Original Article

A Novel Locus for X-linked Retinitis Pigmentosa

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Abstract

Introduction: Retinitis pigmentosa (RP) is the most prevalent group of inherited retinopathies and demonstrates considerable clinical and genetic heterogeneity, with wide variations in disease severity, progression, and gene involvement. We studied a large family with RP to determine the pattern of inheritance and to identify the disease-causing gene/locus. Materials and Methods: Ophthalmic examination was performed on 35 family members to identify affected individuals and carriers and to characterise the disease phenotype. Genetic linkage analysis was performed using short tandem repeat (STR) polymorphic markers encompassing the known loci for X-linked RP (xlRP) including RP2, RP3, RP6, RP23, and RP24. Mutation screening was performed by direct sequencing of PCR-amplified genomic DNA of the RP2 and RPGR genes of the affected individuals. Results: A highly penetrant, X-linked form of RP was observed in this family. Age of onset was from 5 to 8 years and visual acuity ranged from 20/25 in children to light perception in older adults. Linkage analysis and direct sequencing showed that no known loci/genes were associated with the phenotype in this kindred. Conclusion: A novel disease gene locus/loci is responsible for the xlRP phenotype in this family.

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Key words: Genetic linkage, Mutation screening, Retinopathy

Introduction

Retinitis pigmentosa (RP) is characterised by initial night blindness followed by progressive loss of visual fields and eventually, loss of central vision. RP is the most prevalent group of inherited retinopathies, affecting approximately 1 in 3500 individuals.[1,2] RP demonstrates considerable clinical and genetic heterogeneity, with wide variations in disease severity, clinical phenotype, age of onset, rate of progression, mode of inheritance, and number of genes involved. Fundus examination of RP reveals bone spicule pigmentation in the retina (hence the name "retinitis pigmentosa"), narrowing of retinal vessels, depigmentation of the retinal pigment epithelium, and waxy pallor of the optic discs. Histopathologic studies demonstrate initial loss of rod photoreceptors followed by loss of cone photoreceptors. RP patients with advanced disease may demonstrate low amplitude or non-detectable electroretinograms (ERGs) coinciding with severe loss of peripheral visual fields and central vision. Some patients may also develop cystoid macular oedema early in the course of the disease, leading to central vision loss. Despite much research effort and the prevalence of the disease, there is no effective treatment for RP at the present time.

RP may be transmitted in various patterns: the trait is autosomal recessive in approximately 14% of patients, whereas the trait is autosomal dominant in 17% of patients. In addition, 10% of cases are X-linked, and 42% occur as simplex cases. The remaining 17% of cases are syndromic and may involve additional symptoms such as deafness, cerebellar ataxia, and mental retardation (Usher syndrome, Bardet-Biedl syndrome, Batten-Spielmeyer-Vogt disease, and Refsum's disease).

X-linked RP is the most severe form of RP. Affected individuals experience a decrease in peripheral and night vision during the second or third decade of life. Patients tend to present with night blindness and constriction of visual field during the third and fourth decades of life.[3] In contrast, visual acuity and colour vision can be normal until advanced stages of the disease in RP patients.

X-linked RP is genetically heterogeneous, with loci

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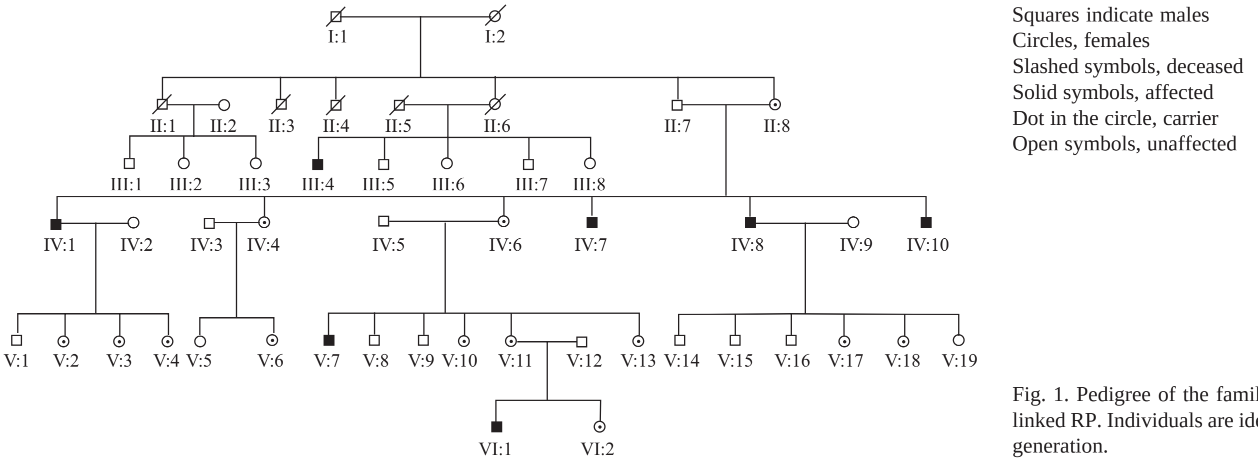

