Clinical and molecular genetic characterisation of a family segregating autosomal dominant retinitis pigmentosa and sensorineural deafness

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Abstract

Aims/background—To characterise clinically a large kindred segregating retinitis pigmentosa and sensorineural hearing impairment in an autosomal dominant pattern and perform genetic linkage studies in this family. Extensive linkage analysis in this family had previously excluded the majority of loci shown to be involved in the aetiologies of RP, some other forms of inherited retinal degeneration, and inherited deafness.

Methods—Members of the family were subjected to detailed ophthalmic and audiological assessment. In addition, some family members underwent skeletal muscle biopsy, electromyography, and electrocardiography. Linkage analysis using anonymous microsatellite markers was performed on DNA samples from all living members of the pedigree.

Results—Patients in this kindred have a retinopathy typical of retinitis pigmentosa in addition to a hearing impairment. Those members of the pedigree examined demonstrated a subclinical myopathy, as evidenced by abnormal skeletal muscle histology, electromyography, and electrocardiography. LOD scores of $Z_{\max} = 3.75$ ($\Theta = 0.10$), $Z_{\max} = 3.41$ ($\Theta = 0.10$), and $Z_{\max} = 3.25$ ($\Theta = 0.15$) respectively were obtained with the markers D9S118, D9S121, and ASS, located on chromosome 9q34-qter, suggesting that the causative gene in this family may lie on the long arm (q) of chromosome 9.

Conclusions—These data indicate that the gene responsible for the phenotype in this kindred is located on chromosome 9q. These data, together with evidence that a murine deafness gene is located in a syntenic area of the mouse genome, should direct the research community to consider this area as a candidate region for retinopathy and/or deafness genes.

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retinographic amplitudes which are reduced or non-detectable.¹ The aetiological basis of most inherited retinal degenerations has remained obscure until recent years.

Molecular genetic research resulting in the localisation and mutation analysis of genes responsible for some forms of RP has helped to elucidate the basic pathophysiology of these inherited retinopathies. To date, mutations in four genes have been shown to be responsible for autosomal dominant or recessive forms of non-syndromic RP—that is, rhodopsin,^{2,3} peripherin/rds,⁴ rom1,⁵ β pde,⁶ and rod α cGMP gated channel.⁷

Five genetic loci for Usher syndrome, the concomitant inheritance of RP and deafness in an autosomal recessive inheritance pattern, have been established—that is, 1q32 (type 2A),⁸ 3q21-q25 (type 3),⁹ 11p15.1 (type 1C),¹⁰ 11q13.5 (type 1B),¹¹ and 14q32 (type 1).¹² Mutations in the myosin VIIa gene have recently been shown to be responsible for symptoms in five families with type IB Usher syndrome.¹³

Here we describe an Irish kindred cosegregating RP and deafness in an autosomal dominant fashion and report molecular genetic findings which suggest that the responsible gene lies on the long arm (q) of chromosome 9.

Patients and methods

Family TCD ZMK 92 (Fig 1) is a five generation Irish kindred in which classic retinitis pigmentosa and partial deafness cosegregate in a manner compatible with autosomal dominant inheritance. Most members of the family were clinically characterised in the research department of the Eye and Ear Hospital, Dublin, using procedures to be described. A number of elderly patients with advanced retinopathy and hearing loss were examined in their homes with direct ophthalmoscopy.

Twenty nine individuals were clinically assessed. DNA was extracted from blood samples drawn from all individuals in the pedigree, including spouses of affected people with offspring, using a standard protocol.¹⁴

A nuclear family unit, consisting of an affected mother and her three affected offspring, two daughters and a son (IV7, V4, V5, and V6 in Fig 1), underwent electromyography (EMG) and electrocardiography (ECG). In addition, the mother had a quadriceps femoris muscle biopsy performed with a local anaes-

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The term retinitis pigmentosa (RP), although semantically incorrect, describes a variety of retinal degenerations characterised by a number of common clinical features such as night blindness, peripheral visual field loss, optic disc pallor, retinal vascular attenuation, intraretinal pigment deposition, and electro-

Figure 1 Family TCD ZMK 92. All living individuals were used in molecular genetic analysis.

