ELECTRONIC LETTER

Evaluation of genotype-phenotype correlations in neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with complete penetrance and extremely variable expression, and an incidence of approximately 1 in 4000 live births. Despite the high incidence of NF1, neither the natural history nor the genetic epidemiology of the disorder are well understood. People with NF1 have reduced reproductive fitness and life expectancy,[1] but the cause of the high mutation rate at the *NF1* locus is unknown.

The most prominent clinical hallmarks of NF1 are café-au-lait (CAL) macules, neurofibromas, Lisch nodules of the iris, and axillary freckling.[2] Other clinical manifestations are abnormalities of the cardiovascular, gastrointestinal, renal, and endocrine systems, facial and body disfigurement, cognitive deficit, and malignancies of the peripheral nerve sheath and central nervous system. About 25% of people with NF1 develop one or more of these clinical complications, which together cause significant morbidity and mortality.[3] The tumours that occur in NF1 are dermal and plexiform neurofibromas, optic gliomas, malignant peripheral nerve sheath tumours (MPNSTs), pheochromocytomas, and rhabdomyosarcomas.[1] Children with NF1 have an increased risk of developing myeloid disease, particularly juvenile chronic myeloid leukaemia. Some 30–40% of NF1 patients develop plexiform neurofibromas[2–4] that become MPNSTs in 5–10% of cases,[5–7] often in pre-existing plexiform neurofibromas.

The *NF1* gene on chromosome 17q11.2 spans more than 350 kb of genomic DNA and contains 60 exons.[8] The 8457 bp open reading frame predicts a protein of 2818 amino acids with an estimated mass of 327 kDa.[9] [10] The *NF1* gene product, neurofibromin, contains a 360 amino acid region with homology to the catalytic domain of the mammalian guanosine triphosphatase activating protein. Neurofibromin is a GTPase activating protein for members of the p21ras (Ras) protein family. Loss of neurofibromin function leads to downstream cell growth activation because neurofibromin negatively regulates Ras output by accelerating the conversion of active Ras-GTP to inactive Ras-GDP. Three genes (*EVI2A*, *EVI2B* and *OMGP*) are embedded within intron 27 but are transcribed in the opposite direction to the *NF1* gene.[11] [12] However, in a comparative study of various *NF1* gross gene deletions, Viskochil[8] concluded that haploinsufficiency of the three embedded genes did not contribute to the clinical features of NF1.

Genetic counselling is problematic in NF1 owing to the marked inter- and intra-familial variation in NF1 expression. The exploration of genotype–phenotype correlations in NF1 is still in its infancy because of the extensive mutational heterogeneity of the gene, and because large scale mutation screening is laborious owing to the size and complexity of the gene. In this study, we characterised constitutional *NF1* gene mutations in 113 NF1 patients, and statistically evaluated genotype–phenotype correlations in 110 of these patients. We also considered atypical cases in detail because allelic heterogeneity is a possible cause of some NF1 phenotypes,

Key points

- Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with complete penetrance and extremely variable expression, with an incidence of approximately 1 in 4000 live births.

- Despite the high incidence of NF1, neither the natural history nor the genetic epidemiology of the disorder is well understood. In this study, we characterised constitutional *NF1* gene mutations in 113 NF1 patients and statistically evaluated genotype–phenotype correlations in 110 of these patients.

- The clinical abnormalities were café-au-lait macules, axillary freckling, number of dermal and nodular neurofibromas, macrocephaly, short stature, ratio of head circumference to height, pectus excavatus, learning disabilities, and plexiform and spinal neurofibromas.

- Logistic regression analysis was used to calculate relative risks (RR) and 95% confidence intervals (CI) for binary outcomes, while linear regression analysis was used for continuous outcomes.

- When compared with people with nonsense or frameshift mutations and adjusted for age at examination, individuals with missense mutations had an RR of Lisch nodules that was lower than unity, although of borderline statistical significance (RR = 0.26, 95% CI = 0.07 to 1.04, p = 0.06).

- This exploratory finding nevertheless merits investigation in other NF1 patient populations.

particularly Watson syndrome and NF Noonan syndrome (NFNS).[13–15]

METHODS

The constitutional *NF1* gene mutations were identified in one laboratory using a battery of different mutation detection techniques: single strand conformational polymorphism analysis, heteroduplex analysis, the protein truncation test, dideoxy fingerprinting, denaturing high performance liquid chromatography, and direct DNA sequencing.[16–20] Clinical data were obtained from medical records of examinations within the previous 2 years, or if recent medical data were

Abbreviations: CAL, café-au-lait; MPNSTs, malignant peripheral nerve sheath tumours; NF, neurofibromatosis; NFNS, neurofibromatosis Noonan syndrome; NIH, (US) National Institutes of Health

unavailable, the patients were re-examined. The examining physician was blinded to the patient's mutation.

