A molecular analysis of individuals with neurofibromatosis type 1 (NF1) and optic pathway gliomas (OPGs), and an assessment of genotype–phenotype correlations

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Andrew Shenton and Mike Baser are both deceased.

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ABSTRACT

Background Neurofibromatosis type 1 (NF1) affects 1 in 2500 people, and 15% of these may develop an optic pathway glioma (OPG). OPGs behave differently in NF1, and, given their frequency, surveillance is important. However, this is difficult because of the additional complications these patients may have, such as learning difficulties. Management is also different given that NF1 results from loss of function of tumour suppressor gene. A genotype–phenotype correlation may help to determine who is at risk of developing these tumours, aid focused screening, and shed light on response to treatments.

Methods As part of a long-term follow-up study of patients with NF1 OPGs, the authors assessed genotype–phenotype correlation. Fluorescein in situ hybridisation was performed to identify large deletions, and then a full gene screen for mutations, by denaturing high-performance liquid chromatography.

Results 80 patients with NF1 OPGs were identified, and molecular analyses were performed in a subset of 29. A clustering of pathogenic changes in the 5′ tertile of the gene was found. The authors combined these results with those for another two NF1 OPG cohorts and collectively found the same trend. When compared with a control population of NF1 patients without an OPG, the OR of a mutation being present in the 5′ tertile was 6.05 (p=0.003) in the NF1 OPG combined cohorts.

Conclusion It is possible that genotype is a significant determinant of the risk of development of OPGs in NF1.

INTRODUCTION

Neurofibromatosis type 1 (NF1; MIM 162200) is a common, autosomal dominant genetic disorder, affecting multiple systems in both males and females of every age and from every ethnic background.[1 2] The *NF1* gene is a tumour suppressor, and loss of gene function due to somatic inactivation of both *NF1* alleles leads to an increased risk of developing benign and malignant tumours. NF1 is a chronic, often progressive, disorder with widespread manifestations, and approximately a third of patients develop serious complications, mainly in childhood. Neurological complications include cognitive impairment and central nervous system tumours, the most common being the optic pathway glioma (OPG).[3 4]

OPGs are detected in 15% of patients with NF1,[5–7] occurring predominantly in early childhood. However, they are only symptomatic in around 5% of NF1 cases.[8 9] Differences have been observed in the natural history and effects of treatment outcome of NF1 OPGs compared with sporadic OPGs,[8 9] but the reasons for this discrepancy are unknown.

Debate exists about the role and nature of visual screening in the detection of NF1 OPGs, and the presence of cognitive impairment makes it difficult for children to cooperate with visual testing. It is difficult to determine clinically and radiologically which tumours will behave more aggressively and require intervention. The identification of the *NF1* gene alterations that predispose to OPG formation would facilitate focused screening of individuals at risk of NF1 OPG. Potentially, genotype–phenotype correlation could predict disease progression and response to treatment.

We performed a long-term follow-up study of 80 patients with NF1 OPGs to determine the natural history of these tumours, treatment outcomes and psychosocial morbidity. These were largely symptomatic OPGs and therefore the ones it would be important to pick up early. This paper focuses on the molecular aspect of the study, evaluating if a genotype–phenotype correlation exists. If present, a genotype–phenotype correlation would enable molecular genetic screening for OPGs early in childhood and potentially enable a greater understanding of the natural history of the tumour to guide management.

METHODS

The molecular study was part of a case controlled study involving patients ascertained from two NF1 clinics over an 18-month period. The Manchester NF1 clinic is based in the regional Genetics Unit at St Mary's Hospital. In addition to diagnosis and management of complications, all patients with NF1 are offered annual review. A database of these patients detailing their age, gender, family history and manifestations is maintained. The second NF1 clinic is based at Guys Hospital, London. This is a neurology-led multidisciplinary service, which also has close links with genetics. A database of name and date of birth was available, and, during the study, details of gender and family history were added for all patients on the database (650 notes were available). Both clinics receive referrals predominately from non-specialists; thus each represents a relatively unbiased population-based

group of NF1 patients. This study was performed before the start of the NCG-funded complex NF1 service. Both the Manchester and Guys NF1 clinics now also see patients with NF1 OPGs referred from specialist centres as part of this NCG service. Ethics approval for our study was sought independently from each centre (Manchester 02/CM/432 and Guys 03/02/13).