Figure 2 Rod isolated responses recorded from the dark adapted eye in response to single flashes of -3.5 log unit intensity.

thetic. This biopsy specimen was subjected to light and electron microscopy.

Patients who were assessed in the research department had best corrected Snellen visual acuity measured. Goldmann perimetry, using IV4e, I4e, and O4e targets, was then performed followed by assessment of colour vision using the Farnsworth Munsell 100 Hue test under standardised conditions.

Electroretinograms (ERGs) were recorded using a protocol based on that proposed by the

Figure 3 Cone isolated responses recorded from the light adapted eye in response to single flashes of maximal intensity.

Figure 4 Dark adaptation profiles to red and blue targets. The normal means and standard deviations were calculated from 23 normal individuals.

International Standardisation Committee.¹⁵ Briefly, the patient's pupils were dilated with tropicamide BP 1% and phenylephrine BP 10%. The subject was then dark adapted for at least 30 minutes. Following corneal anaesthesia induced with benoxinate BP 0.4%, corneal

Figure 5 Pure tone audiometry.

Figure 6 Mid to far retinal periphery of patient IV11, aged 24 years, showing retinal pigment epithelial mottling and pigmentary disturbance.

contact lens electrodes of the Henkes type were inserted under dim red illumination. A Ganzfeld bowl was used to present a series of flashes produced by an OS 5 photostimulator located above and slightly behind the subject's head. The intensities of the flashes were altered by a combination of varying the output of the photostimulator and the interposition of neutral density filters. Wave forms were analysed using the Medelec 'Mistral' data averaging and display system. The intensity of the flash at the patient's eyes was measured using a Gossen 'Mastersix' photometer. The standard flash produced by this protocol (setting 16) was 1.5 candelas (cd)/m² per second. Wave forms were analysed using the standard conventions.¹⁶

Figure 7 Photograph of the posterior pole of patient V2, aged 16 years, showing marked optic disc pallor, extreme vascular attenuation, retinal pigment epithelial thinning, and mid peripheral pigmentary deposits.

The responses of the dark adapted eye to a series of single flashes of increasing intensity, starting with a flash of -3.5 log units intensity, were used to assess rod function. A light of intensity 22 cd/m² was then used to illuminate the interior of the bowl and the patient was light adapted for 10 minutes. Cone responses to a series of maximal intensity flashes, presented singly and at 30 Hz flicker in the presence of the background illumination, were then measured.

Two colour dark adaptometry using a Goldmann-Weekers dark adaptometer and red and blue filters was performed on selected individuals.¹⁷ Following pupillary dilatation as described above, the subject's left eye was bleached by exposure to a light of 2650 apostilbs for 5 minutes. Thresholds to two 2° targets, one blue and the other red, presented at 15° above fixation were measured at 2.5 minute intervals over a 30 minute period. Flashing targets were presented at the rate of 1 second on, 1 second off. The true threshold was taken as the average of the on threshold and the off threshold for each testing.

Anterior segment biomicroscopy and applanation tonometry were then carried out followed by direct and indirect funduscopy. Most individuals had colour retinal photography.

Pure tone audiometry was carried out in the audiology department at the Royal Victoria Eye and Ear Hospital.

DNA ANALYSIS

Microsatellite markers covering extensive regions of the genome were analysed in the pedigree. Two point LOD scores were calculated using the program LIPED for IBM PC/At (October 1987).¹⁸ Data were entered using the data management program LINKSYS (version 4.11).¹⁹

Results

The retinopathy in TCD ZMK 92 is marked by extreme variability in the age of onset and the severity of affection. This ranges from legal blindness (as assessed by loss of central visual acuity and of peripheral visual field) by early 20s in one individual to only mild night vision difficulties in the early 60s in another. Most individuals become aware of mild hearing loss and night vision problems in the third decade with subsequent symptoms as a result of loss of peripheral vision in the fourth decade. The hearing loss and visual symptoms are slowly progressive. Posterior subcapsular lens opacities requiring surgery were noted in some individuals in their late 40s and early 50s.