There were 113 NF1 patients with identified *NF1* gene mutations. Three of these patients were excluded from the statistical analysis of genotype–phenotype correlations: two patients who did not meet the US National Institutes of Health (NIH) diagnostic criteria for NF1[21] and a single patient with an identified *NF1* large gene deletion. The common clinical abnormalities were coded as: Lisch nodules (present/absent), number of CAL macules (0, 1–6, >6), axillary freckling (present/absent), number of dermal and nodular neurofibromas (0, 1–10, 11–100, 101–500, >500), macrocephaly (present/absent), short stature (present/absent), the ratio of head circumference (cm) to height (m) (OFC/height ratio), pectus excavatum (present/absent), learning difficulties (present/absent), plexiform neurofibromas (present/absent), scoliosis (present/absent), and spinal neurofibromas (present/absent). Percentiles for the OFC/height ratio were calculated using charts.[22 23] The prevalence of other clinical abnormalities (glioma, sarcoma, phaeochromocytoma, gastrointestinal neurofibromas, pulmonary stenosis, renal artery stenosis, delayed puberty, aqueduct stenosis, Watson syndrome, Noonan syndrome, epilepsy, bone dysplasia) was too low (5% or less) for meaningful statistical analysis.

Logistic regression analysis was used to calculate relative risks (RR) and 95% confidence intervals (CI) for binary outcomes. Linear regression analysis was used for continuous outcomes. Each regression model had the covariates of the type of constitutional *NF1* mutation and age at examination (as a continuous variable). The mutation covariate was coded as several binary variables (indicators of missense and splice site mutations, each compared to nonsense or frameshift mutations). Age at examination was included as a covariate because the penetrance of many NF1 disease features increases with age.[24 25] A logarithmic transformation was used for age at examination, which ranged from 2 to 70 years. There is a potential bias toward lower age at examination in non-probands relative to probands, but proband status did not contribute significantly to any of the models after including age at examination as a covariate, so probands and non-probands were combined in subsequent analyses. There were eight families with two affected relatives and two families with three affected relatives.

We evaluated genotype–phenotype correlations for each common clinical abnormality individually and for three groups of disease features:[26] (*a*) CAL macules, axillary freckling, and Lisch nodules, (*b*) dermal, nodular, and plexiform neurofibromas, and (*c*) macrocephaly, optic glioma, and other neoplasms. A patient was categorised as belonging to a group if at least two disease features in the group were present.

RESULTS

The identified constitutional *NF1* gene lesions ranged from single base pair substitutions to gross deletions, but the microlesions appeared to be uniformly distributed across the gene (http://www.uwcm.ac.uk/study/medicine/medical_genetics/research/nf1/supplementary_data.html). The prevalence of common clinical abnormalities in this study was generally similar to those of other published studies (table 1). Delayed and precocious puberty occur in NF1,[27 28] but the exact prevalence is unknown. In this study, 6% of males and 5% of females had delayed puberty at16 years of age. One patient had an empty sella turcica without abnormal pituitary function and a second patient with very short stature had isolated growth hormone deficiency with otherwise normal pituitary function. There were no patients with precocious puberty.

Genotype–phenotype correlations

Eighty four patients had ocular examinations. As expected, the relative risk (RR) of Lisch nodules increased with increasing age at examination (table 2). When compared with people with nonsense or frameshift mutations, people with missense mutations had an RR of Lisch nodules that was lower than unity but of borderline statistical significance (RR = 0.26, 95% CI 0.07 to 1.04, p = 0.06, Cox and Snell $R^2 = 0.10$). There were no significant genotype–phenotype correlations for any other individual abnormalities or for any of the three groups of disease features (data not shown).

Atypical cases

There were two patients who did not fulfil the NIH diagnostic criteria for NF1 and who were not included in the statistical analysis of genotype–phenotype correlations. One was a 10 year old child who, in common with his mother, had CAL macules but no other stigmata suggestive of NF1. The *NF1* mutation in the family was a missense substitution (R1809L). The second patient was a 27 year old woman with no family history of NF1 and no CAL spots, freckling, or Lisch nodules. She had a small number (<10) of cutaneous and nodular neurofibromas, confirmed on biopsy. In addition, she had a vestibular schwannoma and a schwannoma of the posterior fossa involving the vagus nerve. A cervical cord ependymoma was resected, leaving the patient paraplegic. Although initially considered as a possible case of NF type 2, a single base pair insertion in exon 37 (6792 ins A) of the *NF1* gene was subsequently identified in this patient.

There were three patients with NFNS. The first NFNS patient was a 6 year old boy from a large Ashkenazi kindred with NF1. He had only four CAL macules on examination but had been developing cutaneous neurofibromas since the age of 2 years, and had intracranial tumours and hydrocephalus secondary to aqueduct stenosis, which had required shunting. There were no Lisch nodules and he was not macrocephalic. He had facial dysmorphism suggestive of Noonan syndrome but had an inactivating splice site mutation in the *NF1* gene (IVS 29+1 G→C).

