Title
An integrated genetic map of the pearl locus of mouse chromosome 13.

Permalink
https://escholarship.org/uc/item/8qd6q5t2

Journal
Genome Research, 6(6)

ISSN
1088-9051

Authors
Seymour, AB
Yanak, BL
O'Brien, EP
et al.

Publication Date
1996-06-01

DOI
10.1101/gr.6.6.538

Peer reviewed
LETTERS

An Integrated Genetic Map of the Pearl Locus of Mouse Chromosome 13

Albert B. Seymour,1,2 Brenda L. Yanak,2 Edward P. O’Brien,3
Michael E. Rusiniak,3 Edward K. Novak,3 Larry H. Pinto,4
Richard T. Swank,3 and Michael B. Gorin1,2,5

1Departments of Human Genetics, and 2Ophthalmology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213; 3Molecular and Cellular Biology Department, Roswell Park Cancer Institute, Buffalo, New York 14263; 4Neurobiology Department, Northwestern University, Evanston, Illinois 60201

We have used a Mus domesticus/spretus congenic animal and two interspecific backcross panels to map genetically 30 sequence-tagged sites (STSs) and 13 genes to the vicinity of the pearl locus on mouse chromosome 13. The STSs defining the mapped region are from D13Mit9 to D13Mit37, spanning 10.6 cM. Genes mapped to this region include Versican (Cspg2), GTPase activating protein (Rasa), dihydrofolate reductase (Dhfr), arylsulfatase (Atsl), thrombin receptor (Cf2r), hexosaminidase b (Hexb), 3-hydroxy-3-methylglutaryl coenzyme A reductase (Hmgcr), microtubule associated protein 5/lb (Mtap5), phosphodiesterase (Pde), phosphatidilinositol 3’ kinase (Pik3r1), rat integrin al subunit (Itgala), collagen receptor a2 subunit (Itga2), and 5-hydroxytryptamine la receptor (Htrla). This high resolution genetic map of the pearl region of chromosome 13 establishes the order of multiple markers, including genes whose human homologs are located within a limited region of human chromosome 5, with respect to the phenotypic anchor marker pearl.

The mouse pearl mutation is a recessive, hypopigmentation mutation that affects retinal development and function, and causes inherited platelet storage pool deficiencies (Balkema et al. 1983; Linden and Pinto 1985; O’Brien et al. 1995). Pearl has been studied as a model of human inherited congenital stationary night blindness and the inherited human Hermansky-Pudlak Syndrome. Originally identified in 1954 in a C3H strain, it has subsequently been transferred into a C57Bl/6J line, C57Bl/6J PIN pe/pe (Avner et al. 1988). The pearl locus was first localized to chromosome 13 by linkage to Arylsulfatase B and Lth1 (Elliott et al. 1985). Because of its heritable visual phenotype, pearl served as an anchor locus for other genes and markers that have been mapped to chromosome 13 (Holcombe et al. 1991; O’Brien et al. 1995; Xu et al. 1996).

In an effort to further localize the pearl locus, a congenic strain, B6/spretus pe+PINN12F6, was developed to introduce a highly polymorphic region suitable for the mapping of new markers to the pearl region (Rikke et al. 1993). The selective breeding of a region of Mus spretus containing the pearl locus into a C57Bl/6J background allowed for the binning of genes and other markers to within or outside a 10.0-cM interval that contains the pearl locus.

Interspecific backcross panels have been developed to allow high resolution recombinant mapping (Copeland and Jenkins 1991). Markers that are in the pearl region have been localized partly by using a 96-mouse interspecific backcross panel of C57Bl/6J and M. spretus from Jackson Laboratories. A 528-mouse backcross panel segregating pearl, muted, and satin phenotypes mapped pearl to within a 1.4-cM region (O’Brien et al. 1995). This panel has subsequently been propagated to 1250 mice and the pearl region is now localized to within 0.5 cM.

We have integrated mapping data from these sources as part of our efforts to define the critical pearl region with respect to the closest flanking markers. This integrated map establishes the or-
order of multiple genes and microsatellite markers on chromosome 13.