Fig. 1. Pedigree of the family with X-linked RP. Individuals are identified by generation.

localised to Xp11.2 (RP2), Xp21.1 (RP3), and Xp21.2-21.3 (RP6), Xp22 (RP23), and Xq26-27 (RP24) (Available at: http://www.sph.uth.tmc.edu/RetNet/disease.htm). Genes for RP2 and RP3 have been cloned.[4-7] The RPGR (RP3) gene encodes a protein with homology to RCC1 (regulator of chromatin condensation-1), a guanine nucleotide exchange factor for the small GTPase Ran, a protein involved in nuclear trafficking. RPGR interacts with a protein termed RPGR-interacting protein (RPGRIP).[8] We investigated 35 members of a large, Caucasian, Utah family with RP to determine the pattern of inheritance and identify the disease-causing gene.

Materials and Methods

Approval for this study was obtained from the Institutional Review Board of the University of Utah, USA, and informed consent was obtained from all participants in accordance with the tenets of the Declaration of Helsinki and guidelines of the National Institutes of Health (NIH) on human subject research. A complete ophthalmic history and examination was performed on 35 individuals in the family and included assessment of visual acuity and detailed examination of the anterior segment and fundus using colour photography. Several affected individuals also underwent fluorescein angiography and electrophysiological studies. Male individuals were diagnosed with RP if they had night blindness, decreased visual fields, and bone spicule pigmentation on fundus examinations. Female carriers were diagnosed based on fundus appearance of mosaic hyperpigmentation in the peripheral retina.

Initial genetic linkage studies were performed on all living affected patients whose disease status could be determined with certainty as well as known carriers. Genomic DNA was extracted from blood samples by standard methods. Genetic linkage analysis was performed using short tandem repeat (STR) polymorphic markers encompassing the known loci for xlRP, including RP2, RP3, RP6, RP23, and RP24.[9,10] Linkage analysis was then used to determine the LOD score in each locus using the LINKAGE software package.[11-14] Mutation screening was performed by direct sequencing of PCR-amplified genomic DNA corresponding to each exon of the RP2 and RPGR genes (including ORF15) of the affected individuals as described previously.[15,16]

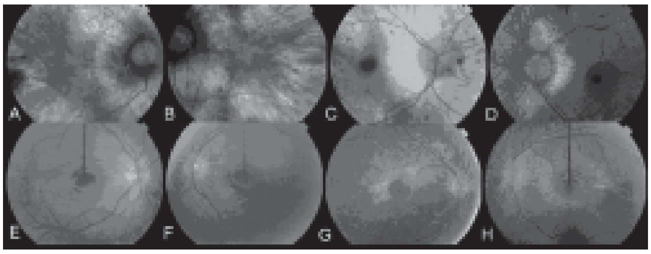

Fig. 2. A,B: Fundus photographs of individual III:4 (51 years old) with extensive pigment change and retinal pigment epithelium (RPE) atrophy. Visual acuity (VA): light perception, OU. C,D: Individual IV:7 (38 years old) with extensive RPE atrophy. VA: light perception, OU. E,F: Individual VI:1 (5 years old) with early pigment changes. VA: 20/20 OU. G,H: Individual IV:6 (female carrier) with depigmented spots in the retina. VA: 20/20, OU.

Results

Ophthalmic examination found 7 affected individuals and 13 carriers among the 35 tested family members. A 5-generation pedigree was compiled and revealed X-linked inheritance (Fig. 1). Age of onset was from 5 to 8 years and visual acuity ranged from 20/25 in children to light perception in older adults. Fundus examination and fluorescein angiography in affected patients demonstrated a typical clinical phenotype of RP, including bone spicule pigmentation in the peripheral retina and extensive retinal and RPE atrophy in the advanced stages of the disease (Fig. 2). The retina of female carriers showed a mosaic pattern of depigmented spots (Fig. 2, G, H). ERGs revealed markedly decreased scotopic and photopic amplitude, consistent with the diagnosis of RP (data not shown).

Genetic linkage studies showed that no known loci/genes (RP2, RP3, RP6, RP23, and RP24) were associated with

