2011

Genetic modulation of horizontal cell number in the mouse retina

Irene Whitney
*University of California, Santa Barbara,*

Mary Raven
*University of California, Santa Barbara,*

Daniel Ciobanu
*University of Nebraska, Lincoln, NE*

Ross Poché
*Baylor College of Medicine, Houston, TX*

Qian Ding
*University of Rochester, Rochester, NY*

*See next page for additional authors*

Follow this and additional works at: [http://digitalcommons.unl.edu/animalscifacpub](http://digitalcommons.unl.edu/animalscifacpub)

Part of the Animal Sciences Commons

Whitney, Irene; Raven, Mary; Ciobanu, Daniel; Poché, Ross; Ding, Qian; Elshatory, Yasser; Gan, Lin; Williams, Robert; and Reese, Benjamin, "Genetic modulation of horizontal cell number in the mouse retina" (2011). *Faculty Papers and Publications in Animal Science.* Paper 757.

[http://digitalcommons.unl.edu/animalscifacpub/757](http://digitalcommons.unl.edu/animalscifacpub/757)
Genetic modulation of horizontal cell number in the mouse retina

Irene E. Whitney,1,2 Mary A. Raven,1,2 Daniel C. Ciobanu, Ross A. Poché, Qian Ding, Yasser Elshatory, Lin Gan, Robert W. Williams,3 and Benjamin E. Reesea

*Neuroscience Research Institute and Departments of Psychological and Brain Sciences and Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106; bAnimal Science Department, University of Nebraska, Lincoln, NE 68583; cDepartment of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030; dDepartment of Ophthalmology, University of Rochester, Rochester, NY 14642; and eCenter of Genomics and Bioinformatics and Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38120

Edited* by John E. Dowling, Harvard University, Cambridge, MA, and approved April 26, 2011 (received for review February 28, 2011)

Neuronal populations display conspicuous variability in their size among individuals, but the genetic sources of this variation are largely undefined. We demonstrate a large and highly heritable variation in neuron number within the mouse retina, affecting a critical population of interneurons, the horizontal cells. Variation in the size of this population maps to the distal end of chromosome (Chr) 13, a region homologous to human Chr 5q11.1–11.2. This region contains two genes known to modulate retinal cell number. Using conditional knock-out mice, we demonstrate that one of these genes, the LIM homeodomain gene Iset-1 (Is1), plays a role in regulating horizontal cell number. Genetic differences in Is1 expression are high during the period of horizontal cell production, and cis-regulation of Is1 expression within the retina is demonstrated directly. We identify a single nucleotide polymorphism in the 5′ UTR of Is1 that creates an E-box sequence as a candidate causal variant contributing to this variation in horizontal cell number.

Results

Mouse Retina Displays a Large and Highly Heritable Variation in Horizontal Cell Number. Mice of the A/J strain contain 9,884 ± 168 horizontal cells, whereas those of the B6/J strain contain 18,471 ± 288 cells (mean and SE; Fig. 1, white and black bars). F1 hybrids (B6AF1) have a value intermediate to the two parental strains, 14,417 ± 263 cells (Fig. 1, light gray bar). Members of the AXB/ BXA strain family have genomes that are a unique mix of A and B haplotypes across chromosomes. These progeny strains have numbers of horizontal cells that are distributed widely across the range defined by the two parental strains (Fig. 1, dark gray bars). Note, however, that variance within any strain is low, relative to that observed across strains, the coefficient of variation for each strain being less than 4%. Resampling the parental strains with additional mice at the end of the experiment confirmed no procedural or criterion drift contaminating our data collection procedures (Fig. 1, second white and black bars). These data show that horizontal cell number is a highly heritable trait, with a narrow-sense additive heritability (h²) of 0.89 (13). Such high heritability makes horizontal cell number a particularly attractive trait for genetic dissection.