Electroretinographic findings demonstrate the wide variability in affected status. The rod isolated responses, in individuals in whom these responses can be recorded, tend to be significantly delayed in latency and reduced in amplitude (Fig 2). Single flash cone responses are similarly delayed in timing and reduced in amplitude (Fig 3).

Two colour dark adaptometry profiles in individuals with impaired but electroretinographically recordable retinal function demon-

Figure 8 Electrocardiogram tracings from patient V6, aged 13 years, showing inferoseptal and inferolateral ST depression and right axis deviation. The ECG lead order is: top row: I, AVR, V1, V4; middle row: II, AVL, V2, V5; and bottom row: III, AVF, V3, V6.

strate a rod mediated profile but at thresholds significantly above normal values (Fig 4).

Audiometric analysis of some members of the family shows a moderate to severe sensorineural deafness particularly to middle range frequencies (Fig 5), with evidence of 'recruitment'—that is, marked distortion and physical discomfort precipitated by loud noise, suggesting that the primary site of hearing impairment may lie in the organ of Corti.

Funduscopy in young members of the pedigree with significantly abnormal ERG findings showed subtle diffuse retinal pigment epithelial thinning and pigmentary mottling (Fig 6). Older affected individuals showed all the classic features of retinitis pigmentosa with waxy disc pallor, arteriolar narrowing, and bone spicule pigmentary deposition in all four retinal quadrants.

Two individuals in this family suffer moderate to severe mental handicap in addition to visual and auditory symptoms. Figure 7 shows the fundus picture of the youngest of these patients. The retinopathy in both cases is distinguished by marked optic disc pallor. The disc pallor noted in other affected individuals is not as striking.

None of the affected individuals in this family was found to have external ocular muscle movement disorders suggestive of the Kearns-Sayre syndrome.

In the four individuals on whom electrocardiography was performed, ECG changes were noted. All showed right axis deviation. Significantly, the youngest affected child, aged 13 years, in addition to having the most compromised retinal function of the three children as measured by ERG, also had the most serious ECG changes. These consisted of inferoseptal and inferolateral ST depression in addition to right axis deviation, suggestive of global concentric hypertrophy (Fig 8). Electromyography demonstrated short, small motor unit action potentials, consistent with myopathy in the mother and two of her three affected children. Electron microscopy of the muscle biopsy obtained from the mother showed an abnormally high number of mitochondria beneath the sarcolemmal membrane (Fig 9A) when compared with a biopsy obtained from an unrelated normal individual (Fig 9B). While no abnormal forms were seen, the significantly increased number of mitochondria seen was consistent with a mitochondrial myopathy.

Figure 9 (A) Electron micrograph of a muscle biopsy from patient IV7 showing excessive numbers of mitochondria under the sarcolemmal membrane. (B) Electron micrograph of a muscle biopsy from a normal individual. In both (A) and (B) the sarcolemmal membrane is indicated by the black arrow, the mitochondria by the white arrow. Magnification $\times 1925$ in each case.

Table 1 Two point LOD score values for a number of genetic markers linked to loci of ophthalmic and auditory significance showing exclusion of these loci in family TCD ZMK 92

