



HAL
open science

NF1 microdeletions in neurofibromatosis type 1: from genotype to phenotype

Eric Pasmant, Audrey Sabbagh, Gill Spurlock, Ingrid Laurendeau, Elisa Grillo, Marie-Josée Hamel, Ludovic Martin, Sébastien Barbarot, Bruno Leheup, Diana Rodriguez, et al.

► To cite this version:

Eric Pasmant, Audrey Sabbagh, Gill Spurlock, Ingrid Laurendeau, Elisa Grillo, et al.. NF1 microdeletions in neurofibromatosis type 1: from genotype to phenotype. *Human Mutation*, 2010, 31 (6), pp.E1506-E1518. 10.1002/humu.21271 . hal-00552390

HAL Id: hal-00552390

<https://hal.science/hal-00552390>

Submitted on 6 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



NF1 microdeletions in neurofibromatosis type 1: from genotype to phenotype

| | |
|-------------------------------|---|
| Journal: | <i>Human Mutation</i> |
| Manuscript ID: | humu-2010-0043.R1 |
| Wiley - Manuscript type: | Mutation in Brief |
| Date Submitted by the Author: | 02-Apr-2010 |
| Complete List of Authors: | <p>pasmant, eric; UMR745 INSERM, Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques Sabbagh, Audrey; Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UMR745 INSERM Spurlock, Gill; Institute of Medical Genetics, Medical Genetics Laurendeau, Ingrid; Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UMR745 INSERM Grillo, Elisa; Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UMR745 INSERM Hamel, Marie-Josée; Hôpital Beaujon, AP-HP, Service de Biochimie et Génétique Moléculaire Martin, Ludovic; University of Angers, Department of Dermatology Barbarot, Sébastien; Hôtel Dieu, CHU de Nantes, Service de Dermatologie LEHEUP, Bruno; CHU de Nancy, Nancy-Université, Service de Médecine Infantile et de Génétique Rodriguez, Diana; UMR 546, Université Paris Pitié Salpêtrière, Inserm Lacombe, Didier; Pellegrin Hospital, Medical Genetics Dolfus, Helene; Faculté de Médecine de Strasbourg, Université de Strasbourg, Laboratoire de Génétique Médicale EA 3949, Pasquier, Laurent; Université de Rennes 1, CNRS UMR6061 Génétique et Développement Isidor, Bertrand; Centre Hospitalier Universitaire de Nantes, Service de Génétique Médicale Ferkal, Salah; AP-HP, Groupe hospitalier Henri Mondor-Albert Chenevier, INSERM, Centre d'Investigation Clinique 006 Soulier, Jean; 3UMR728 INSERM Unité d'immuno-hématologie (UIH) and laboratoire d'hématologie, Hôpital St-Louis, AP-HP Sanson, Marc; Hôpital de la Salpêtrière, Service de Neurologie Mazarin, INSERM UMR-97 Dieux-Coeslier, Anne; Hôpital Jeanne de Flandre, Center for Clinical Genetics Bieche, Ivan; Université Paris Descartes, Faculté des Sciences</p> |

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

| | |
|------------|---|
| | Pharmaceutiques et Biologiques, UMR745 INSERM Parfait, Béatrice; Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UMR745 INSERM Vidaud, Michel; Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UMR745 INSERM Wolkenstein, Pierre; Hopital Henri Mondor, AHP, Service de Dermatologie Upadhyaya, Meena; Cardiff, Medical Genetics Vidaud, Dominique; Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UMR745 INSERM |
| Key Words: | Genotype-phenotype correlation, High-resolution custom CGH, Neurofibromatosis type 1, NF1 locus microdeletion, Standardized phenotype questionnaire, Type-3 NF1 microdeletion |
| | |

For Peer Review



MUTATION IN BRIEF

HUMAN MUTATION

For Peer Review

Received 27 January 2010; accepted revised manuscript 12 April 2010.

