Running title: A New Locus for Autosomal Recessive Retinitis Pigmentosa

A New Locus for Autosomal Recessive Retinitis Pigmentosa Maps to 1p13-1p21

Amalia Martínez-Mir,* Mònica Bayés,* Lluïsa Vilageliu,* Daniel Grinberg,* Carmen Ayuso,§ Teresa del Río,§ Blanca García-Sandoval,§ Elena Bussaglia,# Montserrat Baiget,# Roser Gonzàlez-Duarte,* and Susana Balcells*

* Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Spain; § Departamento de Genética, Fundación Jiménez Díaz, Madrid, Spain; and # Unitat de Genètica Molecular, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Corresponding author: Susana Balcells
Dept. de Genètica
Facultat de Biologia
Universitat de Barcelona
Avda. Diagonal, 645
08071. Barcelona. Spain
Telf.: 34/3/402 15 01
Fax: 34/3/411 09 69

Abstract

Autosomal recessive retinitis pigmentosa (arRP) is characterized by considerable allelic and nonallelic heterogeneity. Mutations have been described in the rhodopsin gene (RHO), the genes encoding the α and β subunits of rod phosphodiesterase (PDEA and PDEB) and the gene encoding the α subunit of the cGMP-gated channel (CNCG). In addition, linkage studies in single extended pedigrees have defined two new arRP loci, at 1q and 6p. In order to identify the disease gene in a Spanish consanguineous arRP family, a linkage analysis was undertaken. After testing 102 polymorphic markers, a significant positive lod score ($Z_{\max}=3.64$ at $\theta=0$) was obtained with marker D1S188 at 1p13-p21, the same region where the Stargardt and fundus flavimaculatus (FFM) loci were previously defined. Exhaustive ophthalmologic examination of the patients clearly distinguished the disease from the Stargardt and FFM phenotypes, and revealed an atypical form of arRP with choroidal atrophy as a distinctive feature.

Retinitis pigmentosa is a clinically and genetically heterogeneous group of retinal degenerations characterized by progressive loss of night and peripheral vision and, eventually, severe visual loss (9). As is the case of the autosomal dominant (adRP) and the X-linked (xLRP) forms of the disease, autosomal recessive retinitis pigmentosa (arRP) is characterized by allelic and nonallelic heterogeneity. Mutations cosegregating with the disease have been described in the rhodopsin gene (RHO) (15, 24), the genes encoding the α and β subunits of rod phosphodiesterase (PDEA and PDEB, respectively) (3, 10, 21, 28, 29) and the gene encoding the α subunit of the cGMP-gated channel (CNCG) (6). In addition, genetic linkage studies in single extended pedigrees have identified two new arRP loci, at 1q (18, 30) and 6p (14).

Among the Spanish population, 39% of the RP pedigrees show an autosomal recessive pattern of inheritance (arRP) (2). Here we report the case of a Spanish consanguineous arRP family (M-33) with six out of seven sibs affected (Fig.1). Ophthalmologic examination of both parents revealed no manifestation of RP; the father (who died aged 72), showed no symptoms other than glaucoma, whereas the mother (aged 82) is totally asymptomatic. This fact, together with the consanguinity (second-cousin marriage), indicate an autosomal recessive pattern of inheritance. Clinical evaluation of the affected members suggested an aggressive form of the disease, with night blindness as the first symptom appearing in the first decade of life (mean age of onset, 8 years), followed by a decrease in visual acuity, starting at 14 years of age. Fundus examination and fluorescein angiographies (Fig.2) of the affected sibs in the fourth decade of life revealed papillary pallor, attenuated vessels, peripheral scattered pigmentation, bone spicule-like pigmentation reaching some areas of the macula and severe atrophy of the retinal pigment epithelium (RPE). At present, the older sibs show a complete scotoma while the youngest (around 45 years of age) still retain central vision and some peripheral islands. Finally, electrophysiological studies revealed abolished rod and mixed responses and a very diminished cone response. Taking into account the clinical and electrophysiological findings and the autosomal recessive pattern of inheritance, we conclude that the family presents an atypical form of RP with the distinctive feature of choriocapillaris atrophy.

In order to identify the disease gene segregating in this family, we performed a linkage analysis. Three sets of markers were analyzed: those defining loci for RP and other eye disorders; those corresponding to the loci of retina-specific genes and a set of anonymous markers from the Weissenbach set.

Primers for the microsatellite markers were from the MapPairs set (Research Genetics, Huntsville, AL, USA) and those for gene-specific polymorphisms were synthesized commercially according to data from GDB (Johns Hopkins University) or from sequence data in the GenBank/EMBL database. DNA was extracted from peripheral blood leukocytes

of all the members of the family (except the father) using standard methods (22). Genomic DNA was amplified by the polymerase chain reaction (PCR) in a total reaction volume of 25 μ l containing 300 ng of genomic DNA, 6.6 pmols of each primer, 1 unit of Dynazyme DNA polymerase (Fynzymes Oy, Espoo, Finland) in the recommended buffer and 200 μ M dNTPs. Reactions were subjected to 35 cycles of 40s at 94°C and 30s at 45-60°C (depending on the primer pair), followed by a final extension step of 5 min at 72°C, in a thermal cycler (Perkin Elmer Cetus, Norwalk, CT, USA). The amplified products were then separated by electrophoresis on 6% non-denaturing polyacrylamide gels and stained with ethidium bromide.

Linkage analysis was carried out using the LINKAGE package, version 5.1 (16). Population allele frequencies for those markers that showed evidence of linkage were calculated in a sample of 76 chromosomes.

