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## Title page

### Title:

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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### Keywords:

NF1, OPG, genotype, phenotype

Abstract:

Background.

Neurofibromatosis type 1 (NF1) affects 1 in 2500 individuals 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 NF1 OPGs patients we assessed genotype-phenotype correlation. FISH was performed to identify large deletions and then a full gene screen for mutations, by DHPLC.

Results

80 NF1 OPG patients were identified and molecular analyses performed in a subset of 29. We found a clustering of pathogenic changes in the 5' tertile of the gene. We combined our results with another two NF1 OPG cohorts and collectively we saw the same trend. When compared to a control population of NF1 patients without an OPG the odds ratio 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, frequently 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 commonest being the optic pathway glioma (OPG) [3,4].

Optic pathway gliomas (OPGs) are detected in 15% of NF1 patients [5, 6, 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 co-operate 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 NF1 OPG patients to determine the natural history of these tumours, treatments outcome 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 NF1 patients 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 multi-disciplinary service which also has close links with Genetics. A database of name and DOB 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 prior to the start of the NCG funded complex NF1 service. Both the Manchester and Guys NF1 clinics now also see NF1 OPG patients referred from specialist centres as part of this NCG service. Ethical 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 fundoscopy, 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 was collected from each patient for molecular and fluorescent *in situ* hybridisation studies (FISH) studies respectively. A two-step procedure was used to detect mutations. Firstly 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) [13,14,15].

#### 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 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 a 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 (Upadhyaya personal communication, 2004). It was not possible to check for the presence of SNPs for the intronic changes, as sequence data was not available, and these were not looked for in the normal controls.

The location of pathogenic changes identified in the *NF1* gene from the NF1 OPG patients was reviewed to evaluate possible genotype phenotype correlation. Our findings were compared to mutations identified in another cohort of NF1 OPG patients (Ars et al. [16]). Both sets of results (our and 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 (Meena Upadhyaya personal communication 2010).

A logistic regression analysis was then used to determine if the odds ratio of developing an OPG was associated with location of mutation. 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-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. 80 NF1 OPG patients were identified from 1331 NF1 patients known to both centres. Of these 47 were eligible for the molecular study and 29 were recruited (subjects).



**Table 1:** NF1 OPG patients identified and data collection

	Patients 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 contact	6	0	
New diagnosis post recruitment	0	2	
Deceased	13	4	
Child	4	10	

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

**Table 2** Sequence changes identified in the *NF1* gene in the NF1 OPG patients

Study number	Location Lower numbering corresponds to NCBI	DNA change	Protein Change	Type *	Pathogenic mutation	Reported #	Presence of SNP (not tested: NT, Not applicable N/A)	Affected first degree relative
GS10	Ex 2 Ex 2	c.98-99delAA		F	Yes	No	N/A	Yes
GS05	Ex 4b Ex 5	c.574C>T	R192X	N	Yes	[18] HGMD	N/A	Yes
MS02	Ex 7 Ex 9	c.1011 ins T		F	Yes	No	N/A	No
MS06	Ex 7 Ex 9	c.1012G> A	D338N	M	Potentially Yes	No	No	No
MS11	Ex 10a Ex 12	c.1306-1307delT C		F	Yes	No	N/A	
MS03	Ex 10a Ex 12	c.1381C> T	R461X	N	Yes	[18]	N/A	No
MS12	Ex 10c Ex 14	c.1544-1545delG G		F	Yes	No	N/A	Yes
GS04	IVS11 IVS15	c.1721+3 A>G		I	Yes	[18,21]	NT	No
MS16	Ex 12a Ex 16	c.1722C> A	S574R	M	Potentially Yes	[22]\$	No	
MS07	Ex 16 Ex 21	c.2446 C>T	R816X	N	Yes	[18]	N/A	No
MS08	IVS 21 IVS 27	c.3709-1G>C		SS	Yes	No	NT	No
MS15	Ex 34 Ex 42	c.6524 dupGA		F	Yes	No	N/A	
GS09	Ex 37 Ex 45	c.6788-6792del TTAC		F	Yes	HGMD	N/A	No
MS14	Ex 42 Ex 50	c.7411C> T	Q2471X	F	Yes	No	N/A	Yes

\* Frameshift-F; Intronic-I; Nonsense-N; Splice site-SS; Missense-M

# Checked if reported in 13, 14, 18, 19, 20, 21, 22 and the HGM (<http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>).

