

Genetics of Retinitis Pigmentosa



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Abstract : Retinitis pigmentosa (RP) is one of the most genetically heterogeneous of hereditary conditions for which molecular pathologies have so far been elucidated. This group of hereditary conditions involving death of retinal photoreceptors, represents the prevalent cause of visual handicap among working populations in developed countries. Here we provide an overview of our research in Italy on the molecular pathologies associated with RP

Key Words : Retinitis pigmentosa, gene, mutations, genotype/phenotype correlations

Introduction :

Retinitis pigmentosa (RP) is a group of clinically and genetically heterogeneous retinal degenerative hereditary diseases. The disease can display X-linked (XLRP), autosomal recessive (AR RP) and autosomal dominant (AD RP) inheritance patterns. The onset of disease is the first two decades of life. Prominent symptoms are night blindness, narrowing of the visual field and pigmentary alterations of the retina. The disease can be isolated or associated with systemic diseases such as Usher syndrome (RP and deafness), Laurence-Moon syndrome (RP, short stature, hypogonadism, ataxia and mental retardation) and/or Bardet-Biedle syndrome (RP, polydactyly, obesity, hypogonadism and mental retardation), Refsum's syndrome (RP, ataxia, polyneuropathy), etc. (Pagon, 1993). Worldwide, about 1.5 million people can be expected to be affected (Bird, 1975; Weleber and Gregory-Evans, 2001). Retinitis pigmentosa is the first cause of irreversible blindness in developed countries among persons under 70 years of age. Epidemiological studies conducted in the United States, China, Switzerland, Slovenia, Denmark, Israel and England show an estimated prevalence of the disease of approximately 1/4000 (Ayuso *et al.*, 1995; Boughman *et al.*, 1980; Bunker *et al.*, 1984; Zhang *et al.*, 2002). Thus far there are no epidemiological data of RP in Italy. Consequently the distribution of the inheritance pattern is unknown. Over the last few years, more than 100 genes causing inherited retinal diseases

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have been mapped to chromosomal locations (RETnet, see, <http://www.sph.uth.tmc.edu/Retnet/disease.htm>). Concerning isolated forms of RP, thirty genetic loci and more than twenty genes are so far identified. It must be underlined, however, that several families have been reported that do not show linkage to any of the previously identified loci, indicating that additional RP genes must exist (Rivolta *et al.*, 2002).

The high number of different genes involved in RP has hampered extensive molecular studies in Italian patients in order to determine the correct molecular diagnosis for this condition.

Genes Involved in Autosomal Dominant RP (AD RP)

To date, 12 genes have been linked to AD RP form (RETnet, <http://www.sph.uth.tmc.edu/Retnet/disease.htm>). Among these, we focused our attention on 9 genes, RHO, RDS, RP1, RP10, RP11, NRL, CRX, FSCN2 and HPRP3.

We found 7 different mutations in 12 of 59 Italian families with AD RP, and specifically 2 different kinds of mutations in RP1 gene for 3 families, 3 different ones in RHO gene for 7 families, and two different ones in NRL gene for 2 families (Ziviello *et al.*, 2005).

The RP1 gene is localised in the pericentric region of chromosome 8 at 8q11-13. RP1 protein is expressed in the photoreceptors cell body and its function is required for the correct orientation and higher order stacking of outer segment disc (Liu *et al.*, 2004).

The two mutations detected in RP1 gene are R677X and Y1053 X. The first, a nonsense mutation found in two different families, appears to be quite common which has been already reported; the second instead was found in only one family.

Based on our study, mutations in RP1 gene seem to account for 5.1% of AD RP (Ziviello *et al.*, 2005).

Five patients from two unrelated families with dominant retinitis pigmentosa showed RP1, R667X gene mutations showed a late onset of disease after the third decade of life. The patients retained a good visual acuity, except the 79 years old patient, and a recordable rod and cone ERG signals, until the beginning of the fourth decade of life, after then only the photopic response was elicitable. Fundus examination revealed a normal retinal appearance in

three patients, while in two patients, after the fifth decade of life, typical fundoscopic signs were present in retinitis pigmentosa.

RHO gene is mapped on 3q21-q24 and consists of 5 exons, organized in 6 fragments. In similar studies, carried out in U.S.A., RHO accounts for 26% of AD RP forms, while in Japan accounts for 2.5% (Jay, 1982; Inglehearn *et al.*, 1998; Sohocki *et al.*, 2001; Wada and Tamai, 2003)

In the RHO gene we found 3 different types of mutations; the first, R135W, found in four individuals, was located in the exon 2. The second P347L, found in two patients, was located in the exon 5, while the third C167R, in the second exon, was found only in one patient. These mutations, already described in other studies (Dryja *et al.*, 1991; Simonelli *et al.*, 1998), are responsible for 11.8% of AD RP cases in Italy.

