Prenatal diagnosis and presymptomatic detection of neurofibromatosis type 1

M Upadhyaya, A Fryer, J MacMillan, W Broadhead, S M Huson, P S Harper

Abstract

A two year experience of DNA diagnosis for NF1 is presented. Twenty-three NF1 families have been analysed using 11 closely linked and intragenic markers. Prenatal testing was undertaken for six families; 11 affected subjects and their partners wished to know if they would be informative for future prenatal testing, seven of whom are so far fully informative. Presymptomatic testing was done for six subjects. Despite the availability of a series of closely linked markers, informativeness could not be achieved in all of the families tested.

Neurofibromatosis type 1 (NF1) is an autosomal dominant condition, affecting around 1 in 3000 subjects. The disease is typically characterised by café au lait spots, neurofibromas, and Lisch nodules. The main problems in NF1 arise secondary to disease complications, which are extremely variable and the occurrence cannot be predicted even within families.¹² The gene for NF1 was mapped to chromosome 17 in 1987^{3,4} and several close flanking markers were defined subsequently.^{5,6} Balanced translocations involving chromosome band 17q11.2 were reported in two unrelated NF1 patients^{7,8}; the breakpoints on chromosome 17 were 60 kb apart.⁹ Positional cloning (reverse genetic) techniques have enabled the gene to be cloned.^{10,11} Using a RNA PCR based assay,¹⁰ it was confirmed that translocations prevented transcription of the NF1 gene on the affected chromosome 17 and NF1 cDNAs were interrupted by both translocation breakpoints.^{11,12} The NF1 gene has been reported to be interrupted by four deletions, 11 kb, 40 kb, 160 kb, and 90 kb,^{11,13} one insertion,¹⁰ and three point mutations.¹⁴ Such specific changes are so far too infrequent to be of clinical application outside these rare specific families.

With the availability of closely linked and intragenic markers, it has been possible to provide prenatal and heterozygote testing for NF1. We report here our experience over a two year period of providing a diagnostic service for NF1 using DNA markers.

Material and methods

The families used in the analysis were referred from clinical genetics centres in Britain and elsewhere in Europe. Blood for DNA samples, clinical data, and pedigree data were provided by the referring centre after full discussion

with us. Chorionic villus tissue or DNA were sent by courier.

Whole blood for DNA extraction was collected in EDTA tubes. The DNA was digested with appropriate restriction enzymes (*MspI*, *RsaI*, *EcoRI*, *PstI*, *BamHI*, *HindIII*, *BglII*, *TaqI*) and fractionated on 0.7 to 0.8% agarose gel before transfer by Southern blotting to Hybond N (Amersham) filters. DNA probes were labelled with ³²P using the primer extension method¹⁵ and hybridised to filters at 65°C. The posthybridisation wash was in 2 × SSC, 0.1% SDS; the stringency of washing was increased as necessary. The hybridised membrane was exposed to x ray film at -70° for between one and seven days.

Clinical requests were of three types: prenatal diagnosis in a continuing pregnancy, suitability for future prenatal diagnosis, and assignment of genotype in clinically normal subjects at risk.

Two point linkage analysis for risk estimation was done using the program MLINK. Allele frequencies were determined for the founder of each NF1 family and the spouses. The recombination fraction and lod scores between the NF1 locus and each marker used for the risk assessment are summarised in table 1.

Results

Twenty-three families were referred for analysis. Prenatal testing was undertaken for six families (table 2), four giving a low risk result and two a high risk; one of these pregnancies was terminated. Eleven affected subjects and their partners wished to know if they would be informative for future prenatal diagnosis, seven of whom are so far fully informative (table 3). Presymptomatic testing was done for six subjects (table 4), this being discussed more fully below.

Particular examples are discussed here and are illustrated in the figure.

CASE 1

The proband (NF 501.3), whose mother was affected, initially wanted to know whether she would be informative for future prenatal testing. Analysis of this family with markers EW206, HHH202, β8.2, and C11.7 did not at this stage give an informative situation. By the time a pregnancy ensued, the availability of additional markers meant that the couple were informative using DNA markers VAW212 and pTH17.19. DNA analysis on a chorionic villus biopsy indicated that the fetus had inherited the grandpaternal (normal) allele and the risk

Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

M Upadhyaya

A Fryer*

J MacMillan

W Broadhead

P S Harper

Department of Medical Genetics, Churchill Hospital, Headington, Oxford OX3 7JL.

S M Huson

*Present address: Genetics Unit, Royal Liverpool Hospital, Prescot Street, Liverpool. Correspondence to Dr Upadhyaya.

Received 18 August 1991. Revised version accepted 25 September 1991.

Table 1 Chromosome 17 DNA markers used for genetic prediction in NF1.

