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Stephen P Daiger
Human Genetics Center, School of Public Health and School of Medicine, The University of Texas Health Science Center, Houston TX

Lori S Sullivan
Human Genetics Center, School of Public Health, The University of Texas Health Science Center, Houston TX

Sara J Bowne
Human Genetics Center, School of Public Health, The University of Texas Health Science Center, Houston TX

Daniel C Koboldt
The Genome Institute, Washington University School of Medicine, St. Louis, MO

Susan H Blanton
John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL

See next page for additional authors

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Recommended Citation

Citation Information: Daiger, Stephen P; Sullivan, Lori S; Bowne, Sara J; Koboldt, Daniel C; Blanton, Susan H; Wheaton, Dianna K; Avery, Cheryl E; Cadena, Elizabeth D; Koenekoop, Robert K; Fulton, Robert S; Wilson, Richard K; Weinstock, George M; Lewis, Richard A; and Birch, David G, "Identification of a Novel Gene on 10q22.1 Causing Autosomal Dominant Retinitis Pigmentosa (adRP)." (2016). Advances in Experimental Medicine and Biology, vol. 854
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Authors

Stephen P Daiger, Lori S Sullivan, Sara J Bowne, Daniel C Koboldt, Susan H Blanton, Dianna K Wheaton, Cheryl E Avery, Elizabeth D Cadena, Robert K Koenekoop, Robert S Fulton, Richard K Wilson, George M Weinstock, Richard A Lewis, and David G Birch

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Adv Exp Med Biol. Author manuscript; available in PMC 2016 June 14.

Published in final edited form as:

Adv Exp Med Biol. 2016 ; 854: 193–200. doi:10.1007/978-3-319-17121-0_26.

Identification of a Novel Gene on 10q22.1 Causing Autosomal Dominant Retinitis Pigmentosa (adRP)

Stephen P. Daiger,
Human Genetics Center, School of Public Health, The University of Texas HSC, 1200 Pressler St., Houston, TX 77030, USA, Ruiz Department of Ophthalmology and Visual Science, Medical School, The University of Texas Health Science Center, Houston, TX 77030, USA

Lori S. Sullivan,
Human Genetics Center, School of Public Health, The University of Texas HSC, 1200 Pressler St., Houston, TX 77030, USA

Sara J. Bowne,
Human Genetics Center, School of Public Health, The University of Texas HSC, 1200 Pressler St., Houston, TX 77030, USA

Daniel C. Koboldt,
The Genome Institute, Washington University School of Medicine, St. Louis, MO 63108, USA

Susan H. Blanton,
John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL 33136, USA, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL 33136, USA

Dianna K. Wheaton,
The Retina Foundation of the Southwest, Dallas, TX 75231, USA

Cheryl E. Avery,
Human Genetics Center, School of Public Health, The University of Texas HSC, 1200 Pressler St., Houston, TX 77030, USA

Elizabeth D. Cadena,
Human Genetics Center, School of Public Health, The University of Texas HSC, 1200 Pressler St., Houston, TX 77030, USA

Robert K. Koenekoop,
McGill Ocular Genetics Laboratory, Departments of Paediatric Surgery, Human Genetics and Ophthalmology, Montreal Children's Hospital, McGill University Health Center, Montreal, QC H3H 1P3, Canada

Robert S. Fulton,
The Genome Institute, Washington University School of Medicine, St. Louis, MO 63108, USA

Richard K. Wilson,
The Genome Institute, Washington University School of Medicine, St. Louis, MO 63108, USA

Correspondence to: Stephen P. Daiger, Stephen.P.Daiger@uth.tmc.edu.

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George M. Weinstock,
Microbial Genomics, The Jackson Laboratory for Genomic Medicine, Farmington, CT 06032, USA

Richard A. Lewis, and
Departments of Ophthalmology, Medicine, Pediatrics and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA

David G. Birch
The Retina Foundation of the Southwest, Dallas, TX 75231, USA

Stephen P. Daiger: Stephen.P.Daiger@uth.tmc.edu; Lori S. Sullivan: lori.s.sullivan@uth.tmc.edu; Sara J. Bowne: sara.j.bowne@uth.tmc.edu; Daniel C. Koboldt: dkoboldt@genome.wustl.edu; Susan H. Blanton: sblanton@med.miami.edu; Dianna K. Wheaton: dwheaton@retinafoundation.org; Cheryl E. Avery: cheryl.e.avery@uth.tmc.edu; Elizabeth D. Cadena: elizabeth.d.cadena@uth.tmc.edu; Robert K. Koenekoop: robkoenekoop@hotmail.com; Robert S. Fulton: rfulton22@wustl.edu; Richard K. Wilson: rwilson@wustl.edu; George M. Weinstock: george.weinstock@jax.org; Richard A. Lewis: rlewis@bcm.tmc.edu; David G. Birch: dbirch@retinafoundation.org