Inclusion criteria for the molecular study were a confirmed diagnosis of NF1 according to the NIH diagnostic criteria, 1988,[10] suspected diagnosis of an OPG (made on clinical and/or radiological features), and aged 16 years or more. In Manchester, the Manchester Children's Tumour registry (detailing cancer diagnoses from 1954 in those under 15 years) was cross-checked. The search for OPG included 'juvenile astrocytoma' and 'neurofibromatosis tumours not specified' to achieve full ascertainment. The OPGs were diagnosed on clinical and radiological features, including visual impairment, changes on funduscopy, pupillary alteration, proptosis or precocious puberty, or radiological evidence such as thickening of the optic nerves with enhancement. Few had histological confirmation, as it is not usual clinical practice to perform biopsies for these tumours. The OPG diagnosis was reviewed during the natural history study (details will be reported separately). An EDTA sample and lithium heparin sample were collected from each patient for molecular and fluorescence in situ hybridisation (FISH) studies, respectively. A two-step procedure was used to detect mutations. First, FISH was performed on all samples to look for any large deletions in the *NF1* gene.[11 12] Then a full gene screen for mutations was carried out, using denaturing high-performance liquid chromatography (DHPLC).[13–15]

Interpreting the findings

Mutations were classified as pathogenic if they resulted in a truncated protein, nonsense frameshift mutations, and splice-site changes. Amino acid changes altering the protein sequence (missense changes) were also classified as pathogenic if no single-nucleotide polymorphism (SNP) was detected at this position (http://www.ncbi.nlm.nih.gov/SNP/), and they were not seen in 1000 NF1 chromosomes and the same number of unaffected control chromosomes. Family studies and functional analyses were not possible during the course of the study, but these may aid our interpretation of these changes further.

Polymorphisms were only considered to be non-pathogenic if they were previously reported as an SNP (http://www.ncbi.nlm.nih.gov/SNP/), were previously reported as a polymorphism, had been found in normal controls, or were seen in association with a pathogenic mutation in an affected individual (MU, unpublished, 2004). It was not possible to check for the presence of SNPs for the intronic changes, as sequence data were not available, and these were not looked for in the normal controls.

The location of pathogenic changes identified in the *NF1* gene from the patients with NF1 OPGs was reviewed to evaluate possible genotype–phenotype correlation. Our findings were compared with mutations identified in another cohort of patients with NF1 OPGs (Ars *et al*[16]). Both sets of results (ours and those of Ars *et al*[16]) were then combined with a larger series of NF1 patients (Castle *et al*[17]). This group (Castle *et al*[17]) provided a control NF1/NF1 OPG group, in whom pathogenic changes had previously been identified in a large cohort of unselected NF1 patients, predominantly from the UK (MU, unpublished, 2010).

A logistic regression analysis was then used to determine if the OR of developing an OPG was associated with mutation location. The outcome was presence or absence of OPG. For ease of analysis, the *NF1* gene was divided into tertiles of approximately equal base pair length, using the *NF1* intron–exon boundaries as dividing points. The 5′ tertile included exons 1–16, the middle tertile exons 17–30 (the GAP(GTPase activating protein) -related domain is in exons 21–27a), and the 3′ tertile exons 31–49. In the logistic regression analysis, the tertiles were represented by indicator variables. The middle tertile was the reference category in comparisons between tertiles.

RESULTS

Table 1 summarises patient ascertainment in the overall long-term follow-up study. Any pathogenic mutation was checked against previous reports.[13 14 18–22] Eighty patients with NF1 OPGs were identified from 1331 patients with NF1 known to both centres. Of these, 47 were eligible for the molecular study and 29 were recruited (subjects).

There were no significant differences (in gender, age at diagnosis of NF1, or family history of NF1) between patients ascertained from the two centres. During reassessment of the OPGs as part of the natural history study, three patients were shown not to have an OPG. Twenty-eight of the 29 subjects had mutation analyses with DHPLC (table 2), as one patient was identified as having a whole gene deletion by FISH.

One patient had a whole gene deletion, and a further 14 had pathogenic changes, seven of which had not been described previously. Of these, 10 were protein truncating (frameshift/nonsense), two intronic changes, and the remainder were missense changes. A total of 20 non-pathogenic changes were seen, 15 previously unreported. Ten were polymorphisms, two silent changes and eight intronic changes. Figure 1 depicts the distribution of the pathogenic changes within the *NF1* gene. Clustering of changes was seen towards the 5′ end of the gene.