Marker	Recombination fraction θ								Locus excluded	Disease excluded
	0.00	0.001	0.05	0.10	0.15	0.20	0.25	0.30		
D1S201	-99.9	-9.19	-2.59	-0.94	-0.17	0.22	0.39	0.44	1p	ad hearing loss
D1S238	-99.9	-9.71	-3.10	-1.45	-0.69	-0.29	-0.1	-0.31	1q	ar RP
D1S213	-99.9	-9.18	-3.48	-1.92	-1.09	-0.58	-0.27	-0.01	1q	ar RP
D1S249	-99.9	-16.71	-5.16	-3.38	-2.39	-1.67	-1.13	-0.72	1q	ar RP, USH1A
D3S196	-99.9	-9.19	-1.67	-0.66	-0.28	-0.14	-0.11	-0.12	3q21-25	USH3
D3S1279	-99.9	-5.10	-1.35	-0.43	-0.02	0.18	0.26	0.27	3q21-25	USH3
D3S47	-99.9	-17.22	-3.92	-1.86	-0.83	-0.24	0.09	0.26	3q, RHO	ad RP
D3S621	-99.9	-10.58	-2.39	-1.20	-0.62	-0.30	0.10	0.02	3q, RHO	ad RP
RDS	-99.9	-17.00	-4.40	-2.37	-1.35	-0.74	-0.37	-0.15	6p, RDS	ad RP
TCTE1	-99.9	-7.67	-2.32	-1.24	-0.68	-0.34	-0.14	-0.03	6p, RDS	ad RP
D4S43	-99.9	-6.90	-2.08	-1.37	-0.96	-0.69	-0.52	-0.41	4p16.3, β PDE	ar RP
D4S412	-99.9	-23.23	-6.51	-3.76	-2.29	-1.37	-0.76	-0.37	4p16.3, β PDE	ar RP
D4S1599	-99.9	-18.91	-5.61	-3.49	-2.36	-1.63	-1.13	-0.76	4p16.3, β PDE	ar RP
D4S231	-99.9	-16.47	-4.73	-2.81	-1.79	-1.14	-0.71	-0.43	4p14-q13, cGMP	ar RP
D4S428	-99.9	-11.22	-2.93	-1.64	-0.97	-0.56	-0.29	-0.12	4p14-q13, cGMP	ar RP
D5S210	-99.9	-5.69	-1.80	0.76	-0.27	-0.07	-0.12	0.15	5q	ad hearing loss
D5S414	-99.9	-12.82	-4.22	-1.98	-0.89	-0.28	0.05	0.20	5q	ad hearing loss
FIB5	-99.9	-12.52	-3.95	-1.75	-0.70	-0.13	0.17	0.28	5q	ad hearing loss
D7S460	-99.9	-14.00	-3.94	-2.21	-1.28	-0.72	-0.37	-0.17	7p	ad RP
D7S493	-99.9	-10.16	-2.02	-0.87	-0.34	-0.76	0.57	0.11	7p	ad cystoid maculopathy
D7S435	-99.9	-17.83	-5.31	-3.03	-1.84	-1.12	-0.64	-0.30	7p	ad RP, ad cystoid maculopathy
D7S486	-99.9	-24.56	-7.74	-5.00	-3.41	-2.40	-1.67	-1.14	7q	ad RP
CFTR	-99.9	-22.62	-6.03	-3.43	-2.10	-1.30	-0.8	-0.49	7q	ad RP
D8S84	-99.9	-6.52	-2.16	-1.35	-0.91	-0.63	-0.43	-0.30	8q12	ad RP
D8S165	-99.9	-2.08	-0.53	-0.37	-0.29	-0.22	-0.16	-0.16	8q12	ad RP
D8S87	-99.9	-6.78	-1.84	-1.08	-0.68	-0.44	-0.28	-0.17	8q12	ad RP
D8S260	-99.9	-31.31	-9.59	-6.05	-4.14	-2.82	-2.018	-1.37	8q12	ad RP
D8S283	-99.9	-22.02	-6.97	-4.47	-3.10	-2.17	-1.50	-1.00	8q12	ad RP
D8S373	-99.9	-27.75	-7.81	-4.63	-2.97	-1.93	-1.23	-0.74	8qter	AMD
D11S527	-99.9	-15.82	-3.79	-1.83	-0.85	-0.28	-0.5	0.23	11q13	VMD2, USH1B
D11S871	-99.9	-14.62	-3.08	-1.35	-0.52	-0.08	0.16	0.24	11q13	VMD2, USH1B
D11S419	-99.9	-7.19	-2.16	-1.32	-0.86	-0.56	-0.36	-0.21	11p	USH1C
INT2	-99.9	-16.2	-4.52	-2.65	-1.65	-1.03	-0.61	-0.33	11q13	USH1B, adFEVR
D11S35	-99.9	-13.31	-1.92	-0.35	0.32	0.61	0.67	0.59	11q13	ad FEVR
D13S158	-99.9	-27.00	-7.17	-4.03	-2.40	-1.41	-0.78	-0.38	13q34	ad Stargardt-like macular degeneration
D13S175	-99.9	-6.78	-2.91	-1.81	-1.21	-0.81	-0.53	-0.33	13cen	ad and ar hearing loss
D17S938	-99.9	-19.2	-4.37	-2.12	-1.03	-0.42	-0.08	0.08	17p	ad RP
D17S796	-99.9	-15.12	-3.59	-1.84	-0.99	-0.51	-0.23	-0.09	17p	ad RP
D17S849	-99.9	-15.59	-3.98	-2.19	-1.29	-0.75	-0.42	-0.22	17p	ad RP
D19S210	-99.9	-14.37	-3.47	-1.80	-0.96	-0.46	-0.15	0.26	19q13.4	ad RP
D22S283	-99.9	-18.9	-5.60	-3.48	-2.36	-1.65	-1.15	-0.78	22q	Sorsby's macular dystrophy

