phenomenon are very rare.⁷ However, because of the seriously confounding influence of non-penetrance on genetic linkage studies, every effort must be made to minimise the potential impact of this problem. At present, this can only be done by using age related diagnostic criteria, as discussed below.

Von Recklinghausen NF: diagnostic criteria

In February 1986, an NF 'task force' was assembled by the American National Institute of Neurological and Communicative Diseases and Stroke. At that meeting, diagnostic criteria for VRNF were adopted. The diagnosis of VRNF is probable, if not certain, when any two of the following are present: (1) a first degree relative with VRNF (independently documented); (2) five or more café au lait spots at least 5 mm in diameter for prepubertal patients, or six or more at least 15 mm in diameter thereafter; (3) freckling in the axillary or inguinal regions; (4) two or more neurofibromas of any type or one plexiform neurofibroma; (5) iris Lisch nodules. Additional supportive findings characteristic of VRNF include optic pathway gliomas and pseudarthrosis (or tibial/fibular bowing). The presence of bilateral acoustic neuromas rules out the diagnosis of VRNF and favours the diagnosis of BANF.

For the purpose of providing consistent criteria for multi-institutional VRNF genetic linkage studies, the above criteria should be used for clinical determination of the presence of the VRNF mutant gene. Since genetic linkage studies inter alia use only persons with an affected first degree relative, isolated cases presenting with only one of the other criteria will not present a diagnostic problem in this particular context.

In general, there will be three types of subjects:

those for whom the diagnosis of VRNF is obvious and unequivocal; those for whom there is no basis for considering VRNF other than the fact of an affected first degree relative; and those who have suggestive, but not sufficient, findings (for example one or two café au lait spots or similar skin lesions). While the diagnosis of VRNF is ordinarily definite by one year of age, because of the need to avoid erroneous assignment of phenotype, for genetic linkage studies one should not score a subject as definitely unaffected until he or she has reached five years of age.

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Von Recklinghausen neurofibromatosis: a linkage study of candidate and random marker genes

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SUMMARY Genotyping, using plasma proteins or DNA polymorphisms or both, was carried

out on 30 families selected through probands with Von Recklinghausen disease. The data provide additional evidence for the exclusion of loci on chromosomes 3 and 5, and chromo-

some arms 1q, 2p, 4p, 4q, 6q, 7p, 9q, 11p, 11q, and 14q. There was no evidence for genetic heterogeneity at D1S1 (DNF15S2) on chromosome arm 3p, using the Morton test for heterogeneity.

This paper is part of an international consortium project organised to establish genetic linkage of Von Recklinghausen neurofibromatosis. The data provide additional evidence for the exclusion of loci on chromosomes 3 and 5 and chromosome arms 1q, 2p, 4p, 4q, 6q, 7p, 9q, 11p, 11q, and 14q.

Materials and methods

The data reported here derive from 15 families from the Baylor NF Program (BNFP), described previously by Dunn *et al.*,¹ six families previously studied by Huson *et al.*,² and nine families from the University of Iowa (UI). All families were selected

through probands with ordinary Von Recklinghausen disease, or NF-I.³ Affected subjects were diagnosed according to the criteria of Riccardi and Carey.³ The number of families and their members participating in this investigation are given in table 1.

Plasma protein typing was carried out on the BNFP and UI families, D1S1 typing was carried out on the BNFP and Cardiff families, and genotyping for the remaining DNA polymorphisms was carried out on the UI families. Standard abbreviations for the marker loci studied are according to Human Gene Mapping 8,⁴ and a specific reference for each locus is given in table 2. Endonuclease and probe (clone) names are available on request. The linkage analyses used the computer programmes LIPED⁵ and LINKAGE.⁶ Recombination fractions (θ) are equal for males and females. In these families, there was no evidence of reduced penetrance and therefore either 99.7% or 100% penetrance was used for the calculation of lod scores.