A QTL for Horizontal Cell Number Resides on Distal Chromosome 13. We mapped the variation in this trait to a narrow locus at the distal tip of Chr 13, peaking at or beyond reference SNP (rs) 4230072 (113.52 Mb) (Fig. 2). This QTL, named “Horizontal cell number” (Horizontal Cell Number).
Subsequently examined horizontal cell number in the cells, bipolar cells, and retinal ganglion cells (17, 18). We compared the mouse retina to lower the number of cholinergic amacrine
117.08 Mb. Recent studies have shown its conditional deletion in either the center or the periphery of the retina.
in horizontal cell number compared with control littermates, in

Fst (Fig. 3 fi

 significance elevated in the central parts of the retina, whereas another LIM homeodomain transcription factor gene, Lim1, is expressed exclusively by the axon-bearing horizontal cells (14, 27), the only type present in the mouse retina (28). Overexpression of Isl1 in postmitotic horizontal cells in the chick retina results in a switching to the axonless fate, presumably due to a repression of

Two Candidate Genes That Modulate Retinal Nerve Cell Number Are Positioned at the QTL. One candidate gene within this QTL is follistatin (Fst), positioned at 115.24 Mb. Administration of follistatin during the neurogenic period has been shown to increase the number of retinal horizontal cells in chicken embryos (14), and so we examined the effect of disrupting Fst gene expression by excising a floxed allele of Fst in cells expressing the Cre gene within the developing mouse retina (15, 16). Effective cre-mediated recombination of Fst within the retina was confirmed by qPCR, yet Fig. 3A shows, for the opposite retinas of these Fst-conditioned knock-out (CKO) mice, no significant change in horizontal cell number compared with control littersmates, in either the center or the periphery of the retina.

Another candidate gene within this QTL is Isl1, positioned at 117.08 Mb. Recent studies have shown its conditional deletion in the mouse retina to lower the number of cholinergic amacrine cells, bipolar cells, and retinal ganglion cells (17, 18). We consequently examined horizontal cell number in the Isl1-CKO retina and in littersmate controls. Horizontal cell density was significantly elevated in the central parts of the Isl1-CKO retina (Fig. 3B and C, Left), being increased by 41%. Densities in the periphery were only marginally increased, by 8% (Fig. 3B and C, Right), likely due to the mosaicism in cre-recombinase activity: Six3-cre drives LacZ reporter expression in the Rosa26 reporter line more thoroughly in the central than in the peripheral parts of the mouse retina (e.g., figure 1e in ref. 19). Conditional deletion of Isl1 therefore increases the number of horizontal cells.

Isl1 Is Not Expressed in Horizontal Cell Precursors. The transcription factor genes Foxn4, Ptf1A, and Prox1, each of which is thought to play a hierarchical role in the specification of retinal cell fate, have all been shown to produce profound deficits in horizontal cell number when knocked out (20–22). How Isl1 interacts with this hierarchy is unknown. Isl1 is not normally expressed by horizontal cells in the mature mouse retina (23, 24), and we have confirmed that it is not expressed in Prox1-expressing precursors, a subset of which will become horizontal cells (20) (Fig. 4): Crossing mice with tamoxifen-dependent cre-recombinase driven by the Isl1 locus (25) with the Rosa26 reporter line expressing LacZ (26), and administering tamoxifen on embryonic (E) days E12.5–E16.5, yielded no Prox1+ cells (magenta) on E18.5 that also expressed the LacZ gene product, β-galactosidase (green).

Isl1 is, however, expressed by one type of horizontal cell in the chick retina, the axonless horizontal cell, whereas another LIM homeodomain transcription factor gene, Lim1, is expressed exclusively by the axon-bearing horizontal cells (14, 27), the only type present in the mouse retina (28). Overexpression of Isl1 in postmitotic horizontal cells in the chick retina results in a switching to the axonless fate, presumably due to a repression of
endogenous *Lim1* by *Isl1* (27). Conversely, the increase in horizontal cells, shown here in the *Isl1* conditional knockout, suggests a role for *Isl1* that is incompatible with *Lim1* expression and horizontal cell differentiation. These two LIM homeodomain transcription factor genes, *Isl1* and *Lim1*, are therefore likely to be mutually inhibitory within the mouse retina, as they are in other systems (29, 30), participating in the production of horizontal cells (27). The phenotypic difference in horizontal cell number between the parental strains, therefore, may arise from allelic variation within a regulatory sequence for *Isl1*.

