211 Genetic Epidemiology
Monday, May 08, 2017 8:30 AM–10:15 AM
Room 309 Paper Session
Program #/Board # Range: 1223–1229
Organizing Section: Clinical/Epidemiologic Research

Program Number: 1223
Presentation Time: 8:30 AM–8:45 AM
Han Chinese families show significant linkage for myopia on 10q26 and suggestive linkage on 9q33
Joan E. Bailey-Wilson[1], Anthony Musolf[1], Claire L. Simpson[2, 1], Bilal A. Moiz[1], Kyle A. Long[1], Deyana D. Lewis[1], Candace D. Middlebrooks[1], Laura Portas[3], Federico Murgia[3], Dwight Stambolian[4]. [1]National Human Genome Research Inst, National Institutes of Health, Baltimore, MD; [2]Department of Genetics, Genomics and Informatics, Univ of Tennessee Health Science Center, Memphis, TN; [3]Institute of Population Genetics, CNR, Li Punti, Italy; [4]Ophthal-Stellar Chance Lab, University of Pennylvania, Philadelphia, PA.
Purpose: Myopia is caused by an overgrowth of the eye which causes light to focus in front of the retina, leading to blurry vision. Myopia has reached epidemic proportions in Southeast Asia, with about 80% of the population affected. The genetic underpinnings that drive myopia are still murky however. This study uses Han Chinese families (living in the U.S.) with a history of myopia to search for linkage between genomic variants and the disease.
Methods: The sample data consisted of 34 Han Chinese families with a history of myopia. Subjects were genotyped on an Illumina Exome Array. We had refractive error measurements on the subjects and these were converted to either affected (≤ -1D), unaffected (≥ 0 D) or unknown (< 0D, > -1 D). Three types of parametric linkage analyses were performed: standard single variant two-point linkage, multipoint linkage, and collapsed haplotype pattern variant linkage (CHP). CHP creates a multi-allelic pseudomarker that corresponds to a genomic region from multiple single variants. This serves to raise information content. Standard two-point linkage analysis is then run on the CHP marker. Family-based association analyses are currently underway, including a family-based test using both rare and common variants and a rare variant version of TDT.
Results: CHP linkage analysis identified a genome-wide significant locus at 10q26.13, centered around *TACC2*. This pseudomarker consisted of several rare exonic SNPs from the *TACC2* gene. CHP analysis also found 6 more suggestive signals in 10q24.2-26.2. Single variant two-point identified 34 suggestive loci on 10q24-26, while multipoint identified 8 suggestive loci in 10q26.11-13. Many of the suggestive markers in both analyses were found in *HTRA1*, a known age-related macular degeneration gene. Several other promising candidate genes, such as *BAG3* and *DOCK1*, are also present in the region. Multipoint analysis also identified a highly suggestive region at 9q33.1. This region includes *TLR4*, a gene known to interact with alpha-crystallin in the retina.
Conclusions: This study identified a genome-wide significant signal on 10q26 and a suggestive signal on 9q33 in Han Chinese families. Both regions contain strong candidate genes. Targeted sequencing and laboratory confirmation for both regions is planned to elucidate the causal variants.
Commercial Relationships: Joan E. Bailey-Wilson, None; **Anthony Musolf**, None; **Claire L. Simpson**, None; **Bilal A. Moiz**, None; **Kyle A. Long**, None; **Deyana D. Lewis**, None; **Candace D. Middlebrooks**, None; **Laura Portas**, None; **Federico Murgia**, None; **Dwight Stambolian**, None
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Presentation Time: 8:45 AM–9:00 AM
Epigenetic markers for early-onset myopia in an epigenome-wide association study
Seang-Mei Saw[2, 1], Cheryl Ngo[3], Pan Hong[4], Wei Jie Seow[1], Stuart W. Tompson[7], Kristina N. Whisenhunt[7], Eranga N. Vithana[2], Yap Seng Chong[5, 4], Fabian Yap[8], Veluchamy A. Barathi[2], Pirro G. Hysi[6], Terri L. Young[7], Neerja Karnani[4]. [1]Saw Swee Hock School of Public Health, National Univ of Singapore, Singapore, Singapore; [2]Singapore Eye Research Institute, Singapore, Singapore; [3]Ophthalmology, National University Hospital, Singapore, Singapore; [4]Singapore Institute of Clinical Sciences, Singapore, Singapore; [5]Obstetrics and Gynaecology, National University of Singapore, Singapore, Singapore; [6]St Thomas' Hospital, London, United Kingdom; [7]Ophthalmology, Wisconsin University, Madison, WI; [8]Kerdang Kerbau Hospital, Singapore, Singapore.
