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

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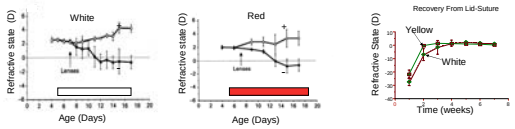
Introduction

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.



Rohrer, Schaeffel, & Zrenner, (1992)

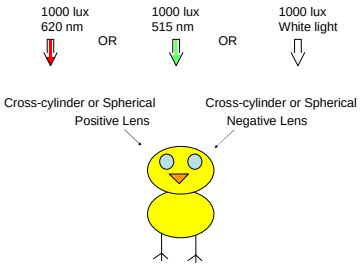
Wildsoet, Howland, Falconer, & Dick, (1993)

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, 2003) and monochromatic light.

Methods

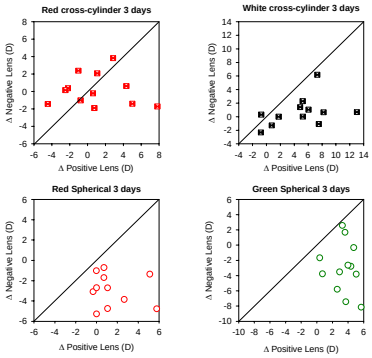
Chicks wore lenses that presented astigmatic defocus (+5/–5 D crossed cylinders) combined with +3 D of spherical defocus over one eye and astigmatic defocus (+4/–4 D crossed cylinders) combined with –2 D over the other eye. Some chicks wore these lenses under white light; others under red monochromatic 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, choroid responses were examined after 3 hours and 24 hours wear.



Results

Change in Refractive Error

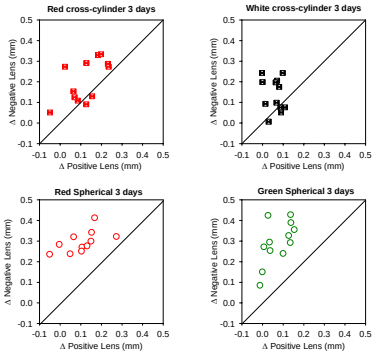
(Points above the diagonal line show the expected difference in compensation for positive vs. negative lenses)



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.

Change in Eye Length

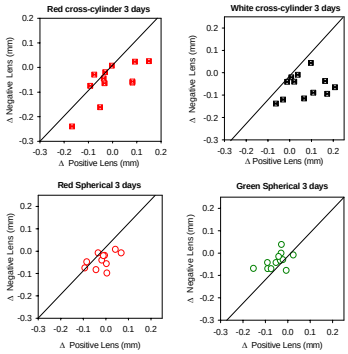
(Points above the diagonal line show the expected difference in compensation for positive vs. negative lenses)



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

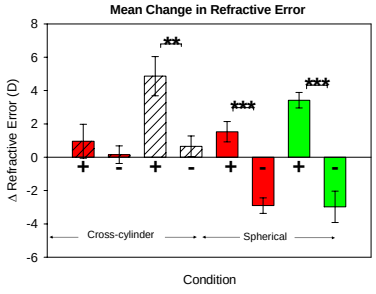
Change in Choroidal Thickness

(Points below the diagonal line show the expected difference in compensation for positive vs. negative lenses)

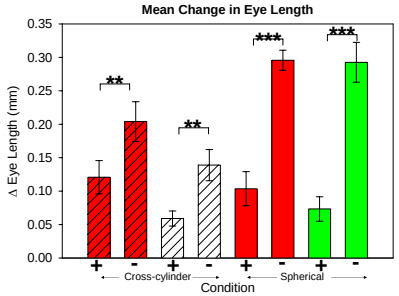


With astigmatic defocus, choroidal compensation was poorer in red than in white light. With spherical defocus, there was compensation in red light, but in green light, the choroidal responses were more transient, being evident at 3 and 24 hours (see below) but not at 3 days.

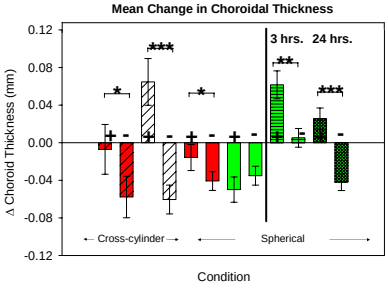
Mean Change in Refractive Error



Mean Change in Eye Length



Mean Change in Choroidal Thickness



Red Cross-cylinder
White Cross-cylinder
Red Spherical
Green Spherical

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.

Acknowledgements

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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.