\$ ? Amino acid substitution or interference with normal splicing

One patient had a whole gene deletion and a further 14 had pathogenic changes, 7 of which had not been described previously. Of these, 10 were protein truncating (Frameshift/nonsense), 2 intronic changes and the remainder were missense changes. A total of 20 non pathogenic changes were seen, 15 previously unreported. Ten were polymorphisms, 2 silent changes and 8 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 to changes seen in previously reported NF1 OPG patients.

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

We compared the molecular data from our patients to the data reported by Ars et al. (table 3, supplementary on line material). All of the changes described in the OPG patients by Ars et al. [16] were shown to change mRNA processing, resulting in truncated protein. Figure 2 shows the distribution of pathogenic changes in the *NF1* gene in both studies (ours and 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 NF1 patients in figure 3. Here Griffiths et al. [33] identified pathogenic NF1 changes in 99 NF1 patients. Six of these had OPGs and so their results are not included in figure 3. It clearly shows that mutations in NF1 patients 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 [13,14,15], 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 seen by Ars et al. [16] who used SSCP/HD analyses, followed by sequencing and characterisation of the changes at the genomic level. They detected 12 mutations in 20 NF1 OPG patients, a mutation detection rate of 60%.

### Assessing genotype-phenotype correlations.

Logistic regression analysis was used to determine if the relative risk of OPG was associated with the location of mutations in the *NF1* gene. NF1 patients with identified

mutations were ascertained from three sources: 111 NF1 patients from Castle et al.[17], of whom 9 had OPGs; 12 NF1 OPG patients from Ars et al. [16]; and 15 patients with OPGs from this study. A sample from one deceased NF1 OPG patient from Manchester, ME13, was shown 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 people 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, of whom 36 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 familiarity due to 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 OPG patients 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 NF1 OPG patients from Ars et al. [16], resulted in a significant distribution of mutations in the 5' third of the gene (odds ratio 6.05, p=0.003), and less so in the 3' region (odds ratio 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 of 14), of the pathogenic changes detected in our study were previously unreported. Although no mutation hotspots have been identified, exons 4a, 4b, 10 a-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 OPG 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-exon 15 may indicate a new genotype-phenotype correlation in NF1 OPG patients, 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 familiarity 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 due to the pathogenic heterogeneity, the complexity of the phenotype, and the 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]; NF1 patients with a 1.4Mb microduplication with mild learning difficulties, teeth and hair characteristics [31] and NF1 Noonan syndrome patients 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 in the 5' end of the *NF1* gene appears to be a true feature of the mutations occurring in the NF1 OPG patients when compared to NF1 patients without OPGs (mutations in 5' third of the gene, odds ratio 6.05,  $p=0.003$ ). Since the delineation of the whole gene deletion group in NF1, this finding is the third molecular finding which may have a significant impact in the management of NF1 patients. However, these findings require further confirmation in larger number of NF1 OPG patients and understanding of the outcome of these changes. Corroboration of these results may allow for 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.

## References

1. The Neurofibromatoses. A pathogenetic and clinical overview. 1st ed. Chapman and Hall, 1994.
2. Neurofibromatosis. Phenotype, Natural history and Pathogenesis. 3rd ed. The John Hopkins University Press, 1999Huson SM, 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(2):143-149.
4. Rosser TL, Packer RJ. Neurocognitive dysfunction in children with neurofibromatosis type 1. *Curr Neurol Neurosci Rep* 2003; 3(2):129-136.
5. Lewis RA, Gerson LP, Axelson KA, Riccardi VM, Whitford RP. von Recklinghausen neurofibromatosis. II. Incidence of optic gliomata. *Ophthalmology* 1984; 91(8):929-935.
6. Listernick R, Charrow J, Greenwald MJ, Esterly NB. Optic gliomas in children with neurofibromatosis type 1. *J Pediatr* 1989; 114(5):788-792.
7. Lund AM, Skovby F. Optic gliomas in children with neurofibromatosis type 1. *Eur J Pediatr* 1991; 150(12):835-838.
8. Singhal S, Birch JM, Kerr B, Lashford L, Evans DG. Neurofibromatosis type 1 and sporadic optic gliomas. *Arch Dis Child* 2002; 87(1):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 Jun 1;24(16):2570-5
10. National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987. *Neurofibromatosis* 1988; 1(3):172-178.
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(1):80-86.
12. Kluwe L, Siebert R, Gesk S, Friedrich RE, Tinschert S, Kehrer-Sawatzki H et al. Screening 500 unselected neurofibromatosis 1 patients for deletions of the NF1 gene. *Hum Mutat* 2004; 23(2):111-116.
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(5):487-497.
14. De Luca A, Buccino A, Gianni D, Mangino M, Giustini S, Richetta A et al. NF1 gene analysis based on DHPLC. *Hum Mutat* 2003; 21(2):171-172
15. Upadhyaya M, Han S, Consoli C, Majounie E, Horan M, Thomas NS et al. 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(2):134-146.