**Cis-Acting Regulatory Variants Control Parental Differences in *Isl1* Expression.** We sequenced the *Isl1* gene and confirmed the presence of four previously documented synonymous sequence differences between A/J and B6/J, identifying no additional SNPs within the coding region. We next examined potential regulatory regions of this gene, including the putative promoter (~1 kb upstream of the transcriptional start site), 5' UTR, the first two introns, and the 3' UTR, using the more complete sequencing of A/J as part of the Mouse Genome Project by the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/resources/mouse/genomes). Sequence analysis revealed numerous possible regulatory SNPs between these two strains, including previously undocumented SNPs (Table S1). To ascertain whether the parental strains differ in their expression of *Isl1*, we measured transcript levels using qPCR during the period of horizontal cell genesis as well as in maturity, finding significantly greater levels of expression in A/J, the strain with fewer horizontal cells (Fig. 5A). We also compared the relationship between *Isl1* expression and horizontal cell number across the two parental and 26 RI strains of the AXB/BXA strain set from our previous microarray analysis of adult whole eye mRNA (6), available on GeneNetwork for public access (accession no. GN210). We found a significant negative correlation between *Isl1* expression and horizontal cell number across these 28 strains (*r* = −0.525, *P* value = 0.00557).

To test directly whether differences in *Isl1* expression between the parental strains are at least partially controlled by a cis-regulatory variant, we examined the allele-specific expression (ASE) of *Isl1* in the retinas of reciprocal F1 offspring of the A/J and B6/J strains. Significant differences between the parental alleles in the F1 animals would indicate cis-modulation, whereas trans-acting regulation of *Isl1* expression would be reflected by the lack of difference between parental allelic expression, because both *A* and *B* alleles of *Isl1* will be equally affected by trans-factors (31). The significance of the difference in allelic expression was evaluated by comparing the deviation of the ratio of allelic expression in the mRNA from retina taking into account the difference in amplification efficiency of the alleles using genomic DNA (gDNA) as a control. In the genome of F1 hybrids, there is a copy of each of the alleles in every somatic cell; the ratio in allelic abundance following amplification of the gDNA is therefore expected to be 1. Deviation from this ratio, however, is frequent, being a result of various factors such as secondary DNA structure, presence of unaccounted DNA polymorphisms that can influence the efficiency of allelic amplification, and the ratio between alleles, which should affect gDNA as well as the mRNA/cDNA equally. Any difference in allelic expression due to *cis*-elements alone should be expressed as a deviation in the ratio of allelic abundance profiled in the mRNA of reciprocal F1 hybrids relative to the gDNA. We found the expression of the *A* allele in *Isl1* to be significantly greater, in both reciprocal hybrids (Fig. 5B), validating the mechanism of *cis*-regulation of this gene.

**An SNP Creates an E-Box in the *Isl1* 5' UTR in B6/J Mice.** We used two predictive programs of transcription factor binding sites, TESS and MATCH, to identify potentially functional consequences of sequence differences between A/J and B6/J in the promoter re-
Figure 4. (A) Cells of the Isl1 lineage born between E12.5 and E16.5, positive for the LacZ reporter gene product β-galactosidase (green), are all positioned in the inner retina on E18.5. (B) Horizontal cell precursors, identified by their expression of Prox1 (magenta), are known to migrate beyond the future horizontal cell stratum in the outer retina, raising the possibility that they too are derived from the Isl1-expressing cells in the inner retina at these early stages. (C) When both channels are viewed together, however, not a single Prox1+ precursor is found to coexpress β-gal. Samples are from the central retina, but identical results were obtained at the far retinal periphery. (Scale bar, 50 μm.)