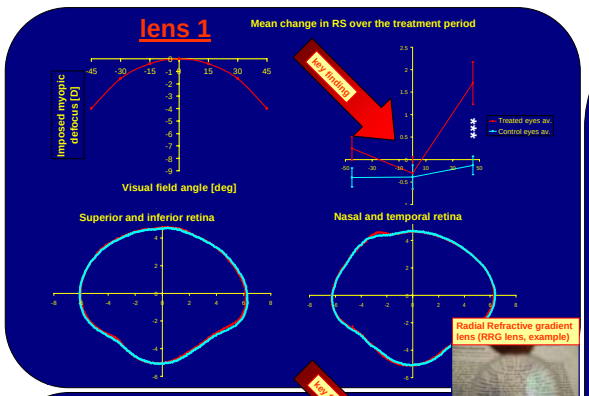
METHODS

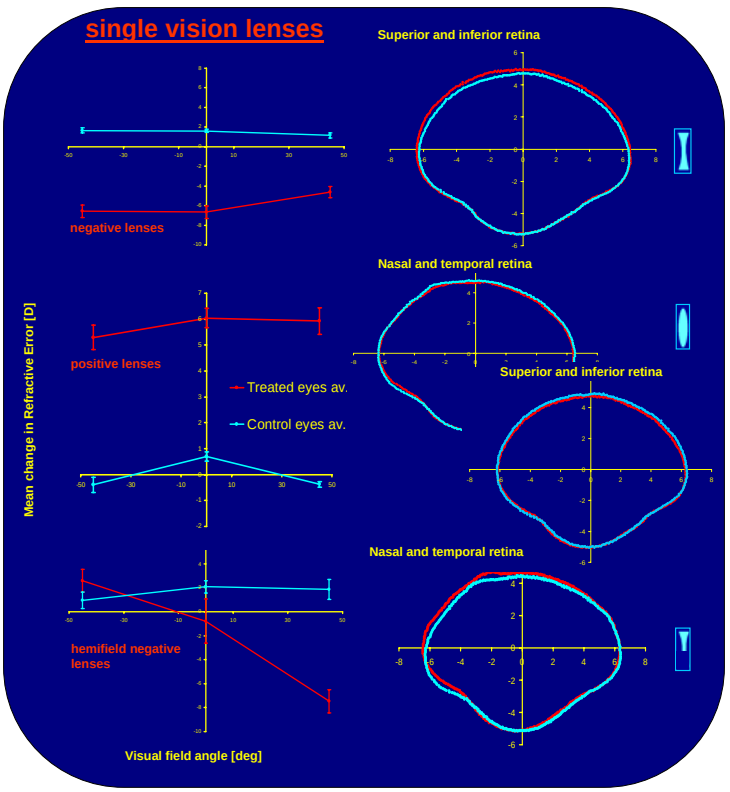
- 54 chickens (*Gallus domesticus)*, **monocularly** treated with:

±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)
(13+21 chicks)

- Infrared photoretinoscopy at -45^0, 0^0 and +45^0 eccentricity – NR,CR,TR
- A-scan ultrasonography
- "Image J" – self-written macro file to trace outlines of excised eyes

RESULTS





CONCLUSIONS

- 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
RODENSTOCK
This project has received funding from the Marie Curie Research Training Network "MyEuropia" MRTN-CT-2006-034021

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.[3]

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 ±1.0D 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.[4]

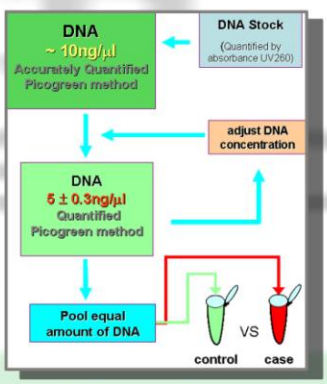

Figure 1. The flowchart shows the procedures in the quantification of DNA samples and the construction of DNA pools.

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.

ESTMATION 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 (Transgenomic) (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.

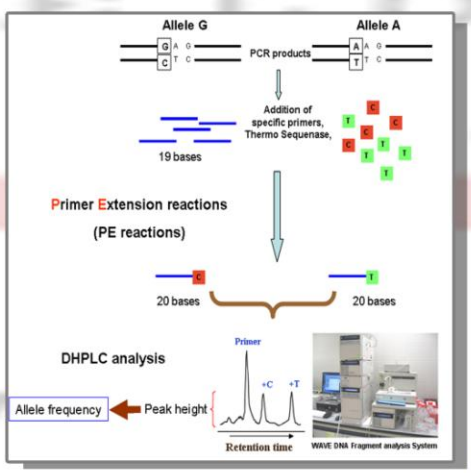

Figure 2. The principle of primer extension coupled with denaturing high performance liquid chromatography (PE-DHPLC) in genotyping single nucleotide polymorphisms.

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 (rs17127311) within the *COL11A1* gene gave a *P* value of 0.0983.

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 no 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.

ACKNOWLEDGMENTS

This study was supported by funds from Research Grant Count (Ref. No. PolyU 5411/06M; account code: B-Q04A) and from The Hong Kong Polytechnic University (J-BB7P, 87MS and 87LV).

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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 -8.0 D or worse) and 300 controls (emmetropes: spherical equivalent within ±1.0D). 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 meaurement 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.

ESTMATION 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.