DISCUSSION

Comparison with changes seen in previously reported patients with NF1 OPGs

Our data suggest a clustering of mutations in the 5′ region of the *NF1* gene. No mutation data were available in patients with NF1 OPGs when we started this study. Subsequently, during the course of our study, Ars *et al* reported 12 mutations in a cohort of 20 patients with NF1 OPGs, but no clinical information was reported.[16]

We compared the molecular data from our patients with the data reported by Ars *et al* (table 3, supplementary online material). All of the changes described in the patients with OPGs by Ars *et al*[16] were shown to change mRNA processing, resulting in truncated protein. Figure 2 shows the distribution of pathogenic

Table 1 Patients identified with neurofibromatosis type 1 (NF1) optic pathway gliomas (OPGs) and data collection

	Patient groups		
	Manchester	Guys	
Total NF1	788	543	
Total NF1 OPG	45	35	
Eligible	28	19	
Seen (subjects)	18	11	Patient group for the molecular study
Declined	4	8	
Unable to be contacted	6	0	
New diagnosis after recruitment	0	2	
Deceased	13	4	
Child	4	10	

Table 2 Sequence changes identified in the *NF1* gene in the patients with neurofibromatosis type 1 (NF1) optic pathway gliomas (OPGs)

Study number	Location*	DNA change	Protein change	Type	Pathogenic mutation	Reported†	Presence of SNP	Affected first-degree relative
GS10	Exon 2 Exon 2	c.98-99delAA		F	Yes	No	NA	Yes
GS05	Exon 4b Exon 5	c.574C→T	R192X	N	Yes	HGMD[18]	NA	Yes
MS02	Exon 7 Exon 9	c.1011 ins T		F	Yes	No	NA	No
MS06	Exon 7 Exon 9	c.1012G→A	D338N	M	Potentially Yes	No	No	No
MS11	Exon 10a Exon 12	c.1306-1307delTC		F	Yes	No	NA	
MS03	Exon 10a Exon 12	c.1381C→T	R461X	N	Yes	18	NA	No
MS12	Exon 10c Exon 14	c.1544-1545delGG		F	Yes	No	NA	Yes
GS04	IVS11 IVS15	c.1721+3 A→G		I	Yes	18 21	NT	No
MS16	Exon 12a Exon 16	c.1722C→A	S574R	M	Potentially Yes	22‡	No	
MS07	Exon 16 Exon 21	c.2446 C→T	R816X	N	Yes	18	NA	No
MS08	IVS 21 IVS 27	c.3709-1G→C		SS	Yes	No	NT	No
MS15	Exon 34 Exon 42	c.6524 dupGA		F	Yes	No	NA	
GS09	Exon 37 Exon 45	c.6788-6792del TTAC		F	Yes	HGMD	NA	No
MS14	Exon 42 Exon 50	c.7411C→T	Q2471X	F	Yes	No	NA	Yes

*Lower numbering corresponds to NCBI.
†Checked if reported in reference[13 14 18–22] and the HwGM (http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html).
‡?Amino acid substitution or interference with normal splicing.
F, frameshift; I, intronic; M, missense; N, nonsense; NA, not applicable; NT, not tested; SNP, single-nucleotide polymorphism; SS, splice site.

changes in the *NF1* gene in both studies (ours and that of Ars *et al*). Both studies show a clustering of mutations at the 5′ end of the gene, most likely to result in protein truncation.

As a comparison we also show the distribution of pathogenic *NF1* gene changes in a control group of patients with NF1 in figure 3. Here Griffiths *et al*[23] identified pathogenic NF1 changes in 99 patients with NF1. Six of these had OPGs, and so their results are not included in figure 3. It clearly shows that mutations in patients with NF1 are distributed throughout the *NF1* gene.

Mutation detection rate

Mutation testing in NF1 has not proven easy, with many past studies reporting mutation detection frequencies of <50%. The large size of the gene, the lack of hotspots, the diverse spectrum of mutational mechanisms, and the presence of homologous loci throughout the genome have hindered both the previous methods available (including heteroduplex analyses, single-stranded conformational polymorphism analyses, and the protein truncation test) to find changes, and, once found, the interpretation of their role. DHPLC has been shown to be an effective mode of mutation detection in NF1,[13–15] and prior FISH analysis will also detect deletions, the largest group of changes missed with DHPLC.

