A Novel Locus for X-linked Retinitis Pigmentosa

Zongzhong Tong, Zhenglin Yang, Jay J Meyer, Allen W McInnes, Lai Xue, Asif M Azimi, Jenn Baird, Yu Zhao, Erik Pearson, Changguan Wang, Yali Chen, Kang Zhang

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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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 xRP, 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

**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
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**