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Consideration for Gene-Environment Interactions as Novel Determinants of Exfoliation Syndrome

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Introduction and Overview

In systemic biology one uses high throughput data from various study types to derive information on better ways to diagnose, treat, and prevent complex diseases like exfoliation syndrome (XFS) (Figure). Here we focus on how analytical epidemiological studies contribute to an improved understanding of XFS and its attendant unwanted consequences (such as glaucoma,¹ cataract,² retinal vein occlusion,³ etc.). Specifically, we concentrate on how the environment may modify the association between genotypes and exfoliation syndrome (XFS). The discovery of gene-environment (GxE) interactions for XFS may form the basis for novel XFS primary prevention strategies. It should be stated at the outset that GxE interactions for XFS have not been confirmed but several are suspected. The discovery of the robust association between *LOXLI* genotypes and XFS⁴ has led directly to the discovery of environmental risk factors for the disease, setting the stage to assess GxE interactions for this condition. This review will cover how the association between *LOXLI* genotypes and XFS was discovered and confirmed. The impact of the geographical distribution of *LOXLI* gene variants on the identification of environmental risk factors will be reviewed. The strongest candidate GxE interactions for XFS will be presented, and the implications these have for understanding the disease and for targeted strategies to reduce disease burden will be described. Finally, the necessary steps required for testing putative interactions between *LOXLI* gene variants and environmental factors will be discussed.

The discovery of *LOXLI* variants in relation to exfoliation syndrome

XFS is a strongly age-related disease,⁵ making it difficult to collect multigenerational families with the disorder, although some evidence of familial aggregation has been reported.⁶ Using a large Finnish family for genome wide linkage study, several genomic regions were suggested to contain genes contributing to XFS.⁷ However, further study has not identified disease-associated genes in these regions, suggesting loci in these regions are not common causes of XFS. Candidate gene studies using selected genes of interest in case

control studies of unrelated individuals have been performed. However, these studies have been mostly underpowered and the results have been inconsistently replicated across populations.⁸

The results from Phase I of The Human Genome Project suggested that an agnostic search for gene variants related to any trait is possible.⁹ First, the human genome contains DNA blocks that tend to travel together during cell reproduction. The size of such blocks differs by ancestry. Second, roughly every 300th base, there is a nucleotide change that occurs commonly (>5% of the time) in the general population, referred to as a common single nucleotide polymorphism (SNP). These realizations combined with improvements in microfabrication and nucleotide chemistry assays allowed for the creation of technologies that can simultaneously genotype several hundred thousand common polymorphic sites strategically located throughout the genome. Thus, these genotyping technologies enabled genome wide association studies (GWAS), which are agnostic searches for common polymorphisms that have associations with complex diseases, like XFS. An adequately powered GWAS using a case control design performed in Iceland demonstrated that *LOXLI* variants were associated with XFS.⁴ The researchers used a simple chi square test to compare the genotype frequency between cases and controls at 304,250 loci throughout the genome. The association between each marker in cases and control was adjusted for inherent population structure that might confound the relation between a genotype and affected status. The typical p-value for statistical significance ($p=0.05$) was adjusted to 1.6×10^{-7} using the Bonferroni method by dividing 0.05 by the number of tests performed (304,250). Using this approach, polymorphic variants located in the genomic region that includes *LOXLI* were found to be statistically associated with XFS. These results were confirmed using a candidate approach in another population of subjects as part of the same publication.

There were two rather remarkable features of the XFS GWAS performed by the Icelandic group. First, the effect size for the rs3825942 *LOXLI* variant found in association with XFS was among the highest seen in a GWAS for common complex disease (odds ratio = 20.1); typically, odds ratios for SNPs in association with common complex diseases are in the 1.2-1.5 range and rarely are they above 2.0. This suggests that the rs3825942 allelic variant represents a locus of major functional significance in XFS. Second, while 99% of cases harbored the at-risk allele, 85% of controls also harbored this allele. This suggests that while the rs3825942 *LOXLI* variant is necessary for disease, it was not sufficient to produce it. Additional factors that could produce XFS include other genes, environmental factors or epigenetic factors.