Purpose: To investigate epigenetic modifications in umbilical cords associated with early-onset myopia in Singapore preschool children.
Methods: Pregnant women from two major hospitals were recruited for the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort. We determined epigenome-wide DNA methylation profiles in umbilical cords using the Infinium HumanMethylation450 BeadChip at birth. Cycloplegic autorefraction was obtained in 3 year old children. Logistic regression models were used to identify the top epigenetic hits for myopia (spherical equivalent (SE) <-0.5) using methylation profiles from fetal cord cells (29 cases, 490 controls), controlling for gestational age, ethnicity, gender and estimated cell types. Expression data from fetal eyes at 12-weeks' (n=8) and 24-weeks' gestation (n=6) were compared with 6 cadaveric adult eyes. The RNA samples were labelled, amplified and hybridized to Illumina HumanHT-12 v4 Expression BeadChips. Mouse gene expression was determined using total RNA isolated from mouse retina (n=12), hybridized and scanned using a Genechip® Scanner 3000 7G.
Results: We identified 5 epigenome-wide significant CpG sites and all 5 showed loss of DNA methylation in myopic cases compared to controls. The nearest genes for the top CpG sites included *CLDN23* ($p = 1.7 \times 10^{-7}$) on chromosome 8, responsible for tight junction-specific obliteration of the intercellular space, *ARL1* on chromosome 12 ($p=2.5 \times 10^{-7}$) regulating intracellular vesicular membrane trafficking and *FGB* on chromosome 4 ($p=3.6 \times 10^{-7}$) that encodes the beta component of fibrinogen. Two other genes were *PQLC1* ($p=8.9 \times 10^{-7}$) and *KRT12* ($p = 1.2 \times 10^{-6}$). CLDN23, ARL1 and PQLC1 were expressed in the sclera, choroid and retina of human and fetal tissues. CLDN23, FGB, PQLC1 and KRT12 were expressed in mouse myopia scleral tissue.
Conclusions: Young children with myopia had loss of DNA methylation for 5 CpG sites that map to genes on chromosomes 8, 12, 4, 18 and 17 which correlate with gene expression alterations. Intrauterine epigenetic mechanisms may play an important role in the development of early-onset myopia.
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Presentation Time: 9:00 AM–9:15 AM
Light processing and regulators are important mechanisms in refractive error
Milly S. Tedja[5], Robert Wojciechowski[1, 2], Pirro G. Hysi[3], Virginie J. Verhoeven[5], Adriana I Iglesias[4], Roxanna Haak[6], Peter J. Van der Spek[6], Christopher J. Hammond[7], Caroline Klaver[5, 8].
[1]Inherited Disease Research Branch, US National Institutes of Health, Baltimore, MD; [2]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [3]Department of Twin Research and Genetic Epidemiology, King's College London School of Medicine, London, United Kingdom; [4]Epidemiology, Genetic Epidemiology Unit, Erasmus Medical Center, Rotterdam, Netherlands; [5]Ophthalmology & Epidemiology, Erasmus Medical Center, Rotterdam, Netherlands; [6]Bio-informatics, Erasmus Medical Center, Rotterdam, Netherlands; [7]Department of Twin Research and Genetic Epidemiology, King's College London School of Medicine, London, United Kingdom; [8]Ophthalmology, Radboud University, Nijmegen, Netherlands.
Purpose: Myopia is a major cause of blindness worldwide. The underlying pathways remain largely unknown. Recently, a large GWAS meta-analysis on refractive error (RE) of CREAM and 23andMe(N=160,420) yielded 152 loci. The next step is to clarify biological pathways including the role of regulatory elements.
