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Report

A Genomewide Scan Identifies Novel Early-Onset Primary Open-Angle Glaucoma Loci on 9q22 and 20p12

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Glaucoma is a leading cause of blindness worldwide. The disease is characterized by a degeneration of the optic nerve, which is usually associated with elevated intraocular pressure. The common form of adult-onset primary open-angle glaucoma is inherited as a complex trait, whereas the rarer early-onset juvenile open-angle glaucoma (JOAG) exhibits autosomal dominant inheritance. Of all cases of JOAG, ~10%–20% are caused by mutations in the myocilin gene. We have identified 25 pedigrees that are affected with typical JOAG and that demonstrate autosomal dominant inheritance. We sequenced the myocilin gene in probands from each family and found mutations in 8% of this population. To identify novel genes responsible for JOAG, we used families that did not have myocilin mutations for a genomewide screen. Markers located on chromosomes 9q22 and 20p12 showed evidence for linkage, identifying two novel loci for early-onset open-angle glaucoma.

Glaucoma is the third leading cause of blindness in the United States and is also a leading cause of blindness worldwide (Dimitrov et al. 2003). The disease is characterized by a degeneration of the optic nerve, which is usually associated with elevated intraocular pressure. The increase in intraocular pressure is probably caused by a reduction in outflow of aqueous humor through the trabecular outflow pathways; however, the molecular mechanisms responsible for normal aqueous humor outflow and impaired outflow in glaucoma are not known (Lutjen-Drecoll et al. 2001). The degeneration of the optic nerve is the result of the loss of individual retinal ganglion cells. The ganglion cells die by an apoptotic mechanism, although the details of this process are not well understood (Farkas and Grosskreutz 2001). Moreover, the relationship between the elevation of intraocular pressure and the apoptosis of retinal ganglion cells is not well defined.

A family history of glaucoma has long been recognized as an important risk factor for the disease, and both Mendelian and non-Mendelian forms of glaucoma have been identified. The common adult-onset primary open-angle glaucoma (POAG) is inherited as a complex trait, whereas the rarer early-onset POAG exhibits autosomal dominant inheritance (Wiggs et al. 1996). Adult-onset POAG is the most common type of glaucoma, and substantial efforts have gone into understanding, diagnosing, and treating the disease. However, the successful identification of the genes responsible for complex heterogeneous disorders such as POAG requires a multipronged study design and very large, well-defined data sets of affected individuals (Wiggs et al. 2000; Schmidt et al. 2002). Despite these difficulties, genetic techniques for complex disorders have proven successful and are one path to the desired genes. Another path is to study simple Mendelian forms of the disease with defined genetic models resulting from defects in a single underlying gene. Mendelian forms of a disease are often rare, but the discovery of the responsible gene can provide information that is applicable to other, more common forms of the disease. Mutant alleles of genes responsible for rare forms of a disease may be one of several factors leading to the development of a more common complex disease.

POAG that develops before the age of 40 years, known as "juvenile-onset POAG" (JOAG) (Wiggs et al.

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Table 1

Markers with Two-Point LOD Scores >1.0

Marker	Location (cM)	Two-Point LOD Score for θ						
		.00	.05	.10	.15	.20	.30	.40
D3S1294	210	−12.30	−.99	.44	.92	***1.01***	.70	.26
D5S617	95	−2.79	.60	***1.00***	***1.03***	.93	.58	.22
D9S1803	101	−3.67	.63	***1.13***	***1.24***	***1.20***	.84	.37
D12S159	45	−2.86	.38	.89	***1.00***	.92	.58	.21
D20S189	34	−3.63	.88	***1.30***	***1.29***	***1.13***	.67	.26
D20S104	37	−6.70	.28	***1.03***	***1.17***	***1.08***	.64	.19

Note.—Two-point LOD scores >1.0 are shown in bold italics.