RESULTS

The congenic B6/spretus pe^PIN12F6 mouse strain contains a 10-cM genomic region of M. spretus DNA surrounding the pearl locus with a small flanking region of C3H genomic DNA from the original pearl strain. The remainder of its genome is derived from C57B1/6J. Forty microsatellites and sequence-tagged sites (STSs) with nine expressed genes were typed in M. spretus C57B1/6J and C3H strains to determine the boundaries of the spretus genomic region containing the pearl gene. STS markers PL2, PL3, D13SH1, D13Gor2, D13Gor3, D13Gor4, D13Mit27, D13Mit28, D13Mit29, D13Mit105, D13Mit106, D13Mit107, D13Mit160, D13Mit161, D13Mit169, D13Mit258, D13Mit104, D13Mit69, D13Mit145, D13Mit191, D13Mit36, D5S39 (human), and mouse genes Dhfr, Hexb, As-1, Hmgcr, Rasa, and Mtap5 were binned within the 10-cM interval. Htrla and Ctla3 were binned telomeric to the 10-cM interval.

Southern hybridization with the probe for Srd5 alpha revealed a 1.7-kb informative allele in the congenic that matched the C3H parental strain. We were unable to distinguish whether the position of Srd5 alpha, based on the Southern blot data, was centromeric or telomeric to the M. spretus region. We have placed it in the proximal C3H region based on the 1993 and 1994 consensus maps (Justice and Stephenson 1993, 1994) and a previous study that localized Srd5 alpha centromeric with respect to As-1 and Rasa (Jenkins et al. 1991), which we have binned into the M. spretus region.

The strain of origin of most alleles could be determined by directly comparing the products of the appropriate parental DNAs. D13Mit36, however, identified an allele in the congenic B6/spretus pe^PIN12F6 mouse that could either be attributed to M. spretus or C57B1/6J. Examination of the locus in the original C57B1/6J PIN pe/pe mouse showed that the D13Mit36 allele corresponded to the C3H allele rather than to the C57B1/6J allele. We concluded that the observed allele for D13Mit36 in the congenic was specific for M. spretus. In contrast, D13Mit37, which is telomeric to D13Mit36, identified a C3H allele in both the congenic and C57B1/6J PIN pe/pe. Taken together, this indicates that the boundary of the M. spretus region in this congenic strain must lie between D13Mit36 and D13Mit37.

We mapped Dhfr, Pik3r1, and several microsatellites onto the Jackson [(C57B1/6J × spretus) F1 × M. spretus] (BSS) interspecific backcross panel. These data are shown in Figure 1. The marker positions were calculated using Map manager v. 2.6. Marker order was determined by minimizing the number of multiple recombination events. Dhfr showed a band shift when analyzed by single-strand conformation polymorphism (SSCP); C57B1/6J revealed a higher mobility band than M. spretus. Pik3r1 revealed a T-C polymorphism at nucleotide 2833 in the untranslated region between C57B1/6J and M. spretus, respectively. This single-base polymorphism was not consistently informative by SSCP analysis, therefore a PCR-based sequence analysis was performed to determine its segregation throughout the backcross panels. The resolution of this panel is 7.9 cM at the 95% confidence level.

We further localized the pearl region with the interspecific backcross panel described by O'Brien et al. (1995). This panel, originally de-
scribed with 528 mice, has been expanded to 1250 mice. It has a resolution of 0.08 cM with a 95% confidence interval up to 0.2 cM. The results from this backcross panel are summarized in Figure 2. These data place the pearl critical region to within a 0.5-cM region with the closest flanking markers being Centromere–D13Mit28, D13Mit29–(0.3 cM)–Pearl–(0.0 cM)–D13Mit160, D13Mit1258, D13Mit104, D13Mit161, D13Mit169, D13Mit108–(0.2 cM)–Cf2r–Telomere. The T–C polymorphism detected in Pik3rI between M. spretus and C57Bl/6J, using the Jackson panel, was also observed between Satin-Muted-Pearl (SaMuPe) and PWK, respectively. Dhfr analysis by SSCP produced higher mobility bands in the PWK DNA when compared with SaMuPe DNA. The Cf2r PCR product differed by 20 bp between PWK (340 bp) and SaMuPe (320 bp), using primers that flank a GA repeat within the (n-2) intron.

