**AMD risk alleles are not implicated in Age-Related Macular Degeneration in Liver Transplantation patients**

**AUTHORS:**

Samir Khandhadia, MBBS, MRCOphth, PhD1, Jane Gibson, PhD,2 Sarah Ennis, PhD,3 Angela J Cree, BSc,4 and Andrew J. Lotery, MD, FRCOphth1,5

**AFFILIATIONS:**

1Southampton Eye Unit, University Hospital Southampton NHS Trust, Southampton, UK

2Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, UK

3Institute of Ophthalmology, University College London, London, UK

4Genomic Informatics, Human Genetics & Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, UK

5Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK

**CORRESPONDENCE TO:**

Professor Andrew Lotery, Clinical Neurosciences Research Group, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, South Block, Mailpoint 806, Level D, University Hospital Southampton, Southampton SO16 6YD, UK

Tel: +44 (0) 23 80795049, Fax: +44 (0) 23 80794120

Email: A.J.Lotery@soton.ac.uk

**SHORT TITLE:**

AMD genes and liver transplantation

**FINANCIAL SUPPORT**

This research was supported by the TFC Frost Charitable Trust, Claygate, UK (registered charity number: 256590), the Gift of Sight charity, Southampton, UK ([www.giftofsight.org.uk)](http://www.giftofsight.org.uk)) (the Gift of Sight charity is an 'Exempt Charity', Inland Revenue reference number X19140, as noted in the Second Schedule of the 1960 Charities Act), an unrestricted educational grant from Novartis Pharmaceuticals, Frimley, UK, funding from Frimley Park NHS Trust, Frimley Park, UK, and the Wellcome Trust (use of the Clinical Research Facility at Queen Elizabeth Hospital, Birmingham, UK; Addenbrookes’ Hospital, Cambridge, UK; and University Hospital Southampton, Southampton, UK). The funding organizations had no role in the design or conduct of this research.

**DONATION OF DONOR TISSUE**

Donor tissue was provided by the Department of Cellular Pathology, Queen Elizabeth Hospital, Birmingham, UK; Tissue Typing / Human Research Tissue Bank, Addenbrooke’s Hospital, Cambridge, UK; Liver Histopathology, Kings College Hospital, London, UK.

**CONFLICTS OF INTEREST**

No conflicting relationship exists for any author.

**PREVIOUS RELATED PUBLICATIONS / PRESENTATIONS**

Khandhadia S, Hakobyan S, Heng LZ, et al. Age-related macular degeneration and modification of systemic complement factor H production through liver transplantation. Ophthalmology 2013;120:1612-8.8

AMD prevalence complement and C3 genotype data was previously presented as posters at the Association for Research in Vision and Ophthalmology (ARVO) meeting in Fort Lauderdale, USA (2012 and 2013)

**KEYWORDS**

Age-related macular degeneration, Liver transplantation, Genetics

**Introduction and Methods**

We previously reported an increased prevalence of age-related macular degeneration (AMD) in liver transplant (LT) patients compared to the general population of similar ethnicity and age (64.6% versus 37.1% in the Rotterdam Study baseline population, p<0.001).1,2 The reasons for the increased prevalence of AMD have not been fully explained. This study aims to determine whether other SNPs in genes associated with AMD in the general population, are also associated with AMD in our LT patient group.

Full methodology of patient recruitment and DNA analysis is described in our previous paper.2 Statistical significance was defined as p≤0.05. Data analysis was carried out using PLINK v1.07 (Centre for Human Genetic Research, Massachusetts General Hospital, USA) and SPSS version 21 (IBM, New York, USA). Ethical committee approval was obtained and the study adhered to the tenets of the Declaration of Helsinki.

**Analysis of SNPs in LT patients with AMD status**

Recipient DNA was analysed for SNPs in 9 genes associated with AMD.3,4 Donor *CFH* rs1061170 status was obtained through measurement of levels of both Y402 and H402 CFH proteins in recipient blood. All SNPs conformed to Hardy-Weinberg Equilibrium (table 1).

Multivariate analysis within the LT group was carried out using binary logistic regression with AMD status (present/absent) as the dependent variable and other covariates added in a forward stepwise method. Only increasing age (P=0.018, OR = 1.060, CI 1.010-1.112) and recipient *CFH* rs1061170 SNP (P=0.049, OR = 1.508, CI 1.002-2.268) were associated with AMD. No association was found between AMD status and the remaining 8 recipient SNPs explored in this LT group study, when controlling for known significant risk factors for AMD (age, gender, smoking status, BMI).

Univariate analysis using the chi square test, showed no significant association between any SNP and AMD status in the LT group, after Bonferroni correction (table 1).