1995), is a rare disorder that results in high intraocular pressure, usually requiring surgical therapy. JOAG is typically inherited as an autosomal dominant trait, and one gene, myocilin (MYOC [MIM 601652]), has been associated with this condition (Adam et al. 1997; Stone et al. 1997; Richards et al. 1998; Wiggs et al. 1998). MYOC was originally identified as a glucocorticoid response protein in cultured human trabecular meshwork cells (Nguyen et al. 1998). The gene is comprised of three exons, and missense mutations associated with JOAG have been found primarily in the third exon that codes for a protein domain with homology to olfactomedin (Rozsa et al. 1998; Fingert et al. 1999; Alward et al. 2002). The function of the normal protein in aqueous humor outflow is not currently known. Several studies have indicated that the mutant forms are associated with a gain of function or dominant negative effect (Kim et al. 2001; Wiggs and Vollrath 2001; Joe et al. 2003).

MYOC mutations may contribute to both JOAG and POAG. The MYOC DNA sequence variants found in patients with glaucoma are associated with a range of disease severity, with some missense mutations causing very severe early-onset disease with autosomal dominant inheritance, whereas other missense mutations and a common truncating mutation (GLN368STOP) are associated with late-onset POAG (Allingham et al. 1998; Shimizu et al. 2000; Mackey et al. 2003). Mutations in MYOC occur in 3%–5% of patients with the adult-onset disease, and the adult-onset mutations—in particular, the GLN368STOP sequence variant—are associated with disease risk and exhibit variable penetrance (Craig et al. 2001; Graul et al. 2002). The MYOC mutations that cause the early-onset disease are highly penetrant, with ~90%–95% of individuals carrying mutations showing evidence of disease by the age of 40 years (Wiggs et al. 1998; de Vasconcellos et al. 2003).

Most cases of JOAG (80%) cannot be explained by mutations in the MYOC gene (Bruttini et al. 2003). We have identified a study cohort of families affected with typical early-onset JOAG demonstrating autosomal dominant inheritance. We sequenced the MYOC gene in the probands from each of these families and found mutations in 8% of this study population (Wiggs et al. 1998). To identify novel genes responsible for the disease, we have used the families that did not have MYOC mutations for a genomewide screen.

For this study, we identified 25 pedigrees affected by JOAG that consisted of a minimum of three affected individuals in two generations (198 total individuals, 105 affected individuals). In this population, juvenile

