Experimental Eye Research 139 (2015) 115–122

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Association of clusterin (*CLU*) variants and exfoliation syndrome: An analysis in two Caucasian studies and a meta-analysis

Bao J. Fan [a], Louis R. Pasquale [a, b], Jae H. Kang [b], Hani Levkovitch-Verbin [c], Jonathan L. Haines [d], Janey L. Wiggs [a, *]

[a] Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA
[b] Channing Division of Network Medicine, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA
[c] Goldschleger Eye Institute, Chaim Sheba Medical Center, Tel Aviv University, Tel Hashomer, Israel
[d] Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form
4 August 2015
Accepted in revised form 5 August 2015
Available online 10 August 2015

Keywords:
Exfoliation syndrome
Glaucoma
Clusterin
Genetic variants
Meta-analysis

ABSTRACT

Exfoliation syndrome (XFS) is an important risk factor for glaucoma (XFG) worldwide. *LOXL1* variants are highly associated with XFS in most populations; however, the high frequency of risk alleles in normal individuals and the reversal of risk alleles in different ethnic populations suggest that other factors contribute to XFS pathogenesis. Clusterin (CLU) is an extracellular matrix chaperone that prevents protein aggregation and is highly expressed in ocular tissues affected by XFS. Studies examining common *CLU* variants for association with XFS have been inconsistent. The purpose of this study was to evaluate *CLU* variants for association with XFS in two independent datasets from the United States (222 cases and 344 controls) and Israel (92 cases and 102 controls). Seven tag SNPs that captured >95% of alleles at r^2 greater than 0.8 across the *CLU* genomic region were genotyped using TaqMan assays. Genotypes for an additional SNP, rs2279590, were imputed using phased haplotypes of HapMap reference CEU samples. Of the 8 *CLU* SNPs selected for the study, none were significantly associated with XFS in either case–control group (age and sex adjusted $P > 0.14$ and 0.36, respectively, in the US and Israeli datasets), or when they were meta-analyzed together (age and sex adjusted $P > 0.13$). Haplotype analysis using all 8 SNPs or only the promoter region SNPs also did not show significant associations of *CLU* with XFS in the combined US and Israeli dataset ($P > 0.28$). Meta-analysis of the data from this study and previous studies in Caucasian populations (1184 cases and 978 controls) resulted in statistically significant association of rs2279590 with XFS (summary OR = 1.18, 95% CI: 1.03–1.33, $P = 0.01$). Significant association between rs2279590 and XFS was also found in Indian populations (summary OR = 0.76, 95% CI: 0.61–0.96; $P = 0.02$); however, significant heterogeneity between the Caucasian and Indian populations possibly due to reversal of the risk allele precluded an overall meta-analysis for rs2279590 ($Q = 0.001$, $I^2 = 91\%$). No significant association was identified for rs3087554 in either Caucasian populations (summary OR = 0.90, 95% CI: 0.77–1.05, $P = 0.17$) or Indian populations (summary OR = 0.89, 95% CI: 0.72–1.10, $P = 0.28$), or in both populations combined (1705 cases and 3713 controls; summary OR = 0.90, 95% CI: 0.79–1.01, $P = 0.08$). Significant heterogeneity precluded the addition of the Japanese data to the meta-analysis for rs3087554 ($Q = 0.006$, $I^2 = 87\%$). Our results suggest that common *CLU* variants may contribute to modest XFS risk but even larger datasets are required to confirm these findings.

1. Introduction

Exfoliation syndrome (XFS) is a complex systemic disease whose most notable feature is the deposition of fibrillar material throughout the anterior segment of the eye. Exfoliation material contains elements of basement membranes and the elastic fiber framework, as well as other macromolecules (Ritch et al., 2003).

Abbreviations: *CLU*, clusterin; XFS, exfoliation syndrome; XFG, exfoliation glaucoma; I^2, heterogeneity index; LD, linkage disequilibrium; *LOXL1*, *lysyl oxidase-like 1*; SNP, single nucleotide polymorphism.

* Corresponding author. Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA.
E-mail address: janey_wiggs@meei.harvard.edu (J.L. Wiggs).

http://dx.doi.org/10.1016/j.exer.2015.08.004
0014-4835/

XFS is a risk factor for developing elevated intraocular pressure (IOP) and glaucoma (Anastasopoulos et al., 2015). While XFS fibrillar material accumulates in the trabecular meshwork outflow pathways, the molecular mechanisms underlying elevated IOP and exfoliation glaucoma (XFG) are not completely known (Sacca and Izzotti, 2014).

Exfoliation syndrome (XFS) and the associated glaucoma (XFG) are genetically complex traits with contributions from both genetic and environmental factors (Sein et al., 2013). A genome-wide association study (GWAS) using unrelated patients and controls from Iceland and Sweden revealed significant association between common *lysyl oxidase-like 1* (*LOXL1*) variants and XFS (Thorleifsson et al., 2007). This association was subsequently replicated in populations worldwide (Wang et al., 2014), including in United States clinic-based samples (Fingert et al., 2007; Aragon-Martin et al., 2008; Challa et al., 2008; Fan et al., 2008; Yang et al., 2008). Overall, these results show that *LOXL1* is a major gene associated with XFS. However, while the risk alleles are present in the majority of cases worldwide, they are also frequently found in control individuals, arguing that other genetic and/or environmental factors are necessary for the disease to be fully manifested. Recently, another GWAS using a discovery sample set of 1484 cases and 1188 controls from Japan and replication datasets of 6901 cases and 20,727 controls from 17 countries showed that common variants in the *calcium channel, voltage-dependent, P/Q type, alpha 1A subunit* (*CACNA1A*) gene were significantly associated with XFS (Aung et al., 2015).

