Association of Adipose and Red Blood Cell Lipids With Severity of Dominant Stargardt Macular Dystrophy (STGD3) Secondary to an ELOVL4 Mutation

Amy F. Hubbard, MS; E. Wayne Askew, PhD; Nanda Singh, PhD; Mark Leppert, PhD; Paul S. Bernstein, MD, PhD

Objective: To determine whether adipose and red blood cell membrane lipids, particularly long-chain polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid, are significantly correlated with phenotype in a family with autosomal dominant Stargardt macular dystrophy (gene locus STGD3). A mutation in the ELOVL4 gene is responsible for the macular dystrophy in this family, and its disease-causing mechanism may be its possible involvement in fatty acid elongation in the retina.

Methods: The subjects included 18 adult family members known to have a 2-base pair deletion in the ELOVL4 gene. Control subjects included 26 family members without the mutation. Each subject received a complete eye examination including fundus photographs, the results of which were used to grade the severity of macular dystrophy on a 3-tier scale. Red blood cell membrane results of which were used to grade the severity and the level of eicosapentaenoic acid (r = -0.55; P = .02) and docosahexaenoic acid (r = -0.48; P = .04).

Results: When adipose lipids were analyzed, there was a significant inverse relationship between phenotypic severity and the level of eicosapentaenoic acid (r = -0.54; P = .04). When red blood cell lipids were analyzed, there were significant inverse relationships between phenotypic severity and levels of eicosapentaenoic acid (r = -0.55; P = .02) and docosahexaenoic acid (r = -0.48; P = .04).

Conclusions: These results indicate that the phenotypic diversity in this family may be related to differences in dietary fat intake as reflected by adipose and red blood cell lipids.

Clinical Relevance: This study demonstrates that dietary factors can influence the severity of an inherited human macular dystrophy.

Arch Ophthalmol. 2006;124:257-263

TARGARDT MACULAR DYSTROPHIES are early-onset disorders characterized by macular atrophy and subretinal flecks that lead to central visual loss, typically in the first or second decade of life.1,2 Autosomal recessive Stargardt macular dystrophy (gene locus STGD1) (Online Mendelian Inheritance in Man [OMIM] 248200) is the most common form; it is due to homozygous or compound heterozygous mutations in the ABCA4 gene (also known as ABCR), whose protein product is involved in the transport of retinoids from the rod outer segments.1,3 Autosomal dominant Stargardt macular dystrophy (gene locus STGD3) (OMIM 600110) is much rarer than autosomal recessive Stargardt macular dystrophy.3 In past years, a number of STGD3 pedigrees linked to chromosome 6q14 had been reported, and in 2001, a 5-base pair (bp) deletion in the ELOVL4 gene was described to be causative for STGD3.4 This 5-bp deletion causes a frameshift and premature termination of the ELOVL4 gene’s protein product.4 All of the previously described families with STGD3 shared this same mutation, and further analysis revealed that all of these families could be traced to a common ancestor.5 Subsequently, we identified a different mutation in ELOVL4 in an unrelated large Utah family (kindred K4175) with STGD3.6 This mutation was in the same location as the 5-bp deletion in exon 6 of the ELOVL4 gene, but there are 2 1-bp deletions 4 nucleotides apart that are predicted to truncate the protein by 51 amino acids.6

The ELOVL4 gene codes for a protein that is highly homologous to proteins found in yeast that are involved in the elongation of fatty acids.4 The ELOVL4 gene, short for “elongation of very long-chain fatty acids,” is therefore named for the proposed function of its protein product. The ELOVL4 gene is expressed abundantly in the photoreceptor cells of the retina and, to a lesser extent, in the brain, lens, testis, and skin.7 Photoreceptor outer seg-