Discussion
Horizontal cells display a conspicuous variation in their number within the mouse retina that is independent of variation in the overall size of the retina itself. This variation is highlyheritable, with surprisingly low within-strain differences—a coefficient of variation of less than 4% for every RI strain examined. Although sets of identified neurons in invertebrates often display this level of tight genetic control (36), there is no precedent for such precision in the mammalian central nervous system. The minimal residual nongenetic variation is presumably due to a combination of intrinsic developmental noise and sampling error (7).

The heritable variation in horizontal cell number is independent of the variation in other types of retinal neurons in these same strains. Cone photoreceptors, for instance, are 70% more abundant in the B6/J strain relative to A/J (6), whereas dopaminergic amacrine cells exhibit a fourfold variation in total number across this same family of RI strains (4). The former trait mapped to a locus on Chr 7, where at least three genes modulating dopaminergic amacrine cell number were shown to be present (4). The variation in horizontal cell number mapped to a locus on Chr 13, where two promising prospective candidates were identified, Fst and Isl1. Despite the documented role for Fst in controlling horizontal cell number in the chick retina (14, 37), no comparable effect was detected in Fst-CKO retinas. Conditional knock-out of Isl1, by contrast, produced a substantial increase in horizontal cells in a retina with drastically reduced numbers of ganglion cells, cholinergic amacrine cells, and bipolar cells (17, 18), indicating a suppressive role for Isl1 upon horizontal cell number. Indeed, Isl1 mRNA levels were greater in the A/J strain with lower numbers of horizontal cells during the period of horizontal cell neurogenesis (38). We found only synonymous SNPs in the coding region of the Isl1 gene, but detected an SNP in the 5′ UTR, which creates a bHLH binding motif in the B6/J strain that may participate in the regulation of Isl1 expression. Consistent with this, we have shown that Isl1 transcript levels are controlled by a cis-acting variant in retinal tissue.

Exactly how the modulation of Isl1 expression affects horizontal cell number remains to be determined. Previously, we had shown that all horizontal cells arise from the Math5+ precursors that also generate the Isl1+ amacrine and ganglion cells as well as certain photoreceptor cells (39, 40). Given that Isl1-lineage cells do not produce horizontal cells (Fig. 4), that Lim1 protein is found only in horizontal cells (24), and that inhibitory relationship between these two genes (29, 30), a mutually exclusive expression pattern of Isl1 and Lim1 among common precursors could participate in determining horizontal cell number. The conditional knockout of Isl1 may alleviate additional precursors of such repression of Lim1, allowing them to differentiate as horizontal cells. In the B6/J retina, the presence of the E-box may ensure further repression of Isl1 to allow more cells to express Lim1, becoming horizontal cells. This E-box–mediated repression of Isl1 expression is likely regulated by a bHLH transcriptional repressor specific to horizontal cell precursors. In other precursors that give rise to Isl1+ cells in the B6/J retina (23), Isl1 expression may not be suppressed, due to the absence of this bHLH transcriptional repressor and/or to the presence of other transcriptional activators.

bHLH transcription factors have been shown to be integral during embryonic development, playing roles in myogenesis, heart development, hematopoiesis, and neurogenesis (34). Of the various bHLH transcription factors predicted to bind the E-box, E2A and NeuroD1 are expressed in developing vertebrate retina (41), and have been reported to play a role in retinal neurogenesis and differentiation (42, 43). In these instances, E2A and NeuroD1 are considered to be proneural bHLH proteins, acting as transcriptional activators. However, they have been described in other tissues to function as transcriptional repressors during development (44, 45). In the present instance, their proposed action upon Isl1 is predicted to be repressive, given the greater number of horizontal cells in B6/J and the greater Isl1 mRNA expression observed in the A/J strain. The creation of a novel E-box due to an SNP, leading to altered gene expression, is not unprecedented (46), including an instance when this resulted in the suppression of gene expression (47). This polymorphism in the Isl1 5′ UTR may therefore contribute to the variation in horizontal cell number documented herein.