**Comparing SNPs between LT and general population group**

Genotype data for the same SNPs were extracted from a genome-wide association study for a local Southampton AMD group (515 AMD cases and 616 controls).5 Three SNPs were not significantly associated with AMD in this group (*CFI* rs10033900, *TIMP3* rs9621532, *LIPC* rs10468017) (table 1, genotype counts in table 2, available at http://www.ophthalmology-retina.org/).

We sought to determine if the lack of association in the LT group was due to a difference between the controls in the LT versus the Southampton general population group, or due to a difference between AMD cases in the LT versus Southampton general population (using the chi square test). In four of the six significant SNPs there was a significant difference in allele frequencies between the two AMD case groups including, *CFH* rs1061170 and *HTRA1/ARMS2* rs10490924.

**Conclusion**

We expected to find the same SNPs associated with AMD in our LT group as in the general population, however this was not the case. We did find a significant association between AMD and recipient *CFH* rs1061170 in the multivariate analysis (as previously reported2), although it did not pass multiple testing correction in the univariate analysis. However, we found no association between the remaining SNPs and AMD status in LT patients.

Three SNPs previously associated with AMD (*CFI* rs100033900, *TIMP3* rs9624532, *LIPC* rs10468017)3, were not found to be significant in our Southampton AMD group. This suggests that the associations with AMD at these particular SNPs are less robust, although other SNPs within these genes may be associated. A limitation of this study was the relatively small sample size of 144 AMD cases and 79 controls in the LT group. We may have been underpowered to detect smaller effects at some of these SNPs. However, it was surprising not to see any association of the *HTRA1/ARMS* SNP rs10490924 with AMD in our LT group. This gene is often the most strongly associated with AMD in the general population3 and in our Southampton AMD group had a p-value of 4.33x10-21 and an odds ratio of 2.419. Furthermore, although the risk allele is the same for the *CFH* rs1061170 SNP the effect size is much reduced in the LT group, with an OR of 1.58 versus 2.013 in the Southampton AMD group.

The lack of association for rs1061170 and rs10490924 in the LT group is due to a significant reduction in the risk allele frequency in AMD cases in the LT group as shown by comparison to the Southampton AMD group case group (P= 0.01, P <0.0001 respectively). The frequency of the risk allele in the control groups remained similar (P=0.575 and 0.463 respectively). There were no significant differences in the frequency of risk alleles between the control groups for all SNPs tested, except *TIMP3* rs9621532 (the minor allele frequency for this rare SNP may not have been accurately assessed in the LT group due to sample size). Therefore the LT group controls are similar to the general population controls but the cases are different, suggesting LT patients may have a different underlying genetic mechanism of developing AMD.

A further limitation of this study was the small number of SNPs studied. The SNPs were chosen based on information derived from the literature that was deemed most inclusive and current at the time this study was conducted.3 Next generation sequencing methods may uncover alternative genetic risk factors unique to LT AMD cases.

Other factors may also affect AMD prevalence in the LT group. Insufficient donor genotyping available to us; therefore it is hard to know whether the SNP of recipients or donors play a role in the pathogenesis of AMD in LT patients. Systemic hepatic protein production will be profoundly affected through liver transplantation. Furthermore, LT patients are systemically unwell prior to transplantation, and undergo compulsory immunosuppression following LT.

In summary, this study shows that not only is AMD more prevalent in an LT group but there appear to be different underlying genetic risk factors. Although *CFH* (rs1061170) is significant it has a smaller effect on AMD risk than in the general population, and strikingly *HTRA1/ARM2* (rs10490924) is not associated with AMD risk in the LT group at all.

**ACKNOWLEDGEMENTS**

The authors thank the Liver Eye Study Team (full details in our previous paper.2

**REFERENCES**

1. Klaver CC, Assink JJ, van LR, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci* 2001;42(10):2237-41.

2. Khandhadia S, Hakobyan S, Heng LZ, et al. Age-related macular degeneration and modification of systemic complement factor H production through liver transplantation. *Ophthalmology* 2013;120(8):1612-8.

3. Yu Y, Bhangale TR, Fagerness J, et al. Common variants near FRK/COL10A1 and VEGFA are associated with advanced age-related macular degeneration. *Hum Mol Genet* 2011;20(18):3699-709.

4. Ennis S, Jomary C, Mullins R, et al. Association between the SERPING1 gene and age-related macular degeneration: a two-stage case-control study. *Lancet* 2008;372(9652):1828-34.

5. Alexander P, Gibson J, Cree AJ, et al. Complement factor I and age-related macular degeneration. *Mol Vis* 2014;20:1253-7.