Abstract

Whole-genome linkage mapping identified a region on chromosome 10q21.3–q22.1 with a maximum LOD score of 3.0 at 0 % recombination in a six-generation family with autosomal dominant retinitis pigmentosa (adRP). All known adRP genes and X-linked RP genes were excluded in the family by a combination of methods. Whole-exome next-generation sequencing revealed a missense mutation in hexokinase 1, HK1 c.2539G > A, p.Glu847Lys, tracking with disease in all affected family members. One severely-affected male is homozygous for this region by linkage analysis and has two copies of the mutation. No other potential mutations were detected in the linkage region nor were any candidates identified elsewhere in the genome. Subsequent testing detected the same mutation in four additional, unrelated adRP families, for a total of five mutations in 404 probands tested (1.2 %). Of the five families, three are from the Acadian population in Louisiana, one is French Canadian and one is Sicilian. Haplotype analysis of the affected chromosome in each family and the homozygous individual revealed a rare, shared haplotype of 450 kb, suggesting an ancient founder mutation. HK1 is a widely-expressed gene, with multiple, abundant retinal transcripts, coding for hexokinase 1. Hexokinase catalyzes phosphorylation of glucose to glusose-6-phospate, the first step in glycolysis. The Glu847Lys mutation is in a highly-conserved site, outside of the active site or known functional sites.

Keywords

Hexokinase; Founder effect; Retinitis pigmentosa; Autosomal dominant retinitis pigmentosa; Next-generation sequencing; Linkage mapping

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26.1 Introduction

Retinitis pigmentosa (RP) has a prevalence of approximately 1 in 4000 and affects more than 1.5 million individuals world-wide (Haim 2002; Daiger et al. 2007). RP is extremely heterogeneous: mutations in more than 60 genes cause syndromic and non-syndromic forms of RP, more than 3100 mutations have been described in these genes, and disease symptoms and progression are highly variable (Daiger et al. 2007; Berger et al. 2010; Wright et al. 2010; RetNet 2014). Our research focuses on finding genes and mutations causing

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autosomal dominant RP (adRP). To date mutations in more than 20 genes are known to cause adRP and these genes and mutations are themselves highly heterogeneous (Daiger et al. 2014a).

In research over the past 25 years we have assembled a cohort of adRP families and applied a wide range of methods to detect the disease-causing mutation in each family, most recently using several next-generation sequencing (NGS) approaches (Sohocki et al. 2001; Sullivan et al. 2006; Daiger et al. 2014a; Daiger et al. 2014b). In one large, six-generation Louisiana family, UTAD003, linkage mapping identified a novel adRP locus on chromosome 10q22. Here we report identification of the disease-causing gene and mutation in this family and evidence of a founder-effect in the gene, hexokinase 1 (HK1), accounting for approximately 1 % of adRP in Americans of European origin and Europeans (Sullivan et al. 2014).

26.2 Materials and Methods

26.2.1 Family Ascertainment and Clinical Characterization

Families in the Houston AdRP Cohort are ascertained and examined by clinical collaborators in Houston, at the Retina Foundation of the Southwest, and in other retinal genetics centers. Clinical examinations include best-corrected visual acuity, visual fields, dark adaptometry, dark-adapted full-field electroretinograms, spectral-domain optical coherence tomography, anterior and indirect ophthalmoscopy, and retinal imaging (Churchill et al. 2013; Sullivan et al. 2014). Genetic testing is conducted in the Laboratory for Molecular Diagnosis of Inherited Eye Diseases, a CLIA-Certified research facility in the Human Genetics Center, School of Public Health, at the University of Texas Health Science Center, Houston. Families in the Cohort have an initial diagnosis of adRP and three or more affected generations with affected females, or two or more generations with male-to-male transmission. Currently, there are 270 families in the Cohort (Daiger et al. 2014a).

The research adhered to the tenets of the Declaration of Helsinki and the study was approved by the Committee for the Protection of Human Subjects at UTHealth, Houston, and by human subjects review boards at participating institutions.

26.2.2 Next-Generation Sequencing (NGS)

Whole-exome NGS of 4 affected and 4 unaffected members of UTAD003 was done at The Genome Institute, Washington Univ., St. Louis (Bowne et al. 2011). Exome capture was done using a customized Agilent SureSelect All Exome Kit v.2.0 or the Nimblegen SeqCap EZ Human Exome Library v.2.0. Illumina paired-end sequencing, alignment, and variant calling were performed using the VarScan and Mendle- Scan software packages developed for this project (Koboldt et al. 2014). Variants were ranked based on segregation, rareness in human populations, predicted functional impact, and expression level in human retinal tissue.