Prominent variation in nerve cell number is predicted to have functional consequences for neural organization and circuitry (36). In the case of the horizontal cells, dendritic field area is modulated in these parental strains of mice to maintain an average dendritic overlap of around 6.0 (12). Because variation in the number of their afferents, the cone photoreceptors (6), is
with mice homozygous for floxed Fst allele (16). Three Fst-CKO mice and three littermate control mice were examined. Isl1-CKO mice and their control littermates were produced as described previously. Briefly, Six3-cre-expressing mice [Tg(Six3-cre):E9FpRTy] (48) were crossed with mice harboring either a floxed allele of Isl1 or an Isl1-lacZ knock-in allele (Isl1tm1Gan) and subsequently bred with mice homozygous for floxed Isl1 allele (18). Twelve Isl1-CKO mice and 11 littermate control mice were examined. All mice used in the present study were between 3 and 12 wk of age.

**Retinal Immunofluorescence and Quantification of Horizontal Cell Number.** Standard procedures for perfusion, dissection, and immunofluorescence were used to prepare retinal wholemounts for quantification of horizontal cell number, as described previously by us (9). Mean horizontal cell density (derived from sampling eight fields totaling ~15% of the retinal surface area) was multiplied by retinal area to estimate total horizontal cell number. Further details are provided in SI Materials and Methods.

**QTL Mapping.** Each of the RI strains has a unique mix of A and B alleles across all chromosomes (with the exception of the Y chromosome and the mitochondrial genome). QTL mapping takes advantage of this nearly random recombination of parental genotypes to estimate the genetic control of a given trait (in our case, horizontal cell number) and the presence of A or B alleles across the genome. Each of the RI strains has been genotyped with high-density microsatellite and SNP markers (49). QTL mapping of horizontal cell number was performed using standard interval mapping and the genotypes of 3,785 informative markers using computational and visualization tools available in GeneNetwork (www.GeneNetwork.org). GeneNetwork implements now standard methods of simple and composite interval mapping and estimates the genomewide P value of a type I error by permutation. The primary horizontal cell data have been permanently deposited in GeneNetwork as phenotype accession identifier GN no. 10132 (horizontal cell number per retina) in the mouse AXB/BXA Published Phenotypes database.

**Lineage Marking.** To study the lineage of Isl1-expressing retinal cells, male tamoxifen-inducible Isl1-cre mice (Isl1tm1Ganfoxesix3ter-Tcry) (25) (kindly provided by Dr. Amy Kiernan, University of Rochester, Rochester, NY) were crossed to females that carried the Rosa reporter allele, R26R-LacZ [Gt(ROSA)26Sor(TKneo)1Bgr] (26) (The Jackson Laboratory). Time-mated pregnant mice were injected intraperitoneally with tamoxifen at 4 mg/30 g body weight at E12.5, E13.5, E14.5, E15.5, and E16.5. Embryos were harvested at E18.5, fixed for 2 h in 4% paraformaldehyde in PBS, and saturated in 30% sucrose dissolved in PBS at 4 °C overnight. Samples were then embedded in OCT and sectioned at 20 μm on a cryostat. The following antibodies were used: chicken anti–α-galactosidase (1:1,000; Abcam) and rabbit anti-Prox1 (1:1,000; Covance). Fluorescence images were captured and analyzed by a Zeiss LSM 510 confocal microscope using a 20× objective.

**Quantitative RT-PCR.** Isl1 transcript levels were quantified during the period of horizontal cell genesis and in maturity using qPCR, as described previously (4) with a few modifications. Further details are provided in SI Materials and Methods.

**Rapid Amplification of the cDNA Ends and cDNA Sequencing.** The Isl1 mouse sequence available in Ensembl (ENSMUST0000036060.4) and the Primer 3 program were used to design primers to amplify and sequence the entire transcript. Further details are provided in SI Materials and Methods.

**Allele-Specific Expression Assay.** An ASE was performed using male and female pooled samples from each reciprocal F1 cross of the A/J and B6/J strains, A86F1 and B6AF1, as previously described (31). Further details are provided in SI Materials and Methods.