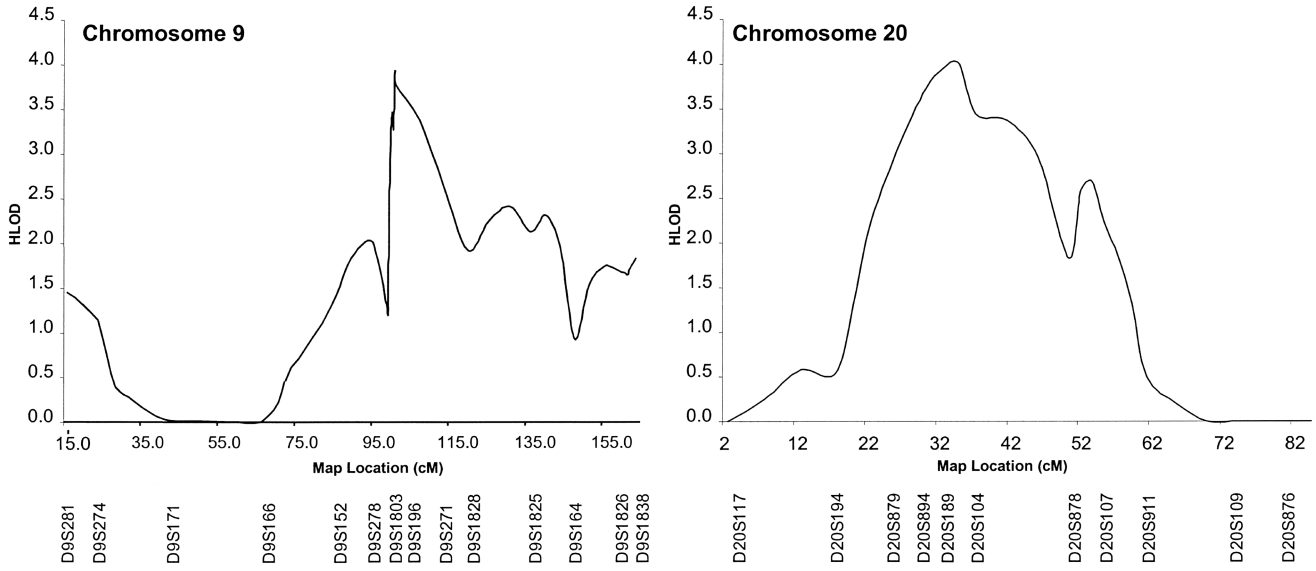


Figure 1 Multipoint linkage analysis. HLOD values were used for multipoint calculations performed by use of the computer program Allegro. The deCODE genetic map was used for the marker locations (Kong et al. 2002).