26.2.3 Linkage Mapping and Haplotype Analysis

DNA samples from nine affected and six unaffected, at-risk, members of the UTAD003 family, and an additional parent, were genotyped at the UCLA Sequencing and Genotyping

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Center with an ABI High Density 5 cM STR marker set. Data from the 811 STR markers were analyzed with the LINKAGE package. For haplotyping, STR markers were selected from the ABI linkage mapping set and haplotypes were determined by inspection and confirmed by segregation analysis (Sullivan et al. 2014).

26.3 Results

26.3.1 Linkage Mapping in UTAD003

UTAD003 is a large Louisiana adRP family with over six known, affected generations (Fig. 26.1). It is one of the 270 AdRP Cohort families in our studies. Probands of families in the cohort have been tested for mutations causing adRP by Sanger sequencing and retinal-capture NGS and, in the absence of male-to-male transmission, for mutations in RPGR and RP2 (Sullivan et al. 2006; Churchill et al. 2013; Wang et al. 2013). No disease-causing mutations were detected in UTAD003 by these methods.

Samples from 19 family members were tested for linkage. Multipoint linkage analysis with affected family members produced a single chromosomal region with a LOD score of 3.0, on chromosome 10q21.3–10q22.1. This region spans approximately 9 Mb and includes 96 putative genes. Subsequently, intragenic and flanking STR markers from the ABI linkage set were tested to refine the linkage region (Sullivan et al. 2014).

Whole-exome NGS revealed a missense mutation in the HK1 gene, c.2539G >A, p.Glu847Lys, tracking with disease in all available, affected members of UTAD003, with two homozygous copies in one severely-affected family member. No other potentially-pathogenic mutations were identified in the linkage region or elsewhere in the genome.

26.3.2 Linkage Mapping in Additional Families

The entire HK1 gene was sequenced in 346 additional, unrelated probands with a diagnosis of adRP (Sullivan et al. 2014). The HK1 Glu847Lys mutation was found in all affected members of two additional families from the AdRP Cohort, UTAD936 and UTAD952, both from Louisiana (Fig. 26.1). No other potential disease-causing mutations were observed in HK1. The exon containing the HK1 mutation was then sequenced in 64 more adRP families, from Canada and Europe, provided by the McGill Ocular Genetics Laboratory, McGill Univ. Health Center, Montreal. The Glu847Lys mutation was observed in all affected members of two of these families, MOGL1 and MOGL2, from Canada and Sicily, respectively (Fig. 26.1). The smallest shared linkage region, including one informative, unaffected, at-risk member of UTAD952, is 55 kb (Fig. 26.2).

26.3.3 Disease Chromosome Haplotypes

Haplotypes defined by SNP markers flanking the HK1 mutation were tested in the five families, including the homozygous member of UTAD003, to determine the degree of sharing identical-by-descent between families (Fig. 26.2—excluding the unaffected individual in UTAD952). Since UTAD003, UTAD936 and UTAD952 derive from Louisiana we expected a common ancestor. In confirmation, the shared region in these families is approximately 500 kb centered on the HK1 mutation. (The homozygous male has distinct

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but overlapping haplotypes.) The Canadian and Sicilian families also share this haplotype with a total overlap of 450 kb. This is consistent with the mutation arising from a common ancestor living 100s of years ago (Sullivan et al. 2014).

26.3.4 Functional Evaluation

At least five alternate transcripts of HK1 are expressed in humans, encoding multiple alternate protein isoforms. Two isoforms predominate in the human retina; both contain the Glu847Lys mutation. Analysis of pathogenicity, e.g., PolyPhen 2, was inconclusive because of the multiple transcripts and several close-related hexokinase genes in vertebrate species. Hexokinase 1 catalyzes the first step in phosphorylation of glucose to glucose-6-phosphate and may play a role in mitochondrial activity. However, the Glu847Lys mutation, though in a highly-conserved site, lies outside of known active sites in the protein, so the pathogenic mechanism of the mutation is not established at present (Sullivan et al. 2014).

26.3.5 Clinical Findings

Affected members of the families display a highly-variable RP phenotype including pericentral RP, an arcuate band of pigmentary degeneration, and/or central areolar choroidal dystrophy. Symptoms by mid-life are mild to moderate. The homozygous male showed symptoms of RP at age 4 and when examined at age 33 had count-finger acuity, severe retinal vascular attenuation, extensive bone spicule accumulation, and macular atrophy in both eyes (Sullivan et al. 2014).

