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Expression and regulation of LOXL1 and elastin-related genes in eyes with exfoliation syndrome (2013)

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LOXL1 genetic variants are significantly associated with exfoliation syndrome (XFS), however the impact of the associated variants on disease development is not yet understood. Initially the associated common missense variants, R141L (Arginine 141 Leucine) and G153D (Glycine 153 Aspartate), were considered to be pathogenic alleles [1]. ‘Flipping’ of the risk allele in certain populations for both missense variants has provided strong evidence that these missense changes are not biologically significant (Figure 1) [2,3]. Additionally, in an *in vitro* assay, the associated missense alleles do not affect *LOXL1* enzymatic activity [4]. Together, these results suggest that other *LOXL1* variant(s), in linkage disequilibrium with the common missense variants, predispose to XFS by affecting protein function and/or gene expression. Several lines of evidence suggest that a reduction in *LOXL1* gene expression contributes to disease development and each are discussed in the following sections.

Using ocular tissue samples (iris, lens, and ciliary body) from 25 XFS / exfoliation glaucoma (XFG) cases and 25 controls, the expression level and localization of *LOXL1*, *LOXL2*, and lysyl oxidase (*LOX*) were investigated and correlated with the *LOXL1* risk genotype and also disease stages (early versus late) [5]. *LOXL1* ocular expression was reduced by approximately 20% per risk allele of rs1048661(R141) [5]. An effect on gene expression was not observed for G153D. Additionally, *LOXL1* was observed to have higher levels of expression in early disease stages compared to late and this was not correlated with risk genotypes. The expression of *LOXL2* and *LOX* were not altered in XFS- derived tissue samples compared with controls.

The fact that common *LOXL1* missense changes (R141L and G153D) are not likely to have biologically relevant effects suggests that other variants in the *LOXL1* genomic region must underlie the significant association observed with disease. Haplotype analysis can be used to identify functional variants within a genomic region. Functional variants could include those in coding and/or noncoding regions. To identify variants associated with disease risk, haplotype analysis using variants distributed throughout the *LOXL1* regulatory regions and

gene coding regions identified a risk haplotype in a Caucasian population from the United States that included a *LOXLI* promoter region variant previously shown to reduce gene expression (rs16958477) [6,7]. These variants and other regulatory elements that could influence *LOXLI* expression are shown in Figure 2. Of particular interest are the *LOXLI-ASI* (antisense RNA) and its promoter, the *LOXLI* promoter elements, and a highly conserved region in the distal 5' *LOXLI* regulatory region. Further work will be necessary to determine if these elements impact disease pathogenesis.

In addition to *LOXLI*, several other factors may contribute to XFS pathogenesis, including TGF- β 1 (transforming growth factor beta 1), oxidative stress, UV light and hypoxia. Factors potentially influencing XFS pathogenesis were tested for their effect on *LOXLI* expression using cultured fibroblasts from Tenon's capsule biopsies from five patients with cataract and XFS and three patients with cataract without XFS [8]. Changes in *LOXLI* expression were also investigated for high and low *LOXLI* risk haplotypes (based on the R141L and G153D polymorphisms) [8]. Each of these disease-associated factors, (TGF- β 1, oxidative stress, UV light and hypoxia), induced significant increases in expression of *LOXLI* and elastic proteins in cells with the low-risk haplotype but had comparatively lower expression in cells with the high-risk haplotype. These results suggest that the high-risk *LOXLI* haplotype is associated with reduced gene expression at baseline and also under conditions where *LOXLI* gene expression increases in response to factors impacting disease pathogenesis.

To investigate the impact of loss of *LOXLI* function on disease development, the phenotypic features of a *LOXLI* null mouse were characterized [9]. The *LOXLI* null mouse was found to have some features of XFS, including lens abnormalities consistent with cataract and disruption of the blood-aqueous barrier [10]. The *LOXLI* null mouse did not have deposition of exfoliation fibrillar material or elevated intraocular pressure and glaucoma. These results suggest that loss of *LOXLI* enzyme activity contributes to disease predisposition, but that other factors, which may be genetic and/or environmental, are necessary for the disease to be fully manifest.

Collectively, these results support the hypothesis that dysregulation of *LOXLI* expression is a contributing factor to exfoliation disease development. Further study defining the regulatory elements necessary for regulation of *LOXLI* expression could point to possible therapeutic targets.

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FIGURE 1.

LOXL1 risk alleles in Caucasians, East Asians, and South Africans. LOXL1 exons are shown as solid dark blue rectangles. The 5' UTR (untranslated region) and 3' UTR are shown as smaller rectangles. The promoter regulatory region is adjacent to the 5'UTR. The location of the two missense alleles initially associated with exfoliation syndrome R141L (Arginine 141 Leucine) and G153D (Glycine 153 Aspartate) are indicated in exon 1. The allele associated with risk in each of three ethnic groups is shown by larger rectangles directly below exon 1: Caucasians- Arginine at position 141 and Glycine at position 153; East Asians- Leucine at position 141 and Glycine at 153; and South Africans-Arginine at position 141 and Aspartate at position 153. The flipping of risk alleles in different populations indicates that these missense changes do not cause disease but that they are markers of nearby variants (in linkage disequilibrium) that are likely to affect gene expression and/or protein function

FIGURE 2.

LOXL1 5' regulatory region. Regulatory elements located in the genomic region 5 to the LOXL1 gene are depicted using a modified screen shot from the UCSC genome browser (<http://genome.ucsc.edu/>). SNPs of interest include rs12914489 (part of the risk haplotype from reference 6), rs16958477 (promoter region SNP with possible functional consequences, see references 6 and 7), rs1048661 (R141L), and rs3825942 (G153D). The location of the LOXL1-AS1 (LOXL1 antisense 1) transcripts are shown directly above the LOXL1 gene transcript (only LOXL1 exon 1 and part of intron 1 are in this figure). GERP (genomic evolutionary rate profile) scores indicating mammalian conservation for DNA sequence are shown below the gene transcripts. GERP scores >1.0 are considered to be evolutionarily conserved, and conserved sequences are more likely to have a biological impact. Layered histone marks for H3K27Ac for 7 common cell lines are shown below the GERP scores on a scale of 0 to 100. These marks (indicated by peaks) are often found near regulatory elements. DNA methylation sites in two cell lines potentially involved in exfoliation syndrome, iris pigment epithelial cells (HIPEpiC) and nonpigmented ciliary body epithelial cells (NHPCEpiC) are shown in red (highly methylated) and in green (unmethylated). The lack of methylation in the promoter region suggests that the gene is expressed. The LOXL1 promoter region is indicated by the H3K4Me3 marks (shown for 7 common cell types) directly below the methylation marks. DNaseI hypersensitivity sites active in iris pigment epithelial cells and nonpigmented ciliary body epithelial cells are shown in the bottom track. These are regions of open chromatin that could be active regulatory sites.