**Statistics.** Student’s t test was used for all comparisons between A/J versus B6/J, and between conditional knock-out mice and their respective littermate controls. A one-tailed Student’s t test with unequal variance examined the difference in allelic expression of Isl1 by testing the deviation in the ratio of allelic abundance (log10) profiled in the mRNA of reciprocal F1 hybrids relative to the gDNA, as previously described (31). A one-tailed test was chosen because the ASE sought to test the direction of the difference detected by qPCR, either confirming it (therefore being cis-regulated) or finding no difference (and therefore trans-regulated).

---

**Materials and Methods**

**Mouse Strains.** B6/J and A/J mice, their F1 progeny (B6AF1), and RI mice from 26 strains in the AXB/BXA strain set were obtained from The Jackson Laboratory. A minimum of three mice per strain were examined, with one exception (Bxa2, n = 2), sampling a single eye from each mouse. The RI strains were originally generated by the continuous inbreeding of F1 progeny (either A86F1 to make AXB strains or B6AF1 to make BXA strains) through successive generations to yield chromosomes that are homozygous but independent of the variation in horizontal cell number across this large family of RI strains, this ratio must be unique for each strain of mouse, yet the horizontal cells modulate their connectivity with the population of afferents to ensure a uniform sampling of every pedicle by six neighboring horizontal cells (12). The genetic determinants of nerve cell number, therefore, by modulating fold changes in the retina from the allelic ratio in the genomic DNA in reciprocal F1 hybrids. (P = 0.0007, Student’s t test). A difference is also detected in maturity (P = 0.0028). (A) ASE confirms cis-regulation of Isl1 in AXB/BXA, evidenced by testing the deviation of the allelic expression changes in the retina from the allelic ratio in the genomic DNA in reciprocal F1 hybrids. (P = 0.007). (C) An SNP in the Isl1 5′ UTR creates an E-box sequence in the B6/J strain. The A SNP is the conserved allele. (Alignment generated with CLC Sequence Viewer).

---

**Figure 5.** (A) Retinal expression of Isl1 during the period of horizontal cell neurogenesis, at E13, shows a nearly fivefold difference favoring AJ (P = 0.0007, Student’s t test). A difference is also detected in maturity (P = 0.0028). (B) ASE confirms cis-regulation of Isl1 in AXB/BXA, evidenced by testing the deviation of the allelic expression changes in the retina from the allelic ratio in the genomic DNA in reciprocal F1 hybrids. (P = 0.007). (C) An SNP in the Isl1 5′ UTR creates an E-box sequence in the B6/J strain. The A SNP is the conserved allele. (Alignment generated with CLC Sequence Viewer).


Supporting Information

Whitney et al. 10.1073/pnas.1103253108

SI Materials and Methods

Retinal Immunofluorescence. Mice were given a lethal dose of sodium pentobarbital (120 mg/kg, i.p.) and, once heavily anesthetized, they were intracardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2 at 20 °C). (All procedures were conducted under authorization by the respective institutional animal care and use committees at the University of California at Santa Barbara, Baylor College of Medicine, and the University of Rochester and conform to the American Veterinary Medical Association’s Guidelines on Euthanasia). Retinas were dissected from the eye, prepared as wholemounts, and then labeled using standard immunofluorescence procedures, as described elsewhere (1). The retinas were incubated with a mouse monoclonal antibody to calbindin D-28 (1:10,000; Sigma) and subsequently incubated with a rabbit antimouse IgG conjugated to Cy2 (1:200; Jackson Labs). Only entire, intact, whole retinas were used for subsequent quantification.