The R135W and P347L rhodopsin mutations particularly yielded severe phenotype which is identified both clinically and functionally.

In 2 families with substitution at aminoacid protein 135, there was interfamilial consistency in phenotype. All 5 affected individuals had early onset of disease, ophthalmoscopic features of typical retinitis pigmentosa and undetectable rod and cone electroretinograms. Later in the course of the disease a macular dystrophy can also be observed.

Five patients from a family with dominant RP and rhodopsin Pro 347 Leu also showed an early onset of disease, blindness at the age of 40 to 60 and extinguished both rod and cone electroretinograms. The ophthalmoscopic findings revealed some interfamilial clinical variability. Three patients showed RP with macular dystrophy, one of which also presented an exudative retinal detachment Coats-like in the left eye, one patient showed a typical RP, while the oldest (83 years old) patient showed retinal detachment in both the eyes.

The mutation C167R identified in five patient of an AD RP family yielded a typical retinitis pigmentosa with a well preserved visual acuity, until the fifth decade of life, but unrecordable ERG after the age of fifteen years. Clinical examination repeated yearly for up to 13 years indicated that the disease was progressing very slowly.

Furthermore we found two different mutations in NRL gene. This gene maps on 14q11.2 and consists of 3 exons. Moreover it encodes a protein termed neural retina leucine zipper (NRL) that is a basic motif-leucine zipper transcription factor preferentially expressed in rod photoreceptors. It acts

synergistically with CRX protein to regulate rhodopsin gene transcription (Mears *et al.*, 2001).

The clinical phenotype of family with a Pro51Leu mutation in the NRL gene was characterised by a typical AD RP phenotype with an early onset of the disease, a severe impairing of photoreceptors' function, cystoid macular edema and peripapillary chorioretinal atrophy.

Genes Involved in X-Linked RP (XLRP)

X linked retinitis pigmentosa (XLRP) is a severe retinal degeneration that typically causes night blindness, loss of peripheral vision within the first or second decades of life, legal blindness by 30 years of age and often complete functional blindness by 40 or 50 (Bird and Heckenlively, 1988; Fishman *et al.*, 1988).

Mutations in the RPGR gene (responsible for RP3) are the most frequent cause of XLRP (Meindl *et al.*, 1996), while mutations in the RP2 gene account for about 10% of XLRP in European and North American families.

We have performed mutation analysis of the RPGR gene in a cohort of 49 Southern European males affected with XLRP (Miano *et al.*, 1999).

Seven different mutations were identified in eight of the 49 families; these include three splice site mutations, two microdeletions and two missense mutations.

Two missense mutation in the RCC1 domain were found in two families. The first (T99N) is Thr>Asn at nucleotide 355 in exon 4; the second (I289V) is Ile>Val nucleotide 924 in exon 8. Both aminoacid substitutions are within residues which are conserved across evolution.

Two different microdeletion were identified among the affected members of three unrelated families. One deletion (Δ T 545), which is a single thymine deletion at base 545 in exon 6, causing a frame-shift and premature termination signal in codon 174. The second (Δ AT 296-297), was a deletion of two base pairs at 296-297 in exon 3, causing a frame-shift and premature termination signal in codon 86.

In three patients, splice site mutations were detected. The first (IVS2-2), it is an A>G transition at position 2, at the first intronic nucleotide of the splice-acceptor site of exon 3.

Six of the observed mutations, as described in others reports, fall in the RCC1 repeats and one falls in the "charged" domain. The amino-terminal

half of predicted RPGR protein contains 6-7 repeats with similarity to repeats within RCC1, a protein which is essential for nucleo-cytoplasmic transport, though might also be involved in protein trafficking through the Golgi apparatus (Rosa *et al.*, 1996; Yan *et al.*, 1998). Most RPGR mutations fall in the RCC1 repeats, providing evidence that it is an important functional domain (Buraczynska *et al.*, 1997; Fujita *et al.*, 1997). Recently, studies to identify proteins that interact with RPGR, showed that the RCC1 like domain of RPGR interacts with phosphodiesterase delta sub-unit (Lorenz *et al.*, 1998). This interaction links RPGR to the visual transduction cascade that regulates the rod phosphodiesterase holoenzyme (Linari *et al.*, 1999).

All studies indicate that RPGR is expressed at surprisingly low levels in the retina, and it is unclear how mutations in this gene cause retinal degeneration.

The large group of XLRP families in which no mutations have been detected in RPGR provides one starting point for the mutations analysis in the RP2 gene.