Methods: We used summary statistics of the aforementioned GWAS meta-analysis, and performed a gene set enrichment analysis on single-nucleotide polymorphisms (SNPs) with a P-value ≤ 1,00E-05 using DEPICT. Pearson correlation coefficients (*r*) were calculated between gene sets to form pathway families (i.e. gene set clusters with $r > 0.4$). To search for regulatory elements, we combined the VISTA enhancer database based on activity experiments conducted in transgenic mice, with POSSUM, a hereditary dysmorphology database based on human data using data mining software Spotfire on the 152 tag SNPs.
Results: Firstly, 66 gene sets were significantly associated with RE with a false discovery rate < 5% and a P-value < 0.05. 55 (83%) gene sets were eye-related. The most significant gene set was the abnormal photoreceptor inner segment morphology (P-value 1.79E-07). The eye-related gene sets consisted of specific pathway families: the retinal outer nuclear layer (n=27; 55%), the detection of light stimulus (n=13; 24%), non-motile cilium (n=3; 5%) and anterior eye segment (n=3; 5%). The correlation between the detection of light stimulus, retinal outer nuclear layer and non-motile cilium pathway families were > 0,6 [range *r* 0,61 – 0,68]. Secondly, 7 SNPs were located in enhancer regions which play a role in eye-development. 2 of these SNPs were specifically related to myopia, and are known to be human non-coding fragments with gene enhancer activity.
Conclusions: Our study strongly supports the importance of light processing as a primary role for the development of RE, and suggests that regulatory elements are major players in this process.
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Presentation Time: 9:15 AM–9:30 AM
Post-GWAS analysis approaches uncover dozens of novel genes associated with myopic refractive error
Stuart MacGregor[1], Milly S. Tedja[2], Robert Wojciechowski[3, 4], Pirro G. Hysi[5], Virginie J. Verhoeven[2], Christopher J. Hammond[5], Adriana I Iglesias[5], David A. Mackey[6], Cornelia van Duijn[2], Caroline Klaver[2]. [1]QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia; [2]Ophthalmology and Epidemiology, Erasmus University, Rotterdam, Netherlands; [3]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [4]Wilmer Eye Institute, Johns Hopkins Medical Institutions, Baltimore, MD; [5]Ophthalmology, Kings College London, London, United Kingdom; [6]Lions Eye Institute, Perth, WA, Australia.
Purpose: Our aim was to identify genes influencing refractive error, a very common eye disorder and an important cause of blindness. To complement ongoing (unpublished) standard per SNP (single nucleotide polymorphism) analysis, we conducted a range of post-GWAS (genome wide association study) approaches using data on myopia related traits in two large datasets.
Methods: We firstly considered refractive error (measured as quantitative trait spherical equivalent) in 44000 samples from the Consortium for Refractive Error And Myopia (CREAM); standard quantitative trait analysis was used. Secondly, we assessed myopia via questionnaire data from 23andMe; 104000 genotyped participants were asked "What age did you first wear glasses for myopia" and a survival analysis approach was used.
We applied three post-GWAS approaches. Firstly, we applied a gene-based test which accumulates evidence for association across all SNPs in/within 50kb of a gene (fastBAT). Secondly, we applied a gene-based test which incorporates eQTL information (Eugene). Genes were declared significant for the gene-based tests if they achieved P<2e-6 (correcting for number of genes). Thirdly, we applied an approach, where 450 ENCODE annotations are tested for enrichment in the single SNP GWAS (fgwas). In this third case, we also re-weighted our results based on the most important annotations to test for additional loci influencing myopia.
Results: Initially, the gene-based approaches were applied to the CREAM dataset. The two tests uncovered 5 genes not significant (P>5e-8) in standard per-SNP tests. We then validated these results using per SNP tests in the meta-analysed CREAM and 23andMe datasets. All of the novel gene-based associations replicated in per SNP tests (P<5-e8) for CREAM+23andMe.
Applying the gene-based approaches to the full CREAM+23andMe data revealed a total of 24 novel associations. The functional annotation approach identified several significantly enriched annotations (most significant was regions defined as being DNase I hypersensitive in fetal brain tissue); reweighting using these annotations uncovered a further 8 loci not significant in per-SNP tests.
Conclusions: Overall, our post-GWAS approach provided evidence for a role of a large number of novel genes in myopic refractive error.