© 2010 WILEY-LISS, INC.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

NF1 Microdeletions in Neurofibromatosis Type 1: From Genotype to Phenotype



Eric Pasmant^{1,2,*}, Audrey Sabbagh^{1,2}, Gill Spurlock³, Ingrid Laurendeau¹, Elisa Grillo¹, Marie-José Hamel², Ludovic Martin⁴, Sébastien Barbarot⁵, Bruno Leheup⁶, Diana Rodriguez⁷, Didier Lacombe⁸, Hélène Dollfus⁹, Laurent Pasquier¹⁰, Bertrand Isidor¹¹, Salah Ferkal¹², Jean Soulier¹³, Marc Sanson¹⁴, Anne Dieux-Coeslier¹⁵, Ivan Bièche^{1,2}, Béatrice Parfait^{1,2}, Michel Vidaud^{1,2}, Pierre Wolkenstein¹⁶, Meena Upadhyaya³, and Dominique Vidaud^{1,2} and the members of the NF France Network

¹UMR745 INSERM, Université Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, 4 av. de l'Observatoire, 75006, Paris, France, ²Service de Biochimie et de Génétique Moléculaire, Hôpital Beaujon, AP-HP, 100 Boulevard du Général Leclerc, 92110, Clichy, France, ³Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, Cf14 4XN, UK, ⁴Service de Dermatologie, CHU d'Angers, Université d'Angers, 49933 Angers Cedex 9, France, ⁵Service de Dermatologie, Hôtel Dieu, CHU de Nantes, 44093, Nantes, France, ⁶Service de Médecine Infantile III et Génétique Clinique, Hôpital d'Enfants CHU de Nancy, Faculté de Médecine Nancy Université Henri Poincaré, Vandoeuvre, France, ⁷AP-HP, Hôpital Armand Trousseau, Service de Neuropédiatrie, Paris, France, ⁸Service de Génétique Médicale, Hôpital Pellegrin, CHU de Bordeaux, Université de Bordeaux 2, 33076, Bordeaux, France, ⁹Laboratoire de Génétique Médicale EA 3949, Equipe Avenir-Inserm, Faculté de Médecine de Strasbourg, Université de Strasbourg, 67000, Strasbourg, France, ¹⁰CNRS UMR6061 Génétique et Développement, Université de Rennes 1, IFR140, Rennes, France, ¹¹Service de Génétique Médicale, Centre Hospitalier Universitaire de Nantes 7, Quai Moncoussu, 44000 Nantes Cedex, France, ¹²INSERM, Centre d'Investigation Clinique 006, AP-HP, Groupe hospitalier Henri Mondor-Albert Chenevier, Créteil, F-94000, France, ¹³INSERM U944, Hôpital Saint-Louis, Paris, France, ¹⁴Service de Neurologie Mazarin, INSERM UMR-975, Hôpital de la Salpêtrière, 75013, Paris, France, ¹⁵Service de Génétique Clinique, Hôpital Jeanne de Flandre, 59000, Lille, France, ¹⁶Département of Dermatologie, AP-HP and Université Paris 12, Hôpital Henri-Mondor, 94000, Créteil, France.

*Correspondence to Eric Pasmant, UMR745 INSERM, Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75006, Paris, France; E-mail: eric.pasmant@etu.univ-paris5.fr.

Communicated by Dominique Stoppa-Lyonnet

ABSTRACT: In 5-10% of patients, neurofibromatosis type 1 (NF1) results from microdeletions that encompass the entire *NF1* gene and a variable number of flanking genes. Two recurrent microdeletion types are found in most cases, with microdeletion breakpoints located in paralogous regions flanking *NF1* (proximal *NF1-REP-a* and distal *NF1-REP-c* for the 1.4 Mb type-1 microdeletion, and *SUZ12* and *SUZ12P* for the 1.2 Mb type-2 microdeletion). A more severe phenotype is usually associated with *NF1* microdeletion patients than in those with intragenic mutations. We characterized *NF1* microdeletions in 70 unrelated *NF1* microdeleted patients using a high-resolution *NF1* custom array comparative genomic hybridization (CGH). Genotype-phenotype correlations were studied in 58 of these microdeletion patients and compared to 389 patients with intragenic truncating *NF1* mutations and phenotyped in the same standardized way. Our results confirmed in an unbiased manner the existence of a contiguous gene syndrome with a significantly higher incidence of learning disabilities and facial dysmorphism in microdeleted

Received 27 January 2010; accepted revised manuscript 12 April 2010.

© 2010 WILEY-LISS, INC.

Comment [c1]: Please provide the first name and affiliation for this author.

Deleted: : Réseau NF

patients compared to patients with intragenic *NF1* mutations. Microdeleted NF1 patients also showed a trend toward significance for childhood overgrowth. High-resolution array-CGH identified a new recurrent ~1.0 Mb microdeletion type, designated as type-3, with breakpoints in the paralogous regions middle *NF1-REP-b* and distal *NF1-REP-c*. ©2010 Wiley-Liss, Inc.