**DISCUSSION**

High resolution genetic mapping is an essential step in the positional cloning of any disease gene. With the concentration of highly polymorphic microsatellites increasing rapidly, this approach becomes increasingly powerful. The specific breeding of mice with visual heritable phenotypes has provided the meiotic events necessary to genetically map markers using the visual phenotype as an anchor locus (Holcombe et al. 1991; O'Brien et al. 1995; Xu et al. 1996). We have used two mapping tools that take advantage of the heritable pearl phenotype and the integration of an interspecific backcross panel of C57B1/6J and M. spretus to construct a genetic map of the pearl region of chromosome 13.

The congenic B6/spretus pe^PINN12F6 enabled us to bin genes and other markers into a limited region including and flanking the pearl locus. The 96-mouse BSS interspecific backcross panel increased the resolution and aided in the ordering of the markers. However, because of the low number of informative meioses, the resolution was only 7.9 cM at the 95% confidence level. The 1250-mouse interspecific backcross panel of PWK and SaMuPe has increased the resolution to 0.2 cM at the 95% confidence level.

This integrated map identifies a number of potential discrepancies with the published consensus map (Justice and Stephenson 1994), primarily not with the order of marker sets, but with the alignment of the pearl locus and genes with respect to the microsatellite markers. We mapped Hexb telomeric to the pearl locus by 0.7 cM, and nonrecombinant with D13Gor3. We have further refined the initial mapping of Pik3rI (Hoyle et al.
1994) to 1.8 cM telomeric to the set of markers nonrecombinant with the pearl locus. D13Mit28 and D13Mit29 are nonrecombinant with each other in this panel and D13Mit105 and D13Mit106 are 0.1 cM and 0.2 cM centromeric to D13Mit28/29, respectively. D13Mit104 has been placed telomeric to D13Mit105 by 0.4 cM and telomeric to D13Mit106 by 0.6 cM. The positions of D13Mit145, D13Mit144, and D13Mit109 have been localized to 0.3, 0.6, and 1.2 cM, respectively, telomeric to D13Mit161. In the Jackson backcross panel we identified a recombination between D13Mit160 and the pearl locus. A similar recombination event has not been observed in the 1250-mouse interspecific backcross panel. Because of the higher resolution of this panel, we have placed D13Mit160 centromeric but closer to D13Mit258, D13Mit104, D13Mit161, D13Mit169, and D13Mit108.

Many of the genes that we placed on this map were potential candidate genes for the pearl mutation, specifically Csgp2, Rasa, Itga2, and Pik3rl. The mapping results have eliminated these, as well as the other mapped genes, as candidate genes based on recombination events from the pearl locus.

All of the genes that we have placed in the region of the pearl locus on mouse chromosome 13 have been associated with a limited region of human chromosome 5. The orders of these genes and their respective genetic distances on the human genome have not been established. Although it is likely that this region of mouse chromosome 13 is syntenic with human 5q13, further human genome physical mapping efforts will be necessary to establish if small rearrangements distinguish these regions of the two genomes.

The human spinal muscular atrophy (SMA) locus is telomeric to the syntenic pearl region of mouse chromosome 13 based on the human chromosome 5 mapping data. MAP1B, the human homolog of Mtap5, which shows linkage to the human SMA locus is 1.6 cM distal to the pearl region. It has been reported that this region of the genome is very unstable with regards to genomic rearrangements (Selig et al. 1995). One pearl lineage has been reported to have a spontaneous reversion rate of 0.5% (Pinto et al. 1985), suggesting that, at least for one pearl allele, the disease phenotype could be caused by a DNA insertion. Elucidation of the actual pearl gene and the characterization of the different pearl mutations may clarify this potential genomic instability with respect to this gene.