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Presentation Time: 9:30 AM–9:45 AM
Meta-analyses of two large GWAS show a high genetic correlation between myopia age-at-onset and refractive error during adulthood and older age
Robert Wojciechowski[1, 2], Pirro G. Hysi[4, 3], Milly S. Tedja[5], Virginie J. Verhoeven[5], Jeremy A. Guggenheim[6], Stuart MacGregor[7], Christopher J. Hammond[3], Caroline Klaver[5]. [1]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Wilmer Eye Institute, Johns Hopkins Medical Institutions, Baltimore, MD; [3]Ophthalmology, King's College London, London, United Kingdom; [4]Twin Research & Genetic Epidemiology, King's College London, London, United Kingdom; [5]Ophthalmology and Epidemiology, Erasmus University, Rotterdam, Netherlands; [6]School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom; [7]Genetic Epidemiology & Statistical Genetics Laboratories, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia.
Purpose: Genes and environment play important roles in refractive development. Myopia age-at-onset (MAO) is a strong determinant of the magnitude of myopia throughout life. Hence, delaying the onset of myopia has become a priority of public health efforts and treatment strategies for myopia. We investigated the genetic correlation between MAO and ocular refraction in two large meta-analyses (MA) of genomewide association studies (GWAS).
Methods: We conducted whole-genome meta-analyses (MA) of two independent studies: CREAM, an international consortium of 30 studies (n=56,368); and two datasets from 23andMe (n=104,293). MA of CREAM GWAS were performed using summary statistics from linear regressions of refractive error (RE) on allele dosage. GWAS of MAO in 23andMe were performed using Cox proportional hazards models. All GWAS were conducted on marker sets imputed to 1000 Genomes reference panels (>10 million markers per study). Normalized CREAM and 23andMe association results were then combined in a MA using equal variances on marker-specific Z-scores. We calculated cross-trait genetic correlations (r) using several methods: 1) LD-score regression (LDr); 2) a thresholded regression of beta statistics (BREGr); 3) a variable threshold bootstrapped regression of beta coefficients from near-independent (r^2<0.1) markers (iBREGr); 4) a trans-ethnic method implemented in the POPCORN software (POPr).
Results: We identified ~200 loci for MAO and RE. Estimated genetic correlations between MAO and RE in European cohorts were high using all methods: LDr=0.94 (with 6.6M SNPs); BREGr=0.95 (8,434 SNPs at p<0.0011 in both MA); iBREGr=0.67 (8,456 SNPs with p<0.0001 in the stage 3 MA). Inter-ethnic POPr for CREAM-Asian (n=12,839) vs 23andMe and CREAM-Asian vs CREAM-European (n=43,529) were 0.79 and 0.80, respectively. We estimated that a 1.1-fold increase in the genetic risk (hazard) of myopia is associated with a -0.15 diopter mean shift in myopia in adulthood.
Conclusions: Using two independent GWAS (n=160,661), we show a high genetic correlation between MAO and RE in adulthood, indicating that the complex polygenic architecture of myopia onset significantly determines relative refractive status later in life. Despite marked differences in myopia incidence between East Asian and European populations, the underlying genetic causes do not differ significantly.
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Presentation Time: 9:45 AM–10:00 AM
Genome-wide association study identifies a new genetic locus at 2q36.3 for polypoidal choroidal vasculopathy in East Asians: The GAMA consortium
Tien Y. Wong[2, 1], Masayuki Yasuda[2], Qiao Fan[1], Chui Ming Gemmy Cheung[2], Masato Akiyama[3], Chiea Chuen Khor[4], Chi Pui Pang[5], Kyu Hyung Park[7], Nagahisa Yoshimura[6], Ching-Yu Cheng[2]. [1]Duke-National University of Singapore School of Medicine, Singapore, Singapore; [2]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore; [3]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan; [4]Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore; [5]Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong; [6]Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; [7]Ophthalmology, Seoul National University Bundang Hospital, Seoul, Korea (the Republic of).
Purpose: Polypoidal choroidal vasculopathy (PCV) is the most common subtype of age-related macular degeneration (AMD) in East Asians. Genome-wide association studies (GWASs) have identified at least 30 genetic variants for AMD. However, it remains unclear whether there is any unique genetic variation for PCV. In this study, we aim to identify susceptibility loci unique to PCV in East Asian populations.