ad = autosomal dominant; ar = autosomal recessive; US = Usher syndrome; AMD = atypical macular dystrophy; VMD = vitelliform macular dystrophy; FEVR = familial exudative vitreoretinopathy.

DNA ANALYSIS

A high resolution karyotype was performed on four affected individuals from the family. No abnormalities were observed (data not shown).

Table 1 shows two point LOD (Log of the Odds) scores obtained in the family for a number of markers linked to various retinitis pigmentosa, Usher syndrome, maculopathy, and deafness loci. A LOD score of -2.0 or less indicates exclusion of the locus concerned as a site likely to harbour the disease gene. Conversely, a score of 3.0 or more provides significant evidence of linkage. Various regions of interest

have been excluded, including areas of linkage to loci of ophthalmic and audiological relevance.

Table 2 shows two point LOD scores obtained with a number of markers on chromosome 9q34-qter. The markers D9S118, D9S121, and ASS showed positive LOD scores of 3.75 at a recombination ratio (θ) of 0.10 , 3.41 ($\theta = 0.10$), and 3.25 ($\theta = 0.15$), respectively, suggesting that the causative gene in this family lies on chromosome 9q. Other, less statistically significant LOD scores were obtained in the same region.

Discussion

The pedigree TCD ZMK 92 is a large kindred segregating retinitis pigmentosa and sensorineural hearing loss in a pattern consistent with autosomal dominant inheritance. To our knowledge this is the first extensive family of this type to be clinically characterised and subjected to molecular genetic analysis.

The association of a pigmentary retinopathy with hearing loss has been known for many decades. The most common syndrome in which this association is found is that described by Usher in 1914, which is characterised by an autosomal recessive mode of inheritance. Deafness in Usher syndrome is typically congenital in onset and may be partial or complete. Karjalainen *et al.*²⁰ described a number of

Table 2 Two point LOD score values for chromosome 9q markers showing significantly positive scores greater than 3.0 for a number of markers