Clusterin (CLU), an extracellular chaperone also known as apolipoprotein J, has been identified as a major component of the fibrillar deposits in XFS (Zenkel et al., 2006; Ovodenko et al., 2007; Doudevski et al., 2014). This ubiquitous glycoprotein is secreted by most cell types and is found in all body fluids (Jones and Jomary, 2002). In the eye, *CLU* is expressed in most ocular cells and tissues, particularly in the ciliary epithelium (Zenkel et al., 2005). *CLU* expression is down regulated in the iris, lens and ciliary processes of patients with XFS compared to non-XFS glaucomatous control subjects (Zenkel et al., 2006). One study found reduced levels of clusterin mRNA and protein in aqueous humor samples from XFS patients compared to non-XFS glaucomatous control subjects (Zenkel et al., 2006), while another study found the opposite result (Doudevski et al., 2014), suggesting that *CLU* dysregulation may contribute to the disease.

Previous genetic studies provide some evidence that common *CLU* variants may contribute to XFS risk, although the association results are not consistent among different studies and populations. In the Blue Mountain Eye Study, the *CLU* single nucleotide polymorphism (SNP) rs3087554 was nominally associated with XFS (86 cases and 2422 controls) at the genotypic level ($P = 0.044$), but not at the allelic level or when the age of controls was restricted to those over 73 years old ($P > 0.07$) (Burdon et al., 2008). No significant association between the rs3087554 variant and XFS was observed in a German (661 cases and 342 controls; $P > 0.08$) and Italian case–control set (209 cases and 190 controls; $P > 0.70$), although a positive association for another *CLU* SNP, rs2279590 (allele A) was reported in the German dataset only (Krumbiegel et al., 2009). A study of 136 cases and 89 controls from India did not find an association of rs3087554 with XFS ($P > 0.06$), but did find a significant association with rs2279590 (Padhy et al., 2014), although the risk allele was 'G' rather than the 'A' allele that was associated with disease risk in the German dataset. Interestingly, in the Indian study, the rs2279590 risk allele 'G' was also associated with elevated mRNA in lens capsules compared with the 'A' allele. However, a recent study of 299 cases and 224 controls from South India did not find significant association of rs3087554 or rs2279590 with XFS ($P > 0.43$; Dubey et al., 2015). Additionally, a recent GWAS in the Japanese dataset of 1484 cases and 1188 controls showed a nominal association of rs3087554 with XFS ($P = 0.029$), although the direction of effect for the minor allele 'G' is in the opposite direction compared with the effect in Caucasians with European ancestry (Aung et al., 2015). rs2279590 was not included in the Japanese study.

Clusterin's role as an important extracellular matrix chaperone required for prevention of extracellular protein aggregation makes it an interesting candidate genetic risk factor for XFS and XFG. To help clarify its relation to XFS, the purpose of this study was to evaluate common *CLU* variants for association with XFS (including cases with and without glaucoma) in two independent Caucasian case–control datasets from the United States (US) and Israel. Additionally, we performed a meta-analysis that included these two datasets as well as published results from Australia (Burdon et al., 2008), Germany (Krumbiegel et al., 2009), Italy (Krumbiegel et al., 2009), India (Padhy et al., 2014; Dubey et al., 2015) and Japan (Aung et al., 2015).

2. Materials and methods

2.1. Patients and control subjects

This study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Boards of the Massachusetts Eye and Ear Infirmary (Boston, US) and the Goldschleger Eye Institute (Tel-Hashomer, Israel). Informed consent was obtained from all patients and controls after explanation of the nature and possible consequences of the study.

After we obtained informed consent, we recruited cases and controls from the Massachusetts Eye and Ear Infirmary (US), including 222 unrelated patients affected by XFS and 344 control subjects. Of the 222 cases with XFS, 110 also had glaucoma (XFG). A second independent dataset using cases and controls from Israel at the Goldschleger Eye Institute included 92 unrelated cases with XFS and 102 control subjects. Of the 92 patients with XFS, 67 patients also had XFG.

XFS patients had evidence of characteristic fibrillar material on the lens capsule or pupillary margin. XFG was additionally defined as: intraocular pressure >22 mm Hg (for at least one eye) on two occasions or intraocular pressure >19 mm Hg (for at least one eye) on treatment with two or more glaucoma medications; evidence of optic nerve damage in at least one eye based on clinical exam or visual field with changes consistent with nerve fiber layer loss on at least one reliable test. Control patients had no evidence of XFS or glaucoma based on clinical exam.

In the US dataset, the average age of the XFS cases and controls was 68.4 and 64.5 years, respectively ($P < 0.0001$; Table 1). 60.4% and 54.1% of XFS cases and controls were female ($P > 0.14$). In the Israeli dataset, the average age of the XFS patients and controls was 75.5 and 66.0 years, respectively ($P < 0.0001$). 51.1% of XFS cases were female while 41.2% of the controls were female ($P > 0.14$). All the cases and controls in the US and Israeli datasets were of self-reported Caucasian ancestry. Demographic features of the XFG subgroup (those XFS patients who also had evidence of glaucoma) were similar to the XFS cases overall for both datasets (69.0 years and 55.5% female for the US dataset and 75.0 years and 47.8% female for the Israeli dataset).

2.2. Genotyping

We selected seven tag SNPs that captured >95% of alleles at r^2 greater than 0.8 across the *CLU* genomic region, including all exons, introns, the 5′UTR, the 3′UTR, and the 7 kb proximal promoter region. Tag SNPs were selected according to the HapMap CEU (Utah