Methods: We conducted a GWAS of 1,062 PCV cases and 1,152 typical noevascular AMD (tAMD) cases versus 5,275 non-AMD controls, comprising individuals of East Asian ancestry recruited in the Genetics of AMD in Asians (GAMA) Consortium. Genetic variants were genotyped using the Illumina HumanOmniExpress bead chips and imputed with multi-ethnic 1000 genome data as reference panels. For the replication, we genotyped a total of 895 PCV cases, 721 tAMD cases and 5,734 non-AMD controls from 3 independent sample collections in East Asians.
Results: At the discovery stage, three loci showed genome-wide significant association with PCV ($P < 5.0 \times 10^{-8}$), including known loci of *ARMS2* (rs11200634, P =9.97 x 10^{-14}) and *CFH* (rs514591, P = 1.45 x 10^{-26}), and a novel locus at 2q36.3 (odds ratio [OR] = 1.45, $P = 2.88 \times 10^{-8}$). We replicate the 2q36.3 regions in three independent sample collections (OR = 1.26, $P = 3.35\times10^{-4}$; *overall* $P = 1.18 \times 10^{-10}$). For tAMD, the association at the 2q36.3 locus was marginal (overall OR = 1.18; $P = 1.02\times10^{-3}$), with smaller effect size compared to PCV (Pdif = 0.048). The new genetic locus is involved in the structure of basement membrane in RPE and choriocapillaris.
Conclusions: We found a new genetic locus associated with PCV susceptibility and this locus is more closely involved in the pathogenesis of PCV than that of tAMD.
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Program Number: 1229
Presentation Time: 10:00 AM–10:15 AM
Evaluation of the myocilin mutation Gln368Stop demonstrates reduced penetrance for glaucoma in European populations
Abhishek Nag[1], Adriana I Iglesias[2], Pieter W. Bonnemaijer[2], Cornelia van Duijn[2], Caroline Klaver[2], Pirro G. Hysi[1], Christopher J. Hammond[1]. [1]King's College London, London, United Kingdom; [2]Erasmus Medical Centre, Rotterdam, Netherlands.

Purpose: Sequence variations in the myocilin (*MYOC*) gene account for ~2–4% of the glaucoma cases. One particular *MYOC* mutation, Gln368Stop (dbSNP accession number: rs74315329), is the commonest genetic mutation causing primary open-angle glaucoma (POAG), by raising the intraocular pressure (IOP). Previous studies have reported that this mutation has a penetrance as high as 90-100% with respect to ocular hypertension or POAG. The objective of our study was to evaluate the penetrance of the Gln368Stop *MYOC* mutation i.e. the risk allele of rs74315329 on IOP (as a proxy for POAG) using data from large-scale European population panels (directly sequenced and imputation-based).
Methods: In the TwinsUK study (N=6,092; mean age = 57 years), carriers of the risk allele of rs74315329 were identified using sequencing data, while the same in the Rotterdam study (N=11,189; mean age = 64 years) were identified using imputed data based on the Haplotype Reference Consortium panel. The penetrance of the risk allele of rs74315329 was estimated using IOP measurements (high IOP or ocular hypertension was defined as mean IOP > 21 mm Hg), history of IOP-lowering medication and / or visual field testing information / a diagnosis of glaucoma.
Results: In the TwinsUK, one out of the eight risk allele carriers for rs74315329 had high IOP and a diagnosis of glaucoma. In the Rotterdam study, 6 out of the 31 risk allele carriers for rs74315329 had high IOP or a history of IOP-lowering medication (two of the six risk allele carriers had a diagnosis of glaucoma). For rs74315329, this corresponds to a penetrance of 12.5% and 19.4% in relation to ocular hypertension, in the TwinsUK and the Rotterdam study, respectively. This suggests a much lower penetrance for rs74315329 for ocular hypertension (and hence, POAG) observed in our study, in comparison to that reported previously.
Conclusions: We report findings of the largest study to date evaluating the penetrance of the Gln368Stop *MYOC* mutation i.e. rs74315329 using large-scale population-based sequencing panels, which we believe might represent a more “realistic” measure of its penetrance compared to previous estimates. The significance of this finding is that higher numbers of healthy individuals in the population are expected to be carriers of this mutation, which in turn reduces the utility of identifying carriers of this mutation as a screening tool for glaucoma.
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