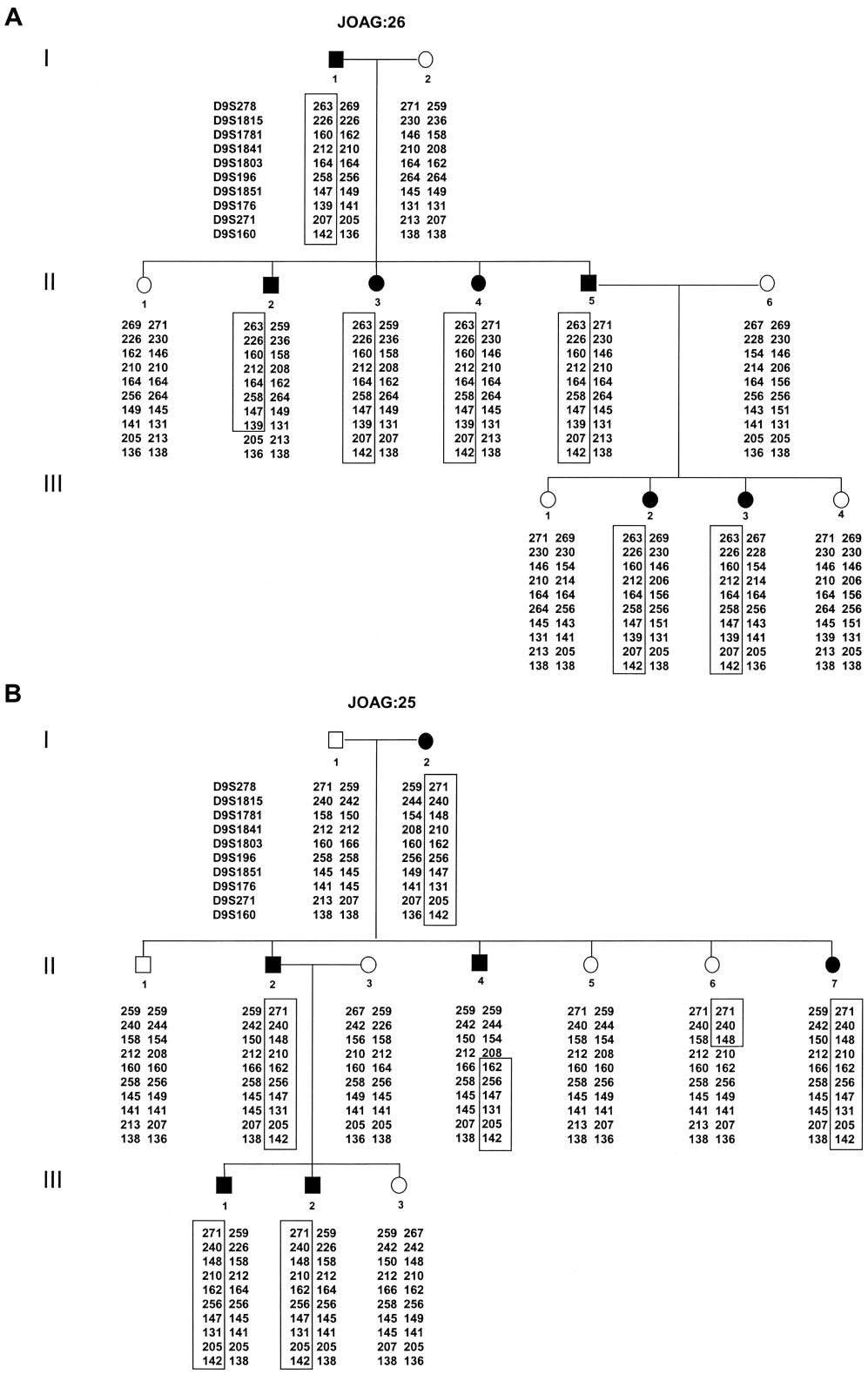
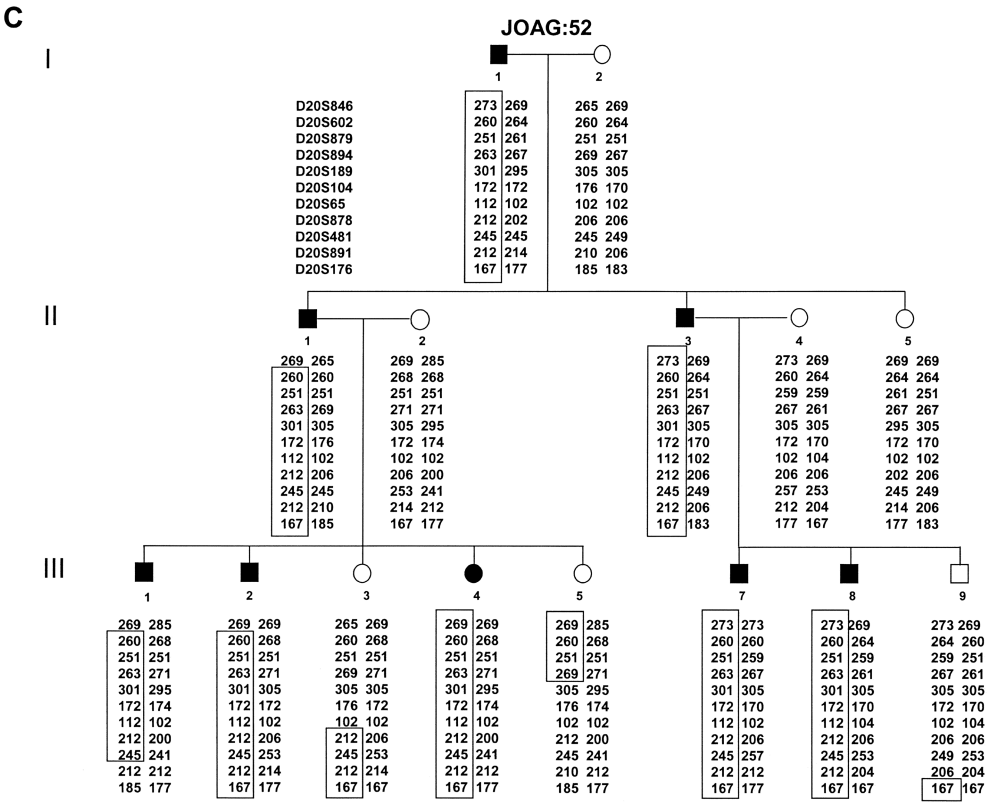


Figure 2 Haplotypes for selected pedigrees using markers from chromosome 9 (pedigrees 25 and 26) and chromosome 20 (pedigree 52). Segregating haplotypes are shown in the rectangles. Marker locations are according to Kong et al. (2002).



glaucoma is diagnosed if patients display the following characteristics: onset of the disease before age 35 years, intraocular pressure >22 mmHg in both eyes, glaucomatous optic-nerve damage in both eyes, and visual-field loss in at least one eye. Patients with clinical findings consistent with anterior-segment-dysgenesis syndromes were not included in this study. A proband from each pedigree had been previously screened for MYOC mutations. This study was approved by the Massachusetts Eye and Ear Infirmary institutional review board, and informed consent was obtained from individuals participating in the study.