The second NFNS patient was a 20 year old man who fulfilled the NIH diagnostic criteria for NF1, and had a pectus excavatum, learning difficulties, and mild hydrocephalus. He was also thought to have aqueduct stenosis causing hydrocephalus, which did not require shunting at the time of examination. The *NF1* mutation was a microdeletion (2133 del CC). There was no evident cardiac abnormality in these two patients.

The third NFNS patient was a 10 year old boy who was born prematurely and had facial dysmorphism, widely spaced nipples, and an atrial–septal defect. His *NF1* mutation was a microinsertion (4093 ins TG).

There were three patients with Watson syndrome. Two had mild to moderate pulmonary stenosis that was documented on echocardiography. Neither had a history of significant learning difficulties, but they did not complete their schooling. One patient had a novel 3 bp deletion (AAT) within exon 17 of the *NF1* gene leading to a loss of ATG (methionine) in exon 17,[29] and the other had a paternally inherited nonsense mutation in exon 29 (S1745X). This patient's father also had mild pulmonary stenosis with stigmata of NF1. The third patient was a 49 year old man with significant learning difficulties, behavioural problems associated with epilepsy, and pulmonary stenosis. He had an intragenic *NF1* gene deletion of >20 bp.

Another atypical patient was a 31 year old woman who had stigmata of NF1 almost exclusively in the cranial region. There were four CAL macules on the head and neck only, and multiple large histologically confirmed neurofibromas on the scalp and behind the ears. There was also macrocephaly but

Table 1 Prevalence of common clinical features of NF1 in this study and in previously published studies

Clinical feature	Prevalence in this study (%)	Prevalence in previous studies (%)	References
CAL macules	98	100 (at 15 years of age)	2 23 27
Axillary freckling	41	85	71 72
Neurofibromas	81	98–100	27 72
Lisch nodules	82	92–95	27 73
Macrocephaly	40	45	27 74
Short stature	50	30–40	27 72
OFC/height ratio⩾95%	97	Unknown	None
Pectus excavatus	12	Up to 30	71 72
Learning difficulty	35	30–60	75 76
Plexiform neurofibromas	37	27	27

no other manifestations of NF1 in the rest of the body. In addition, the proband was diagnosed with Van der Woude syndrome at the age of 6 months, with a cleft palate and lip pits, which were surgically treated. The *NF1* mutation was a microdeletion in exon 22 (3731 del T).

DISCUSSION

NF1 patients with missense mutations had an RR of Lisch nodules that was less than 1, and of borderline statistical significance when compared to NF1 patients with nonsense or frameshift mutations and adjusted for age at examination. This exploratory finding merits investigation in other NF1 patient populations, although it is difficult to evaluate the pathogenicity of missense mutations without functional studies. In this study, multiple statistical comparisons were made for the different clinical abnormalities and different mutation types, but as these analyses were exploratory, not confirmatory, we chose to test associations at the $\alpha = 0.05$ level.

From the clinical viewpoint, our findings are notable for two reasons. The OFC/height ratio was at or above the 95th percentile in 97% of cases, but the prevalence of macrocephaly was 40% and the prevalence of short stature was 50%. This suggests that an increase in the OFC/height ratio is a useful clinical indicator of NF1, even in the absence of absolute macrocephaly or short stature. The finding of delayed puberty in 6% of males and 5% of females at 16 years of age confirms that this is an NF1 related phenomenon.

We did not find genotype–phenotype correlations for other common clinical features of NF1, similar to the findings of most other studies of genotype–phenotype correlations in NF1.[4][30–33]

The only exception is the correlation of large *NF1* gene deletions (up to 1.5 Mb genomic DNA) with an earlier age of onset of cutaneous neurofibromas, learning disability, dysmorphic features, and developmental delay.[18][34–38] However, not all patients with a gross *NF1* gene deletion have this NF1 phenotype,[18][36] and flanking DNA sequence (or the lack of it) may affect the phenotype. Easton *et al.*[30] modelled the genetic contributions to eight traits in 175 people with NF1 (CAL

macules, neurofibromas, head circumference, plexiform neurofibromas, optic gliomas, scoliosis, epilepsy, and remedial education). There were no significant correlations between the traits, indicating that the phenotypic expression of NF1 was largely determined by trait specific loci unlinked to the *NF1* gene.

We were not able to evaluate genotype–phenotype correlations for some of the most clinically significant disease features of NF1, such as the various types of cancer. The RR of these complications is very high compared with the general population, but the prevalence in NF1 is relatively low, and there were too few patients with these complications in this study to support statistical analysis.