Table 1. Exclusion of Linkage for Known X-linked RP Loci/Genes

Loci/genes	Marker	LOD scores at different recombination fractions (θ)							
		0	0.01	0.02	0.05	0.1	0.2	0.3	0.4
RP6	DXS1226	-∞	-3.61	-2.73	-1.60	-0.81	-0.17	0.06	0.10
(Xp21.3)	DXS1214	-∞	-3.61	-2.73	-1.60	-0.82	-0.17	0.06	0.10
RP3/RPGR, RP15	DXS9907	-∞	-3.61	-2.73	-1.60	-0.82	-0.17	0.06	0.10
(Xp21.1)	DXS1068	-∞	-1.61	-1.04	-0.32	0.132	0.42	0.43	0.28
RP2	DXS1003	-∞	-4.50	-3.60	-2.44	-1.58	-0.78	-0.37	-0.13
(Xp11.3)	DXS1208	-∞	-1.62	-1.04	-0.34	0.08	0.33	0.28	0.11
RP23	DXS1223	-∞	-2.50	-1.92	-1.16	-0.63	-0.18	-0.00	0.04
(Xp22)	DXS7161	-∞	-3.91	-3.02	-1.88	-1.07	-0.37	-0.08	0.02
RP24	DXS8094	-∞	-1.92	-1.35	-0.65	-0.20	0.06	0.10	0.06
(Xq26-27)	DXS8043	-∞	-3.61	-2.74	-1.62	-0.86	-0.26	-0.05	-0.00
	DXS1227	-∞	-3.90	-3.02	-1.88	-1.07	-0.37	-0.08	0.02

the phenotype in this kindred (Table 1). Direct sequencing of the RP2 and RPGR genes (including ORF15) showed no mutations in any of the affected individuals in this family.

Discussion

It is important to study the genetic basis of RP because it is the most common cause of inherited retinal degeneration with visual impairment, affecting 1.5 million individuals worldwide. The primary intention of this study was to identify a novel disease gene locus leading to a severe, childhood X-linked form of RP (xlRP) with the ultimate goal of elucidating the underlying molecular mechanisms that lead to retinal degeneration. We identified a large family with a highly penetrant, X-linked form of RP for our study. Clinical features in this family ranged from severe vision loss in elderly patients to asymptomatic young boys. Female carriers showed a characteristic mosaic pattern of depigmentation in the retina.

An initial candidate gene-directed scan excluded all known loci for xlRP as causal genes in this xlRP family. Therefore, we hypothesise that a novel disease gene locus/loci is responsible for the xlRP phenotype in this family. This finding provides further evidence of the genetic heterogeneity of x-linked RP. Identification of this novel gene for xlRP will provide new insight into the pathogenesis of RP and may reveal new avenues for therapy.

REFERENCES

1. Humphries P, Kenna P, Farrar GJ. On the molecular genetics of retinitis pigmentosa. Science 1992;256:804-8.
2. Rivolta C, Sharon D, DeAngelis MM, Dryga TP. Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. Hum Mol Genet 2002;11:1219-27. Erratum in: Hum Mol Genet 2003;12:583-4.
3. Bird AC. X-linked retinitis pigmentosa. Br J Ophthalmol 1975;59: 177-99.
4. Schwahn U, Lenzner S, Dong J, Feil S, Hinzmann B, van Duijnhoven G, et al. Positional cloning of the gene for X-linked retinitis pigmentosa 2. Nat Genet 1998;19:327-32.
5. Meindl A, Dry K, Herrmann K, Manson F, Ciccodicola A, Edgar A, et al. A gene (RPGR) with homology to the RCC1 guanine nucleotide exchange factor is mutated in X-linked retinitis pigmentosa (RP3). Nat Genet 1996;13:35-42.
6. Roepman R, van Duijnhoven G, Rosenberg T, Pinckers AJ, Bleeker-Wagemakers LM, Bergen AA, et al. Positional cloning of the gene for X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1. Hum Mol Genet 1996;5:1035-41.
7. Vervoort R, Lennon A, Bird AC, Tulloch B, Axton R, Miano MG, et al. Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. Nat Genet 2000;25:462-6.
8. Hong DH, Yue G, Adamian M, Li T. Retinitis pigmentosa GTPase regulator (RPGRr)-interacting protein is stably associated with the photoreceptor ciliary axoneme and anchors RPGR to the connecting cilium. J Biol Chem 2001;276:12091-9.
9. Kniazeva M, Chiang MF, Morgan B, Anduze AL, Zack DJ, Han M, et al. A new locus for autosomal dominant stargardt-like disease maps to chromosome 4. Am J Hum Genet 1999;64:1394-9.
10. Kniazeva M, Traboulsi EI, Yu Z, Stefko ST, Gorin MB, Shugart YY, et al. A new locus for dominant drusen and macular degeneration maps to chromosome 6q14. Am J Ophthalmol 2000;130:197-202.
11. Lathrop GM, Chotai J, Ott J, Lalouel JM. Tests of gene order from three-locus linkage data. Ann Hum Genet 1987;51:235-49.
12. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci U S A 1984;81:3443-6.
13. Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 1985;37:482-98.
14. Ott J. Analysis of Human Genetic Linkage. 3rd ed. Baltimore, MD: Johns Hopkins University, 1999.
15. Zhang K, Kniazeva M, Han M, Li W, Yu Z, Yang Z, et al. A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. Nat Genet 2001;27:89-93.
16. Yang Z, Peachey NS, Moshfeghi DM, Thirumalaichary S, Chorich L, Shugart YY, et al. Mutations in the RPGR gene cause X-linked cone dystrophy. Hum Mol Genet 2002;11:605-11.