The mutation detection rate was 52% by DHPLC, and with FISH this was increased further, to 54%, for the 28 NF1 OPG patients tested. No additional analyses such as RNA studies, functional analyses or family studies were performed. These studies might have shown that some of the intronic changes or polymorphisms are disease-causing mutations. However, our results are comparable to those of Ars *et al*[16] who used single strand conformation polymorphism/hetero-duplex (SSCP/HD) analyses, followed by sequencing and characterisation of the changes at the genomic level. They detected 12 mutations in 20 patients with NF1 OPGs, a mutation detection rate of 60%.

Assessing genotype–phenotype correlations

Logistic regression analysis was used to determine if the RR of OPG was associated with the location of mutations in the *NF1*

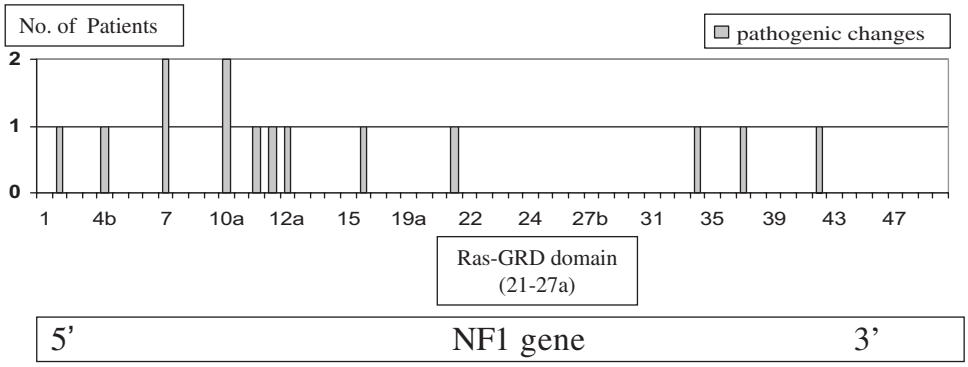

Figure 1 Distribution of pathogenic changes in the *NF1* gene, detected by denaturing high-performance liquid chromatography.

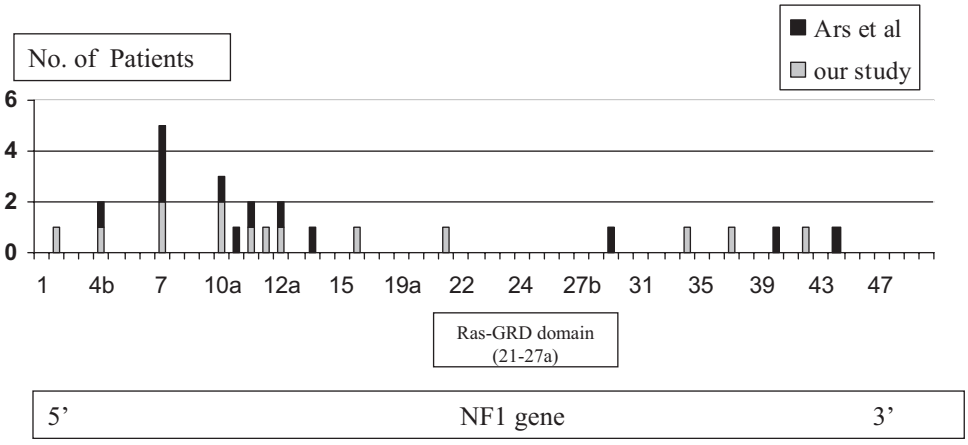

Figure 2 A diagrammatic representation of the location of mutations seen in both our study and that of Ars *et al*.[16]

gene. Patients with NF1 with identified mutations were ascertained from three sources: 111 patients with NF1 from Castle *et al*,[17] nine of whom had OPGs; 12 patients with NF1 OPGs from Ars *et al*[16]; and 15 patients with OPGs from this study. A sample from one deceased NF1 OPG patient from Manchester, ME13, showed him to have the same mutation as his monozygotic twin, MS14, as would be expected. This result was also included in this analysis, as part of our NF1 OPG cohort (hence increasing our sample from 14 to 15 patients).

