LETTER TO JMG

Genotype-phenotype correlations for cataracts in neurofibromatosis 2

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Neurofibromatosis 2 (NF2) is an autosomal dominant disease that is caused by inactivating mutations of the *NF2* tumour suppressor gene.[1 2] Multiple central and peripheral nervous system tumours and ocular abnormalities are common in NF2; bilateral vestibular schwannomas are pathognomonic for the disease. Genotype-phenotype correlations are well established for NF2 associated tumours. In general, constitutional nonsense or frameshift *NF2* mutations are associated with severe NF2 (that is, earlier onset of symptoms and more tumours), splice site mutations with variable disease severity, and missense mutations with mild disease.

Genotype-phenotype correlations have not been reported for the non-tumour manifestations of NF2. The most common of these manifestations is presenile cataracts, which occur in about 60–80% of people with NF2.[3–5] In animal models, lens fibre cells that are more differentiated express less *Nf2* protein than the epithelial regions of the lens, suggesting that the *Nf2* protein may play a role in lens epithelial cell migration or elongation.[6] The purpose of this study was to determine if there were genotype-phenotype correlations for cataracts in NF2.

Key points

- Genotype-phenotype correlations have not been established for the non-tumour manifestations of neurofibromatosis 2 (NF2), such as cataracts.
- When compared to people with classical NF2 and nonsense or frameshift mutations, the relative risk of cataracts was significantly less than 1 in somatic mosaics, in people with large deletions, and in people with new unfound mutations and onset of symptoms at ages ⩾20 years, who probably have somatic mosaicism or large deletions.
- These results extend the genotype-phenotype correlations that have been reported for the tumour manifestations of NF2.

PATIENTS AND METHODS

The study was based on the United Kingdom NF2 registry in the Department of Medical Genetics, St Mary's Hospital, Manchester. NF2 patients are ascertained by contacting neurosurgeons, otolaryngologists, neurologists, paediatricians, dermatologists, and geneticists throughout the United Kingdom, augmented in the North West Region by the Regional Cancer Registry. The study was subject to continuing ethics committee evaluation and patients consented to participation. Patients were screened for constitutional *NF2* mutations using single strand conformational polymorphism analysis (SSCP) as previously described,[7] and examined for cataracts using slitlamp biomicroscopy at the time of diagnosis of NF2. For this study, cataracts were defined as present or absent (that is, posterior subcapsular cataracts and cortical cataracts were aggregated). There were 255 people from 190 families (159 people with new mutations and 96 inherited cases; 132 females and 123 males) (table 1).

For univariate analyses, Fisher's exact test was used for binary variables and the two-tailed *t* test was used for continuous variables. A multivariate probit model with an exchangeable correlation structure within families was used with various sets of covariates to account for possible familial dependence.[8] From a regression coefficient β, an approximate relative risk ($RR = \exp\{2^* \beta\}$) and confidence interval (CI) for presence of cataracts can be calculated. In the probit model, the reference group in comparisons between people with different types of *NF2* mutations was people who had constitutional nonsense or frameshift *NF2* mutations.

There is a potential bias towards a lower age at onset of symptoms or age at diagnosis in inherited cases owing to the family history of the disease. In the study group as a whole, there were no significant differences in these ages between people with new mutations and inherited cases for any type of *NF2* mutation. Also, using a probit model, the RR of cataracts was not significantly associated with age at diagnosis (see below). Therefore, for all mutation categories except unfound mutations, we combined people with new mutations and inherited cases. In the large group of people with unfound mutations, we retained the division between those with new mutations and inherited disease because people with new unfound mutations may be somatic mosaics. We used age at onset of symptoms to categorise people with new unfound mutations by disease severity (severe disease, onset of symptoms at ages <20 years; mild disease, onset of symptoms at ages ⩾20 years).[3]

RESULTS

As expected, the mean age at onset of symptoms and age at diagnosis were higher in people with non-truncating mutations and in somatic mosaics than in people with classical NF2 and nonsense or frameshift mutations (table 1). The overall prevalence of cataracts was 33%, but the prevalence of cataracts was significantly lower in somatic mosaics and in people with new unfound mutations and onset of symptoms at ages ⩾20 years than in people with classical NF2 and nonsense or frameshift mutations. In people with cataracts, 29% were diagnosed with cataracts at ages <10 years, and 47% at ages <20 years (mean 23 (SE 2) years). Seventy percent were diagnosed with cataracts before their first non-ocular sign or symptom.

In the multivariate probit model summarised in table 2, the RR of cataracts did not significantly increase with increasing age at diagnosis, after accounting for the type of constitutional *NF2* mutation. This was also the case in the other probit models (data not shown). This is probably due to the

Table 1 Characteristics of study population by type of *NF2* mutation

	Nonsense or frameshift					Unfound (mutations)			
							People with new mutations (age of onset (y))		
	Classical	Somatic mosaic	Splice site	Missense	Large deletion	Inherited cases	<20 years	⩾20 years	Total
No. of people/families	73/56	17/17	47/25	15/6	25/14	14/11	23/23	41/41	255/190
Age (y), mean (SD)									
Onset of symptoms†	16 (9)	32 (12)	23 (12)	30 (14)	21 (9)	25 (16)	12 (6)	34 (9)	22 (12)
Diagnosis	21 (12)	38 (12)	27 (15)	38 (21)	23 (9)	29 (17)	22 (12)	40 (11)	28 (15)
Current	27 (12)	44 (12)	32 (16)	45 (21)	30(11)	35 (20)	29 (12)	46 (13)	34 (16)
Intracranial meningiomas (%)	56	59	34	27	52	14	70	51	48
Cataracts (%)	45	18	38	27	28	36	39	10	33