KEY WORDS: Genotype-phenotype correlation; High-resolution custom CGH; Neurofibromatosis type 1; *NF1* locus microdeletion; Standardized phenotype questionnaire; Type-3 *NF1* microdeletion

INTRODUCTION

Neurofibromatosis type 1 (NF1; MIM# 162200) is an autosomal disorder with an estimated incidence of 1 in 3500 live births (Carey et al., 1986). NF1 is due to autosomal dominant loss-of-function mutations of the *NF1* gene (neurofibromin 1; NM_000267), a tumour suppressor gene located at 17q11.2 and containing 60 translated exons distributed over ~300 kb. In 5-10% of patients, NF1 is caused by genomic microdeletions that encompass the entire *NF1* gene and a variable number of immediately flanking genes (Kluwe et al., 2004). The majority of such NF1 patients have one of two recurrent types: type-1 and type-2 microdeletions. The typical type-1 microdeletion is 1.4 Mb long, contains *NF1* and at least 14 additional protein-coding genes, four pseudogenes and two microRNAs (Supp. Figure S1) and is caused by non-allelic homologous recombination (NAHR) between paralogous sequences flanking the *NF1* gene: the *NF1* proximal and distal low-copy repeats (LCRs): *NF1-REP-a* and *NF1-REP-c* (Dorchner et al., 2000; López Correa et al., 2000; Jenne et al., 2001, 2003; Forbes et al., 2004; Raedt et al., 2006). The typical type-2 microdeletion is smaller (1.2 Mb) and has breakpoints located in the *SUZ12* gene (suppressor of zeste 12 homolog; NM_015355) and its pseudogene *SUZ12P* (Petek et al., 2003; Kehrer-Sawatzki et al., 2004; Steinmann et al., 2007; Roehl et al., 2010). Even less frequent, atypical *NF1* microdeletions with non-recurring breakpoints have also been reported (Riva et al., 2000; Kehrer-Sawatzki et al., 2003, 2008; Venturin et al., 2004a; Mantripragada et al., 2006; Pasmant et al., 2008).

The main features of NF1 include multiple café-au-lait (CAL) spots, skin-fold freckling, Lisch nodules, and benign peripheral nerve sheath tumours (dermal and plexiform neurofibromas) whose occurrence, number, and size vary greatly from one patient to another, even from within the same NF1 family (Friedman, 1999). More than half of NF1 patients will also develop plexiform neurofibromas, of which about 5-10% will progress into a malignant peripheral nerve sheath tumour (MPNST) (Evans et al., 2002).

While NF1 represents a simply determined Mendelian disorder with complete penetrance, it is however characterized by highly variable expressivity in both the number of major features and the occurrence of complications (Friedman, 1999; Sabbagh et al., 2009). A more severe clinical phenotype has been reported in NF1 patients carrying genomic microdeletions that involve the entire *NF1* gene, compare to patients with intragenic *NF1* mutations. This contiguous gene syndrome does appear to include dysmorphic features (Upadhyaya et al., 1998; López Correa et al., 1999, 2000; Streubel et al., 1999; Riva et al., 2000; Castle et al., 2003; Venturin et al., 2004b), learning disabilities (Tonsgard et al., 1997; Upadhyaya et al., 1998; Korf et al., 1999; López Correa et al., 1999, 2000; Streubel et al., 1999; Riva et al., 2000; Castle et al., 2003; Descheemaeker et al., 2004; Venturin et al., 2004b), cardiovascular malformations (Riva et al., 2000; Venturin et al., 2004b, 2005; Mensink et al., 2006), childhood overgrowth (Spiegel et al., 2005), a higher tumour burden and earlier onset of benign neurofibromas (Leppig et al., 1997; López Correa et al., 2000; Castle et al., 2003), and probably, a higher incidence of MPNSTs (De Raedt et al., 2003) and other malignancies (López Correa et al., 2000). While many reports of *NF1* microdeleted patients (currently > 150 cases) (Mensink et al., 2006) have been published since the initial study in 1992 (Kayes et al., 1992), to date, little is known about this genotype-phenotype correlation. The limited number of microdeletions characterized per study, the imprecise definition of their boundaries, and the lack of appropriate control patients, had made it difficult to establish reliable genotype-phenotype correlations.

To further explore the different *NF1* locus rearrangements, and the putative association with such a contiguous gene syndrome phenotype, a large cohort of 70 unrelated *NF1* microdeleted patients was studied. Their microdeletions were accurately characterized using a high-resolution *NF1* custom array comparative genomic hybridization (CGH) targeting the 17q11.2 region. The genotype-phenotype correlation in 58 of these microdeletion patients was statistically evaluated, taking 389 unrelated patients with intragenic *NF1* truncating mutations as a reference group. All NF1 patients were clinically assessed utilising the same standardized

4 Pasmant et al.

questionnaire collection protocol. Our results demonstrate that *NF1* microdeletion patients have a significantly higher incidence of learning disabilities and facial dysmorphism than patients with intragenic *NF1* mutation, after adjusting for age and sex.