Identical *NF1* gene mutations can occur in unrelated patients with very different phenotypes.[11][19][38–41] Owing to the absence of strong genotype–phenotype correlations,[30] *NF1* mutation analysis has very limited predictive utility for specific sequelae. *NF1* mutation analysis has greater diagnostic utility because mutation detection can confirm the aetiology of the disease in the relatively few families and individuals in whom the clinical phenotype does not fulfil the NIH diagnostic criteria. A provisional diagnosis of NF1 improves clinical management by triggering the initiation of routine blood pressure monitoring in patients from an early age and alerting medical care providers to the possibility of clinical complications. A firm diagnosis allows counselling regarding mode of inheritance, recurrence risk, and potentially, prenatal diagnosis.

Allelic heterogeneity is a possible cause of some of the multiple phenotypes in NF1, particularly Watson syndrome (multiple CAL macules, pulmonic stenosis, and dull intelligence),[13] NFNS,[15] and familial spinal neurofibromatosis.[42] To date, however there is no evidence to support allelic heterogeneity as a cause for any of these three atypical phenotypes, nor did we find such evidence in the present study, as detailed below.

Allelic heterogeneity is not likely to be responsible for Watson syndrome because the three patients in the present study with Watson syndrome had *NF1* mutations of differing types and locations. Divergent *NF1* gene mutations have been

Table 2 Results of logistic regression with presence of Lisch nodules as the outcome

Covariate	RR	95% CI	P
Age at examination (per unit increase in logarithm of age)	8.53	1.43 to 50.83	0.02
Type of constitutional *NF1* mutation (compared with nonsense or frameshift mutations)			
Missense mutation	0.26	0.07 to 1.04	0.06
Splice site mutation	0.55	0.09 to 3.27	0.51

CI, confidence interval; RR, relative risk.

identified: an 80 kb deletion in a Watson syndrome patient,[16] and a 42 bp duplication that segregated in a family with a phenotype suggestive of NFNS.[43]

About 12–13% of NF1 patients who are specifically examined for Noonan syndrome have a clinical phenotype that is similar to Noonan syndrome,[14 15] but Carey,[44] and Carey and Viskochil[32] reviewed these patients, and concluded that they did not have typical Noonan syndrome, as features of Noonan syndrome without cardiovascular malformation have been observed in many NF1 patients. The three patients in the present study with features of Noonan syndrome had NF1 mutations of differing types and locations. As two of these patients had hydrocephalus associated with features of aqueduct stenosis, the dysmorphic features of Noonan syndrome could be a secondary effect, and NFNS may not be a distinct clinical entity. There is clinical overlap between Noonan syndrome and NF1, but the Noonan syndrome gene (PTPN11) has been mapped to 12q24.1 and there is no suggestion of locus heterogeneity for NF1.

Spinal neurofibromas have been reported in up to 38% of NF1 patients, but only 5% of these patients have spinal neurofibromatosis.[42] All patients in our study who had familial spinal neurofibromatosis also fulfilled the NIH diagnostic criteria for NF1, and had Lisch nodules and dermal neurofibromas, as has been previously reported.[42 45] Ars et al[42] described a frameshift mutation in exon 46 (8042 ins A) that cosegregated with spinal neurofibromatosis. In another study, two unrelated NF1 patients with spinal neurofibromatosis had an NF1 missense mutation in exon 33 (L2067P) and an NF1 splice site mutation (IVS31-5 A→G).[46] A recurrent NF1 splice site mutation (IVS19b-3 C→G) was identified in a patient with spinal neurofibromatosis.[47] In the present study, patients with spinal neurofibromatosis also had different types of mutations. These findings indicate that specific NF1 gene mutations are not associated with spinal neurofibromatosis.

One NF1 patient in this study also had Van der Woude syndrome, a dominantly inherited disorder with locus heterogeneity. The majority of Van der Woude patients have linkage to the 1q32–41 locus,[48] but there is a second locus on 1p34.[49] A modifier locus exists on 17p11.2,[50] but there is no evidence to suggest an association with the NF1 locus at 17q11.2. This patient may be segregating two distinct and unrelated autosomal dominant conditions.

The marked clinical variability between multiple affected relatives in NF1 families also could be due to the nature, timing, or location of the "second hit" mutations at the NF1 locus in dermal and plexiform neurofibromas,[51–55] MPSNTs,[56–58] pheochromocytomas,[59] pilocytic astrocytomas,[60] and juvenile chronic myelogenous leukaemia cells.[61] Variable expressivity has also been observed in other tumour prone genetic conditions such as neurofibromatosis 2, tuberous sclerosis 1 and 2, and familial adenomatous polyposis.[62 63]

Somatic mosaicism, which has been documented in over 60 monogenic disorders,[64] is another potential cause of inter-individual phenotypic variation. Somatic mosaicism can occur sufficiently early in embryonic life to involve both somatic and germline cells, and such individuals may also be at risk of having affected children.[65] Somatic mosaicism in NF1 is thought to be the cause of segmental neurofibromatosis.[66] There is inter-individual variation in mitotic recombination,[67] and the genes that control this process may be an important cause of clinical variability within NF1 families. Serra et al[68] reported that mitotic recombination was the causal mechanism for the loss of heterozygosity of an NF1 constitutional mutation.