Author Affiliations:
Departments of Foods and Nutrition (Ms Hubbard and Dr Askew), Human Genetics (Drs Singh and Leppert), and Ophthalmology and Visual Sciences (Dr Bernstein), University of Utah, Salt Lake City.
gene product. Recently, there was a case report\textsuperscript{12} of im-
Elolvl4
orders associated with DHA in the retina, possibly exacerbating the macular dis-
would have a high risk of developing a local deficiency of
supply DHA to the retina. However, current intakes of
intake can modify the severity of the macular dystrophy
mutation were consuming an adequate sup-
muta-
tervential trial has recently been completed,\textsuperscript{13} but no
previous studies to our knowledge have investigated the relationship between dietary fats and the severity of any inherited macular dystrophies. On the other hand, sev-
eral studies\textsuperscript{14-19} have investigated the relationship be-
tween dietary fats and the incidence or severity of age-
related macular degeneration. Of these studies, a few\textsuperscript{14-16,18}
have found significant inverse relationships between DHA or fish consumption and the incidence or severity of age-
related macular degeneration.

These previous age-related macular degeneration studies generally relied solely on a food frequency question-
aire (FFQ) to indicate individuals’ dietary fat intakes. An
FFQ assesses nutrient intakes by determining how fre-
quently a person consumes certain foods known to be the
major sources of particular nutrients.\textsuperscript{20} The main limita-
tions of FFQs are that they are time-consuming to admin-
ister and that they rely solely on subject recall to describe
their typical long-term diet. Studies based on FFQs usu-
ally require rather large populations on the order of hun-
dreds or even thousands of subjects to reduce the inher-
ent individual variability of this subjective measure. Thus,
there has been considerable interest in the development of
surrogate objective biomarkers that capture information on
the long-term intake of nutrients but exhibit less variabil-
ity. In the case of dietary fat intake, 2 techniques have proven
to be most useful—red blood cell membrane analysis and
fat biopsy analysis. Both tests are easily and rapidly per-
formed on subjects, and they have been successfully cross-
validated against FFQs. Red blood cell membrane fatty ac-
ids appear to be good indicators of dietary intake for
saturated and unsaturated fatty acids over the past 6 to 8
weeks.\textsuperscript{21-24} Adipose tissue aspirates are indicative of the ha-
itual diet over the last 2 to 3 years, especially with regard
to dietary intake of essential polyunsaturated fatty acids such as
ω-3 and ω-6 fatty acids.\textsuperscript{25-30}

Given the proposed involvement of the Elolvl4 gene
in fatty acid elongation, individuals with an Elolvl4 mu-
tation who consume an adequate supply of preformed
dietary DHA or an appropriate precursor may have a less
severe form of macular dystrophy. In this study, we have
examined all of the available adult members of Utah kind-
dred K4175, and we have assessed dietary fat intake by
FFQ as well as lipid analyses of red blood cell mem-
branes and fat biopsies to determine whether dietary fat
intake can modify the severity of the macular dystrophy
phenotype in those individuals with an Elolvl4 mutation.
Understanding the role of diet in a genetically de-
fined macular dystrophy may also provide clues as to the
etiology and treatment of more common macular disor-
ders such as age-related macular degeneration.

<table>
<thead>
<tr>
<th>Enzymatic Activity</th>
<th>ω-3 (n-3) Series</th>
<th>ω-6 (n-6) Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ6-Desaturation</td>
<td>α-Linolenic Acid (18:3)</td>
<td>Linoleic Acid (18:2)</td>
</tr>
<tr>
<td>Elongation</td>
<td>Octadecatetraenoic Acid (18:4)</td>
<td>γ-Linolenic Acid (18:3)</td>
</tr>
<tr>
<td>Δ5-Desaturation</td>
<td>Eicosatetraenoic Acid (20:4)</td>
<td>Eicosatrienoic Acid (20:3)</td>
</tr>
<tr>
<td>Elongation</td>
<td>Eicosapentaenoic Acid (20:5)</td>
<td>Arachidonic Acid (20:4)</td>
</tr>
<tr>
<td>Δ6-Desaturation</td>
<td>Docosapentaenoic Acid (22:5)</td>
<td>Docosatetraenoic Acid (22:4)</td>
</tr>
<tr>
<td>β-Oxidation</td>
<td>Tetracosapentaenoic Acid (24:5)</td>
<td>Tetracosatetraenoic Acid (24:4)</td>
</tr>
<tr>
<td></td>
<td>Docosahexaenoic Acid (22:6)</td>
<td>Docosapentaenoic Acid (22:5)</td>
</tr>
</tbody>
</table>

Figure 1. Main pathways of essential fatty acid elongation.\textsuperscript{10}

If the Elolvl4 protein product assists in fatty acid elon-
gation, then individuals with a mutation in this gene con-
suming an intake of DHA average for American persons
would have a high risk of developing a local deficiency of
DHA in the retina, possibly exacerbating the macular dis-
orders associated with Sttg3. However, if individuals with
an Elolvl4 mutation were consuming an adequate sup-
ply of preformed dietary DHA or one of its immediate pre-
cursors, it could be directly used in the retina, bypassing
the step in fatty acid elongation mediated by the Elolvl4
gene product. Recently, there was a case report\textsuperscript{12} of
improvement of visual function in response to DHA supple-
mentation in a patient with an Elolvl4 mutation.

A relative deficiency of DHA has been implicated in
X-linked retinitis pigmentosa. A placebo-controlled in-

METHODS

SUBJECTS

The subjects in this study included 44 genetically related adult
family members of Utah kindred K4175 identified at the Moran
Eye Center, University of Utah, Salt Lake City. This family has
been found to have autosomal dominant Stargardt macular dys-
trophy caused by a mutation of 2 noncontiguous 1-bp dele-
tions in the Elolvl4 gene.\textsuperscript{6} All of the subjects read and signed
a consent form that was approved by the institutional review board and complied with the Declaration of Helsinki.

Blood collected from the 44 family members was genotyped using the method described by Bernstein et al.2 The ELOVL4 variants were identified using denaturing high-performance liquid chromatography analysis and direct sequencing at the Department of Human Genetics, University of Utah. The subjects received a complete eye examination that included best-corrected visual acuity, dilated fundus examination, and fundus photography.

**RED BLOOD CELL MEMBRANE AND ADIPOSE TISSUE FATTY ACID ANALYSES**

Two milliliters of blood was drawn from an antecubital vein in the arm to examine red blood cell fatty acids and to estimate fatty acid intake over the past 6 to 8 weeks.24 An adipose sample was collected from a site on the buttocks to assess dietary fat intake over the past 2 to 3 years according to the method by Beynen and Katan.25 Adipose tissue samples were collected from only 35 of the 44 family members because some subjects lived out of state and it is not a commonly performed procedure. The packed red blood cells and adipose tissue samples were transferred to freezing vials and frozen at −80°C until they were shipped on dry ice to the Kennedy Krieger Institute, Baltimore, Md, for analysis.

At the Kennedy Krieger Institute, the red blood cell and adipose tissue samples were analyzed according to the method by Lagerstedt et al31 using capillary gas chromatography with electron-capture negative-ion mass spectrometry. This method was developed to quantify long-chain fatty acids and can identify 14 saturated and 25 unsaturated fatty acids. These fatty acids range from C8 to C26, including transunsaturated and branched-chain fatty acids. Individual lipids were expressed as a percentage of the total lipids analyzed. Since ELOVL4 has very restricted expression in the body—in the retina, lens, brain, testis, and skin”—the use of red blood cell membrane and adipose tissue fatty acids as biomarkers of long-term fat intake should be valid even in the presence of this mutation.

**DIETARY AND LIFESTYLE SURVEYS**

Family members also completed a semiquantitative FFQ to assess dietary fat intake.24 This questionnaire has been extensively validated against diet records and adipose tissue aspires.26 An additional self-administered questionnaire to identify potential confounding demographic variables was also completed by the family members. This questionnaire consisted of questions about past and present cigarette use, dietary supplements not indicated on the FFQ (including any type of essential fatty acids or fish oil), eye color, height, and weight.

**PHENOTYPIC GRADING OF MACULOPATHY**

The 18 family members who were positive for the ELOVL4 deletion were divided into 3 phenotypic grades based on the severity of their maculopathy (Table 1). Initially, the grades were assigned by one of us (P.S.B.) who had examined most of the subjects and who was aware of the clinical characteristics of all of the subjects. The grades were then confirmed in a masked manner by 3 other retina specialists (Kang Zhang, MD, PhD, Michael Teske, MD, and Albert Vitale, MD) at the Moran Eye Center who were given photographs but no clinical history. There was unanimous agreement between all of the graders for 13 subjects. In 2 cases, the majority of the masked graders agreed with the initial grader, and the initial grades were left unchanged (subjects II:10 and III:11). In 1 case, the masked graders unanimously disagreed with the initial grader, and the grade for that subject was modified to agree with their assessment (subject IV:14). In 1 case, 2 of the 3 masked graders disagreed with the assessment by the initial grader, but the original grade for this subject was left unchanged (subject III:5). One subject had no photographs, and she was graded based on the clinical examination alone (subject IV:24). To minimize the possibility of bias, all of the phenotypic grades were finalized prior to the availability of any of the lipid analysis data.

**STATISTICAL ANALYSES**

Spearman rank correlation was used to assess the correlation between phenotype and dietary intake of fatty acids or other nutrients as measured by red blood cell samples, adipose tissue samples, or the FFQ. If necessary, the Kruskal-Wallis test was then used to examine equality of distributions among the phenotypes for the various nutrients. To analyze the difference in fatty acid levels in red blood cell membranes and adipose tissue between the subjects with the ELOVL4 deletion and a population without the deletion, the Wilcoxon rank sum test for unpaired data was used. Statistical significance was set at $P<.05$. Statistical analyses were conducted using Stata version 8 software (Stata Corp, College Station, Tex).

**RESULTS**

**CLINICAL CHARACTERISTICS OF FAMILY MEMBERS WITH ELOVL4 MUTATIONS**

Of the 44 enrolled adult family members of Utah kindred K4175, 18 were found to carry the family’s truncating deletion in ELOVL4 (Figure 2). Using the grading scheme outlined in Table 1, 8 of these subjects were assigned a moderate phenotypic grade of 2, and 4 of these subjects were assigned a severe phenotypic grade of 3 (Table 2). The remaining 6 family members with an ELOVL4 deletion had minimal to no symptoms; however, fundus photographs and clinical examinations revealed that most but not all of these subjects had at least some macular changes, and they were all assigned phenotypic grades of 1.

Among the 12 family members who had been clinically diagnosed with autosomal dominant Stargardt macular dystrophy, the ages at onset varied from 6 to 63 years, but onset most commonly occurred in the second decade of life. Their fundus photographs varied, showing macular atrophy and flecks to butterfly-pattern dystro-
Their visual acuities also differed, with 6 of the family members considered legally blind but others able to drive and perform job functions. Many of these affected family members have been followed up for more than 6 years at the Moran Eye Center, and only rarely has any clinical progression been detected.

**RED BLOOD CELL MEMBRANE AND ADIPOSE TISSUE ANALYSES OF FATTY ACIDS**

Initially, we compared the red blood cell membrane and adipose tissue EPA and DHA levels of the 18 family members with *ELOVL4* mutations vs the 26 family controls to determine whether there were obvious deficiencies in systemic levels of long-chain ω-3 fatty acids similar to those seen in patients with X-linked retinitis pigmentosa.\(^1\) Table 3 shows that this is clearly not the case. In fact, in all of the cases, the average levels of DHA and EPA were higher in the subjects with *ELOVL4* deletions. Interestingly, the family as a whole had much lower average red blood cell EPA and DHA levels relative to the Kennedy Krieger Institute laboratory control values, which was consistent with the very low median fish intakes of 1 to 3 servings of fish per month that were noted in the FFQs.

The somewhat higher levels of EPA and DHA in the *ELOVL4* deletion group vs the family controls likely reflect different average dietary fat intakes. Since Utah kindred K4175 as a whole has a milder phenotype than other described families with the *ELOVL4* mutation,\(^5\)\(^,\)\(^6\) we hypothesized that some of the family members of Utah kindred K4175 with the mutation may be practicing a particularly healthy, protective lifestyle with a diet higher...
in EPA and/or DHA intake. To investigate this further, we explored whether biomarkers of long-term fat intake correlated with phenotypic severity in the 18 subjects with ELOVL4 deletions. When red blood cell lipids were analyzed, significant inverse relationships were found between the phenotype and levels of EPA \( (r = -0.55; \ P = .02) \) and DHA \( (r = -0.48; \ P = .04) \), ie, as the levels of EPA and DHA increased, phenotypic severity decreased (Figure 4 and Table 4). When adipose tissue lipids were analyzed, data were available for only 14 of the 18 subjects, causing a substantial imbalance between the 3 phenotypic groups. Despite this imbalance, a significant inverse relationship was found between the phenotype and the level of EPA \( (r = -0.54; \ P = .04) \), although not between the phenotype and the level of DHA \( (r = -0.33; \ P > .05) \). In all of the cases, the most severe phenotype (grade 3) had the lowest average EPA and DHA levels, although these levels would be considered to be within the normal range as defined by the family controls. There was also a marginally significant positive relationship between the phenotype and the ratio of arachidonic acid to DHA in red blood cells \( (r = 0.46; \ P = .05) \), ie, as the ratio of arachidonic acid to DHA increased, phenotypic severity increased. There were no significant correlations between the phenotype and total saturated fatty acids, total monounsaturated fatty acids, total transunsaturated fatty acids, total ω-6 fatty acids, total ω-3 fatty acids, linoleic acid, arachidonic acid, ALA, or the ratio of total ω-6 to ω-3 fatty acids \( (all \ P > .05) \) as measured by red blood cell samples or adipose tissue samples (Table 4).

### FFQ and Lifestyle Questionnaire Analyses

There were no significant correlations between the phenotype of the 18 family members with ELOVL4 mutations and the intake of individual fatty acids, total calories, total animal fat, total vegetable fat, total fat, total polyunsaturated fat, cholesterol, lutein and zeaxanthin combined, zinc, vitamin C, vitamin E, servings of tuna per month, or servings of dark-meat fish per month \( (all \ P > .05) \) as measured by the FFQ. None of the subjects reported receiving any type of essential fatty acid or fish oil supplement. As discussed in the introduction, the lack of significance of FFQ data is not surprising because FFQ analysis is generally better suited for the study of much larger populations.

The ages of the 18 subjects with ELOVL4 mutations ranged from 21 to 71 years, with the average age being 38 years, which was well older than the median age at onset of 13 years in this family. Ten \( (55\%) \) of the subjects were female; 8 \( (45\%) \) of the subjects were male. The majority of the subjects \( (11 \text{ subjects} [61\%]) \) had a desirable body mass index of less than 25, with 7 \( (39\%) \) considered overweight according to the Quetelet index for body mass. \( ^{20} \) There were no significant relationships found between the phenotype and the demographic variables of age, age at onset, sex, or body mass index \( (all \ P > .05) \) (Table 2).

All of the subjects had light-colored eyes. Only 1 subject reported being a current cigarette smoker, and 2 reported smoking in the past. Only 1 family member consumed any alcohol \( (2-4 \text{ glasses of beer per week}) \). No statistical tests were conducted using these variables owing to the severe imbalances between categories.

### Table 3. Red Blood Cell Membrane and Adipose Tissue Lipids in Utah Kindred K4175

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Subjects, No.</th>
<th>EPA, %, Mean ± SD</th>
<th>DHA, %, Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell membrane lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELOVL4 deletion present</td>
<td>18</td>
<td>0.40 ± 0.09*</td>
<td>2.8 ± 0.5*</td>
</tr>
<tr>
<td>Family controls</td>
<td>26</td>
<td>0.27 ± 0.12</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Laboratory controls†</td>
<td>19</td>
<td>0.61 ± 0.26</td>
<td>4.2 ± 1.1</td>
</tr>
<tr>
<td>Adipose tissue lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELOVL4 deletion present</td>
<td>14</td>
<td>0.047 ± 0.019</td>
<td>0.095 ± 0.031</td>
</tr>
<tr>
<td>Family controls</td>
<td>21</td>
<td>0.036 ± 0.017</td>
<td>0.087 ± 0.049</td>
</tr>
</tbody>
</table>

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

*Levels of EPA and DHA are significantly higher in the red blood cell membranes of individuals with an ELOVL4 deletion relative to the family controls \( (P<.01) \) and significantly lower than in the laboratory controls \( (P<.01) \).

†Laboratory control values for red blood cell membrane lipids were provided by the Kennedy Krieger Institute, Baltimore, Md. No further demographic information on this population is available. No laboratory control values are available for adipose tissue lipids.
sue and red blood cell membrane lipids. Interestingly, this result appears to be driven by unusually high DHA and EPA levels in the family members with mild and moderate phenotypes (grades 1 and 2) relative to family controls, with a significant inverse relationship between phenotype and DHA percentage in subjects with an ELOVL4 mutation \((r = -0.54; P = .04)\) (B), mean ± SD percentage of EPA in adipose tissue, with a significant inverse relationship between phenotype and EPA percentage in subjects with an ELOVL4 mutation \((r = -0.55; P = .02)\) (A), mean ± SD percentage of docosahexaenoic acid (DHA) in red blood cell membranes, with a significant inverse relationship between phenotype and DHA percentage in subjects with an ELOVL4 mutation \((r = -0.48; P = .04)\) (C), and mean ± SD percentage of DHA in adipose tissue, with no significant relationship between phenotype and DHA percentage in subjects with an ELOVL4 mutation \((r = -0.33; P > .05)\) (D). See Table 1 for definition of phenotypic grades.

![Graphs A, B, C, D](https://archopht.jamanetwork.com/)
tion of ELOVL4 in retinal long-chain fatty acid biosynthesis is still unknown, but our results suggest that it is likely to be involved prior to EPA in the metabolic pathway shown in Figure 2.

This study indicates that phenotypic expression of a genetically defined macular dystrophy can be influenced by a dietary factor. Although this family was relatively large, this study would benefit from extension to even more individuals with ELOVL4 mutations, namely the very large, extended family with a 5-bp deletion in the ELOVL4 gene, to show that DHA and EPA are protective against STGD3 in an even more convincing manner. Prospective studies using DHA and/or EPA supplements in patients with an ELOVL4 mutation should also be considered a biochemically rational intervention against this disorder.

Submitted for Publication: October 1, 2004; final revision received December 29, 2004; accepted January 3, 2005.

Correspondence: Paul S. Bernstein, MD, PhD, Moran Eye Center, University of Utah, 50 N Medical Dr, Salt Lake City, UT 84132 (paul.bernstein@hsc.utah.edu).

Financial Disclosure: None.

Funding/Sponsorship: This study was supported by a center grant from the Foundation Fighting Blindness, Owings Mills, Md, and by a departmental grant from Research to Prevent Blindness, Inc, New York, NY. Dr Bernstein is a Sybil B. Harrington Research to Prevent Blindness scholar in macular degeneration research.

Previous Presentation: Presented in part at the annual meeting of the Macula Society; February 26, 2004; Las Vegas, Nev; and the annual meeting of the Association for Research in Vision and Ophthalmology; April 26, 2004; Fort Lauderdale, Fla.

Acknowledgment: We greatly appreciate the assistance of Kang Zhang, MD, PhD, Michael Teske, MD, and Albert Vitale, MD (fundus photograph grading) and of Clint Thompson, PhD (statistical analyses). Barry Fallsen, OD, facilitated the eye examinations of the Northern California branch of the family.

REFERENCES