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Phospholipase C: A candidate for human retinal degeneration Robert E. Anderson[1], Abboud J. Ghalayini[1], Richard A. Alvarez[1], Robert Nordquist[1], and Wolfgang Baehr[2]. [1]Dean A. McGee Eye Institute and Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Cullen Eye Institute, Baylor College of Medicine, Houston, TX.

The retina contains three families of phospholipase C (β, δ, and γ), a receptor-activated enzyme that generates two intracellular second messengers, diacylglycerol and 1,4,5-inositol trisphosphate. We have characterized the cDNA and partial gene structure for human PLCβ4, a β isoform which shares highest sequence homology with the *norpA* PLC from Drosophila. A mutation in the *norpA* PLC leads to a retinal degeneration in the fly. Immunoblot and immunocytochemical analyses were carried out on bovine, monkey, and human retinas and retinal membranes with a peptide-specific antibodies (Ab) to the C-terminus of bovine PLCβ4. Immunoblot analysis of whole human retina membranes showed a 130 kDa immunoreactive protein and a less abundant 150-160 kDa protein. Immunocytochemical studies showed immunoreaction in the photoreceptor cell layer and the outer plexiform cell layer of bovine retina. The composite cDNA sequence derived from several overlapping cDNA clones from a human retinal library predicts a human PLCβ4 polypeptide of 1022 amino acid residues (m.w. 117,000). This PLCβ4 variant lacks a 165 amino acid N-terminal domain characteristic for the rat brain isoforms, but has a distinct putative exon 1 unique for human and bovine retina isoforms. Human genomic DNA was amplified with exon specific primers to map intron/exon junctions. The β4 gene contains an intron-less putative exon 1 fragment containing PI and the translation start codon. In addition exon 2 (95 bp) and exon 3 (58 bp), as well as intron a (1 kb) and intron b (0.6 kb) have been mapped. Exon 3 corresponds to the P3 slice variant. Somatic cell hybrids and deletion panels were used to localize the PLCβ4 gene to the short arm of chromosome 20. The gene was further sublocalized to 20p12 by fluorescence in situ hybridization.

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EXCLUSION OF ROM-1 AND RECOVERIN GENES IN 16 FAMILIES AFFECTED WITH ADRP

M.J.TRUJILLO; J.BENITEZ; M.ROBLEDO; C.RAMOS
B. MARTINEZ DELGADO & C.AYUSO.
Department of Genetics.FUNDACION JIMENEZ DIAZ.
MADRID (SPAIN).

Retinitis Pigmentosa is a very heterogenous retinal degeneration with different patterns of inheritance and several genes involved.
An approach to the genetic study in RP is direct analysis of candidate genes.

We have selected 16 unrelated families with ADRP, where mutations in RHO and RDS genes had been ruled out, and 30 control individualds.

We analyzed the coding sequences of ROM-1 and Recoverin genes, using the single strand conformation polymorphism (SSCP) and direct sequencing analysis of the exons. SSCP revealed abnormal pattern of migration in the second exon of ROM-1 in three cases.
Direct sequencing showed 2 previously described polymorphisms : an insertion T in 966/967 position which corresponds to the 4th intron and a G to C change in 1071 position which does not alter the aminoacide sequence.
No mutation was observed in Recoverin gene in any patients.

In our ADRP patients without RHO or RDS mutations, ROM-1 and Recoverin genes are not involved in the pathogenesis of RP.

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CYTOGENETIC STUDIES IN RETINITIS PIGMENTOSA PATIENTS

C.AYUSO[1], R.SANZ[1], M.A.CARRIAZO[1], M.A.ANAVITARTE[2], M.E.QUEREJETA[2], M.J.TRUJILLO[1], E.ARRANZ[1], C.RAMOS[1].
[1] Department of Genetics /FUNDACION JIMENEZ DIAZ /MADRID (SPAIN)
[2] Genetics Unit/ POLICLINICA GUIPUZCOA/ SAN SEBASTIAN (SPAIN)

We have performed cytogenetic analyses in 108 unrelated patients affected with different forms of Retinitis Pigmentosa (RP).

We carried out karyotype in blood lymphocytes according to standard procedures, including GTG banding techniques. Fluorescent in situ hybridization (FISH) was also performed in some selected cases.

108 RP patients were studied including 19 SRP, 4 XLRP, 37 ARRP, 11 ADRP, 12 Usher1, 12 Usher2 and 13 patients with other Syndromes.

We have found 4 chromosomal abnormalities (3.7%): A de novo X;4 balanced translocation (Xq21;4p16), a familiar pericentric inversion of chromosome 10, an extra unidentified marker and a fragility on chromosome 3 (Fra3p12).

In the first case, the translocation seems to be responsible of the patient phenotype (retinal degeneration, progressive neurosensorial deafness and primary amenorrhea). The familiar inversion could explain the RP in the second patient, only if another mutation has occurred within the homologous region of the other 10 chromosome.

In the other two cases, molecular analysis and familiar studies can help to elucidate wether chromosomal abnormalities are involved in RP pathogenesis or they are coincidental.

The routine cytogenetic analysis in patients having mendelian disorders with a complex genetic basis, as RP is, could be a feasible help in the search of new causative genes.

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TITLE: A MOLECULAR GENETIC STUDY ON RETINITIS PIGMENTOSA POPULATION FROM GALICIA
BLANCO M.I.[1] CAREO M.V.[2] CARRACEDO A.[2] CAPEANS C.[1] SALGADO M.[1]
1 Department of Ophthalmology, University of Santiago
2 Department of Molecular Biology, University of Santiago.

PURPOSE
Searching for mutations in the rhodopsin gene in 15 unrelated galician pedigrees with autosomal dominant Retinitis Pigmentosa.

METHODS
Mutations in the rhodopsin gene (exons 1 to 5) were investigated after PCR amplification of the exons by using SSCP (single strand conformation polymorphism) analysis in polyacrylamide gels. SSCP analysis were optimized using different gels concentrations and electrophoretic conditions.
Wherever we observed a variant band by the SSCP technique, we searched for the responsible DNA sequence variation by sequencing on an automatic sequencer.

RESULTS
A single mutation in the exon 1 on the rhodopsin gene was found in two independent families among the 15 families studied. Sequencing family data will be reported.

CONCLUSION
Mutations on the rhodopsine gene were identified in two related patients with Retinitis Pigmentosa, suggesting that this gene is a common causing gene in retinal degeneration.