Thirteen patients from Castle *et al*[17] were excluded from the analysis (seven with large deletions, five with non-OPGs (ie, gliomas outside of the optic pathway) and one with missing information on glioma status). The resultant group was 125 patients, 36 of whom had an OPG. There were 104 families with one patient, nine families with two patients (including one pair of monozygotic twins), and one family with three patients. It was not possible to adjust for familiality because of the small number of families with more than one patient, but the type of *NF1* mutation (familial or de novo) has only a minor influence on the variability of clinical expression in NF1.[24] Therefore, each patient was treated as a separate observation.

Mutations in the patients with OPGs from this study (15 patients including the results on the deceased twin discussed above) were analysed with those found in the control group of nine patients from Castle *et al*.[17] Clustering in the 5′ region was apparent, but was not significantly different from the control population (Castle *et al* dataset[17]).

Addition of the patients with NF1 OPGs from Ars *et al*[16] resulted in a significant distribution of mutations in the 5′ third of the gene (OR 6.05, p=0.003), and less so in the 3′ region (OR 3.30, p=0.066). These results suggest that, in the NF1 OPG group, more pathogenic changes are seen 5′.

Genotype–phenotype correlations for OPGs in NF1

Half (7/14) of the pathogenic changes detected in our study were previously unreported. Although no mutation hotspots have been identified, exons 4a, 4b, 10a–c and 37 appear to harbour more mutations,[18 20] and five of our mutations (including two unreported) were found in these exons. Further support for the 5′ predilection is provided by a report of siblings with OPGs and an exon 4b nonsense mutation (c484CAG→TAG; Q162X).[25] The localisation of mutations at the 5′ end of the gene encompassing largely exon 1 to exon 15 may indicate a new genotype–phenotype correlation in patients with NF1 OPGs, although further evaluation of this is required.

Earlier research into such correlations has been limited partly by the difficulty in finding mutations. Moreover, the large phenotypic variability within NF1 families has indicated that there are likely to be other modifying influences as well as the underlying *NF1* gene change. These include the role of modifier genes (epistasis), epigenetic factors and environmental factors.[24 26 27] We did not adjust for familiality in the one family with identical twins with OPG, although clearly they will have carried any modifier genes in common.[24] However, the contribution of each of these factors in determining the NF1 phenotype is unknown, and may vary for different disease traits. Furthermore, when a genotype–phenotype correlation in NF1 does exist, it may be difficult to interpret because of the pathogenic heterogeneity, the complexity of the phenotype, and the

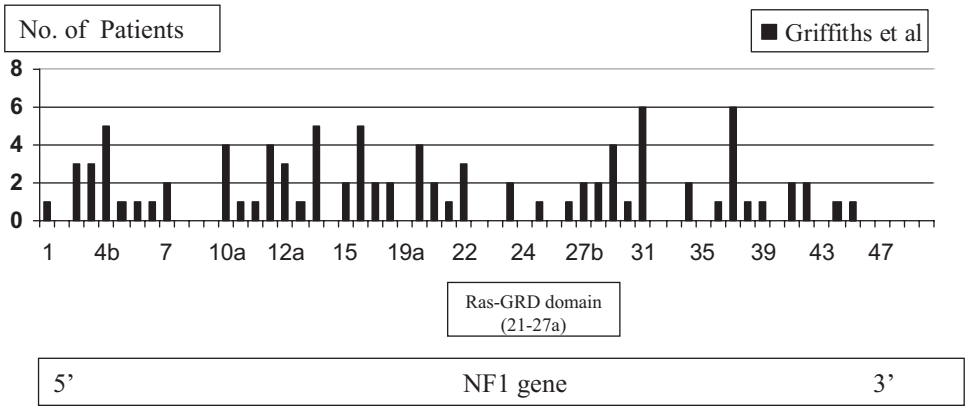

Figure 3 A diagrammatic representation of the location of mutations seen in a control group (Griffiths *et al*[23]).

small number of patients examined. However, recently a number of correlations have been suggested including: a mild phenotype of café au lait patches only in patients with an in-frame deletion[28]; multiple spinal neurofibromas with few other features[29 30]; patients with NF1 with a 1.4 Mb microduplication with mild learning difficulties, teeth and hair characteristics[31]; and patients with NF1 and Noonan syndrome with potential genotype–phenotype correlations with *NF1* gene alterations.[32] It may be that the genotype is a main determinant of the development of OPGs in NF1. However, further evidence is still required to confirm our findings and the other potential genotype–phenotype correlations.[28 29 31 32]