16. Ars E, Kruyer H, Morell M, Pros E, Serra E, Ravella A et al. Recurrent mutations in the NF1 gene are common among neurofibromatosis type 1 patients. *J Med Genet* 2003; 40(6):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(10):e109.
18. Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kucukceylan N et al. 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(3):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(2):241-242.
20. Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N et al. 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(6):541-555.
21. Purandare SM, Lanyon WG, Connor JM. Characterisation of inherited and sporadic mutations in neurofibromatosis type-1. *Hum Mol Genet* 1994; 3(7):1109-1115.
22. Mattocks C, Baralle D, Tarpey P, French-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(4):e48.
23. Osborn MJ, Upadhyaya M. Evaluation of the protein truncation test and mutation detection in the NF1 gene: mutational analysis of 15 known and 40 unknown mutations. *Hum Genet* 1999; 105(4):327-332.
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(2):305-313
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. *ediatr Blood Cancer*. 2008 Mar;50(3):713-5.
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(5B):271-281.
27. Szudek J, Joe H, Friedman JM. Analysis of intrafamilial phenotypic variation in neurofibromatosis 1 (NF1). *Genet Epidemiol* 2002; 23(2):150-164.
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 Jan;80(1):140-51. Epub 2006 Dec 8.

29. Kluwe L, Tatagiba M, Fünsterer C, Mautner V: NF1 mutations and clinical spectrum in patients with spinal neurofibromas. *J Med Genet.* 2003; 40(5):368-371.
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 Jul; 10(3):251-63. Epub 2009 Feb 17.
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(3):305-311.
32. Alessandro De Luca, Irene Bottillo, Anna Sarkozy, Claudio Carta, Cinzia Neri, Emanuele Bellacchio, Annalisa Schirinzi, Emanuela Conti, Giuseppe Zampino, Agatino Battaglia, Silvia Majore, Maria M. Rinaldi, Massimo Carella, Bruno Marino, Antonio Pizzuti, Maria Cristina Digilio, Marco Tartaglia, Bruno Dallapiccola. NF1 Gene Mutations Represent the Major Molecular Event Underlying Neurofibromatosis-Noonan Syndrome. *Am J hum Genet* 2005;77(6):1092-1101.
33. Sian Griffiths, Peter Thompson, Ian Frayling, Meena Upadhyaya. Molecular diagnosis of neurofibromatosis type 1: 2 years experience. *Familial cancer* 2007;6:21-34.

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Competing interest: None to declare.

Figure 1: Distribution of pathogenic changes in the NF1 gene, detected by DHPLC.