Our study gives further support to the probable importance of modifying loci, as suggested by Easton et al[30] This hypothesis is supported by the observation of many investigators that identical NF1 gene mutations often give rise to very different phenotypes in unrelated patients. GDNF is a candidate modifier gene in NF1,[69] and preliminary work in a mouse model of NF1 has also suggested the existence of modifying genes.[70] The identification of modifying genes in humans will be difficult and will require large cohorts of patients identified through multicentre collaborations, but the study of modifying genes may eventually allow more accurate prediction of specific clinical features and complications of NF1 that could possibly lead to new therapeutic approaches.

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REFERENCES

1 **Friedman J**. Epidemiology of neurofibromatosis type 1. Am J Med Genet 1999;**89**:1–6.
2 **Friedman JM**, Gutmann DH, MacCollin M, Riccardi Y. Neurofibromatosis: phenotype, natural history, and pathogenesis. Baltimore: Johns Hopkins University Press, 1999.
3 **Huson SM**, Compston DAS, Harper PS. A genetic study of von Recklinghausen neurofibromatosis in Southeast Wales.1: Guidelines for genetic counselling. J Med Genet 1989;**26**:712–21.
4 **Upadhyaya M**, Cooper DN. The mutation spectrum in neurofibromatosis type 1 and its underlying mechanism. Chapter 5. In: Upadhyaya M, Cooper D, eds. Neurofibromatosis type 1: from genotype to phenotype. Oxford: BIOS Scientific, 1998:65–87.
5 **Rasmussen SA**, Overman J, Thomson SA, Colman SD, Abernathy CR, Trimpert RE, Moose R, Virdi G, Roux K, Bauer M, Rojiani AM, Maria BL, Muir D, Wallace MR. Genes Chromosomes Cancer 2000;**28**:425–31.
6 **Evans DG**, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. J Med Genet 2002;**39**:311–14.
7 **Ferner R**, Gutmann DH. International consensus statement on malignant peripheral nerve sheath tumours in neurofibromatosis 1. Cancer Res 2002;**62**:1573–7.
8 **Viskochil D**. Gene structure and expression. Chapter 3. In: Upadhyaya M, Cooper D, eds. Neurofibromatosis type 1: from genotype to phenotype. Oxford: BIOS Scientific, 1998:39–56.
9 **Xu GF**, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, Dunn D, Stevens J, Gesteland R, White R, Weiss R. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 1990;**62**:599–608.
10 **Marchuk DA**, Saulino AM, Tavakkol R, Swaroop M, Wallace MR, Andersen LB, Mitchell AL, Gutmann DH, Boguski M, Collins FS. cDNA cloning of the type 1 neurofibromatosis gene: complete sequence of NF1 gene product. Genomics 1991;**11**:931–40.
11 **Cawthon RM**, Weiss R, Xu GF, Viskochil D, Culver M, Stevens J, Robertson M, Dunn D, Gesteland R, O'Connell P, White R. A major segment of the neurofibromatosis type 1 gene: DNA sequence, genomic structure and point mutations. Cell 1990;**62**:193–201.
12 **Viskochil D**, Cawthon R, O'Connell P, Xu GF, Stevens J, Culver M, Carey J, White R. The gene encoding the oligodendrocyte- myelin glycoprotein is embedded within the neurofibromatosis type 1 gene. Mol Cell Biol 1991;**11**:906–12.
13 **Allanson JE**, Upadhyaya M, Watson GH, Partington M, MacKenzie A, Lahey M, MacLeod H, Sarfarazi M, Broadhead W, Harper PS, Huson SM. Watson syndrome: is it a subtype of type 1 neurofibromatosis? J Med Genet 1991;**28**:752–6.
14 **Buehning L**, Curry CJ. Neurofibromatosis-Noonan syndrome. Pediatr Dermatol 1995;**12**:267–71.
15 **Colley A**, Donnai D, Evans D. Neurofibromatosis/Noonan phenotype: a variable feature of type 1 neurofibromatosis. Clin Genet 1996;**49**:59–64.
16 **Upadhyaya M**, Shen M, Cherryson A, Farnham J, Maynard J, Huson SM, Harper PS. Analysis of mutations at the neurofibromatosis 1 (NF1) locus. Hum Mol Genet 1992;**1**:735–40.