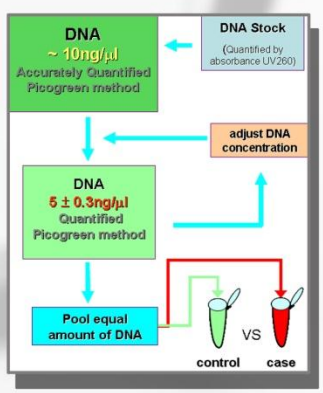

Figure 1. The flowchart shows the procedures in the quantification of DNA samples and the construction of DNA pools.

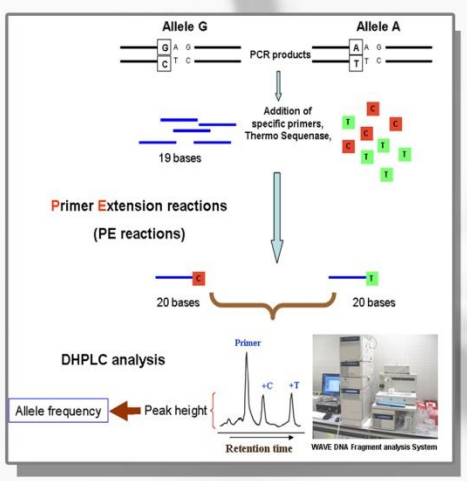

Figure 2. The principle of primer extension coupled with denaturing high performance liquid chromatography (PE-DHPLC) in genotyping single nucleotide polymorphisms.

STATISTICAL ANALYSIS

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.

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 5 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.

Table 1. The SNPs from *ACAN* and *COL1A2* showing significant *P* vaues for nested ANOVA.

Gene	SNP	*P* values for nested ANOVA
ACAN	rs12439075	0.0862
	rs2280468	0.0762
	rs2293087	0.0338
	rs4932438	0.0142
	rs953065	0.0785
	rs3784757	0.0346
	rs1516793	0.0982
	rs1516794	0.0542
COL1A2	rs3763469	0.0463
	rs11770203	0.0194
	rs406226	0.0163
	rs42523	0.0609
	rs42526	0.0405
	rs10487254	0.0456
	rs2521205	0.0112
	rs7805430	0.0428

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 poolings 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.
- Undeniably, 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

This study was supported by funds from Research Grant Count (Ref. No. PolyU 5411/06M; account code: B-Q04A) and from The Hong Kong Polytechnic University (J-BB7P, 87MS and 87LV).

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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

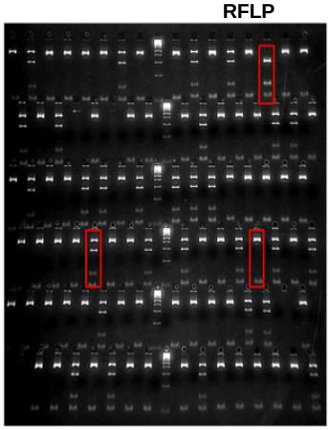
Subjects Selection

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

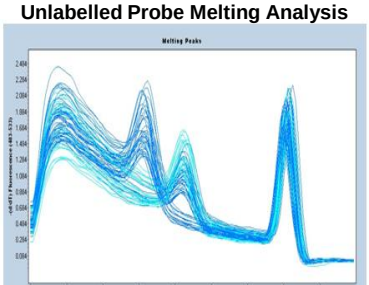
SNP Selection and Genotyping

From the 5 selected candidate genes, 26 tag single nucleotide polymorphisms (SNPs) were identified from the International HapMap database. The selection criteria of TagSNPs were r^2 > 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].

RFLP



Unlabelled Probe Melting Analysis



Statistics

Genotypes were tested for Hardy-Weinberg equilibrium (HWE). Analysis was performed for individual SNPs and haplotypes using BEAGLE [7] and PLINK [8]. Permutation was used to correct multiple comparisons.

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 1

Gene	Marker	Genotype	Freq. (Case)	Freq. (Control)	P Value Genotype Difference	P Value Allele Difference	Model	P Value
VIP	M01	GG GT TT	189 101 10	216 75 9	0.0580	0.0347*	A D	0.0346* 0.0186*
VIPR2	M06	TT TC CC	203 81 16	174 95 31	0.0172 *	0.0024*	A D	0.0047* 0.0143*
	M07	CC CT TT	241 55 4	220 68 12	0.0422 *	0.0123*	A D	0.0168* 0.0422*
	M011	AA AG GG	158 117 25	135 120 45	0.0229*	0.0075 *	A D	0.0102* 0.0603

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 2

Marker(s)	Allele Sequence	Count	Frequency	P Value	P_c Value
M11	G	377	153 (40.8%)	0.0089	
M12M11	GG	304	153 (50.3%)	0.0140	
M13M12M11	GGG	153	153 (100%)	2.87×10^{-5}	0.0001

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_c<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.

ACKNOWLEDGEMENTS

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