26.4 Discussion and Conclusion

The Glu847Lys missense mutation in the HK1 gene on 10q22.1 causes retinal dystrophy in five independently-ascertained families with adRP, including a homozygous patient. The five families share a 450 kb haplotype suggesting the variant arose as an ancient founder mutation. The mutation has a frequency of 1 % in American, Canadian and European adRP families. The HK1 transcript is abundant in mammalian retina, with at least five alternate transcripts. All of the transcripts are predicted to contain the mutation, at a highly conserved site. The hexokinase gene family (HK1–HK4) encodes proteins involved in the phosphorylation of glucose, an essential step in glycolysis. The glycolytic pathway plays a central role in photo-receptor and retinal cell metabolism. In addition, the hexokinase 1 protein is known to interact with mitochondrial membranes, as a modulator of apoptosis. The HK1 mutation may cause retinal disease as a result of perturbations in glycolysis and/or mitochondrial activity. Rare recessive, null, mutations in HK1 cause early-onset, non-spherocytic hemolytic anemia, which was not observed in these patients, and the Glu847Lys missense mutation is outside of any known active site. Thus the HK1 adRP mutation may act through a unique biological mechanism.

Acknowledgments

We dedicate this report to the memory of Dr. Mary Kay Pelias who worked closely with the Louisiana families throughout her career. This work was supported by NIH grant EY007142, by grant HG003079 from the NHGRI, by a Wynn-Gund TRAP Award, and by the Foundation Fighting Blindness.

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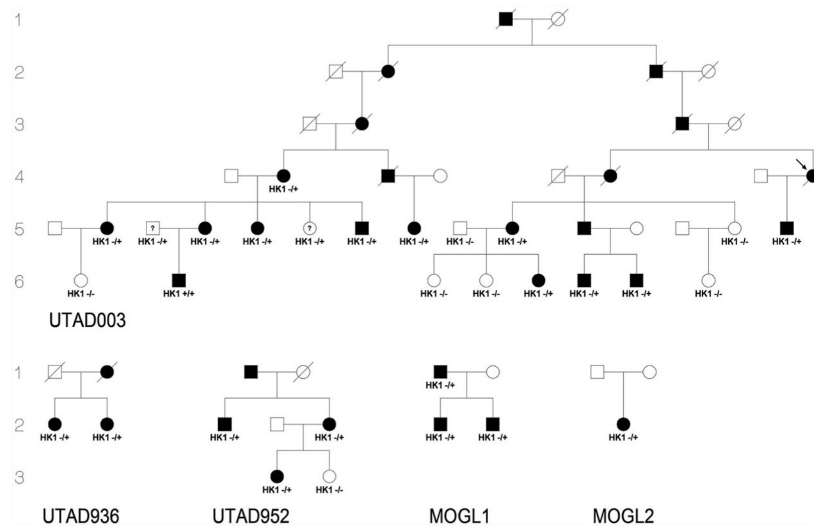

Fig. 26.1.

Pedigrees of five adRP families with the HK1 Glu847Lys missense mutation. *Squares* males; *circles* females; *blackened symbols* affected. All individuals with an HK1 genotype indicated were tested. $HK1^{-/+}$, heterozygous for the mutation; $HK1^{+/+}$, homozygous for the mutation; $HK1^{-/-}$, no mutation

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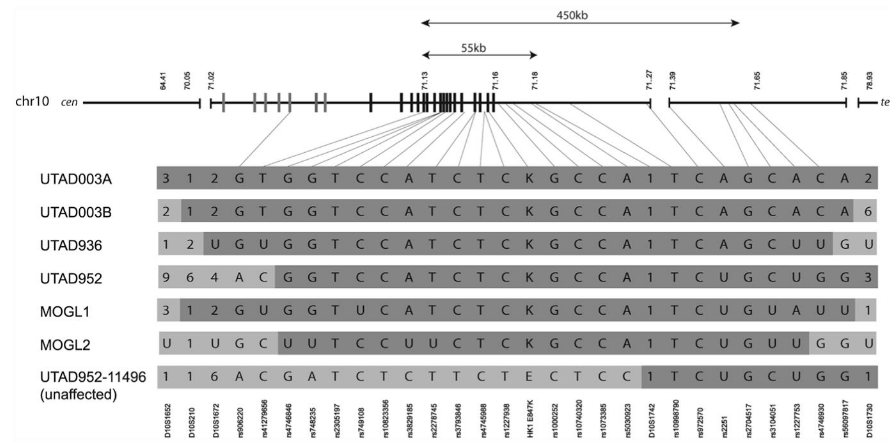

Fig. 26.2.

Chromosomal haplotypes in *cis* to the HK1 Glu847Lys mutation, including two distinct haplotypes in the homozygous individual in UTAD003, and an unaffected, at-risk individual in UTAD952. Exons of HK1 and distances (in kb) of chromosome 10q21.1 are shown at the top of the figure. SNP and markers defining the haplotype are listed at *bottom*. Observed SNP alleles are listed in each bar. *Dark gray* region of bars, shared SNP alleles; *light gray* region of bars, alleles not shared. The shared haplotype across all families is 450 kb (*top arrows*), whereas the shortest region of linkage overlap, including the unaffected member of UTAD952, is 55 kb (*second arrows*)

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