Results

Results of the linkage analysis of the plasma protein coding loci and the DNA polymorphisms are presented in table 2. There was no evidence for genetic heterogeneity at D1S1 (DFN15S2) on chromosome arm 3p, using the Morton test for heterogeneity (data not shown).

TABLE 1 Patient study population.

	Houston	Cardiff	Iowa	Total
No of families	15	6	9	30
No of persons	82	85	79	246
No of affected	51	53	37	141
No of informative meioses	43	44	32	119

TABLE 2 Summed lod scores for linkage between NF-I and eight protein coding loci and 14 DNA polymorphisms for recombination fractions 0.05 to 0.40.

Protein	Assignment	Excl	Recombination fraction					Refs
			0.05	0.10	0.20	0.30	0.40	
A2HS	3	0.20	-4.20	-2.59	-0.98	-0.32	-0.04	7
APOA4	11q13→qter	0.10	-2.15	-1.17	-0.35	-0.11	-0.04	8
PI	14q32.1	0.15	-7.64	-4.48	-1.83	-0.73	-0.24	9
C6	LG4	0.05	-0.97	-0.32	0.08	0.13	0.07	10
PLG	6q25→qter	0.25	-5.00	-3.18	-1.62	-0.83	-0.33	11
ORM1	9q	0.10	-2.22	-1.32	-0.53	-0.19	-0.06	12
F13A	6q23→qter	0.20	-3.99	-2.57	-1.29	-0.65	-0.26	13
F13B	1q	0.10	-3.53	-1.83	-0.48	-0.02	0.07	14
DNA gene								
AT3	1q23→q25	0.00	-0.11	0.06	0.21	0.22	0.14	15
TGFA	2p13	0.15	-2.29	-1.47	-0.75	-0.36	-0.14	16
D4S35	4pter→q12	0.30	-5.86	-4.00	-2.19	-1.19	-0.50	17
ALB	4q11→q13	0.05	-1.58	-0.72	-0.04	0.14	0.11	17
GC	4q12→q13	0.20	-3.36	-2.21	-1.25	-0.67	-0.28	17
GIFN	4q	0.10	-1.74	-1.15	-0.52	-0.20	-0.05	17
ADH3	4q21→q25	0.00	-0.10	0.09	0.21	0.21	0.14	17
EGF	4q25→q27	0.15	-2.05	-1.36	-0.70	-0.35	-0.13	17
FG	4q26→q28	0.00	-0.39	-0.25	-0.09	-0.03	0.00	17
GRL	5	0.00	-0.15	0.11	0.29	0.28	0.17	18
PLG	6q25→qter	0.20	-4.12	-2.72	-1.35	-0.64	-0.23	19
EGFR	7p11→p13	0.00	-0.75	-0.47	-0.21	-0.08	-0.02	20
INS	11p15	0.25	-4.33	-3.08	-1.73	-0.93	-0.39	21
D1S1	3p	0.20	-7.26	-4.07	-1.50	-0.56	-0.21	22

Protein=protein coding loci; DNA gene=DNA polymorphism loci; Assignment=chromosomal region or linkage group assignment; Excl=exclusion interval, based on a lod score of -1.00 or less; GIFN=GIFN31.7. D1S1 is also known as DNF15S2.⁴

Discussion

The possibility that NF-I is linked to markers on chromosome 4q appears to have been excluded by the data in table 2, a conclusion consistent with the findings of other investigators. In addition, the genes for TGFA, EGF, GRL, and EGFR, which may be considered 'candidate genes' for NF-I, show no evidence of close linkage. Eleven other loci selected on the basis of convenience also show no evidence of linkage.

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Linkage analysis of British and Indian families with Von Recklinghausen neurofibromatosis

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SUMMARY Linkage analysis has been undertaken in two British and three South African Indian families with Von Recklinghausen neurofibromatosis. Eleven polymorphic DNA

probes were studied, including both random DNA sequences and candidate oncogenes. Although no evidence for linkage of these probes to the disease was detected, substantial exclusion regions were established on six of the chromosomes studied.

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