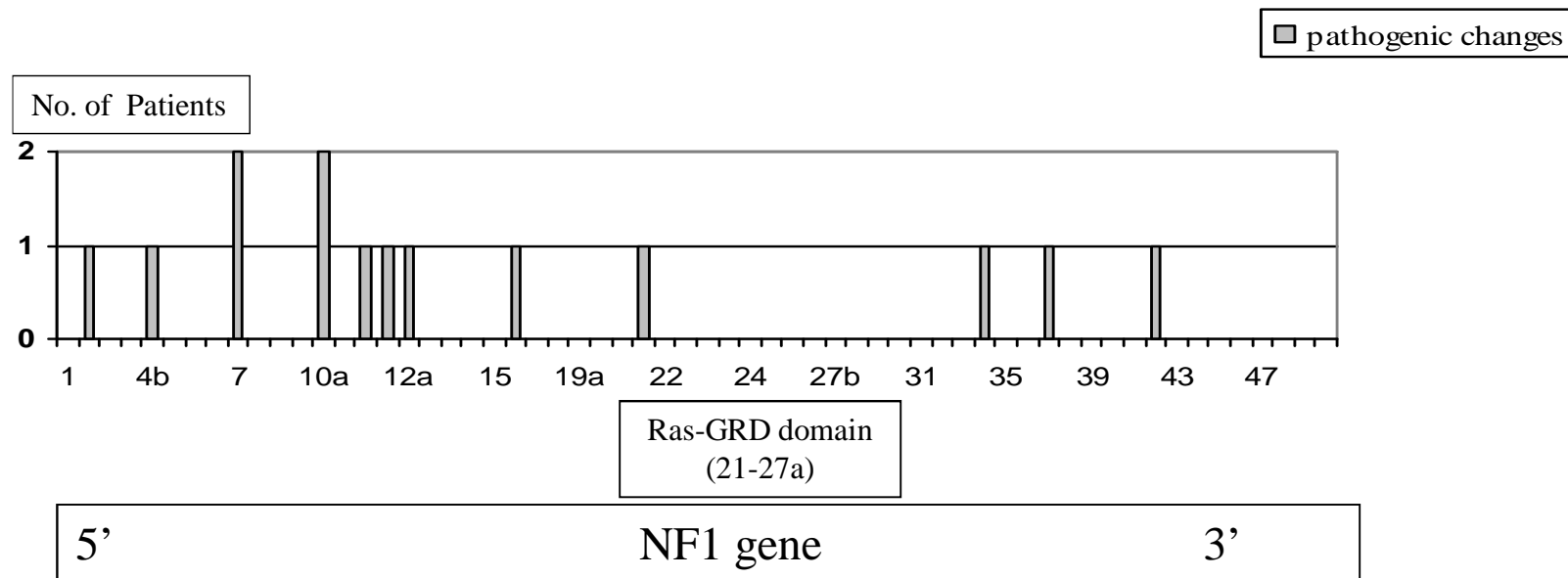


Figure 2: A diagrammatic representation of the location of mutations seen in both our study and that of Ars et al. [16].

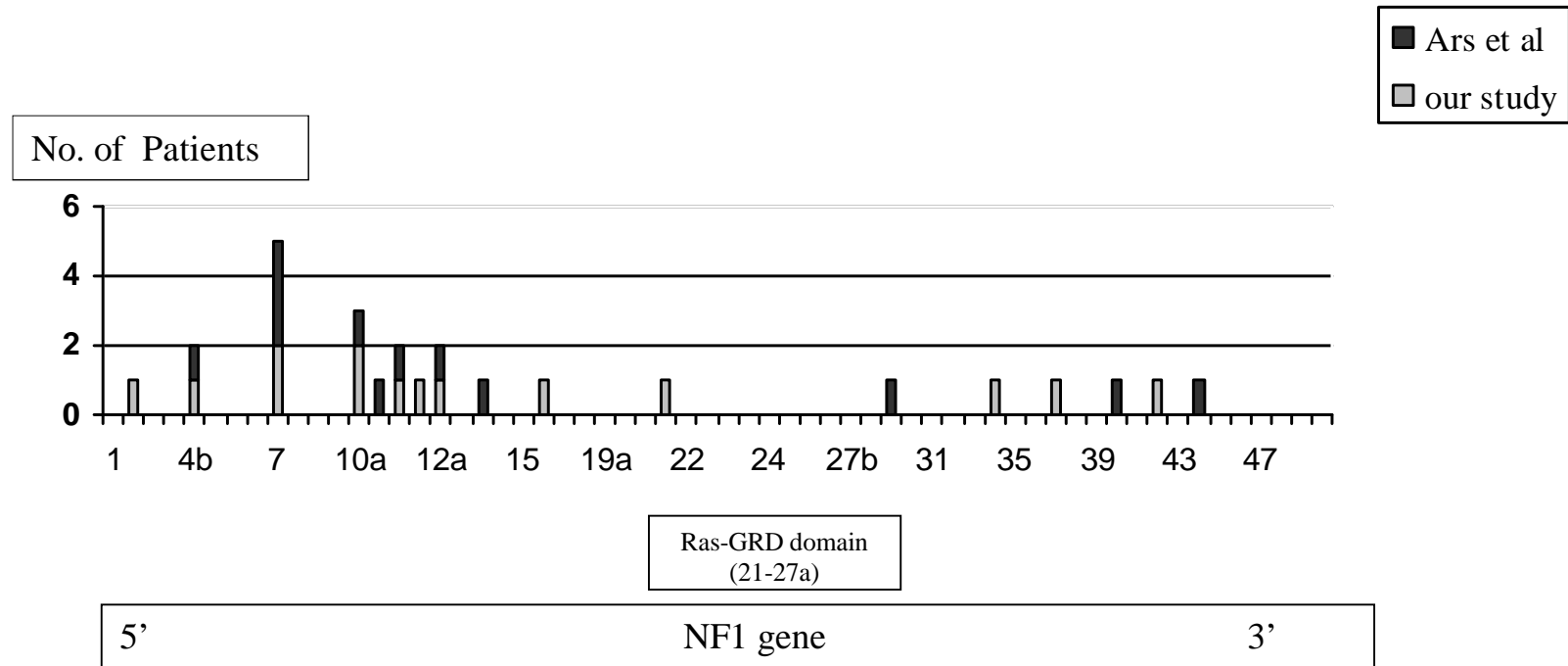


Figure 3: A diagrammatic representation of the location of mutations seen in a control group (Griffiths et al. [33]).