17 **Osborn M**, Upadhyaya M. Evaluation of the protein truncation test and mutation detection in the NF1 gene: mutation analysis of 15 known and 40 unknown mutations. *Hum Genet* 1999;**105**:327–32.

18 **Upadhyaya M**, Ruggieri M, Maynard J, Osborn M, Hartog C, Mudd S, Penttinen M, Cordeiro I, Ponder M, Ponder BA, Krawczak M, Cooper DN. Gross deletions of the neurofibromatosis type 1 (NF1) gene are predominantly of maternal origin and commonly associated with learning disability, dysmorphic features and developmental delay. *Hum Genet* 1998;**102**:591–97.

19 **Upadhyaya M**, Osborn MJ, Maynard J, Kim MR, Tamanoi F, Cooper DN. Mutational and functional analysis of the neurofibromatosis type 1(NF1) gene. *Hum Genet* 1997;**99**:88–92.

20 **Han S**, Cooper DN, Upadhyaya M. Evaluation of denaturing high performance liquid chromatography (DHPLC) for the mutational analysis of the neurofibromatosis type 1 (NF1) gene. *Hum Genet* 2001;**109**:487–97.

21 **National Institutes of Health**. Consensus development conference statement: neurofibromatosis. *Arch Neurol* Chicago, 1988;**45**:575–8.

22 **Szudek J**, Birch P, Friedman J. Growth in North American white children with neurofibromatosis 1 (NF1). *J Med Genet* 2000;**37**:933–8.

23 **Najjar M**, Rowland M. *Anthropometric reference data and prevalence of overweight, United States, 1976–80. Vital and health statistics 1981*, Series 11, No.238. DHHS Publications (PHS) 87-1688, Public Health Service. Washington: US Government Printing Office, 1987.

24 **Huson S**. Neurofibromatosis type 1: historical perspective and introductory overview, Chapter 1. In: Upadhyaya M, Cooper DN, eds. *Neurofibromatosis type 1: from genotype to phenotype*. Oxford: BIOS Scientific, 1998:1–20.

25 **DeBella K**, Szudek J, Friedman J. Use of the National Institutes of Health criteria for diagnosis of neurofibromatosis 1 in children. *Pediatrics* 2000;**105**:608–614.

26 **Szudek J**, Evans DGR, Friedman JF. Patterns of association of clinical features in neurofibromatosis 1 (NF1). *Hum Genet* 2003;**112**:289–97.

27 **Listernick R**, Louis DN, Packer RJ, Gutmann DH. Optic pathway gliomas in children with neurofibromatosis type 1: consensus statement from the NF1 optic pathway glioma task force. *Ann Neurol* 1997;**41**:143–9.

28 **Huson S**, Harper P, Compston D. Von Recklinghausen neurofibromatosis: a clinical and population study in south-east Wales. *Brain* 1988;**111**:1355–81.

29 **Shen M**, Harper P, Upadhyaya M. Neurofibromatosis type 1 (NF1): the search for mutations by PCR-heteroduplex analysis on Hydrolink gels. *Hum Mol Genet* 1993;**2**:1861–4.

30 **Easton DF**, Ponder MA, Huson SM, Ponder BA. An analysis of variation in expression of neurofibromatosis type 1 (NF1): evidence for modifying genes. *Am J Hum Genet* 1993;**53**:305–13.

31 **Easton D**. Genotype-phenotype relationships in neurofibromatosis type 1. Chapter 10. n: Upadhyaya M, Cooper DN, eds. *Neurofibromatosis type 1: from genotype to phenotype*. Oxford: BIOS Scientific, 1998:167–74.

32 **Carey J**, Viskochil D. Neurofibromatosis type 1: A model condition for the study of the molecular basis of variable expressivity in human disorder. *Am J Med Genet* 1999;**89**:7–13.

33 **Szudek J**, Joe H, Friedman JM. Analysis of intrafamilial phenotypic variation in neurofibromatosis type 1 (NF1). *Genet Epidemiol* 2002;**23**:150–64.

34 **Kayes LM**, Burke W, Riccardi VM, Bennett R, Ehrlich P, Rubenstein A, Stephens K. Deletions spanning the neurofibromatosis 1 gene: identification and phenotype of five patients. *Am J Hum Genet* 1994;**54**:424–36.

35 **Wu BL**, Austin MA, Schneider GH, Boles RG, Korf BR. Deletion of the entire *NF1* gene detected by the FISH: four deletion patients associated with severe manifestations. *Am J Med Genet* 1995;**59**:528–35.

36 **Tonsgard JH**, Yelavarthi KK, Cushner S, Short MP, Lindgren V. Do NF1 gene deletions result in a characteristic phenotype? *Am J Med Genet* 1997;**73**:80–6.

37 **Riva P**, Corrado L, Natacci F, Castorina P, Wu BL, Schneider GH, Clementi M, Tenconi R, Korf BR, Larizza L. NF1 microdeletion syndrome: refined FISH characterisation of sporadic and familial deletions with locus-specific probes. *Am J Hum Genet* 2000;**66**:100–9.