Quantification of Horizontal Cell Number. Images of the mosaic of horizontal cells in these wholemounts were obtained using a Nikon FXA fluorescence photomicroscope and an Olympus DP11 digital camera. Labeled horizontal cells were sampled near the optic nerve head (central samples) and near the retinal periphery (peripheral samples) in the four quadrants of each wholemounted retina, from which a mean density for each sample was determined. Each sampled field occupied 0.225 mm² of retinal area (aspect ratio of 1:1.25), so that ~15% of the entire retinal surface was analyzed. Retinal areas were also measured (Biqquant Nova Prime; R&M Biometrics), and multiplied by mean density to estimate the total number of horizontal cells per retina. (All procedures are identical to those we have used elsewhere to compare horizontal cell Biometrics), and multiplied by mean density to estimate the total

Retinal areas were also measured (Bioquant Nova Prime; R&M Biometrics). Only entire, intact, whole retinas were used for subsequent quantification.

Quantitative RT-PCR. Time-mated pregnant mice were heavily sedated and embryos removed for the dissection of retinal tissue. The Thieier stages of embryonic mouse development were used to collect gestationally matched A/J and B6/J embryos on embryonic (E) day 13. Each embryonic sample consisted of pooled retinas from a minimum of five embryos per litter. Retinal tissue was also collected from adult A/J and B6/J mice ranging in age from 114 to 123 d old, averaging 121 d; here, each sample consisted of two pooled retinas from one animal. Following the extraction and verification of RNA integrity, single-strand cDNA was synthesized for quantitative real time RT-PCR that was performed with the BioRad MyQ Single Color Real-Time PCR Detection system. Primers 5’ TGAAGTGACCTCGCCTTGCAAAG 3’ forward and 5’ CATGCTGTGTTGGGTTATCTGCGGG 3’ reverse were used in the amplification of Isl1 transcripts. Normalized amounts were generated using four housekeeping genes for both the embryonic and adult time points.

Rapid Amplification of the cDNA Ends and cDNA Sequencing. RNA was extracted from postnatal day 10 B6/J and A/J retinas, as described previously (2), and used for first strand cDNA synthesis (GE Healthcare), which was amplified (GoTag Flexi DNA polymerase; Promega) using pairs of primers that covered the Isl1 transcript. The 5’ and 3’ rapid amplification of cDNA ends (RACE) was performed using the FirstChoice RLM-RACE kit (Ambion), according to the manufacturer’s protocols, followed by sequencing of the PCR products using dye terminators and capillary electrophoresis (ABI3130; Applied Biosystems). Sequencer software (Gene Codes, version 4.10.1) was used to assemble the sequences and to identify polymorphisms.

Allele-Specific Expression Assay. Samples from each reciprocal F1 cross of the A/J and B6/J strains, AB6F1 and B6AF1, were used, each sample consisting of pooled retinas from two animals averaging 44 d of age. The RNA was extracted from retinas as described previously (2), and genomic DNA was extracted from the corresponding tail clips using the DNeasy Blood and Tissue kit (Qiagen). The DNA samples were used for normalization and assayed at the same time with the RNA pools. First strand cDNA was synthesized using the iScript cDNA synthesis kit (BioRad); cDNA and genomic DNA were amplified (GoTag Flexi DNA polymerase; Promega) using primers located in the same exon (5’ AAGCAGCCGGAGAAGACCAC 3’ and 5’ TCCCTCATGAGCGCATCTGG 3’). Each sample was amplified and analyzed in duplicate. Excess of dNTPs and primers from PCR products were degraded using ExoSap-IT (United States Biochemical). Single-base extension of the PCR products was performed using SNaPshot (Applied Biosystems) and an extension primer (5’ TCCGCAAGGTGTGCAGCTGCTTCTC 3’) located next to a synonymous SNP (accession ID 50976547). The extension products were treated with calf intestinal phosphatase (New England Biolabs) to degrade excess of labeled ddNTPs followed by capillary electrophoresis (ABI3130; Applied Biosystems). Quantification of allelic expression was analyzed using Peak Scanner (Applied Biosystems) using the peak height corresponding to each allele.