The high resolution mapping and the abundance of markers in this region allow the initiation of physical mapping techniques to identify the gene responsible for the pearl phenotype as well as providing a set of markers that can be used in the mapping of other genes to this region of mouse chromosome 13.

**METHODS**

The interspecific backcross panel of PWK and SaMuPe has been described previously (O’Brien et al. 1995). The pearl (pearl PIN<sup>pejpe</sup>) and congenic B6/spretus pe<sup>pej</sup>N12F6 have been described previously (Avner et al. 1988; Rikke et al. 1993). The C3H, C57Bl/6J mice and the 96-mouse backcross panel of B6, were purchased from Jackson Laboratories.

Genomic DNA was isolated from mouse spleen and kidney according to standard protocols (Sambrook et al. 1989; Taylor and Rowe 1989). For Southern blotting, 10 μg of mouse genomic DNA was digested with EcoRI, PstI, BamHI, BglII, or HindIII according to manufacturer’s conditions. The digests were electrophoresed on 1.0% agarose (FMG), stained with EtBr, and transferred to Hybond N+ filters (Amersham). The DNA was immobilized at 80°C for 2 hr. The hybridizations were performed as described previously (Xu et al. 1996). Markers were determined to be within the pearl-containing M. spretus region of the congenic animal if the hybridization patterns of the congenic animal were identical to those of M. spretus and distinct from the C57Bl/6J background.

Candidate genes that have been mapped previously to mouse chromosome 13 or localized to a presumed syntenic region of human chromosome 5 were used to determine their position with respect to the pearl locus. The probes are described in Table 1. All probes were excised from the vector with the appropriate enzyme, separated by gel electrophoresis on 1.0% agarose gel, excised, and purified with Geneclean (Bio 101, Inc.). The DNA Probes were labeled by the random hexamer method with [α-<sup>32</sup>P]dCTP (Feinberg and Vogelstein 1983; NEN/Dupont). D13Mit series primer sets were purchased from Research Genetics. The forward primer for each set was end-labeled with T4 kinase (GIBCO) and [γ-<sup>32</sup>P]ATP (NEN/Dupont). The PCR conditions were 94°C for 2 min followed by 35 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min with 1 unit of Taq polymerase in 25 mm TAPS (pH 9.3), 1.5 mm MgCl₂, 50 mm KCl, 1 mm DTT, and 0.2 mM each of dATP, dCTP, dGTP, and dTTP in a final volume of 20 μl. Products were electrophoresed on a 6.0% polyacrylamide, 7 μm denaturing sequencing gel, dried, and analyzed by autoradiography.

