A novel mutation of the RP1 gene (Lys778ter) associated with autosomal dominant retinitis pigmentosa

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Background: Besides the three known genes (RHO, RDS/Peripherin, NRL) involved in autosomal dominant retinitis pigmentosa (adRP), a fourth gene, RP1, has been recently identified. Initial reports suggest that mutations in the RP1 gene are the second most frequent cause of adRP. The clinical findings were described in a family with adRP and a novel mutation in the RP1 gene.

Method: Index patients from 15 independent families with adRP in which RHO mutations had been excluded in previous examinations were screened for mutations in the RP1 gene by means of direct DNA sequencing. Evaluation of the RP1 phenotype in patients included funduscopy, kinetic perimetry, dark adapted final threshold test, standard electroretinography and, in one case, multifocal electroretinography.

Results: One novel nonsense mutation (Lys778ter) in one of these 15 patients was detected. Co-segregation of the mutation with the disease phenotype could be established in the index patient’s family. The phenotype comprises variable expression of clinical disease probably including one case of incomplete penetrance, a onset of symptoms beginning in adulthood, and evidence of regionally varying retinal function loss.

Conclusion: The lys778ter mutation localises inside the critical region harbouring all mutations described so far. The ophthalmic findings support previous observations that variation of disease expression appears as a typical feature of the RP1 phenotype.

PATIENTS AND METHODS

Recruitment of patients, DNA isolation, and mutation analysis

The entire coding region (exons 2, 3, and 4) of the RP1 gene was sequenced in 13 German patients from independent families, one Spanish patient, and one patient originating from Italy, all suffering from autosomal dominant retinitis pigmentosa, in which mutations in the rhodopsin gene had been excluded in previous screenings.

All patients had been diagnosed at the Universitäts-Augenklinik Tübingen on the basis of typical symptoms and signs—that is, bilateral progressive disease, night blindness and peripheral visual field loss, fundus findings of optic disc pallor, attenuated retinal vessel and peripheral pigmentedary changes, and a severely abnormal or extinguished rod ERG.

Inheritance was considered autosomal dominant when the disease phenotype was observed in at least two subsequent generations.

DNA was isolated from peripheral venous blood samples using the procedure of Miller et al. Coding exons 2, 3, 4 and flanking intronic/untranslated regions were amplified from total genomic DNA by means of the polymerase chain reaction (PCR). The sequences of the oligonucleotide primer pairs were...
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RESULTS
DNA analysis

RP1 mutations were ruled out in 14 of the 15 patients under analysis, one patient (Fig 1B, IV:1) showed a heterozygous A to T transition in exon 4 resulting in a novel nonsense mutation Lys778ter (AAA TAA) (Fig 1A). This mutant allele is predicted to encode a severely shortened RP1 protein of 777 amino acids. Individuals not carrying the mutation (II:1; II:3; II:4; III:4, age range 48–67) do not show signs of RP on funduscopy, Goldmann perimeter (all) or scotopic, and photopic flash ERG (performed in II:4 only), according to external medical records.

Individual II:2 was reported to disclose typical symptoms of retinitis pigmentosa from the end of her fifth decade of life. Medical records were not available. Case II:2, a 60 year old female, carries the mutation, but has no visual complaints, nor, according to outside medical records, does she show clinical signs of RP—that is, her fundus appears normal and she has normal visual fields and visual acuity. Case II:3, a 68 year old female, likewise is unaware of typical symptoms related to the mutation. However, the mutation was also detected in one apparently healthy family member (II-2).

In addition to this novel mutation we detected six polymorphisms, of which all except the first one had already been described. They are as follows: Arg872His (CGT →CAT) (31%), Asn985Tyr (AAT →TAT) (31%), Ala1670Thr (GCA → ACA) (19%), Ser1691Pro (TCT →CTT) (25%), Gln1725Gln (CAA →CAAG) (31%) and Cys2033Tyr (TGT →TAT) (56%).

Phenotype of the Lys778ter mutation

Figure 1B shows the pedigree of the family investigated. Four individuals not carrying the mutation (II:1; II:3; II:4; III:4, age range 48–67) do not show signs of RP on funduscopy, Goldmann perimeter (all) or scotopic, and photopic flash ERG (performed in II:4 only), according to external medical records.