Blood samples were collected from consenting family members, and genomic DNA was prepared from lymphocyte pellets. Initially, 238 microsatellite repeat markers spanning the human genome at ~10-cM intervals were analyzed. Marker locations were based on the deCODE genetic map (Kong et al. 2002). Genotyping was performed by use of PCR amplification with incorporation of P^{32} and visualization of alleles after autoradiography or by PCR amplification without radioactivity, with visualization of alleles by a florescent imager after staining with SYBR Green dye. Marker-allele sizes and frequencies were obtained from the database of the Centre d'Etude du Polymorphisme Humain (CEPH) or the Genome Database. All families were genotyped by use of a single method for a given marker.

The two-point and multipoint LOD scores, which assumed an autosomal dominant model, were calculated by use of the Allegro software package (Gudbjartsson et al. 2000), initially assuming homogeneity and then allowing for genetic heterogeneity (HLOD). For the genomic-screening analyses, only affected pedigree members and spouses were included. For the follow-up studies, unaffected individuals were included.

Markers located in five regions on chromosomes 3, 5, 9, 12, and 20 demonstrated initially interesting results (two-point LOD score >1.0) (table 1). The results of the complete genome scan are provided as an online-only supplement. Flanking microsatellite markers that are located ~5 cM on either side of those generating suggestive results were selected for further analyses using the entire pedigree set, including unaffected individuals who are without evidence of the disease by the age of 40 years. Markers flanking the peak markers on chromosomes 9 and 20 continued to show interesting results with two-point LOD scores >2.0, assuming homogeneity. An increase in LOD scores in both regions was seen when the calculations were repeated, allowing for genetic heterogeneity (alpha = 0.9) (table 2). Multipoint analyses were performed using the HLOD values for all markers in each suggestive region. Multipoint analyses of chromosomes 9 and 20 resulted in higher HLOD scores for markers located in these regions, with a peak score of 4 on chromosome 9 between markers D9S1803 and D9S196, and a peak score of 4 on chromosome 20 be-

tween markers D20S189 and D20S104 (fig. 1). Of the 25 families originally enrolled in this study, 15 were of sufficient size and pedigree structure that haplotypes could be constructed by use of markers located within these regions. Of these 15 families, 7 families have haplotypes consistent with linkage to the chromosome 9 region, 5 families have haplotypes consistent with linkage to the chromosome 20 region, and 3 families have haplotypes consistent with linkage to both regions. The haplotypes for pedigree 26 (chromosome 9), pedigree 25 (chromosome 9), and pedigree 52 (chromosome 20) are shown in figure 2. Critical recombinants identify a 9-cM region on chromosome 9 between markers D9S1841 and D9S271 and a 19-cM region on chromosome 20 between markers D20S894 and D20S878 (fig. 3).

These results provide evidence for two new loci containing genes responsible for JOAG. Although these regions are of considerable size, the annotated human genome sequence makes it possible to compile a comprehensive list of candidate genes located in each region. By use of a variety of databases (Stanford Microarray Database, NEIBank, and UniGene), we have identified 15 genes in the chromosome 9 region and 23 genes in the chromosome 20 region that have significant ocular expression. We are currently sequencing these genes in affected family members.

The *VSX* gene (MIM 605020) is one of the interesting candidate genes located in the chromosome 20 region. Mutations in *VSX* have been shown to be responsible for some cases of posterior polymorphous dystrophy, a disease that can be associated with early-onset glaucoma (Heon et al. 2002). We have sequenced this gene and determined that DNA sequence variants are not present in the patients with early-onset glaucoma in our population. Other interesting candidate genes located in the chromosome 20 region are currently under investigation.

