 6 SNPs formed were adjacent to each other and formed one cluster while 4 others formed another cluster.
For the collagen gene COL1A2, P values were <0.05 for 7 SNPs, and 0.05-0.10 for 1 SNP; and 3 of these 8 SNPs formed were adjacent to each other.

DISCUSSION
• A rapid screening strategy based on DNA pooling and analysis of primer-extended products by DHPLC genotyping system was developed.
• A 15-fold reduction in genotyping work: great saving in time, expense and DNA samples.
• Seventy-eight tag SNPs from 8 candidate genes expressed in sclera ECM were examined. Finally, 16 SNPs from ACAN and COL1A2 genes showed significant differences between cases and controls.
• Follow-up individual genotyping of the samples forming the DNA pools will be conducted for confirmation.
• Less stringent threshold significant level (P value <0.10) was applied and no attempt was made to correct for multiple comparisons. This exploratory nature would allow to identify probable susceptibility genes for further investigation.
• Undoubtedly, the effect of haplotypes of the SNPs would be missed in the DNA pooling strategy. The concern is particularly important for the SNPs individually demonstrating no effect but showing significant effect once haplotype is tested.
• The strategy provides a cost-saving approach not only beneficial for laboratory with limited resource, but also reduces the expenditure of genome-wide association study (GWAS). Thus, GWAS becomes more affordable and popular.

CONCLUSION
In total, 16 SNPs from two candidate genes (ACAN and COL1A2) showed significant differences between cases and controls. These initial positive findings should be confirmed by genotyping these SNPs for individual samples forming the original DNA pools. The DNA pooling strategy proved to be an efficient and cost-saving approach for initial screen of candidate genes.

ACKNOWLEDGMENTS
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REFERENCES
Genetic susceptibility to high myopia: investigating candidate genes involved in the early part of potential biological pathways

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INTRODUCTION

Myopia is the most common eye disorder worldwide, with the highest prevalence in East Asia.¹ In order to control the progression of myopia, the underlying pathway should be understood. It is well established that visual experience alters ocular growth and the changes seem to be mediated locally. Genes responsive to the visual signals are probably involved in the earlier part of potential biological pathways concerned. Five functional candidate genes were selected based on this hypothesis to investigate their potential association with high myopia: early growth response 1 (EGR1),² v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS),³ jun oncogene (JUN),³ vasoactive intestinal peptide (VIP),⁴ and vasoactive intestinal peptide receptor 2 (VIPR2)⁵.

METHODS

Subjects Selection
Case: Dioptr ≤ -8.00, n = 300
Control : Dioptr ± 0.75, n=300
Age: 18-45 yrs

SNP Selection and Genotyping
From the 5 selected candidate genes, 26 tag single nucleotide polymorphisms (SNPs) were identified from the International HapMap database. The selection criteria of TaqSNPs were r² > 0.8 and minor allele frequency (MAF) >0.10 for the Han Chinese population by the Tagger software. Genotypes were obtained by restriction fragment length polymorphism (RFLP) or unlabelled probe melting analysis ⁶.

RESULTS

Single Marker Analysis
The genotypes of the TagSNPs and were all in HWE(genotype rate = 100%). Four TagSNPs were associated with high myopia with nominal p values < 0.05 as shown in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Genotype</th>
<th>Freq. (Case)</th>
<th>Control (Freq.)</th>
<th>P Value</th>
<th>P Allele</th>
<th>Model</th>
<th>P Value</th>
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<tr>
<td>VIP</td>
<td>M01</td>
<td>GG</td>
<td>189</td>
<td>101</td>
<td>0.0586</td>
<td>0.0347</td>
<td>A</td>
<td>0.185</td>
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<tr>
<td>VIPR2</td>
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<td>TT</td>
<td>203</td>
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<td>0.0112</td>
<td>0.0024</td>
<td>A</td>
<td>0.0425</td>
</tr>
<tr>
<td>VIPR2</td>
<td>M07</td>
<td>CT</td>
<td>28</td>
<td>24</td>
<td>0.0442</td>
<td>0.0132</td>
<td>A</td>
<td>0.0143</td>
</tr>
<tr>
<td>VIPR2</td>
<td>M11</td>
<td>AA</td>
<td>377</td>
<td>153 (40.8%)</td>
<td>0.0089</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIPR2</td>
<td>M12</td>
<td>GG</td>
<td>304</td>
<td>153 (50.3%)</td>
<td>0.0140</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

A: additive genetic model, D: Dominant genetic model
*: The positive signals did not survive after multiple testing correction

Haplotype Analysis
The 2 SNPs haplotypes of VIPR2 were associated with high myopia significantly as shown in Table 2.

<table>
<thead>
<tr>
<th>Marker(s)</th>
<th>Allele Sequence</th>
<th>Count</th>
<th>Frequency</th>
<th>P Value</th>
<th>Pᵢ Value</th>
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<tr>
<td>M11</td>
<td>G</td>
<td>377</td>
<td>153 (40.8%)</td>
<td>0.0089</td>
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<tr>
<td>M12</td>
<td>GG</td>
<td>304</td>
<td>153 (50.3%)</td>
<td>0.0140</td>
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<tr>
<td>M11M12</td>
<td>GGG</td>
<td>153</td>
<td>153 (100%)</td>
<td>2.87 x 10⁻⁴</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The G-G haplotype (M11-M13) of VIPR2 was strongly associated with high myopia.

DISCUSSIONS & CONCLUSIONS

Four of the candidate genes tested (EGR1, FOS, JUN and VIP) were unlikely to play significant roles in genetic susceptibility to high myopia in Chinese. However, VIPR2 haplotypes (M11-M13) were found to be significantly associated with high myopia (P<0.001). This indicates that certain functional causal variants in the VIPR2 gene contribute to myopia susceptibility and remains to be identified.

This initial positive finding should be confirmed by replication studies using independent samples and followed by fine mapping of the true causal variant.

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References: