

Alterations of the 5' Untranslated Region of *SLC16A12* Lead to Age-Related Cataract

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PURPOSE. Knowledge of genetic factors predisposing to age-related cataract is very limited. The aim of this study was to identify DNA sequences that either lead to or predispose for this disease.

METHODS. The candidate gene *SLC16A12*, which encodes a solute carrier of the monocarboxylate transporter family, was sequenced in 484 patients with cataract (134 with juvenile cataract, 350 with age-related cataract) and 190 control subjects. Expression studies included luciferase reporter assay and RT-PCR experiments.

RESULTS. One patient with age-related cataract showed a novel heterozygous mutation (c.-17A>G) in the 5' untranslated region (5'UTR). This mutation is in *cis* with the minor G-allele of the single nucleotide polymorphism (SNP) rs3740030 (c.-42T/G), also within the 5'UTR. Using a luciferase reporter assay system, a construct with the patient's haplotype caused a significant upregulation of luciferase activity. In comparison, the SNP G-allele alone promoted less activity, but that amount was still significantly higher than the amount of the common T-allele. Analysis of *SLC16A12* transcripts in surrogate tissue demonstrated striking allele-specific differences causing 5'UTR heterogeneity with respect to sequence and quantity. These differences in gene expression were mirrored in an allele-specific predisposition to age-related cataract, as determined in a Swiss population (odds ratio approximately 2.2; confidence intervals, 1.23–4.3).

CONCLUSIONS. The monocarboxylate transporter *SLC16A12* may contribute to age-related cataract. Sequences within the 5'UTR modulate translational efficiency with pathogenic consequences. (*Invest Ophthalmol Vis Sci.* 2010;51:3354–3361) DOI:10.1167/iovs.10-5193

Cataract is the clouding of the eye's lens that impairs normal vision. It is estimated that cataract accounts for 17 million cases of blindness worldwide, with approximately half of all cases occurring in Asia and Africa.^{1,2} Different criteria—age of onset, morphologic features, and mode of inheritance—can be used to classify the various forms of cataracts. Based on the age of onset, one distinguishes between childhood (congenital and juvenile) cataract and age-related cataract (ARC), but this criterion does not necessarily indicate etiology.³ Genetic predisposition plays a crucial role in childhood cataract.^{1,2} Congenital and juvenile forms of cataract show wide heterogeneity with respect to genetic and phenotypic aspects.⁴ A number of mutations in approximately 20 genes have been described as causing childhood cataract.^{3,5–7}

Approximately 80% of all cataracts are age-related and idiopathic. Depending on the location of the opacity within the lens, ARC is termed cortical, nuclear, or subcapsular. There are also forms of mixed cataract that feature more than one morphological sign. In general, maintenance of an intact, transparent lens requires balanced homeostasis of metabolic components.⁸ ARC is considered a multifactorial disease in which environmental components and genetic predisposition contribute to the development of the pathologic condition. Interactions between these factors are likely, and knowledge of the cause of ARC may provide crucial information for the prevention of and potential therapy for the disease. Among environmental risk factors are smoking, exposure to UV-B radiation, and alcohol.⁹ In addition, physiological conditions such as age, sex, diabetes, high body mass index, persistent intraocular inflammation, prolonged corticosteroid administration, and oxidative damage seem to promote the development of ARC.⁹ In light of this complexity, knowledge of genetic risk factors is still scarce. Variants of the detoxifying enzymes arylamine *N*-acetyltransferase-2 and glutathione-S-transferase (GST) were found to be associated with ARC.^{10–12} Furthermore, two sequence variants in the vicinity of the 3' end of the gene for Eph-receptor tyrosine kinase type A2 (*EPHA2*) were shown to associate with both childhood and age-related forms of cataract.¹³ Recently, a mutation in the gene encoding α A-crystallin was reported to be associated with ARC because of the loss of chaperone-like activity.¹⁴ SNP-based allele frequencies and, consequently, association with a disease phenotype vary often among different ethnic groups, exemplified by a sequence variant in the gene encoding galactokinase, an enzyme involved in galactose metabolism^{15,16} and GST.^{10,11,17} Similarly, heat-shock transcription factor 4 may be involved in ARC in an Asian population.¹⁸

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To the list of genes involved in childhood cataract¹⁹ we recently added *SLC16A12*, which encodes a monocarboxylate transporter.⁶ Although the substrate of this transporter is not yet known, its importance for the establishment or maintenance of homeostasis was suggested because a premature termination codon in *SLC16A12* leads to juvenile cataract and renal glucosuria.⁶ Based on this proposed function of the transporter, we speculated that insufficient activity could interfere with the maintenance of homeostatic conditions within the lens and could lead to ARC. Now we report the effects on ARC of two sequence alterations in the 5' untranslated region (5'UTR) of *SLC16A12*. The importance of 5'UTR sequences for translational regulation has been demonstrated in several other genes.^{20–23} Approximately 10% of all coding genes are regulated at the mRNA level and, hence, influence translational efficiency. Disturbance of the regulation of the translational machinery leads to perturbed cellular metabolism and may tilt the physiological balance from healthy to diseased states, as occurs in breast cancer, Alzheimer's disease, bipolar affective disorder, fragile X-syndrome, and others.^{20,22} Our work focuses on sequence alterations within the 5'UTR of *SLC16A12* and offers an explanation for the development of ARC.

MATERIALS AND METHODS

Patients

Patients with childhood cataract and ARC, including cortical, nuclear, posterior subcapsular, and mixed types of cataract, were seeking ophthalmologic examination in Switzerland. Subjects from among the general population in Switzerland served as controls. Information on patient's condition of hypertension, diabetes mellitus, smoking behavior, alcohol consumption, and exposure to UV-B radiation was not available. Patients gave written informed consent for participation in scientific research. All experiments involving human subjects were conducted according to the principles expressed in the Declaration of Helsinki.

DNA Analysis

DNA was prepared either by the precipitation method (Gentra Kit; Qiagen, Hilden, Germany) or by magnetic bead technology (Chemagen, Aachen, Germany). For PCR, approximately 50 ng template DNA and primers (Table 1) were cycled 35 times with annealing and extension temperatures of 60°C and 72°C, respectively, lasting 1 minute each. DNA sequencing was performed with commercially available technology (Applied Biosystems, Rotkreuz, Switzerland).

RNA Analysis

RNA from vascular smooth muscle cell (VSMC) cultures was isolated (All Prep DNA RNA Mini Kit; Qiagen, Hilden, Germany). RNA was

evaluated with a bioanalyzer (2100 Bioanalyzer; Agilent Technologies, Palo Alto, CA). Two-step RT-PCR required cDNA synthesis (Superscript III; Invitrogen, Basel, Switzerland). Standard RT-PCR conditions were applied (Hotfire *Taq* Polymerase; Solis Biodyne, Tartu, Estonia) for 1 minute at an annealing temperature of 58°C, 1 minute elongation time at 72°C, and 39 cycles. PCR products were analyzed by 1% agarose gel electrophoresis. Quantitative sequencing of the *SLC16A12* c.-42T>G variant was performed as described.²⁴ Briefly, to determine the correction factors, genomic DNA (gDNA) was amplified in duplicate, and each amplicon was sequenced in eight different reactions. For RNA, one-step RT-PCR (Qiagen) was performed in triplicate, and each amplicon was sequenced eight times. Potential splice sites were sought with the online tool ESEfinder 3.0 (http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese_finder.cgi?process=home).^{25,26}

In Silico Analysis of 5'UTR Variants on RNA Folding

Putative RNA folding structures were predicted using Mfold with standard settings (<http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1.cgi>).²⁷ RNA structures were predicted for *SLC16A12* (CL reverse NM_213606) and for its 5'UTR alone.

Plasmids, Vectors, and Cloning

Exon 3 of *SLC16A12* contains part of the 5'UTR and the initiation ATG followed by 36 encoded amino acids. A fragment of the 118-bp *SLC16A12* sequence (5'-GCCAGGTAGCGTTCTATGCCAACCTTGAA-TGCCATCAGGAAGTCACTGGACAGCAAACCTTCCAAGATCATAA-CCT[G/T]GGCTGTTGGAGCAACCTGGAAAAG[A/G]AGAAAAA-GAAAAACC-3') was cloned in front of the luciferase gene in pGL3 control vector (Promega, Madison, WI) with *Hind*III and *Nco*I restriction enzymes. For this purpose, exon 3 was PCR amplified using an upstream primer (Table 1) that contained a *Hind*III restriction site at its 5' end and a downstream primer (Table 1). Genomic DNA heterozygous for the SNP rs3740030 and genomic DNA from the cataract patient with c.-42G/T and c. -17A/G served as templates. PCR amplicons and vector pGL3 were digested with restriction enzymes *Hind*III and *Nco*I and were ligated with DNA ligase (Promega). Constructs were verified by DNA sequence analysis.

Mammalian Cell Culture Experiments

Cells (10⁴ HEK293T) were seeded on 96-well plates in 0.5 mL DMEM, 10% FBS, and 1% penicillin/streptomycin and were transfected 24 hours later. Plasmid DNA (615 ng [600 ng pFirefly construct, 15 ng pRenilla construct]) dissolved in 2 μL calcium chloride (2.5 M) and 10 μL HeBS (2×) was used for each transfection (20 μL transfection mix/well). Cells were harvested 48 hours after transfection, and Luciferase activities were measured with a Luciferase reporter assay system (Dual Glo; Promega Dual Glo) and a luminometer (Luminoskan Ascent; Thermo-Labsystems, Egelsbach, Germany).

TABLE 1. Primer Characteristics

Primer Name	Sequence (5'-3')	Purpose
Intronic primers 3F	gtctgccccagctctagtattca	Genomic DNA sequencing
Intronic primers 3R	cgaaatacacacacaccaca	Genomic DNA sequencing
CL forward	ATGCaagcttGCCAGGTAGCGTTCTATGCC	Cloning
CL reverse	CGGAAATACACACACACCACA	Cloning
SLC16A12RT_ex1_2f	cggggctcagATACAGGAT	RT-PCR
SLC16A12RT_ex4r	aaacagccagccacaatcat	RT-PCR
RTPRC_2F	GTGTGACCATGCTCTGTGct	RT-PCR
RTPRC_4R	AAGACAAAGCCCCAAGAAT	RT-PCR
RTPCR_3F	caggaagtcactggacagca	RT-PCR
RTPCR_5R	gcatgattcccacttgacag	RT-PCR
cSLC16A12_1F	CCCTCTTCCCTCTCCCTGA	RT-PCR
cSLC16A12_3R	TGTGCAGATGGTAACAAGGAAAC	RT-PCR
SLC16A12RTqex2f	TTAGCCTCCCAAAGTGATGG	RT-PCR
SLC16A12RTqex4r	CGTTTGTGCGTAATCCCTGAG	RT-PCR

Isolation and Cultivation of Vascular Smooth Muscle Cells

Vascular smooth muscle cells were isolated from radial arteries of patients undergoing coronary artery bypass grafting, as previously described.²⁸ Identification was made by immunofluorescent staining for smooth muscle α -actin (no. 1148818; Roche Diagnostics, Mannheim, Germany). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and were used up to passage 10.

Statistical Analysis

DNA Analysis. For statistical calculation of odds ratio and significance, an open access Internet portal was used (<http://faculty.vassar.edu/lowry/odds2x2.html>; June 2009). Calculations were based on a $P = 0.05$ level of significance.

RNA Analysis. For the arithmetic mean of replicates (gDNA, $n = 4$; cDNA, $n = 6$), upper and lower confidence limits were determined using critical values of paired t -test distribution.

Mammalian Cell Culture Experiments. Averages and confidence intervals (paired t -test) were calculated and normalized on T-allele activity. Tissue culture experiments were performed twice using three technical replicas per measurement.

RESULTS

Identification of Mutation in the 5'UTR of *SLC16A12* and Patient Characteristics

For mutation screening, we investigated genomic DNA of 350 patients with ARC and 134 patients with childhood cataract. DNA sequences from all exons and adjacent intron regions of *SLC16A12* were analyzed. A 79-year-old woman with ARC was found to carry a heterozygous sequence alteration in the 5'UTR (c.-17A>G; Fig. 1), but coding and approximately 50 nucleotides flanking intron sequences were unchanged. The sequence change was not found in any of the other 483 patients or in the 380 alleles of control subjects. This uniqueness makes the change unlikely to be a normal sequence variant; rather, it indicates pathogenicity. Hence, we will refer to it from now on as mutation. The patient was also heterozygous at an annotated SNP rs3740030 (c.-42T/G) that lies just upstream of the mutation. Cloning and sequencing of the patient's exon 3 showed that the mutation and the G-allele are on the same chromosome.

The patient was seen by an ophthalmologist for cataract. The lens of her left eye showed nuclear and subcapsular opac-

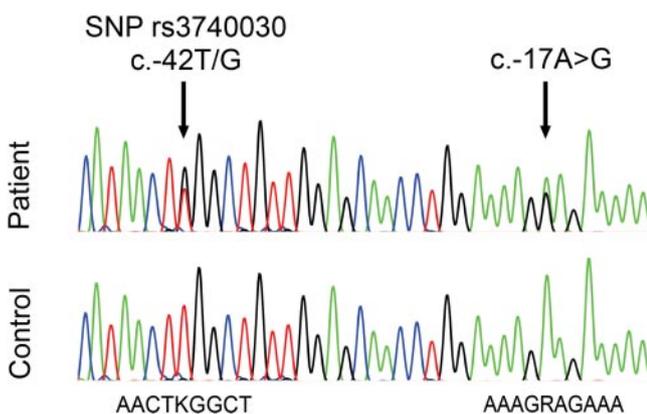


FIGURE 1. Electropherogram showing genomic DNA of 5'UTR section from control subject and ARC patient. Nucleotides of the immediate surroundings of SNP rs3740030 (c.-42) and the mutation (c.-17) are given. K = T and G; R = A and G.

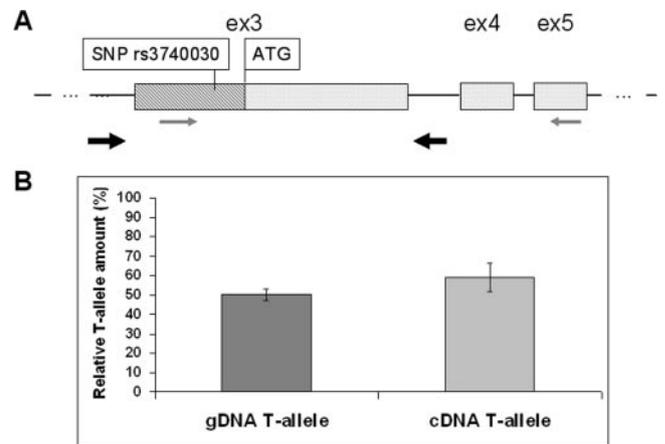


FIGURE 2. Quantitative sequencing analysis. (A) Drawing of exons (ex) 3, 4, and 5 of *SLC16A12*. Exon 3 contains the coding region beginning with ATG (light gray box) and 5'UTR (hatched box) containing SNP rs3740030. gDNA and transcript representing cDNA were obtained from vascular smooth muscle cells of a subject heterozygous for SNP rs3740030 (c.-42T/G). RT-PCR primers are indicated by thin arrows in exons 3 and exon 5. Locations of primers for genomic DNA amplification are indicated with thick arrows in intronic regions flanking exon 3. (B) Quantitative analysis expressed as relative amount of T-allele containing transcripts, given in percentages, for gDNA and mRNA (cDNA). Confidence intervals are shown for eight technical replicas.

ities plus pseudoexfoliation of the lens capsule that was removed when she was 75. At age 79, her right eye was also subjected to surgery. The right lens showed nuclear brunescence sclerosis with pulverulent anterior and posterior cortical opacities plus pseudoexfoliation of the lens capsule. No information about other family members was available.

Quantitative Assessment of *SLC16A12* Transcripts

Because the minor G-allele and the mutation reside within the 5'UTR of *SLC16A12*, it is likely that these changes affect translational rather than transcriptional efficiency. To assess the latter possibility, we determined the amount of *SLC16A12* mRNA by quantitative sequencing experiments, a method that requires RNA from heterozygous tissue. It is based on comparing allele frequencies of complementary DNA (cDNA) with those of gDNA from the same sample.²⁴ Given that no tissue was available from the cataract patient, we focused our attention on measuring SNP allele-specific transcripts in surrogate cells. Among available tissues known to express *SLC16A12*, vascular smooth muscle cells from heterozygous donors served for this purpose. RNA was subjected to one-step RT-PCR using primers in exons 3 and 5, to encompass the SNP (Fig. 2A). As expected, the contribution of the T-allele in genomic DNA was 50% ($\pm 3\%$). No significant difference was measured for the T-allele in the cDNA (57% $\pm 9\%$; Fig. 2B), indicating that the two SNP alleles were present at comparable amounts. We concluded that regulatory mechanisms of translation rather than differential transcription were affected by the patient's sequence alterations.

Influence of *SLC16A12* 5'UTR Sequences at c.-42 and c.-17 on Translational Efficiency

To investigate whether the SNP and the patient's c.-17A>G mutation had effects on translation, constructs containing 5'UTR sequences in front of the luciferase gene were tested in HEK cells, by measuring luciferase reporter activity (Fig. 3). This assay is well suited to simulate translational activity

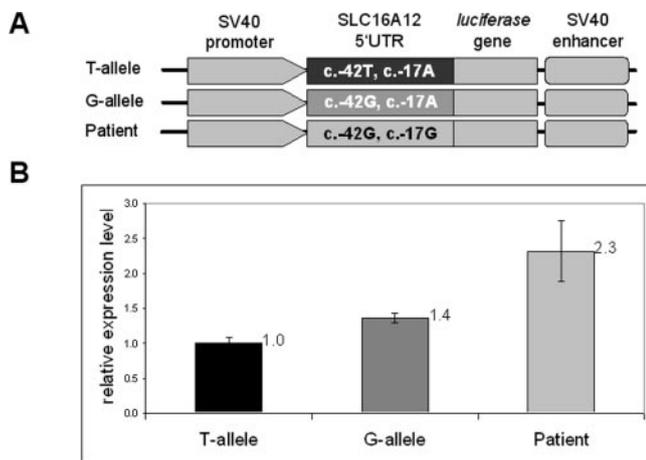


FIGURE 3. Luciferase reporter activity. **(A)** Schematic representation of cloned constructs. T-allele (c.-42T) and G-allele (c.-42G) contain 122 bp of *SLC16A12* 5'UTR. Patient construct contains c.-42G and c.-17G. **(B)** Relative luciferase activity. Values obtained for the T-allele were set to 1, to which other values were normalized. Displayed are the means (numerically) and confidence intervals (graphically) of three technical replicates. Results are from two independent experiments. The presence of the G-allele alone shows 1.4-fold and the presence of the G-allele in combination with the mutation (patient) shows 2.3-fold elevated luciferase expression levels.

changes because all constructs contain the same promoter, making it likely that transcriptional efficiency is the same among all constructs (Fig. 3A). Statistically significant differences in luciferase activity were observed among the three *SLC16A12* constructs (Fig. 3B). The construct that mimicked the patient's haplotype yielded the highest luciferase activity compared with both SNP alleles (2.315 ± 0.433 -fold higher). Furthermore, the SNP alleles yielded significantly different luciferase activities such that the minor G-allele led to higher levels than did the more common T-allele (1.362 ± 0.07 -fold higher). These results demonstrate a significant effect of 5'UTR

sequences and suggest an enhanced potential of *SLC16A12* translation.

Effects of 5'UTR on RNA Folding by In Silico Analysis

One possible mechanism to achieve such regulation is through RNA folding. We simulated the influence of the 5'UTR sequence variants on secondary structures using the method Mfold,²⁷ which predicts a structure based on free energy minimization. RNA structures of the entire *SLC16A12* mRNA, (5'UTR, coding sequences, and 3'UTR) were predicted (Figs. 4A-C). Differences between the T- and G-alleles of SNP rs3740030 affect some branches near the centers of the molecules (Figs. 4A, 4B). The patient's mutation in combination with the SNP G-allele caused a strikingly different RNA structure (Fig. 4C). In addition, 5'UTR sequences alone folded in an allele-specific manner (Figs. 4D-F). Taken together, our experimental data, in combination with the bioinformatic predictions, indicate an effect on nucleotide-specific RNA folding properties.

SNP Allele-Specific Sequence Heterogeneity of 5'UTR *SLC16A12* Transcripts

The 5'UTR contains sequences from exons 1 and 2 and part of exon 3. To assess potential splicing effects on the generation of the 5'UTR, we initially applied the online tool ESEfinder 3.0. Splice factor SC35 was predicted to bind to the region immediately adjacent to the SNP, independently of the allele-specific nucleotide (Fig. 5). Interestingly, splice factor SRp40 was predicted to bind exclusively to G-allele transcripts and not to the T-allele transcripts. No binding sites for any of the ESEfinder 3.0-specific splice factors were found at the c.-17.

These observations supported the existence of alternative and allele-specific splicing of *SLC16A12* transcripts generating 5'UTR heterogeneity.

To test these predictions, we performed RT-PCR experiments with various primer combinations on cDNA from vascular smooth muscle cells of donors who were either homozy-

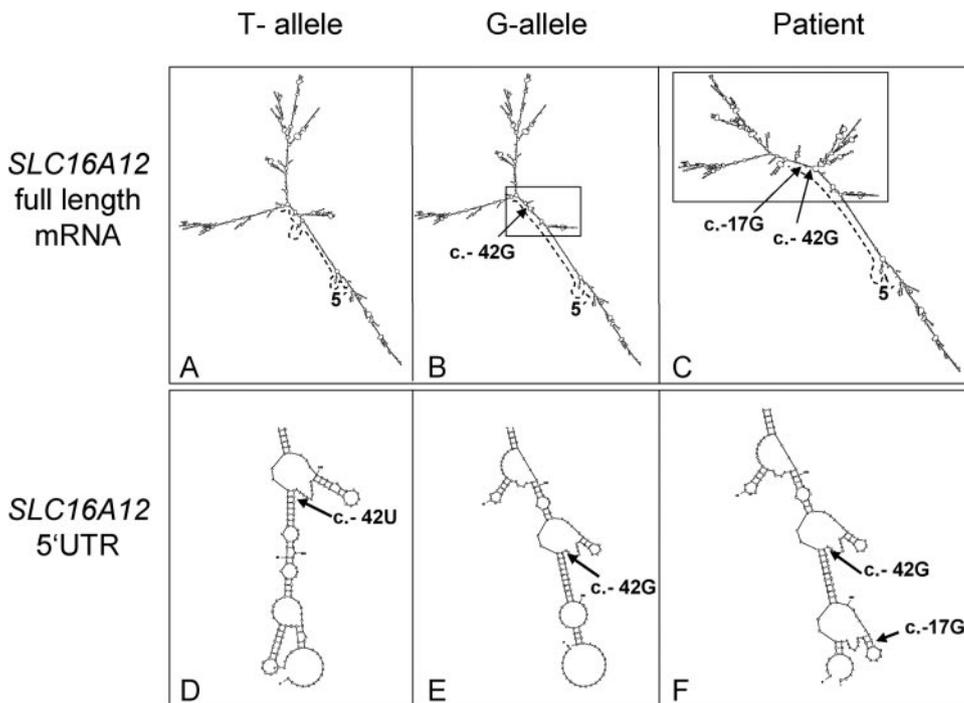


FIGURE 4. Predicted *SLC16A12* RNA foldings. T-allele and G-allele refer to the SNP rs3740030 (c.-42T/G). Patient has sequences c.-42G and c.-17G. **(A-C)** RNA foldings for the entire *SLC16A12* transcript. Differences in mRNA structure are highlighted (box). The 5'-end is marked (5'). Dotted line: 5'UTR. Arrows: positions c.-42G, c.-42U, and c.-17G. **(D-F)** Predicted RNA foldings for 5'UTR sequences only.

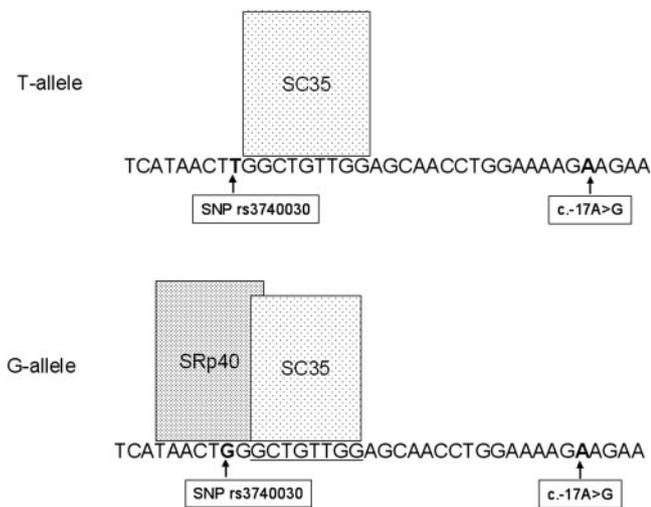


FIGURE 5. Prediction of splice factor binding site for *SLC16A12* 5'UTR. ESEfinder predictions were applied to cDNA from T- and G-alleles of SNP rs3740030. Factor SC35 binds independently of the allele sequence. In contrast, the binding of splice factor SRp40 is predicted only for the G-allele sequence. At the c.-17 position, no splice factor binding sites are predicted for G or A.

gous T/T or heterozygous G/T with respect to the SNP rs3740030 (Fig. 6). Primer combination III (exons 3–5) yielded amplicons of highly similar intensity (Fig. 6), confirming our quantitative transcript data that showed no allele-specific effect (Fig. 2). SNP nucleotide identity, T or G/T, was confirmed by sequencing. Amplification with primer combinations I yielded fragments displaying heterogeneities in size, sequence, and intensity. Surprisingly, in heterozygous samples, these were greatly reduced, and only two samples showed weakly staining single fragments (Fig. 6B). DNA sequence analysis of sample 5 (primer combination II) revealed the expected presence of exons 2, 3, and 4 but unexpectedly only the T nucleotide of the SNP. Sample 8 (primer combination I) contained a short fragment intron 1 sequence in addition to exons 1, 2, and 3. At the SNP position, only the G-nucleotide was found. The weak staining of this fragment suggested reduced levels of this transcript, which may be the reason for chance amplification. Sequence heterogeneity in the homozygous samples resulted from some fragments containing exons 1, 2, and 3, some had an additional short segment of intron 1, and yet others lacked exon 2. Primer combination II amplified fragments containing sequences from exons 2, 3, and 4 (DNA sequence data not shown). These striking differences between the homozygous and heterozygous samples within the 5'UTR region indicated the possibility of an allele-specific splicing mechanism that led to severe reductions in transcripts containing exons 1 and 2 in heterozygous tissues.

Affect of Allele Specificity on the Risk for Cataract

Assessment of the genotype for SNP rs3740030 in 484 cataract patients (350 with ARC, 134 with childhood cataract), most of whom were of Caucasian background, and in 190 ethnically matched control subjects confirmed an overrepresentation of the T-allele (Table 2), as had been reported for Caucasian populations of European descent (HapMap: T-allele frequency of 91.6%). Furthermore, we noticed overrepresentation of the G-allele in patient groups compared with control subjects (juvenile cataract, 6.72%; ARC, 8.71%, control subjects, 4.21%) that was statistically significant only for the ARC patient group ($P = 0.00601768$; $\chi^2 = 7.545$). To

assess the extent to which the G-allele could increase the risk for cataract, odds ratios were calculated. Persons with either the G-allele only or the G/T or G/G genotype are at an approximately 2.2-fold increased risk for ARC (Table 3). Conversely, the T-allele or the T/T genotype confers protection against ARC. Given that the control population originated from the general Swiss population, we cannot exclude the possibility that an individual of this control population may develop ARC. In such case, we would predict that the odds ratio shifts even further toward a risk association with the G-allele. Odds ratios for juvenile cataract were statistically not significant (Table 3).

DISCUSSION

The complex disease ARC is influenced by a multitude of environmental and genetic factors. Knowledge of the identity of the genetic contributions is limited and focuses on the identification of rare variants or the association of sequence variants in affected families and patient populations^{11–14,16,18} and on whole genome scans.²⁹ Previously, we showed that a mutation in the monocarboxylate transporter *SLC16A12* causes juvenile cataract, likely through a disturbance of solute homeostasis.⁶ Given that maintenance of homeostasis within the aging lens is likely to be essential,⁸ this transporter is a prime candidate for ARC. The data presented here support this hypothesis. First, a point mutation and an SNP within the 5'UTR was found in a 79-year-old woman with ARC. Second, in vitro and ex vivo experiments provide evidence that these sequences affect expression of *SLC16A12* by modulating translational efficiency. Third, the SNP within the 5'UTR is likely associated with ARC within a Swiss population.

The c.-17A>G alteration in the patient with ARC was unique among our patient cohort ($n = 484$) and was also absent in our control population ($n = 180$), suggesting that it is a mutation rather than an SNP. Supportively, no sequence variation at position c.-17 of *SLC16A12* has been reported in

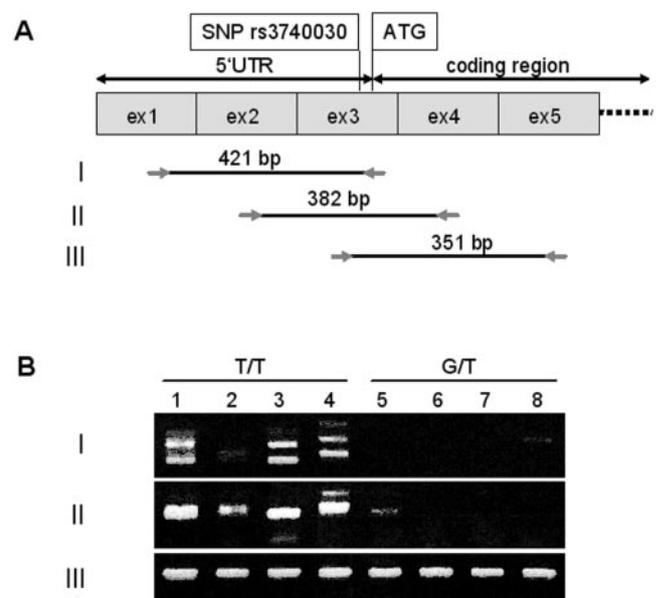


FIGURE 6. *SLC16A12* transcript analysis by RT-PCR. (A) Schematic representation of exons 1 through 5 (size not to scale). Position of SNP rs3740030 and the ATG initiation codon as well as the 5'UTR and coding regions are indicated above the exons. Primer combinations (I, II, III) and their respective sizes (bp) are shown. (B) Electrophoretically separated RT-PCR products from homozygous T/T (1–4) and heterozygous G/T (5–8) donors of vascular smooth muscle cells.

TABLE 2. Genotype and Allele Frequencies of SNP rs3740030

	Genotypes				Alleles				
	TT	TG	GG	Total	T	G	Total	T (%)	G (%)
Control	175	14	1	190	364	16	380	95.79	4.21
Juvenile	116	18	0	134	250	18	268	93.28	6.72
Age-related	291	57	2	350	639	61	700	91.29	8.71

Number of subjects identified with respective genotypes and alleles. Allele frequencies are given in percentages.

available DNA sequence databases to date. The second alteration found *in cis* in the age-related patient is the only annotated exonic SNP rs3740030 (c.-42T/G). Its allele frequency is population dependent. In contrast to a Caucasian population of European descent in which the minor allele is relatively seldom (8.4%), its frequency is higher in a Han Chinese population (27.4%) (HapMap, January 2010). It would be intriguing to investigate whether this is related to the incidence rate of cataract in the Chinese population.³⁰ Based on our data in a Swiss population, the minor G-allele was more frequent in ARC patients than in the control group. This increased frequency translates into an approximately 2.2-fold increased risk and renders SNP rs3740030 a potential modifier in the development of ARC. Numerous examples from genome-wide association studies demonstrate a population-specific effect. ARC is no exception. For example, variants of GST have been investigated in various ethnically different cataract patient populations with varying results. Although no association was found in an Italian population,¹⁷ the opposite was true for an Estonian¹¹ and a Turkish¹⁰ population. The SNP in *SLC16A12* studied here may underlie similar conditions because the frequency in the Swiss control population was lower than that reported by HapMap. In addition, in preliminary studies, we were unable to verify the association in a British control population.

Based on the location of the opacity within the lens, one distinguishes between nuclear (the most frequent form), cortical, posterior subcapsular, and mixed-type cataract.³¹ Some risk factors have been reported as associated specifically with one of the subtypes of ARC,^{10,11,13} whereas association of the N-acetyltransferase 2 isoform "slow" acetylator¹² and of kinesin light-chain *KLC1* sequence variants³² have not been. We did not differentiate between subtypes; therefore, the association of SNP rs3740030 in *SLC16A12* could account for all ARC in our study, but we cannot exclude that the increased risk would also be subtype specific.

The underlying molecular basis for ARC can be explained by altered expression levels of *SLC16A12*. Luciferase activity increases from the common T-allele to the minor G-allele to the patient haplotype (G-allele and mutation), thus offering an explanation for predisposition to and manifestation of the disease. Most likely the pathogenic mechanism acts posttran-

scriptionally because equal amounts of the SNP allele-specific *SLC16A12* transcripts were found and 5'UTR regions are known to harbor control elements that regulate translation. This regulation may occur by way of RNA folding, upstream open-reading frames (uORFs), differential splicing, or specific sequence elements. We found no evidence for the latter. In contrast, the RNA-folding models demonstrated visible allele-specific differences, with a most drastic change for the patient allele. These structural changes correlate with changes in luciferase reporter activity. In all likelihood, no single mechanism is responsible for the observed effects. In addition, or complementary, to RNA folding, uORFs may be involved. Alterations in uORFs of other genes (*TPO*, *HTR3A*, *BRAC1*, *TGF β 3*) were reported to cause various diseases by enhancing gene expression through elevation of the level of translation.^{22,33} The *SLC16A12* 5'UTR contains four uORFs. One of them, which is not in frame with *SLC16A12*, harbors both sequence alterations discussed here. Given that this uORF expands into the coding frame, it may have an inhibitory effect on guiding ribosomes beyond the initiation of the ATG codon.³⁴⁻⁴³ The additional feature that the SNP G-allele changes the coding frame of that uORF slightly (p.L21W) could also influence translational efficiency.

Differential splicing of the 5'UTR may also explain the allele-specific effects. Examples have been reported for the TFII-I transcription factor⁴⁴ and for the estrogen receptor β gene.⁴⁵ For the SNP rs3740030, the action of splice factor SRp40, which is predicted to bind exclusively to transcripts generated from the minor G-allele, may cause the observed allele-specific 5'UTR sequence heterogeneity. Furthermore, the skipping of exon 2 causes shortening of the 5'UTR and elimination of another uORF, thereby increasing the likelihood of influencing the translational regulation. Taken together, at least three different mechanisms may apply, singly or in concert, to explain the altered translational efficiency associated with the SNP and the mutation within the 5'UTR.

Knowledge of the exact function of the monocarboxylate transporter *SLC16A12* requires identification of its substrates, which is currently under investigation. Nevertheless, the *SLC16A12* nonsense mutation leads to juvenile cataract⁶ and nucleotide changes in the 5'UTR associate with ARC, demonstrating the importance of this transporter. The adult lens

TABLE 3. Association of SNP rs3740030 with Cataract

	Allele					Genotype				
	Odds Ratio	CI	P	χ^2	Odds Ratio	CI	P	χ^2		
Juvenile	1.638	0.8196	3.2736	0.158341	1.99	1.8103	0.8774	3.7352	0.104862	2.63
Age-related	2.1718	1.2339	3.8225	0.006001	7.55	2.3654	1.3021	4.297	0.003836	8.36

Odds ratio, confidence interval (CI), *P*, and χ^2 are shown. Statistical significance was reached if odds ratio and CI were >1, *P* < 0.05, and χ^2 > 3.84. Analyzed were 190 control subjects, 134 patients with juvenile cataract, and 350 patients with age-related cataract.

structure demands a well-functioning transport system for metabolites to supply nutrients and to remove waste products,⁸ and the solute carrier SLC16A12 may have an important function in that activity. Complete removal of the transporter leads to congenital cataract and renal glucosuria.⁶ In contrast, alterations in the amount of the transporter, as described here, may lead to imbalanced solute homeostasis, which over time leads to ARC. Given *SLC16A12* expression in tissues other than the lens, it seems feasible that other organs could experience such consequences. Renal glucosuria is a prime candidate of such a condition but other, as yet not described, physiological conditions may become manifest.

We have provided initial examples that *SLC16A12* 5'UTR alterations are involved in the manifestation of and the predisposition to ARC by modifying translational efficiency. Physiological consequences are likely to challenge tissue homeostasis and to transform a healthy lens into a diseased lens.

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