The eight members of the family were typed for 102 polymorphic markers. Significant exclusion was obtained for all loci and gene markers except for those corresponding to Stargardt's disease and late onset fundus flavimaculatus (FFM), at 1p13-p21 (7, 12). Haplotypes for the most informative markers in this region are shown in Figure 1. Recombination events in individuals II.3 and II.7 locate the disease locus within a 10.3 cM interval between markers D1S167 and D1S497. This interval includes a homozygosity region of at least 3.5 cM (D1S435 through D1S236). Two-point lod scores between the disease and the markers are summarized in Table 1. Maximum lod scores were obtained at $\theta=0.0$ for all markers from D1S435 to D1S236. However, the highest value corresponded to D1S188 ($Z_{\max}=3.64$), since a low-frequency allele segregates in the family.

A candidate gene, GNAT2 (α subunit of cone transducin), is physically located at 1p13 (4, 20). However, Gerber et al. (8) showed that this gene is not responsible for Stargardt's disease. To test the possible involvement of GNAT2 in arRP in family M-33, we analyzed an A-to-G transition in exon five of the gene (25). Lack of cosegregation between this polymorphism and the disease ruled out GNAT2 as a cause of arRP in the family. Recently, Anderson et al. (1) constructed a YAC contig of the region encompassing the Stargardt locus but failed to place either GNAT2 or another candidate, the retinal pigment epithelium-specific protein gene (RPE65), to any YAC in this region.

The linkage data presented here identify a locus for an atypical form of arRP, with choroidal atrophy as a distinctive feature, at 1p13-p21. This is the same region where the Stargardt and FFM loci have been defined. A comparison of the minimal criteria for diagnosis of Stargardt's disease (12) with the clinical findings in family M-33 is shown in Table 2. Although the early age of onset of the disease in M-33 is similar to that of Stargardt, the ERG patterns are quite distinct, particularly the abolished rod response. Additionally, fundus findings in M-33 show features of RP that are clearly different from Stargardt's. The above arguments can also be applied to rule out FFM in the family, since its clinical findings

are basically the same as those for Stargardt except for a delayed age of onset and a slower progression of the disease (7).

The fact that three retinal degenerations map to the same genomic region is reminiscent of the situation at region 11q13, where loci for several distinct eye disorders have been mapped (13, 17, 19, 26, 27). An interesting question is whether these three traits are caused by mutations in the same gene. Allelic heterogeneity has been documented. For instance, mutations at the peripherin/RDS gene have been associated with adRP, retinitis pigmentosa punctata albescens and several types of macular dystrophies (11, 23, 30, 31). Moreover, mutations at the PDEB and RHO genes may lead either to RP or to congenital stationary night blindness. The alternative to allelic heterogeneity would be a situation in which different genes, clustered together in region 1p13-p21, were responsible for these three clinically distinct entities. The identification of the causative genes may clarify this issue.

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FIG. 1. Pedigree of family M-33. Black symbols represent affected individuals. Haplotypes for the most informative markers from the 1p region are shown. The haplotype most likely segregating with the disease is boxed.

FIG. 2. Characteristic clinical features of family M-33. (a) Fundus photograph of the left eye of individual II.1; note the choriocapillaris atrophy allowing visualization of the large choroidal vessels; (b) Fluorescein angiography of individual II.6 showing the severe choriocapillaris atrophy.

TABLE 1

**Two-point lod scores between ARRP and Markers on Chromosome
1p13-p21**

Lod score (Z) at recombination fraction (θ)									
Marker	0	0.01	0.05	0.10	0.20	0.30	0.40	$\theta_{\max}$	$Z_{\max}$
D1S167	$-\infty$	0.688	1.132	1.122	0.838	0.492	0.176	0.07	1.153
D1S435	2.673	2.606	2.340	2.015	1.401	0.837	0.320	0.00	2.673
D1S188	3.648	3.569	3.251	2.848	2.036	1.247	0.522	0.00	3.648
D1S2849	2.639	2.573	2.311	1.991	1.386	0.829	0.318	0.00	2.639
D1S2868	2.567	2.502	2.247	1.936	1.349	0.809	0.309	0.00	2.567
D1S424	2.714	2.646	2.377	2.047	1.422	0.848	0.324	0.00	2.714
D1S236	2.639	2.573	2.311	1.991	1.386	0.829	0.318	0.00	2.639
D1S497	$-\infty$	1.648	2.059	1.995	1.557	0.980	0.369	0.06	2.065

TABLE 2**Comparison between the minimal criteria for diagnosis of Stargardt's disease and the clinical findings in family M33**

Phenotypic trait	Stargardt's disease	M33 phenotype
Loss of visual acuity	7-12 years of age	14 years of age
Alteration of the macula	prevalent	some bone-spicule spots
Aspect of peripheral retina	normal	pigmented
Calibre of retinal vessels	normal	attenuated
Pigmented bone-spicules	absent	present
Fluorescein angiography	abnormal choroid	diffuse choriocapillaris atrophy
Visual field	central scotoma	total scotoma (older sibs), retention of central visual field (younger sibs)
Color vision	abnormal	n.a. ^a
ERG	normal in early stage and abnormal photopic ERG in advanced stage	abolished scotopic and mixed ERG, decreased photopic ERG

^a n.a.: not available due to the serious visual impairment of the patients.

D1S454	2122	2122	2113	2122	2113	2122	2122
D1S207	2 2	2 2	2 1	2 2	2 1	2 2	2 2
D1S167	5 53	5 5	535	5 5	5 7	5 5	5 5
D1S435	3 13	3 3	133	3 3	3 4	3 3	3 3
D1S188	134	1313	4713	1313	1322	1313	1313
D1S2849	477	4747	2 47	4747	4773	4747	4747
D1S2868	2 2	2 2	122	2 2	2 1	2 2	3 2
D1S424	1212	1212	12	1212	1231	1212	2211
D1S236							
D1S497							
D1S223							
D1S206							