38 **Lopez-Correa C**, Dorschner M, Brems H, Lazaro C, Clementi M, Upadhyaya M, Dooijes D, Moog U, Kehrer-Sawatzki H, Rutkowski JL, Fryns JP, Marynen P, Stephens K, Legius E. Recombination hotspot in NF1 microdeletion patients. *Hum Mol Genet* 2001;**10**:1387–92.

39 **Ainsworth P**, Rodenhiser D, Costa M. Identification and characterization of sporadic and inherited mutations in exon 31 of the neurofibromatosis (NF1) gene. *Hum Genet* 1993;**91**:151–6.

40 **Horiuchi T**, Hatta N, Matsumoto M, Ohtsuka H, Collins FS, Kobayashi Y, Fujita S. Nonsense mutations at Arg-1947 in two cases of familial neurofibromatosis type 1 in Japanese. *Hum Genet* 1994;**93**:81–3.

41 **Shen M**, Harper P, Upadhyaya M. Molecular genetics of neurofibromatosis type 1. *J Med Genet* 1996;**33**:2–17.

42 **Ars E**, Kruyer H, Gaona A, Casquero P, Rosell J, Volpini V, Serra E, Lazaro C, Estivill X. A clinical variant of neurofibromatosis type 1: familial spinal neurofibromatosis with a frameshift mutation in the gene. *Am J Hum Genet* 1998;**62**:834–41.

43 **Tassabehji M**, Strachan T, Sharland M, Colley A, Donnai D, Harris R, Thakker N. Tandem duplication within a neurofibromatosis type 1 (NF1) gene exon in a family with features of Watson syndrome and Noonan syndrome. *Am J Hum Genet* 1993;**53**:90–5.

44 **Carey JC**. Neurofibromatosis-Noonan syndrome. *Am J Med Genet* 1998;**75**:263–264.

45 **Pulst SM**, Riccardi VM, Fain P. Korenberg JR Familial spinal neurofibromatosis: clinical and DNA linkage analysis *Neurology* 1991;**41**:1923–5.

46 **Kaufmann D**, Muller R, Bartelt B, Wolf M, Kunzi-Rapp K, Hanemann CO, Fahsold R, Hein C, Vogel W, Assum G. Spinal neurofibromatosis without café-au-lait macules in two families with null mutations of the gene. *Am J Hum Genet* 2001;**69**:1395–400.

47 **Wimmer K**, Muhlbauer M, Eckart M, Callens T, Rehder H, Birkner T, Leroy JG, Fonatsch C, Messiaen L. A patient severely affected by spinal neurofibromas carries a recurrent splice site mutation in the NF1 gene. *Eur J Hum Genet* 2000;**10**:334–8.

48 **Sander A**, Schmelzle R, Murray J. Evidence for a microdeletion in 1q32—41 involving the gene responsible for van der Woude sydrome. *Hum Mol Genet* 1994;**3**:575–8.

49 **Koillinen H**, Wong FK, Rautio J, Ollikainen V, Karsten A, Larson O, Teh BT, Huggare J, Lahermo P, Larsson C, Kere J. Mapping of the second locus for the van der Woude syndrome to chromosome 1p34. *Eur J Hum Genet* 2001;**9**:747–52.

50 **Sertie AL**, Sousa AV, Steman S, Pavanello RC, Passos-Bueno MR. Linkage analysis in a large Brazilian family with van der Woude sydrome suggests the existence of a susceptibility locus for cleft palate at 17p11.2–p11.1. *Am J Hum Genet* 1999;**65**:433–40.

51 **Sawada S**, Florell S, Purandare SM, Ota M, Stephens K, Viskochil D. Identification of *NF1* mutations in both alleles of a dermal neurofibroma. *Nat Genet* 1996;**14**:110–12.

52 **Serra E**, Puig S, Otero D, Gaona A, Kruyer H, Ars E, Estivill X, Lazaro C. Confirmation of a double-hit model for the NF1 gene in benign neurofibromas. *Am J Hum Genet* 1997;**61**:512–19.

53 **Eisenbarth I**, Beyer K, Krone W, Assum G. Toward a survey of somatic mutation of the NF1 gene in benign neurofibromas of patients with neurofibromatosis type 1. *Am J Hum Genet* 2000;**66**:393–401.

54 **John AM**, Ruggieri M, Ferner R, Upadhyaya M. A search for evidence of somatic mutations in the NF1 gene. *J Med Genet* 2000;**37**:44–9.

55 **Rutkowski JL**, Wu K, Gutmann DH, Boyer PJ, Legius E. Genetic and cellular defects contributing to benign tumor formation in neurofibromatosis type 1. *Hum Mol Genet* 2000;**9**:1059–66.