LOXL1 gene variants around the world and the latitude effect

Individual researchers worldwide with archived DNA samples from XFS case – control sets embarked on efforts to confirm the results reported in Iceland.¹⁰⁻¹⁸ Since the effect size for the associated alleles were large, and these researchers were not hampered by having to adjust for multiple comparisons, which requires stringent p-values, a sample with as few as 36 cases and 30 controls confirmed the results found in Iceland.¹⁹ Furthermore, the results were relatively consistent in populations worldwide. Interestingly, the risk alleles were found to be reversed in a South African population^{17,20} and in Asian populations.^{13,21-26}

These latter results suggest that the *LOXLI* alleles associated with disease risk are not causative but represent the genomic region containing genetic variants that contribute to disease risk. It is interesting that the ratio of cases and controls with XFS-associated risk alleles was consistently in the ~95%:80% range for these candidate gene replication studies, even though they were derived from populations with widely varying disease prevalence. For example, this ratio was seen in Caucasians from Finland²⁷ and Australia,¹⁴ even though the respective XFS prevalence at these locations was ~22%²⁸ and 1%.²⁹

While the ratio of cases and controls with *LOXLI* alleles associated with increased risk of XFS was fairly constant in various geographic regions, the disease prevalence seemed to increase as a function of latitude. For example, crude prevalence estimates for XFS in Kandy District, Sri Lanka (7° N), Tamil Nadu, India (13° N), Thessaloniki, Greece (40.7° N) and three towns in Finland (64° N) were 1.1%,³⁰ 6%,³¹ 11.9%³² and 22%,²⁸ respectively. Yet one could not conclude whether this distribution of point prevalence estimates reflects a true latitude effect, chance or confounding by other important factors that vary with latitude. Stein et al.,³³ using a healthcare relational database, which captured billing data on insured participants from throughout the continental United States (US), provided intriguing data that higher latitude was a risk factor for XFS. The continental US spans 15 degrees of latitude, and a multivariable model controlling for demographic features, ocular disease and systemic illness demonstrated that relative to living in the middle tier of the US, living in the Northern tier was associated with an increased risk of XFS and living in the Southern tier was associated with a reduced risk of XFS. Even when the analyses were limited to participants of African, Hispanic and Asian heritage, the aforementioned trend remained. Since the healthcare relational database was de-identified, the authors could not explore whether consistent residence in a particular US tier was related to XFS. In the Nurses Health Study and Health Professional Study, Kang et al.⁵ collected zip code level data regarding geographic residence throughout the life of participants drawn from throughout the US. These authors generated a population at risk for exfoliation glaucoma (XFG) and evaluated the relation between consistent residence in the northern, middle and southern tier of the US and XFG. Again, they found that the risk in the Northern tier of the US was higher than in the Southern tier. Furthermore, the same researchers found that self-reported Scandinavian ancestry was not a risk factor for XFS. Finally, when the researchers modeled where participants lived throughout life, residence in the Northern tier during the teenage years and young adulthood was most strongly associated with XFS. This research raised the important question of what factors were responsible for this latitude effect.

Environmental factors driving the latitude effect

Vitamin D

Multiple sclerosis is a disease with a latitude effect similar to XFS.^{34,35} While the reason for higher risk of multiple sclerosis in more Northern geographic locations is not entirely clear, researchers believe that higher solar irradiation and circulating vitamin D levels at lower latitudes contribute to the formation of an intact immune system that mitigates against the

development of this neuro-inflammatory disorder.³⁶ However, in relation to XFS, one study did not find a link between circulating vitamin D levels and XFS.³⁷

Microbes and exfoliation syndrome

Variation in the distribution of inciting microbes could explain the geographic distribution of XFS. Perhaps the best-studied microbial infection in relation to XFS is *Helicobacter pylori* but this microbe does not necessarily have a north-south distribution gradient. In Greece, where XFS is fairly common as demonstrated in the Thessaloniki Eye Study,³² Kountouras and colleagues found more gastric mucosa biopsy-proven *H. pylori* infection³⁸ as well as higher anti-IgG antibody levels in the serum and aqueous humor of XFS cases versus controls.³⁹ However, a study in India⁴⁰ where XFS is also common,³¹ researchers did not find differences in anti-*H. pylori* IgG levels in the serum and aqueous humor in XFS patients versus controls. The relation between *H. pylori* infection and XFS remains controversial and more studies regarding how microbial infection might explain the geographical distribution of XFS are needed.