The results of this study support the hypothesis that multiple genes can give rise to JOAG. The identification of genes and protein products responsible for JOAG will

Table 2

Two-Point LOD Scores for Markers Located in Peak Regions on Chromosomes 9 and 20

Chromosome and Marker	Location (cM)	Two-Point LOD Score, Assuming	
		Homogeneity	Heterogeneity
Chromosome 9:			
D9S1781	95	1.31	1.70
D9S1803	96	2.10	2.20
D9S196	***97***	***2.40***	***2.70***
D9S271	105	1.10	1.20
Chromosome 20:			
D20S879	28	0.70	0.90
D20S894	32	2.10	2.90
D20S189	***34***	***2.37***	***3.15***
D20S104	37	2.10	2.33

Note.—Markers with peak two-point LOD scores are shown in bold italics.

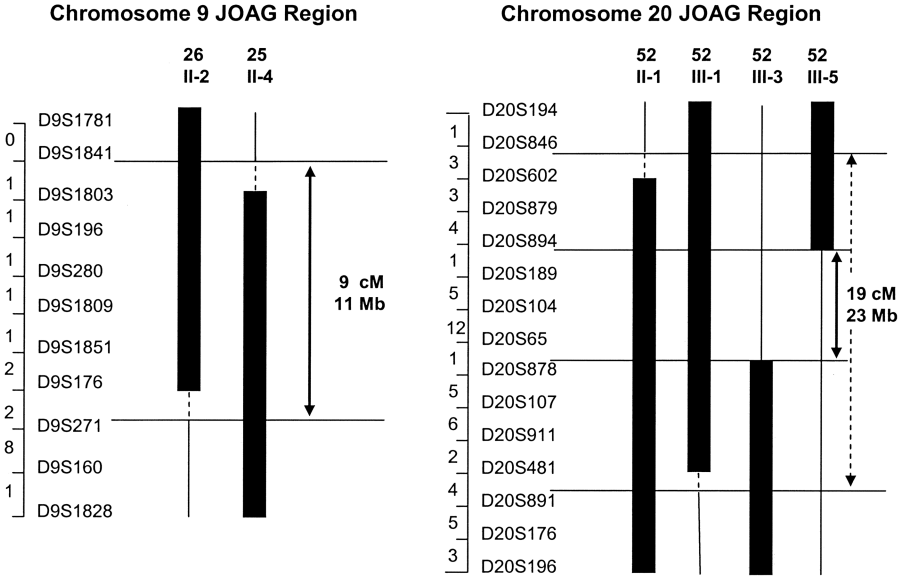


Figure 3 Recombination events defining the chromosome 9 and chromosome 20 regions. Solid rectangles indicate the nonrecombinant region for each individual. The critical recombination events are shown by horizontal lines. Each individual in the chromosome 9 region is affected (unaffected individuals did not have relevant recombination events in this region). In the chromosome 20 region, individuals 52 II-1 and 52 III-1 are affected, whereas individuals 52 III-3 and 52 III-5 are unaffected. When only the affected recombinant individuals are used, a critical region that extends from markers D20S846 to D20S891 (48 cM, 39 Mb) can be identified (indicated by the *dashed arrow*).

help define the underlying biochemical abnormalities responsible for the disease. A better understanding of the underlying molecular defects, as well as the development of transgenic animal models based on the predisposing gene defects, may lead to more effective and specific therapies. Allelic variants of early-onset–glaucoma genes may also contribute to the susceptibility of adult-onset POAG and/or may suggest proteins that can contribute to this devastating disease. Defining gene defects that predispose to adult-onset glaucoma will help identify individuals at risk for the disease, thus allowing for appropriate treatment and prevention of blindness.

Acknowledgments

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Electronic-Database Information

The URLs for data presented herein are as follows:

Centre d'Etude du Polymorphisme Humain (CEPH), http://www.cephb.fr/cephdb/
Genome Database (GDB), http://www.gdb.org/
NEIBank, http://neibank.nei.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MYOC and *VSX*)
Stanford Microarray Database, http://genome-www5.stanford.edu/
UniGene, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene

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