CONCLUSIONS

The apparent localisation of mutations at the 5′ end of the *NF1* gene appears to be a true feature of the mutations in NF1 patients with OPGs when compared with NF1 patients without OPGs (mutations in 5′ third of the gene, OR 6.05, p=0.003). Since the delineation of the whole gene deletion group in NF1, this is the third molecular finding that may have a significant impact in the management of NF1 patients. However, these findings require further confirmation in a larger number of patients with NF1 OPGs and understanding of the outcome of these changes. Corroboration of these results may allow a more targeted approach to screening for OPGs, by first identifying those patients at risk. Potentially, further clinical and molecular studies may permit the early identification of aggressive tumours and facilitate targeted treatment.

Competing interests None.

Ethics approval This study was conducted with the approval of the Manchester 02/CM/432, Guys 03/02/13.

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REFERENCES

1. **Anon.** *The Neurofibromatoses. A Pathogenetic and Clinical Overview*. 1st edn. London: Chapman and Hall, 1994.
2. **Anon.** *Neurofibromatosis. Phenotype, Natural history and Pathogenesis*. 3rd edn. Baltimore: The John Hopkins University Press, 1999, 1994 310 /id.
3. **Listernick R,** Louis DN, Packer RJ, Gutmann DH. Optic pathway gliomas in children with neurofibromatosis 1: consensus statement from the NF1 Optic Pathway Glioma Task Force. *Ann Neurol* 1997;**41**:143–9.
4. **Rosser TL,** Packer RJ. Neurocognitive dysfunction in children with neurofibromatosis type 1. *Curr Neurol Neurosci Rep* 2003;**3**:129–36.
5. **Lewis RA,** Gerson LP, Axelson KA, Riccardi VM, Whitford RP. von Recklinghausen neurofibromatosis. II. Incidence of optic gliomata. *Ophthalmology* 1984;**91**:929–35.
6. **Listernick R,** Charrow J, Greenwald MJ, Esterly NB. Optic gliomas in children with neurofibromatosis type 1. *J Pediatr* 1989;**114**:788–92.
7. **Lund AM,** Skovby F. Optic gliomas in children with neurofibromatosis type 1. *Eur J Pediatr* 1991;**150**:835–8.
8. **Singhal S,** Birch JM, Kerr B, Lashford L, Evans DG. Neurofibromatosis type 1 and sporadic optic gliomas. *Arch Dis Child* 2002;**87**:65–70.
9. **Sharif S,** Ferner R, Birch JM, Gillespie JE, Gattamaneni HR, Baser ME, Evans DG. Second primary tumors in neurofibromatosis 1 patients treated for optic glioma: substantial risks after radiotherapy. *J Clin Oncol* 2006;**24**:2570–5.
10. **Anon.** National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, MD, USA, July 13–15, 1987. *Neurofibromatosis* 1988;**1**:172–8.
11. **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.
12. **Kluwe L,** Siebert R, Gesk S, Friedrich RE, Tinschert S, Kehrer-Sawatzki H, Mautner VF. Screening 500 unselected neurofibromatosis 1 patients for deletions of the NF1 gene. *Hum Mutat* 2004;**23**:111–16.
13. **Han SS,** Cooper DN, Upadhyaya MN. 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.
14. **De Luca A,** Buccino A, Gianni D, Mangino M, Giustini S, Richetta A, Divona L, Calvieri S, Mingarelli R, Dallapiccola B. NF1 gene analysis based on DHPLC. *Hum Mutat* 2003;**21**:171–2.
15. **Upadhyaya M,** Han S, Consoli C, Majounie E, Horan M, Thomas NS, Potts C, Griffiths S, Ruggieri M, von Deimling A, Cooper DN. Characterization of the somatic mutational spectrum of the neurofibromatosis type 1 (NF1) gene in neurofibromatosis patients with benign and malignant tumors. *Hum Mutat* 2004;**23**:134–46.
16. **Ars E,** Kruyer H, Morell M, Pros E, Serra E, Ravella A, Estivill X, Lázaro C. Recurrent mutations in the NF1 gene are common among neurofibromatosis type 1 patients. *J Med Genet* 2003;**40**:e82.