Climatic factors

Climatic factors including average ambient temperature, number of sunny days, and precipitation represent candidate environmental factors to explore in light of the latitude effect described above. Stein et al.³³ found that lower ambient temperature was associated with an increased risk of XFS. It is possible that lower ambient temperature could result in lower ocular temperature that could contribute to precipitation of exfoliation material in the anterior ocular segment. Of course, XFS does occur commonly in warmer climates, thus other climatic factors must also be involved.

Taylor was one of the first investigators to suggest that solar exposure might contribute to XFS based on his ocular feature survey in an Aboriginal Australian population.⁴¹ Thomas et al. suggested XFS was more common in those engaged in outdoor occupations among people studied in southern India.⁴² This topic received renewed attention in 2011 when Stein et al.³³ found that increased number of sunny days was associated with increased risk of XFS in the continental United States. Two subsequent studies – a clinic-based study⁴³ with prevalent cases and another population-based study using incident cases⁴⁴ – noted that more time spent outdoors during summer was associated with increased risk of XFS. It is possible that this association is related to solar radiation. Yet, one needs to reconcile how solar exposure might explain the increased risk of XFS as a function of higher latitude considering that solar exposure to the horizon is clearly more abundant at lower latitudes.⁴⁵ While solar exposure to the horizon is higher in the Southern US tier, Pasquale et al. postulated that it is the ocular solar exposure from reflected surfaces such as water and snow that may incite XFS via long-term damage to the uveal tract.⁴³ It is well known that snow, water and sand are better reflectors of solar radiation than soil and grass.⁴⁶ There is considerable indirect evidence to support the concept that reflected light into the eye is important in XFS. First, in a study on the Island of Rab in the Adriatic Sea, the rate of XFS was higher in fisherman than in urban dwellers.⁴⁷ Second, the rate of XFS among Aboriginal people residing in an Australian desert-like environment was much higher than in urban-dwelling Australians.^{29,48} Third, native indigenous Polynesians have less XFS than

Aboriginal Australians even though they live at the same latitude but the former inhabit a more tropical environment where there may be less solar reflection.⁴⁹ Fourth, the rate of XFS in the African country of Ghana is very low (no cases detected in 198 consecutive glaucoma patients examined);⁵⁰ yet, XFS and XFG is commonly seen in the clinical setting in Ethiopia which is located at the same latitude.⁵¹ This could be due to many factors but Ghana is a tropical green environment, and Ethiopia is a mountainous, arid climate with more abundant sunshine.

UVA and UVB radiation passes through the solar shield and can also pass through the cornea to impact the anterior uveal tract and lens. If solar radiation is related to XFS pathology the other solar ophthalmopathies, including climatic keratopathy, pterygia and pingeculae, should also be associated with XFS. Indeed, several investigators have found a positive association between these entities and XFS.^{48,52}

Interestingly, further away from the equator, light rays have a more angulated entry to the terrain because they are at a lower height in the sky and thus have a greater opportunity to redirect into the eye after contacting reflective surfaces. Thus while overall UV radiation to the horizon is highest at the equator, reflected light into the eye may be more abundant further away from the equator. Ocular UV exposure may be enhanced by the higher snow pack that is more abundant further away from the equator.

UV radiation is known to upregulate LOXL1 expression in Tenon's capsule fibroblasts and trigger formation of extracellular deposits that resemble exfoliation material.⁵³ Ocular UV radiation serves as a leading environmental factor that could modify the relation between *LOXL1* gene variants and XFS.