MATERIAL AND METHODS

Study samples

A initial panel of 21 *NF1* microdeleted patients was identified through a comprehensive mutation screening of 561 *NF1* index cases, enrolled between 2002 and 2005 in the French Clinical Research Program entitled “Study of expressivity of neurofibromatosis 1: constitution of a phenotype-genotype database” (coordinator: Pr. P. Wolkenstein, Henri Mondor Hospital, Créteil, France) to constitute the so-called ‘NF-France database’, as previously described (Sabbagh et al. 2008). The mutation screening of the *NF1* gene was performed using a variety of *NF1* gene screening methodologies that included, DNA and RNA sequencing, for small lesions, polymorphic microsatellite marker analysis and multiplex ligation-dependent probe amplification (MLPA) or real-time PCR-based gene-dosage analysis to permit microdeletion assessment, as previously described (Pasmant et al., 2008; Upadhyaya et al., 2008). Among the 561 *NF1* index cases, 512 individuals presented a *NF1* alteration, 2 showed a *SPRED1* (sprouty-related, EVH1 domain containing 1; NM_152594) mutation, and no *NF1* and *SPRED1* mutation was identified in the 47 remaining patients, as previously described (Pasmant et al., 2009b). Among the 512 index cases with a fully characterized *NF1* mutation, 21 patients (4%) presented a *NF1* microdeletion, in accordance with previous data (Kluwe et al., 2004). A second cohort of 49 *NF1* microdeleted patients was selected from routine *NF1* genotyping at Beaujon Hospital (Clichy, France) and at Institute of Medical Genetics (Cardiff, UK).

In total, 70 unrelated *NF1* patients harbouring a *NF1* microdeletion were included in this present study, of which 50 patients (79%) had a *de novo* microdeletion and 13 patients (21%) had inherited their microdeletion. Relevant inheritance data was unavailable for the remaining 7 patients.

The reference control group for this genotype-phenotype correlation study involved the use of non-deleted *NF1* patients with characterized *NF1* intragenic truncating mutations selected from the NF-France database. Only patients with constitutional *NF1* nonsense or frameshift mutations were included as it is assumed all such mutations would lead to the premature truncation of neurofibromin. A total of 389 *NF1* patients met this criterion and were included in the reference group of non-deleted patients. Patients with missense mutations, in-frame deletions or insertions were excluded from analyses since the functional consequences of such mutations are difficult to assess without functional studies. The 389 patients *NF1* molecular alterations were characterized in the same laboratory (Beaujon Hospital, Clichy, France).

For the 70 microdeleted and 389 non-deleted *NF1* patients, the full clinical available information was recorded utilising a standardized questionnaire. All patients included in this study fulfilled the National Institutes of Health (NIH) diagnostic criteria for *NF1* (Ferner et al., 2007) and their written informed consent was obtained.

Phenotypes

Fourteen major clinical features of *NF1* were selected for consideration for the genotype-phenotype association study, as previously described (Sabbagh et al., 2008). Four were quantitative traits: number of CAL spots (a continuous variable), number of plexiform neurofibromas (a continuous variable), and number of cutaneous and subcutaneous neurofibromas (each classified into one of four semi quantitative categories based on the number of neurofibromas: 0, 1–9, 10–99 and ≥ 100). The other ten clinical features were coded as binary variables: presence/absence of skin-fold freckling, blue-red macules, Lisch nodules, macrocephaly, childhood overgrowth, facial dysmorphism, scoliosis, learning disabilities, optic gliomas, and MPNST development. Patients with missing data for a particular feature were coded as “unknown”, and were not then considered in models involving that feature. Most features were identified by physical examination, with Lisch nodules being diagnosed, or excluded, by slit-lamp examination; individuals not given a slit lamp examination were coded as “unknown”. The presence or absence of optic gliomas was determined by cranial MRI or CT examination with individuals not given cranial imaging being coded as “unknown”. Childhood overgrowth was evaluated only in *NF1* patients under the age of 18 and was defined as height and/or head circumference at least two standard deviations above the age- and sex-matched population mean. A facial dysmorphism was diagnosed if two or more of the following signs were observed: coarse face, flat occiput/brachycephaly, facial asymmetry, prominent forehead, frontal bossing, ptosis,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