Probe	Locus	Enzyme	Rare allele frequency	Location	θ	Z	CI
NF1c2	EV12B	EcoRI	0.47	Intragenic	0	6.51	0-0.03
pRC9.4	EV12A	BamHI	0.48	Intragenic	0	4.86	0-0.09
88.2	CRYB1	MspI	0.40	Proximal	0	5.53	0-0.10
C11.7	D17S83	BamHI	0.37	Distal	0.02	7.04	0-0.11
HHH202	D17S33	RsaI	0.36	Proximal	0.01	16.6	0-0.08
pTH.17.19	D17S82	BglII	0.48	Proximal	0.03	5.6	
VAW212R2i	D17S117	PstI	0.27	Proximal	0.02	6.99	0-0.12
VAW215R2B	D17S120	TaqI	0.20	Proximal	0.00	2.89	0-0.12
VAW211R3	D17S117	MspI	0.08	Proximal	*		
210MI	D17S117	MspI	0.27	Distal	*		
EW206	D17S57	MspI	0.30	Distal	0.03	4.2	0-0.13

* Closely linked (D Barker, personal communication).

Table 2 Results of requests for prenatal diagnosis in NF1.

Case	Family	Probe	Result	Outcome
1	NF 501	VAW212R2i	Low risk (3%)	Continuation of pregnancy
2	NF 502	88.2	Low risk (2%)	Continuation of pregnancy
3	NF 506	HHH202	High risk (96%)	Continuation of pregnancy
4	NF 507	VAW212R2i	Low risk (3%)	Continuation of pregnancy*
5	NF 511	pRC9.4	High risk (99%)	Termination of pregnancy†
6	NF 521	210MI	Low risk (3%)	Continuation of pregnancy

* A twin pregnancy (single placenta) was diagnosed at 18 weeks' gestation and went to term.

† The pregnancy was terminated before the typing result was available as the karyotype showed a 45,X (Turner) fetus.

Table 3 Requests for suitability for future prenatal diagnosis in NF1.

Result	No
Couple fully informative	7
Couple partially (50%) informative	1
Couple uninformative at present	3
Total requests	11

Table 4 Results of requests for assignment of genotype status in NF1.

Case	Family	Age (y)	Result
1	NF 519	Fetus*	Unaffected
2	NF 514	1	Unaffected
3	NF 522	3	Awaited
4	NF 503	14	Unaffected
5	NF 510	16	Unaffected
6	NF 523	16	Unaffected

* Amniocentesis for maternal age; subsequently requested status in the third trimester.

to the pregnancy was reduced from 50% to 4%. A healthy son has since been born.

CASE 2

The proband (NF 502:3) wished to know whether she would be informative for future prenatal testing. The family was analysed initially using closely linked markers and also with distant markers so that haplotypes could be constructed. The couple were partially informative for future prenatal diagnosis using probe 88.2. The couple were counselled on the significance of these DNA results and in a subsequent pregnancy they opted to have prenatal diagnosis; the risk to the fetus was reduced from 50% to 4%.

CASE 3

This family (NF 506) was referred to us from Finland at 10 weeks' gestation (Dr M Ryynanen). The couple was informative using flanking DNA markers HHH202, proximal to the disease locus, and C11.7, a distal marker. Chorion villus biopsy was obtained at 12 weeks and the fetus had inherited the high risk allele. It should be noted that it is a phase unknown situation and a recombination has taken place between C11.7 and the disease. The risk was calculated allowing for uncertainty of phase. The parents decided after counselling to continue the pregnancy and a female child with multiple café au lait spots was born.

CASE 4

This family (NF 507), referred by Dr E Thompson, was analysed using six DNA markers and not found to be informative. The proband (NF 507:2) presented at 8 weeks' gestation requesting prenatal diagnosis. She was found to be informative using DNA marker VAW212 and the fetus sampled carried the low risk allele. Ultrasound scan at 18 weeks' gestation unexpectedly showed a twin pregnancy with single placenta. The pregnancy continued to term with the birth of healthy twins. No further DNA studies have been undertaken on these infants.

CASE 5

This pregnancy was referred at 8 weeks' gestation from Israel (Dr J Zlotogora); the proband (NF 511:2) was considered to be a new mutation. Her clinical features included café au lait spots and neurofibromas. She had an affected son who had café au lait spots only. The couple requested prenatal diagnosis and were informative using intragenic marker pRC9.4. Chorion biopsy was taken at 12 weeks and DNA was sent in ethanol. The genotype of the fetus was the same as that of the affected sib giving a risk approaching 100%. The pregnancy was terminated before the DNA result was available because the karyotype showed a 45,X (Turner) fetus.

CASE 6

In this family, the proband (NF 513:4) had an affected father and unaffected daughter. Because of the lack of availability of prenatal diagnosis, her husband had had a vasectomy in the past. If informative for prenatal diagnosis the husband was going to have a reversal of sterilisation. This family has been analysed using 10 DNA markers, but an informative situation could not be reached.