†Excludes 15 inherited cases who were asymptomatic at the time of diagnosis of NF2.
Comparisons to people with classical NF2 and nonsense or frameshift mutations (p values are computed based on an assumption of independence, which is violated to a slight degree owing to families with multiple affected relatives):
Somatic mosaics: age at onset, age at diagnosis, current age, $p<0.001$; cataracts, $p=0.053$.
Splice site mutations: age at onset, $p=0.001$; age at diagnosis, $p=0.023$; current age, $p=0.043$; intracranial meningiomas, $p=0.024$.
Missense mutations: age at onset, $p=0.003$; age at diagnosis, $p=0.006$; current age, $p<0.001$; intracranial meningiomas, $p=0.049$.
Large deletions: age at onset, $p=0.032$.
Unfound mutations:
Inherited cases: intracranial meningiomas, $p=0.007$.
People with new mutations and age at onset 20 years: cataracts, $p<0.001$.
SD, standard deviation.

relatively young study population (mean age at diagnosis 28 years (SE 1); only 5% diagnosed at ages >55 years and 2% at ages >60 years), since the prevalence of posterior subcapsular and cortical cataracts in people aged <55 years in the general population is very low.[10]

The RR of cataracts was less than 1 in all mutation groups as compared to people with classical NF2 and nonsense or frameshift mutations. The RR was significantly lower than 1 in somatic mosaics (RR = 0.20, 95% CI = 0.10 to 0.40), in people with large deletions (RR = 0.39, 95% CI = 0.16 to 0.98), and in people with new unfound mutations and onset of symptoms at ages ⩾20 years (RR = 0.09, 95% CI = 0.03 to 0.28). The RR of cataracts in people with missense mutations was lower than 1 but not statistically significantly so (RR = 0.38, 95% CI = 0.14 to 1.08).

DISCUSSION

The low RR of cataracts in somatic mosaics, in people with large deletions, and in people with new unfound mutations and older onset of symptoms is consistent with the generally mild disease in NF2 patients with each of these types of mutations or conditions. The low RR of cataracts in people with new unfound mutations and older onset of symptoms could be due to several types of mutations or conditions that are unlikely to be identified by SSCP, and that are known to be associated or likely to be associated with mild NF2. These mutations or conditions are somatic mosaicism; large deletions, insertions, or other rearrangements; mutations in the 3 or 5 untranslated regions, the promoter region, or untranscribed transcriptional control elements; intronic mutations that are not covered by conventional SSCP primers; or other epigenetic events causing loss of *NF2* expression, such as methylation.

Somatic mosaicism and large deletions are the most likely of these possibilities. In the present study, 17 (18%) of the 92 patients with new mutations and identified constitutional *NF2* mutations were somatic mosaics. The estimated prevalence of somatic mosaicism in NF2 patients with new mutations is 25–30%.[11 12] Some of the NF2 patients with new unfound mutations and mild disease may be somatic mosaics in whom conventional DNA sequencing of lymphocyte DNA PCR product has failed to identify a difference from the normal sequence because the mutant allele is present at too low a level to be detected. Constitutional *NF2* large deletions

Table 2 Multivariate probit model for cataracts

Covariate	Estimated prevalence of cataracts from model (%)	Parameter estimate (SE)	RR	95% CI
Age at diagnosis (per year)		0.15 (0.58)	1.00	0.98 to 1.02
Exchangeable dependence (familial correlation)		0.11 (0.15)		
Type of *NF2* mutation				
Nonsense or frameshift				
Classical NF2	45	Reference group	Reference group	
Somatic mosaic	17	−0.82 (0.18)	0.20	0.10 to 0.40
Splice site	39	−0.17 (0.19)	0.71	0.34 to 1.50
Missense	28	−0.48 (0.26)	0.38	0.14 to 1.08
Large deletion	28	−0.47 (0.23)	0.39	0.16 to 0.98
Unfound				
People with new mutations				
Age at onset <20 years	39	−0.15 (0.15)	0.74	0.40 to 1.36
Age at onset ⩾20 years	9	−1.20 (0.28)	0.09	0.03 to 0.28
Inherited cases	36	−0.23 (0.34)	0.63	0.16 to 2.45

RR = relative risk, CI = confidence interval, SE = standard error.

have been found in 21% of NF2 families using microarray comparative genomic hybridisation,[13] and in 32% of NF2 families using multiple mutation screening methods.[14]

The intrafamilial correlation for cataracts was weak (and statistically insignificant) in all multivariate probit models that were tried, although there were relatively few families with multiple affected relatives. Several other clinical features of NF2 (age at onset of symptoms, age at diagnosis, and number of intracranial meningiomas) have strong familial correlations.[15] The prevalence of cataracts in the present study was lower than in other studies,[3–5] probably because the population-based United Kingdom NF2 registry is less heavily weighted towards NF2 patients with severe disease than studies of patients from tertiary referral clinics,[4 5] and because some cataract examinations were done by medical specialists other than ophthalmologists. Non-ophthalmologists may miss faint cataracts, but in such cases it is unlikely that faint cataracts are missed more frequently in people with mild NF2 than in those with severe NF2 (that is, it will not bias genotype-phenotype correlations). In a previous study based on the UK NF2 registry, all patients were examined using slitlamp biomicroscopy by a non-ophthalmologist, and the prevalence of cataracts was similar in mild cases (35%) and in severe cases (40%).[9]

The genotype-phenotype correlations for cataracts in the present study extend the correlations that have been reported for the tumour manifestations of NF2. The high prevalence of cataracts in young NF2 patients, and their frequent occurrence before the tumour manifestations of NF2, underline the importance of non-8th nerve signs and symptoms of NF2 in children and adolescents as a useful aid to diagnosis in this age group.[16]

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