56 **Legius E**, Marchuk DA, Collins FS, Glover TW. Somatic deletion of the neurofibromatosis type 1 gene in a neurofibrosarcoma supports a tumour suppressor gene hypothesis. *Nat Genet* 1993;**3**:122–6.

57 **Lothe RA**, Slettan A, Saeter G, Brogger A, Borresen AL, Nesland JM. Alterations at chromosome 17 loci in peripheral nerve sheath tumours. *J Neuropathol Exp Neurol* 1995;**54**:65–73.

58 **Rasmussen SA**, Overman J, Thomson SA, Colman SD, Abernathy CR, Trimpert RE, Moose R, Virdi G, Roux K, Bauer M, Rojiani AM, Maria BL, Muir D, Wallace MR. Chromosome 17 loss-of-heterozygosity studies in benign and malignant tumours in neurofibromatosis type 1. *Genes Chromosome Cancer* 2000;**28**:425–31.

59 **Xu W**, Mulligan LM, Ponder MA, Liu L, Smith BA, Mathew CG, Ponder BA. Loss of NF1 alleles in phaeochromocytomas from patients with type 1 neurofibromatosis. *Genes Chromosomes Cancer* 1992;**4**:337–42.

60 **Gutmann DH**, Donahoe J, Brown T, James CD, Perry A. Loss of neurofibromatosis 1 (NF1) gene expression in NF1-associated pilocytic astrocytomas. *Neuropathol Appl Neurobiol* 2000;**26**:361–7.

61 **Shannon KM**, O'Connell P, Martin GA, Paderanga D, Olson K, Dinndorf P, McCormick F. Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. *New Engl J Med* 1994;**330**:597–601.

62 **Evans DG**, Wallace AJ, Wu CL, Trueman L, Ramsden RT, Strachan T. Somatic mosaicism: a common cause of classic disease in tumor-prone syndromes? Lessons from type 2 neurofibromatosis. *Am J Hum Genet* 1998;**63**:727–36.

63 **Verhoef S**, Bakker L, Tempelaars AM, Hesseling-Janssen AL, Mazurczak T, Jozwiak S, Fois A, Bartalini G, Zonnenberg BA, van Essen AJ, Lindhout D, Halley DJ, van den Ouweland AM. High rate of mosaicism in tuberous sclerosis complex. *Am J Hum Genet* 1999;**64**:1632–7.

64 **Gottlieb B**, Beitel LK, Trifiro MA. Somatic mosaicism and variable expressivity. *Trends Genet* 2001;**17**:79–82.

65 **Ruggieri M**, Huson S. The clinical and diagnostic implications of mosaicism in the neurofibromatoses. *Neurology* 2000;**56**:1433–43.

66 **Ruggieri M**. The different forms of neurofibromatosis. *Childs Nerv Syst* 1999;**15**:295–308.

67 **Holt D**, Dreimanis M, Pfeiffer M, Firgaira F, Morley A, Turner D. Interindividual variation in mitotic recombination. *Am J Hum Genet* 1999;**65**:1423–7.

68 **Serra E**, Rosenbaum T, Nadal M, Winner U, Ars E, Estivill X, Lazaro C. Mitotic recombination effects homozygosity for NF1 germline mutations in neurofibromas. *Nat Genet* 2001;**28**:294–6.

69 **Bahuau M**, Pelet A, Vidaud D, Lamireau T, LeBail B, Munnich A, Vidaud M, Lyonnet S, Lacombe D. GDNF as a candidate modifier in a type 1 neurofibromatosis (NF1) enteric phenotype. *J Med Genet* 2001;**38**:638–43.

70 **Reilly K**, Christy E, Loisel D, Ledger J, Bronson R, Jacks T. The role of modifier genes in NF1-associated tumours in mice. Presented at NNFF International Consortium for the Molecular Bioloby of NF1 and NF2, Aspen, Colorado, USA, 1992.

71 **Riccardi V**. *Neurofibromatosis: phenotype, natural history and pathogenesis*, 2nd ed. Baltimore: John Hopkins University Press, 1992.

72 **Friedman J**, Birch P. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. *Am J Med Genet* 1997;**70**:138–43.

73 **Lewis R**, Riccardi V. von Recklinghausen neurofibromatosis: prevalence of iris hamartoma. *Ophthalmology* 1981;**88**:348–54.

74 **Holt J**, Kuhns M. Macrocranium and macrocephaly in neurofibromatosis. *Skeletal Radiol* 1976;**1**:25–8.

75 **Ferner R**, Hughes R, Weinman J. Intellectual impairment in neurofibromatosis 1. *J Neurol Sci* 1996;**138**:125–33.

76 **Ozonoff S**. Cognitive impairment on neurofibromatosis type 1. *Am J Med Genet* 1999;**89**:45–52.