Coffee consumption

There is a rough correlation between coffee consumption and latitude with Scandinavian people being the highest consumers of coffee in the world (4 cups per day per person).⁵⁴ Coffee consumption increases homocysteine levels,^{55,56} and homocysteine levels are consistently higher in serum,⁵⁷⁻⁵⁹ aqueous humor⁶⁰ and tear samples⁶¹ taken from XFS patients compared to age matched controls. Homocysteine injected into the internal carotid artery induced cerebrovascular leakage that may be mediated by matrix metalloproteinase 9 activity.⁶² Elevated plasma and aqueous homocysteine may also contribute to oxidative stress, vascular leakage and disorderly extracellular matrix metabolism in XFS. Kang et al. found that heavy coffee consumption was associated with increased risk of XFG and XFG suspects.⁶³ Most of the risk was attributable to people with a positive family history of glaucoma, suggesting perhaps the relation between *LOXL1* gene variants and XFS is also modified by heavy coffee consumption.

Dietary folate levels

Low serum folate levels, but not levels of vitamin B6 and B12, are associated with increased risk of XFS.⁶⁴ Low folate levels can also increase homocysteine levels. Lower folate levels could be related to common genetic variants in enzymes involved in homocysteine metabolism but such variants have not been linked to XFS thus far.^{64,65} Kang et al. found

that among health professionals, ~50% were not getting their recommended allowance of dietary folate.⁶⁶ Furthermore, there was a trend toward reduced cumulatively averaged dietary folate intake and incident XFG or XFG suspect status. Fresh fruit and vegetables are a major source of dietary folate and they are more available closer to the equator. Serum folate levels may also modify the relation between *LOXLI* variants and XFS.

Discovery of gene-environment interactions in XFS

In analytical epidemiology, a GxE interaction exists if one can demonstrate that the relation between a genotype and a phenotype is modified by an environmental exposure. Given that genetic effects in complex disease are modest and environmental exposure data can be imprecisely measured, large sample sizes are typically needed to discover a GxE interaction for common complex diseases. If the search for GxE interactions is an agnostic one, then one also has to account the multiple comparisons made, leading to further expansion of the sample size needed to convincingly show that a GxE interaction is a true positive finding in relation to an outcome of interest. Therefore, candidate approaches represent feasible approaches to discover GxE interactions, particularly if there is a plausible biological argument for them. Case-control study designs, as opposed to cohort analyses, are cost-effective because both environmental exposure and genotype data are needed to evaluate an GxE interaction, and both types of data may not be available in larger cohorts.

Given the robust nature of the association between *LOXLI* variants and XFS, it is an obvious candidate for the gene component of any GxE interaction in relation to XFS. The environmental exposure should be readily measurable, and there should be compelling biologic evidence that it might modify the relation between the gene and the trait of interest. Given the extensive evidence that ocular UV radiation is important in this disorder, it is an attractive environmental exposure; however, one needs a good surrogate measure for capturing this exposure, as there is no instrument that measures ocular UV exposure directly. Time spent outdoors as recalled throughout life might be a reasonable surrogate for this measure, and this can be captured with a validated instrument that is particularly suited for gene association studies as it is relatively simple and can be reproduced by other groups, allowing for data to be pooled across study groups. The PhenX Toolkit (<https://www.phenxtoolkit.org>) provides a validated method to measure time spent outdoors during periods when UV radiation to the horizon is highest.⁶⁷ Systematic collection of geographical residence data allows us to introduce this parameter as a covariate in data analysis. Since interviewer bias and recall bias can cause spurious findings, it is best that trained interviewers who are masked to XFS status administer questionnaires.