The D13Gor primers were described previously (Xu et al. 1996). The Pik3rl primers are specific for nucleotides 2752–2935 in the 3' untranslated region of phosphatidylinositol 3' kinase regulatory subunit resulting in a product of 184 bp. The primer sequences are as follows. Pik3rlF 5'-TTCTCTCACTTCAAGCCACCCAAG-3'; Pik3rlR 5'-AGGTTAGAAGCTCTGTCATCCAAC-3'. The Dhfr primers were OD3PF 5'-ACAGGGATCTCTTAGTCAAGTC-3' and OD3PR 5'-GAGGCTCTAGTGGAGGAGG-3'. Thrombin receptor (Gfr2), D13SH1, PL2, and PL3 primer sequences...
<table>
<thead>
<tr>
<th>Gene</th>
<th>Probe name</th>
<th>Enzyme a</th>
<th>Informative alleles d</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTPase-activating protein</td>
<td>Rasa</td>
<td>EcoRI</td>
<td>14.5, 9.4</td>
<td>Hsieh et al. (1989)</td>
</tr>
<tr>
<td>Arylsulfatase B</td>
<td>As1</td>
<td>BamHI</td>
<td>22, 9.0, 7.5</td>
<td>Schuchman et al. (1990)</td>
</tr>
<tr>
<td>Hexosaminidase B</td>
<td>HexB</td>
<td>BamHI</td>
<td>16.4, 5.4</td>
<td>Bapat et al. (1988)</td>
</tr>
<tr>
<td>3-hydroxy-3-methylglutaryl</td>
<td>Hmgcr</td>
<td>N.D.</td>
<td>4.5</td>
<td>Sundaresan et al. (1989)</td>
</tr>
<tr>
<td>coenzyme A reductase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microtubule-associated protein 5/1B</td>
<td>Mtap5</td>
<td>EcoRI</td>
<td>4.5</td>
<td>Garner et al. (1990)</td>
</tr>
<tr>
<td>Cyclic nucleotide phosphodiesterase</td>
<td>Pde</td>
<td>N.D.</td>
<td>N.D.</td>
<td>Miltovich et al. (1994)</td>
</tr>
<tr>
<td>Collagen receptor a2 subunit</td>
<td>Itga2</td>
<td>N.D.</td>
<td>N.D.</td>
<td>Takada and Hemler (1989)</td>
</tr>
<tr>
<td>Rat integrin a1-subunit</td>
<td>Itga1</td>
<td>N.D.</td>
<td>9.6</td>
<td>Ignatius et al. (1990)</td>
</tr>
<tr>
<td>5-hydroxytryptamine</td>
<td>Htr1a</td>
<td>EcoRI</td>
<td>1.2</td>
<td>Koblika et al. (1987)</td>
</tr>
<tr>
<td>(serotonin) 1α receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroid 5α-reductase</td>
<td>Srd5alpha</td>
<td>BglII</td>
<td>1.7</td>
<td>Jenkins et al. (1991)</td>
</tr>
<tr>
<td>Cytotoxic T lymphocyte</td>
<td>Ctlα3</td>
<td>EcoRI</td>
<td>24.5, 13.5</td>
<td>Gershenfeld and Weismann (1986)</td>
</tr>
<tr>
<td>associated sequence 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Enzyme used to digest genomic DNA to identify polymorphism.

Genomic DNA from the congenic strain was compared to that of C57 and Spretus as described in the text.

Genomic DNA from PWK was compared to SaMuPe to observe crossovers in the backcross panel.

Allele sizes in kilobases (kb).

(N.D.) Not determined.

have been described previously (Casavant and Hardies 1993; Rikke et al. 1993; Poirier et al. 1996). The PCR conditions for \( D h f r \) were identical to those for the D13Mit series. The PCR conditions for \( P i k 3 r 1 \) required an annealing temperature of 60°C.

SSCP analysis for \( D h f r \) was done per standard protocol with a few modifications (Dracopoli et al. 1994). Samples were denatured in 98% formamide/0.025% bromphenol blue/0.025% xylene cyanol and 0.65 mm NaOH at 95°C for 5 min and electrophoresed on a 0.5 × MDE gel (Hydrolink) overnight at 4.0°C. The gel was dried and analyzed by autoradiography.

DNA sequencing of PCR products was performed using a cycle sequencing kit (Epicentre). Primers were end-labeled as described above. The products were separated on a 6.0% 7 M urea denaturing sequencing gel, dried, and analyzed by autoradiography.

ACKNOWLEDGMENTS

We thank Drs. Lucy Rowe and Mary Barter at Jackson Laboratory for assistance in the mapping on the Jackson backcross panel, and we are grateful to Dr. Stephen C. Hardies at The University of Texas Health Science Center for providing \( D h f r \) primers (OD3PF, OD3PR) and critically reading this manuscript. This work was supported by National Institutes of Health (NIH) grant EY9192, National Eye Institute Core grant EYO8098, and Eye and Ear Foundation of Pittsburgh (M.B.G.), NIH grant EY01221 (L.H.P.) and National Heart, Lung, and Blood Institute grant HL31698 (R.T.S.).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

REFERENCES


Received February 26, 1996; accepted in revised form April 16, 1996.
An integrated genetic map of the pearl locus of mouse chromosome 13.


*Genome Res.* 1996 6: 538-544

Access the most recent version at doi:10.1101/gr.6.6.538