Table S1. SNPs and short indels between B6/J and A/J within the coding and putative regulatory regions of *Isl1*

<table>
<thead>
<tr>
<th>Location</th>
<th>Base pair (mm9)</th>
<th>Reference SNP accession ID</th>
<th>B/A allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter</td>
<td>117101122</td>
<td>50480348</td>
<td>T/G</td>
</tr>
<tr>
<td>Promoter</td>
<td>117101115</td>
<td>549680099</td>
<td>C/T</td>
</tr>
<tr>
<td>Promoter</td>
<td>117101086</td>
<td>51306857</td>
<td>T/C</td>
</tr>
<tr>
<td>Promoter</td>
<td>117101009</td>
<td>549678357</td>
<td>A/G</td>
</tr>
<tr>
<td>Promoter</td>
<td>117100884</td>
<td>51567098</td>
<td>C/G</td>
</tr>
<tr>
<td>Promoter</td>
<td>117100098</td>
<td>30049912</td>
<td>A/G</td>
</tr>
<tr>
<td>Promoter</td>
<td>117100063</td>
<td>46344459</td>
<td>C/T</td>
</tr>
<tr>
<td>5′ UTR</td>
<td>117099762</td>
<td>545658554</td>
<td>T/G</td>
</tr>
<tr>
<td>5′ UTR</td>
<td>117099749</td>
<td>NA</td>
<td>T/C</td>
</tr>
<tr>
<td>Intron 1</td>
<td>117099549</td>
<td>NA</td>
<td>G/-</td>
</tr>
<tr>
<td>Intron 1</td>
<td>117099447</td>
<td>NA</td>
<td>T/GorC</td>
</tr>
<tr>
<td>Intron 1</td>
<td>117099003</td>
<td>NA</td>
<td>A/-</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117098742</td>
<td>30022127</td>
<td>C/G</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117098293</td>
<td>29532860</td>
<td>G/A</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117098279</td>
<td>29247586</td>
<td>A/T</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097993</td>
<td>NA</td>
<td>C/G</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097984</td>
<td>NA</td>
<td>T/G</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097982</td>
<td>NA</td>
<td>A/-</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097770</td>
<td>51985069</td>
<td>A/G</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097700</td>
<td>49007764</td>
<td>A/G</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097690</td>
<td>49652582</td>
<td>T/A</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097497</td>
<td>46219885</td>
<td>C/T</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097437</td>
<td>48981166</td>
<td>G/C</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097223</td>
<td>50326404</td>
<td>A/G</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117097088</td>
<td>NA</td>
<td>-/T</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096950</td>
<td>NA</td>
<td>C/-</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096949</td>
<td>NA</td>
<td>T/-</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096744</td>
<td>29972543</td>
<td>G/T</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096717</td>
<td>29775327</td>
<td>G/A</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096568</td>
<td>29783182</td>
<td>G/C</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096351</td>
<td>29730164</td>
<td>C/A</td>
</tr>
<tr>
<td>Intron 2</td>
<td>117096127</td>
<td>29229242</td>
<td>T/C</td>
</tr>
<tr>
<td>Exon 3</td>
<td>11709622</td>
<td>13466915</td>
<td>T/C</td>
</tr>
<tr>
<td>Exon 4</td>
<td>117093450</td>
<td>29525872</td>
<td>C/T</td>
</tr>
<tr>
<td>Exon 4</td>
<td>117093287</td>
<td>50976547</td>
<td>G/A</td>
</tr>
<tr>
<td>Exon 6</td>
<td>117089739</td>
<td>2957061</td>
<td>G/T</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>117089521</td>
<td>29635194</td>
<td>T/G</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>117089225</td>
<td>46842279</td>
<td>C/T</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>117089182</td>
<td>49620560</td>
<td>A/G</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>117089161</td>
<td>NA</td>
<td>T/G</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>11708923-18</td>
<td>NA</td>
<td>-/CCAACT</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>117088752</td>
<td>NA</td>
<td>-/T</td>
</tr>
<tr>
<td>3′ UTR</td>
<td>117088661</td>
<td>NA</td>
<td>T/-</td>
</tr>
</tbody>
</table>

NA, not annotated. Hyphen indicates absence of base.