The effect sizes of the genetic variant, the prevalence of the environmental exposure and the magnitude of the anticipated interaction effect size all determine sample size. Also the number of genotypes assessed dictates the p-value cutoff. In this instance, we would plan to use the *LOXLI* polymorphic locus with strongest effect size (rs3825942 with OR = 20) and use logistic regression analysis in a 1-degree of freedom test according to the following equation:

$$XFS = \beta_0 + \beta_1(\text{age}) + \beta_2(\text{weighted lifetime residential latitude}) + \beta_3(\text{average lifetime number of hours spent outdoors during teenage years in the summer}) + \beta_4(rs3825942 \text{ } LOXL1 \text{ genotype}) + \beta_5(rs3825942 \text{ } LOXL1 \text{ genotype} \times \text{average lifetime spent outc})$$

The β_5 term provides the effect size and significance level for the GxE interaction in a multiplicative scale. Power calculations for sample sizes needed to have an 80% chance to confirm a significant GxE term are provided in the Table. Assuming a 1:1 unmatched case-control set and disease prevalence of 4%, between 50 and 100 cases and 50 and 100 controls are needed to confirm an interaction between the *LOXL1* rs3825942 risk allele and >11 hours per week spent outdoors during the teenage years.⁴⁴ This calculation assumes the effect size for the interaction term ranges from 1.4 to 2.0. This is a modest sample size but the sample size could be higher depending on the precision in recalling time spent outdoors.

Conclusions

LOXL1 genetic variants have contributed to the discovery of environmental factors associated with XFS. The realization that the genotype – phenotype association is modified by environmental factors allows one to consider pathophysiologic mechanisms in disease. It may be that some environmental factors such as UV radiation contribute to alterations of the anterior uveal tract in an individual already genetically predisposed to disease development. Dysregulation of *LOXL1*, in response to the environmental insult could lead to abnormal *LOXL1* protein activity that may contribute to the formation of exfoliation material. It is known that UV radiation causes up-regulation of the exfoliation material components including *LOXL1* in vitro.⁵³ Colder temperatures may enhance precipitation of material out of solution and dietary factors contributing to higher homocysteine levels exacerbate the process by prompting disorganization of the extracellular matrix components over time. If these mechanisms are further elucidated and confirmed, then primary prevention strategies could be envisioned. Regardless of *LOXL1* genotype, protection against ocular UV exposure and diets that enhance folate levels (higher in fruits and vegetables) are beneficial for overall health. Furthermore, moderating coffee consumption over long periods may also be advantageous. Data regarding time spent outdoors in relation to XFS would suggest that ocular protection from UV rays should begin in the teenage years. Since pharmacological approaches to halt or disassemble accumulating exfoliation material have yet to emerge, these lifestyle modifications may represent cost effective ways to reduce the burden from this condition in the future.

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Figure.

A systems biology approach is required to assimilate how genes and environment contribute to the formation of exfoliation material in the human eye as illustrated in the clinical photograph with the white arrow that points to a sheet of exfoliation material in the pupillary axis near the lens capsule. The histopathological correlate on the far right illustrates the cuboidal lens epithelial monolayer and lens capsule as well as the overlying collection of exfoliation material admixed with pigment granules. Biological, genetic, epidemiologic and clinical studies are needed to leverage 'omic level data to understand exfoliation syndrome pathogenesis. Ultimately randomized trials will discern whether interventions successfully treat or prevent exfoliation syndrome.

Table

Sample size calculation to detect an interaction between a *LOXLI* single nucleotide polymorphism and average weighted time spent outdoors during the summer in exfoliation syndrome

Effect size for the GxE* interaction	Number of cases
1.4	99
1.5	85
1.6	75
1.7	67
1.8	61
1.9	55
2.0	51

The gene environment interaction is between *LOXLI* rs3825942 and time spent outdoors during teenage years. The outcome is exfoliation syndrome. Calculations assume a disease prevalence of 4%, an equal number of unmatched controls, an allele frequency for rs3825942 of 0.99, and an effect size for rs3825942 equal to 20. The calculation further assumes that 13% will spend >11 hours per week outdoors during the teenage years and 80% power to detect an rs3825942 X time spent outdoor >11/week. Power calculations determined by quanto (<http://biostats.usc.edu/software>).