Marker	Recombination fraction θ								
	0.00	0.001	0.05	0.10	0.15	0.20	0.25	0.30	0.40
D9S121	-99.9	0.57	3.40	3.41	3.14	2.74	2.28	1.76	0.66
D9S118	-99.9	-0.93	3.53	3.75	3.57	3.21	2.73	2.18	0.95
ASS	-99.9	-3.47	2.70	3.24	3.25	3.02	2.63	2.14	1.58
ABL1	-99.9	-2.79	1.79	2.14	2.10	1.89	1.58	1.20	0.43
AFMc016xh9	-99.9	-3.34	1.32	1.77	1.83	1.72	1.51	1.24	0.56
D9S159	-99.9	-7.36	0.59	1.52	1.79	1.78	1.60	1.30	0.47
D9S164	-99.9	-3.79	0.90	1.38	1.49	1.43	1.29	1.08	0.56
D9S260	-99.9	-1.89	1.14	1.38	1.35	1.22	1.02	0.79	0.28
D9S290	-99.9	-10.1	-0.42	0.81	1.27	1.38	1.29	1.08	0.44
D9S66	-99.9	-10.7	-1.01	0.30	0.84	1.05	1.06	0.95	0.49
D9S158	-99.9	-10.7	-1.10	0.30	0.84	1.05	1.07	0.96	0.50
AFMb030zg9	-99.9	-6.33	-1.30	0.02	0.75	0.96	0.84	0.65	0.22

cases of Usher syndrome in which the hearing loss had its onset at puberty and was progressive (type III Usher syndrome), a pattern which is also found in our family. Karjalainen's cases however had an autosomal recessive inheritance pattern.

As may be seen in Figure 1, there is no evidence of male to male transmission in TCD ZMK 92. This is explained by the fact that most affected males in this family have not married and produced offspring. The possibility of an X linked gene operating in this pedigree was discounted by significant exclusion obtained with X chromosome markers.²¹ The clinical status of patient I1 (Fig 1) is anecdotal, the individual in question having died at the end of the last century. If this individual were unaffected it would raise the possibility of mitochondrial inheritance in this pedigree.

Significantly positive LOD scores—that is, >3.0 , were observed on chromosome 9q with the markers D9S118, D9S121, and ASS at a recombination ratio (θ) of 0.10 in each case. Analysis of this family is ongoing in order to find markers at 0 recombination. The number of microsatellite markers in the area is limited, as this region of 9q is sparsely mapped. Presently, the human genetic linkage map²² leaves a 14 centiMorgan (cM) gap between D9S158, the most distal marker, and D9S164, the next most distal one. In addition, the informativeness of markers within the family is reduced by the fact that the spouses of two ancestral affected people were siblings (Fig 1).

Mice homozygous for the 'whirler' (wi) mutation exhibit the 'shaker-waltzer' syndrome consisting of deafness, circling movements, and tossing of the head. Defects in the membranous labyrinth are thought to be responsible for the deafness. It may be of significance that the whirler locus is located on mouse chromosome 4, which is syntenic with human chromosome 9q32-q34.²³ The human homologue of this gene would be a candidate for the pathology noted in family TCD ZMK 92. Alternatively, a family of genes with ophthalmic and/or auditory functions may be located in this region.

The findings of abnormalities on ECG, EMG, and skeletal muscle biopsy were unexpected. A number of possible explanations present themselves for consideration. The causative gene, at present unknown, may have a function in muscle physiology. Individuals with Duchenne muscular dystrophy (DMD) have been documented as having abnormal retinal electrophysiology.²⁴ It has been postulated that the DMD protein is also functional in retinal cells. Mutated myosins have been shown to be responsible for type 1B Usher syndrome.¹³ Myosins are one of the structural proteins involved in muscle contraction. It is possible that mutations in similar contractile proteins might cause hearing and vision problems in addition to muscle abnormalities.

An intriguing possibility in our family is co-segregation of genomic and mitochondrial DNA mutations. A similar possibility has been invoked for the variation in phenotype noted in Leber's hereditary optic atrophy.²⁵ We are pres-

ently examining the mitochondrial DNA in order to investigate this possibility.

The data presented in this paper will be of benefit to the research community in directing attention to chromosome 9q as a candidate region for genes responsible for human retinopathies and/or hearing deficits.

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