Five genetic alterations at the RP2 locus were identified in the 42 XLRP families, which were excluded for RPGR mutations. Such alterations were found to depend on mutations in exons 2 and 3 (Miano *et al.*, 2001). Our results confirm previous data showing that only 10% of European XLRP families display RP2 mutations (Hardcastle *et al.*, 1999). Two missense mutations were found in two Italian XLRP families, both mutations fall in exon 2: the former at nucleotide 354, resulting in Arg to Leu substitution at residue 118 (R118L); the latter at nucleotide 414, determining a Glu to Gly substitution (E138G). A third missense mutation falls in exon 3 at nucleotide 846, resulting in an Arg to Trp substitution (R282W).

R118L and E138G mutations fall in the RP2 region showing homology to the tubulin folding cofactor C or CFCHD domain (Chapple *et al.*, 2000). Also, the microinsertion mutation falls in exon 2 and it consists of a single Thmine insertion at nucleotide 303 of the coding sequence (303insT). Microinsertion 303insT results in a frameshift at amino acid residue 101 and, because of anticipated stop codon, it gives rise to a considerably truncated protein of 123 residues, which lacks half of the CFCHD domain and the C-terminal part of RP2. In one Italian family, the splice site mutation (IVS1-2A>G), was found to cosegregate with the disease. It is an A>G transition at position -2, at the first intronic nucleotide in the splice-acceptor site of exon 2.

Because mutation R282W falls in exon 3, i.e. in an RP2 region other than CFCHD, we further analyzed RP2 sequence, searching for significant homologies with protein domain of known functions. The RP2 region (residues 207–244) shows homology (63% positives: 31% identity + 32 % similarity) to pig γ subunit of T-complex protein 1 (also named CCT) which is able to associate with *in vitro* assembled microtubules (Melki *et al.*, 1997, Yokota *et al.*, 1999), i.e. behaves as microtubule-associated protein (MAP). Thus, we name RP2 region 207-244 as MAP homologous domain or MAPHD. Furthermore, RP2 region (residues 250–317) shows homology (52% positives: 30% identity + 22% similarity) to the N-terminal domain of a nucleoside diphosphate kinase (NDK, EC 2.7.4.6) family called nm23 (Postel, 1998), suggesting the name NM23 homologous domain (NM23HD). The mutation R282W changes an arginine residue conserved with respect to the homologous N-terminal region of a murine and human nm23- M6 (Zhu J *et al.*, 1999).

Genes Involved in Autosomal Recessive RP (AR RP)

Recessive RP was the last category to be studied, because in most cases there is a little or no family structure to provide clues as to the whereabouts of the mutation.

However, until now mutations in at least 19 genes have been associated to AR RP.

The genes more frequently involved in AR RP, as determined in studies performed in the US, are the genes encoding the subunits α and β of the cGMP phosphodiesterase, RHO, and the cGMP gated ion channel protein CNGC, another member of the phototransduction cascade (Wang *et al.*, 2001). In addition recent studies demonstrated that the USH2A gene, which was first identified as a gene for a syndromic form of RP, i.e., Usher syndrome, and ABCA4 responsible for Stargardt's disease are allelic with AR RP (Aller *et al.*, 2004; Klevering *et al.*, 2004). Mutation screening indicates that each of these loci accounts for about the 5% of all simplex and recessive RP cases. It is conceivable that many more recessive RP loci remain to be found.

Conclusion

The continued analysis of families segregating RP, and the search for genes involved in RP, has resulted and will result in the identification of many different mutations and more genes which are involved. These data complicate the heterogeneity present in RP. At the same time genotype/phenotype correlations and knowledge of the frequency of the different retinal diseases

will help to direct and improve the service which clinical geneticists can offer to these patients.

It is therefore important that the process of mutation screening in retinal dystrophy patients and their families continues to fulfilling the aim of unraveling and understanding the genetic background and the subsequent disease mechanism. This will aid the prognoses in RP.

Moreover this approach will reveal new proteins with important function in the eye, which will lead to greater understanding of normal eye function and may offer new avenues for therapeutic intervention.

Proof of principle for gene therapy for RP has been established in a number of different animal models.

Many animal models of RP, in fact, are available and have led to a better understanding of the disease and to the development of therapeutic strategies aimed at curing the specific genetic disorder (gene therapy), slowing down or even stopping the process of photoreceptor degeneration (growth factors or calcium blockers applications, vitamin supplementation), preserving the cones implicated in the central visual function (identification of endogenous cone viability factors) or even replacing the lost cells (transplantation, use of stem or precursor cells). While much more progress needs to be made before moving from the laboratory to the clinic, gene therapy now holds many promises for slowing or even preventing blindness due to RP.

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