CASE 7

This family (NF 519, table 4) was referred for DNA analysis during the first trimester of pregnancy. The DNA analysis was done rapidly and showed that the couple were suitable for prenatal diagnosis using an intragenic

Family pedigrees. A, β 8.2 (*CRYB1*). B, C11.7 (*D17S83*). F, HHH202 (*D17S33*). M, VAW212R2i (*D17S117*). N, pRC9.4 (*EV12A*). R, NF1c2 (*EV12B*).

probe. They decided against prenatal diagnosis but a subsequent amniocentesis was carried out because of advanced maternal age and DNA from amniocytes was banked. Presymptomatic testing of the banked fetal DNA was done in the third trimester at the family's request because of excessive anxiety of the mother. The fetus carried the low risk allele. A healthy son has since been born.

CASE 8

In this family, the mother (NF 514:3, table 4) had NF1 with secondary complications of severe kyphoscoliosis and seizures. The parents wanted to know the genetic status of their 15 month old daughter who did not have any signs of NF1. The couple were informative using DNA marker VAW212 and the risk of the daughter having the disease was reduced from 50% to 4%.

Discussion

In undertaking linkage studies for NF1, we were aware that the first likely application of linked DNA markers would be for prenatal testing. In Cardiff, where a NF clinic was set up in 1987 offering annual follow up and information concerning future prenatal diagnosis, the application of the linkage approach has been found to be useful for prenatal and presymptomatic diagnosis in affected families. As with any dominant condition, at least two affected subjects were ideally needed in separate generations so that the haplotype associated with NF1 could be determined. Despite the availability of a battery of closely linked and intragenic markers, informativeness could

not be achieved in all the families tested, as shown by family NF 513. The inadequate structure of some families and lack of heterozygosity remain problems. RFLPs associated with the cDNAs for NF1 may improve this situation.¹⁶ The intragenic recombination for NF1 is not known.

The clinical criteria for the diagnosis of NF1 patients have been defined in relation to other forms of NF¹⁷ and are important to establish before tests are offered. As penetrance is almost 100% by the age of 5 years,¹⁸ clinical diagnosis after this age is not a problem; molecular testing should not be needed for clinically unaffected adults. Despite the absence of any abnormal clinical features in the adolescents included in this study, the parents still wanted the reassurance that a low risk DNA result would provide. However, in many paediatric situations, a sufficiently precise diagnosis may not be possible and knowledge of the child's genotype may be helpful. The analysis of DNA samples from asymptomatic children at 50% prior risk of inheriting the gene for NF1 is, however, a contentious issue, as is the general topic of testing children for genetic disorders, especially those of later onset.¹⁹ Some clinicians feel that it is important to make the diagnosis early so that parents can be offered counselling and guidance and that children can be followed for such complications as optic glioma and hypertension. Families which have experienced severe manifestations in a previous child may be especially concerned; the reassurance brought by the prediction of an unaffected genotype in a younger sib must be weighed against any perceived harm that an 'affected' assignment might bring in an asymptomatic child. We believe that each

family situation is different and each request should be discussed fully with the family. The results of DNA studies should always be combined with clinical examination in order to reduce errors in the assignment of genetic status.

One study involving presymptomatic detection and informativeness for prenatal testing in NF1 has already been reported.²⁰ The results reported in our paper are the first to include prenatal diagnosis. We have found the demand for prenatal diagnosis to be limited. In addition to the six pregnancies described here, two families were referred for analysis during pregnancy, were informative for DNA markers, but decided against prenatal testing. The main reason for not choosing to have prenatal testing in the majority of cases was that the parents would only want to consider a test which would predict disease severity. Our series also shows that it cannot be guaranteed that all couples will be informative for prenatal diagnosis, even with available closely linked markers. In addition it is preferable to perform DNA informativeness analysis before a pregnancy is undertaken. The observed outcome of the completed pregnancies have not shown any error, but since the disease is not fully penetrant until the age of 5 the final outcome is still awaited. However, in family NF 506, where DNA analysis predicted a high risk pregnancy, a female child with multiple café au lait spots was born.

Direct analysis of specific mutations or deletions is currently possible for only a very small proportion of families with NF1, but this is likely to change rapidly as the detailed structure of this large gene becomes better understood. The detection of specific mutations will permit specific prenatal diagnosis independent of family structure and will be of particular help for the large numbers of isolated cases. Mutation analysis will also establish whether cases of NF1 with unusual phenotype are indeed determined by the same locus and will thus allow genetic testing to be undertaken for these families.

We thank Drs F Collins, P O'Connell, and D Barker for the DNA probes and Dr M Sarfarazi for linkage analysis. We are grateful to

Action Research for the Crippled Child for financial support and the British Neurofibromatosis Association (LINK) for their help. We also thank the clinicians and laboratories who referred samples, in particular our colleagues in the West of Britain Molecular Genetics Consortium.

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