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RP. However, external records reveal that, on funduscopy, she has peripheral pigmentary degeneration characteristic of RP, and that kinetic visual fields (Goldmann target III/4e) of both eyes are constricted to about 20–30 degrees. Visual acuity is 20/40 in both eyes. There is nuclear cataract and asteroid hyalosis in both eyes.

ERG records are not available for II:2 or II:5.

Case II:6, a 72 year old male, noticed peripheral vision problems as the presenting symptom at about age 45, followed by night blindness at about age 50. At that time the diagnosis of RP was established. Central visual function—that is, reading acuity, contrast sensitivity, and colour vision have been deteriorating since the last decade, mainly in the left eye.

On examination, visual acuity was 20/30 right eye and light perception left eye. Perimetry revealed only a small central island (right eye) and no comprehensive field left eye. Dark adapted final threshold tested at 6 degrees nasally in the right visual field was elevated by about 2 log units for the 500 nm, and 1 log unit for the 656 nm target. The threshold difference between the blue-green and red target revealed residual rod function at this retinal location. On Ganzfeld ERG both rod and cone signals were within noise level. Biomicroscopy showed small lens opacities in both eyes, and typical funduscopic signs of advanced stage RP affecting all peripheral quadrants.

Case III:2, the daughter of II:6, is a 49 year old woman with a history of night blindness since about age 35, and peripheral field loss since about age 40. In the past 5 years she has experienced a rapid decline of visual acuity in both eyes.

Clinical examination disclosed a visual acuity of 20/400 in both eyes. On Goldmann perimetry there were severely constricted central islands and small field remnants in the outer temporal periphery in both eyes. The dark adapted final threshold tested at a central retinal location (2 degrees nasally right eye) showed an elevation by about 3 log units for 500 nm and 2 log units for 656 nm, and pure cone mediation evidenced by equal 500 and 656 nm thresholds. Rod and cone ERG signals were non-detectable. Biomicroscopy revealed dense asteroid hyalosis right eye and cortical cataract left eye,
and fundus findings typical of advanced RP in all retinal quadrants of both eyes.

Case IV:1, the affected 22 year old son of III:1 has a history of esotropia and amblyopia right eye. He reported problems with dim light conditions and a decrease of visual acuity beginning at age 18. Peripheral visual field defects had been detected 1 year ago. Clinical evaluation revealed a visual acuity of 20/400 right eye and 20/70 left eye. Visual field was near normal to the V/4e target in the left eye, but showed depressions in the temporal, mainly the upper temporal, fields to V/4e in right eye, and to the III/4e and I/4e targets in both eyes, respectively (Fig 2). Dark adaptation final threshold, determined at 20 degrees nasally in the left visual field, was clearly rod mediated and showed only a slight elevation (0.6 log units above normal for 500 and 656 nm). On flash ERG (Fig 2), isolated rod b-waves were very small in the right, and not detectable in the left eye, scotopic mixed b-wave responses were clearly discernable from noise but markedly reduced (about 20% of the median in right eye and 10% in left eye, respectively).

Photopic cone signals (b-waves and 30 Hz flicker signals) were slightly better preserved than scotopic b-waves (about 25% of amplitude median right eye, and 20% left eye), their peak times were markedly delayed.

Multifocal ERG signals (Fig 3) were reduced all over the test field in both eyes. The topography of abnormalities paralleled the visual field findings in that amplitude losses tended to be more severe in the temporal than in the nasal fields. The smallest amplitudes were found in the macular and perimacular regions (rings 1 and 2), and amplitudes increased towards the periphery of the field. Likewise, the timing was prolonged at all eccentricities; however, the strongest delay was observed in the two most central regions, whereas the timing of the responses shortened with eccentricity, reaching near normal values at the field border of the right eye.

Biomicroscopy of anterior segments was unremarkable. Funduscopy (Fig 2) showed cystoid macular oedema in both eyes. Peripherally, vessel attenuation, RPE atrophy, and bone spicules were prominent in the nasal fundus, whereas the temporal fundi showed only slight RPE changes and barely any pigmentation.