17. **Castle B,** Baser ME, Huson SM, Cooper DN, Upadhyaya M. Evaluation of genotype-phenotype correlations in neurofibromatosis type 1. *J Med Genet* 2003;**40**:e109.
18. **Fahsold R,** Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kücükceylan N, Abdel-Nour M, Gewies A, Peters H, Kaufmann D, Buske A, Tinschert S, Nürnberg P. Minor lesion mutational spectrum of the entire NF1 gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. *Am J Hum Genet* 2000;**66**:790–818.
19. **Rodenhiser D,** Hovland K. A novel RsaI polymorphism within intron 39 of the neurofibromatosis type 1 (NF1) gene. *Hum Genet* 1995;**95**:241–2.
20. **Messiaen LM,** Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. *Hum Mutat* 2000;**15**:541–55.
21. **Purandare SM,** Lanyon WG, Connor JM. Characterisation of inherited and sporadic mutations in neurofibromatosis type-1. *Hum Mol Genet* 1994;**3**:1109–15.
22. **Mattocks C,** Baralle D, Tarpey P, ffrench-Constant C, Bobrow M, Whittaker J. Automated comparative sequence analysis identifies mutations in 89% of NF1 patients and confirms a mutation cluster in exons 11-17 distinct from the GAP related domain. *J Med Genet* 2004;**41**:e48.
23. **Griffiths S,** Thompson P, Frayling I, Upadhyaya M. Molecular diagnosis of neurofibromatosis type 1: 2 years experience. *Fam cancer* 2007;**6**:21–34.
24. **Easton DF,** Ponder MA, Huson SM, Ponder BA. An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for modifying genes. *Am J Hum Genet* 1993;**53**:305–13.
25. **Kebudi R,** Tuncer S, Upadhyaya M, Peksayar G, Spurlock G, Yazici H. A novel mutation in the NF1 gene in two siblings with neurofibromatosis type 1 and bilateral optic pathway glioma. *Pediatr Blood Cancer* 2008;**50**:713–15.
26. **Carey JC,** Laub JM, Hall BD. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. *Birth Defects Orig Artic Ser* 1979;**15**:271–81.
27. **Szudek J,** Joe H, Friedman JM. Analysis of intrafamilial phenotypic variation in neurofibromatosis 1 (NF1). *Genet Epidemiol* 2002;**23**:150–64.
28. **Upadhyaya M,** Huson SM, Davies M, Thomas N, Chuzhanova N, Giovannini S, Evans DG, Howard E, Kerr B, Griffiths S, Consoli C, Side L, Adams D, Pierpont M, Hachen R, Barnicoat A, Li H, Wallace P, Van Biervliet JP, Stevenson D, Viskochil D, Baralle D, Haan E, Riccardi V, Turnpenny P, Lazaro C, Messiaen L. An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the NF1 gene (c.2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation. *Am J Hum Genet* 2007;**80**:140–51.
29. **Kluwe L,** Tatagiba M, Fünsterer C, Mautner V. NF1 mutations and clinical spectrum in patients with spinal neurofibromas. *J Med Genet* 2003;**40**:368–71.
30. **Upadhyaya M,** Spurlock G, Kluwe L, Chuzhanova N, Bennett E, Thomas N, Guha A, Mautner V. The spectrum of somatic and germline NF1 mutations in NF1 patients with spinal neurofibromas. *Neurogenetics* 2009;**10**:251–63.
31. **Grisart B,** Rack K, Vidrequin S, Hilbert P, Deltenre P, Verellen-Dumoulin C, Destrée A. NF1 microduplication first clinical report: association with mild mental retardation, early onset of baldness and dental enamel hypoplasia? *Eur J Hum Genet* 2008;**16**:305–11.
32. **De Luca A,** Bottillo I, Sarkozy A, Carta C, Neri C, Bellacchio E, Schirinzi A, Conti E, Zampino G, Battaglia A, Majore S, Rinaldi MM, Carella M, Marino B, Pizzuti A, Digilio MC, Tartaglia M, Dallapiccola B. NF1 gene mutations represent the major molecular event underlying neurofibromatosis-Noonan syndrome. *Am J hum Genet* 2005;**77**:1092–101.

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