DISCUSSION

We have analysed patients from 15 independent families showing typical clinical features of autosomal dominant RP. In previous studies all of them had been screened for mutations in the rhodopsin gene and found to be negative. Even though mutations in the most affected gene in adRP, rhodopsin, have been excluded, we could detect only one mutation in these patients, mainly originating from Germany, when screening the entire coding region of the RP1 gene. This fact leads to the assumption that the frequency of mutations in the RP1 gene in German patients might be relatively small. The current sample size, however, is too low to draw more exact conclusions about the proportion of adRP cases that are caused by RP1 mutations.

The RP1 gene is a very long gene consisting of approximately 7 kb. Because of its enormous size and the low frequency of mutations the ratio between the effort of screening the entire gene and the probability to find a mutation seems to be quite small.

A different study reports that mutations in the RP1 gene are clustered in a small region in exon 4 spanning codons 650 to 796. The novel Lys778ter mutation localises inside this mutation cluster region. But recently other studies have found mutations outside this critical segment and have therefore proposed to extend the region of mutational screening.22 23 In conclusion we suggest to analyse an interval spanning from codons 500 to 1053.

The phenotype in the Lys778ter family shows a few features that deserve to be mentioned and may help to define common characteristics of RP1 mutation phenotypes.

First of all, there is considerable variation of disease expression, as to subjective onset of symptoms (range: age 18 up to “asymptomatic” at age 68) and clinical disease. Fundus findings and peripheral visual field loss typical of RP were observed at the earliest in 22 year old patient IV:1. The other extreme is 60 year old patient II:2, who on the basis of the clinical findings available might be classified as a case of non-penetrance. It cannot be excluded, however, that this latter patient would have shown minimal disease expression on ERG testing. Jacobson et al23 found slight reductions in rod ERG maximum amplitude in clinically otherwise normal heterozygote carriers of the RP1 Arg677ter mutation. Variation of disease expression, including cases of non-penetrance, has been reported before among RP1 families22 23 and could also be observed in a larger sample of patients carrying the same Arg677ter mutation,22 thus emerging as a typical feature of RP1 associated RP.

Despite the variability of disease severity displayed among carriers of the Lys778ter mutation attempts can be made to describe general features of the phenotype. Onset of symptoms did not occur before adulthood. Night blindness, if the presenting symptom, did precede visual field problems by only a few years. In comparison, a number of adRP phenotypes caused by rhodopsin mutations have earlier, childhood, onset
of night blindness." Some families with Arg677ter, or a 2280–2284 del mutation of RP1 have likewise been classified as "late onset" phenotypes.\textsuperscript{19} Autosomal dominant forms of RP may clinically differ as to the retinal topography of disease and the relation of rod to cone function loss.\textsuperscript{20}–\textsuperscript{21} Although the data are limited, some conclusions may be drawn from the clinical, psychophysical, and ERG findings in the Lys778ter family. In two older individuals (II:6 and III:2), the common unspecific features of advanced stage disease appear funduscopically, by concentric visual field loss, and by flat ERGs. In an earlier disease stage, however, (IV:1) a regional predeletion of retinal damage is revealed. Peripheral fundus changes are visible mainly in the nasal regions, and both multifocal ERG and perimetry show losses in the corresponding temporal or the upper temporal field, respectively. Based on the standard ERG findings, the rod system overall appears to be substantially affected, and more affected than the cone system. Psychophysical rod final thresholds, however, give evidence of near normal sensitivity at least in single retinal locations. Interestingly, rod function is psychophysically detectable even in late stage disease (72 year old individual II:6) with small residual island fields. These findings argue rather for a regionally evolving pattern of rod disease in RP1 Lys778ter than a primary diffuse rod defect.

Our impressions are paralleled by the detailed investigations of Jacobson et al\textsuperscript{19} on a group of patients carrying the Arg677ter or various RP1 frameshift mutations. They describe a common pattern, comprising regional retinal variation of disease with predilection of the inferonasal part, both for the rod and the cone system, and with rod function being more affected than cone function at all disease stages.

Moreover, they concluded from the cross sectional data and from individual follow up measurements, that progression of rod function loss over time is faster than that of cone function from individual follow up measurements, that progression of affected than cone function at all disease stages.\textsuperscript{22–24} Interestingly, rod function is threshold, however, give evidence of near normal sensitivity of night blindness.

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REFERENCES