downslanting deep set eyes, eversion of the lateral eyelid, epicanthic folds, high and broad nasal bridge, bulbous nasal tip, large and low set ears, malar hypoplasia, wide and prominent philtrum, micrognathia, small pointed chin and low posterior hairline. For scoliosis, only scoliotic curves of $>10^\circ$ were taken into account in our analysis. The diagnosis of learning disabilities was performed on specific testing of cognitive abilities and/or history of scholar difficulties leading to repeating at least one level. Learning deficits most frequently involved visual spatial, visual motor integration skills and language-based skills. The prevalence of many other clinical abnormalities (epilepsy, hydrocephalus, medullary compression by neurofibroma, cerebrovascular complications, renal artery stenosis, pseudoarthrosis, congenital pseudoarthrosis of the tibia, dysplastic vertebrae, sphenoid wing dysplasia, xanthogranuloma) were too low ($\leq 5\%$) for meaningful statistical analysis.

The 70 microdeletion and the 389 control patients' phenotypes, collected through a standardized NF1 questionnaire have been deposited in NCBI's Gene Expression Omnibus (GEO) (Edgar et al., 2002) and are accessible through GEO accession number GSE19730 (at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19730>).

Characterization of NF1 microdeletions

The 70 NF1 patients' microdeletions were accurately characterized by using a custom high-resolution oligonucleotide array-CGH targeting the 17q11.2 region. The theoretical (design) and practical aspects (procedure) of this custom array-CGH are described in detail elsewhere (Pasmant et al., 2009a). Briefly, we designed a specific array-CGH for the *NF1* gene and its flanking regions by using the custom Agilent Human Genome 15K Microarray (Agilent Technologies, Santa Clara, CA, USA). The array comprised a total of 14,207 oligonucleotide probes spanning the whole of chromosome 17, including 12 314 probes spanning a ~8 Mb interval surrounding the *NF1* locus (~300 kb). This 8-Mb interval spanned the *NF1* gene region and included the largest *NF1* atypical microdeletions described in the literature (Riva P et al., 2000; Kehrer-Sawatzki et al., 2003; Venturin et al., 2004a; Pasmant et al., 2008). Details of the microarray design, including the 14,207 oligonucleotide probe chromosome locations, has been deposited in NCBI's GEO (Edgar et al., 2002) and are accessible through the GEO accession number GSE19730.

Statistical Analysis

Multiple logistic regression was performed to test the association of each clinical feature individually with the type of constitutional *NF1* mutation (microdeletion vs. intragenic mutation). Each regression model had the covariates of sex and age at examination (as a continuous variable) to control for potential confounding. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated when appropriate. We applied the Bonferroni correction, by which the nominal alpha value is adjusted based upon the number of tests performed, to account for multiple testing. All statistical analyses were performed with StatView 5.0 software (Cary, NC).

RESULTS

Array-CGH characterization of the 70 NF1 microdeletions

All 70 *NF1* microdeletions were accurately characterized with our *NF1* customised array-CGH platform and consisted of 77% type-1 (54/70), 9% type-2 (6/70), 4% type-3 (new recurrent type) (3/70), and 10% atypical microdeletions (7/70) (Table 1). An estimation of the length of each microdeletion as well as their gene content, was obtained by using the know locations of the last proximal and first distal non-deleted probes (Table 1; Supp. Table S1). A new recurrent ~1.0 Mb microdeletion type, designated here as type-3 microdeletion, was identified in three unrelated NF1 patients (GUE, OLI, and N2603), involving *NF1-REP-b* (but not *NF1-REP-a*) and *NF1-REP-c*. Figure 1 shows the relevant array-CGH profiles for the three recurrent types. The 14,207 array-CGH log₂ normalized ratio data of the 70 patients discussed in this publication have been deposited in NCBI's GEO (Edgar et al., 2002) and are accessible through GEO accession number GSE19730 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19730>).

6 Pasmant et al.

Table 1. Distribution of *NF1* microdeletions types in the 70 studied patients.

| | Length | Number of deleted genes (not including <i>NF1</i>) | Inheritance |
|-------------------------|--------|--|---|
| 54 type-1 (77%) | 1.4 Mb | 14 | <i>de novo</i> : 85% (40/47) inherited: 15% (7/47) |
| 6 type-2 (9%) | 1.2 Mb | 13 | <i>de novo</i> : 67% (4/6) inherited: 33% (2/6) |
| 3 type-3 (4%) | 1.0 Mb | 8 | <i>de novo</i> : 100% (3/3) |
| 7 atypical (10%) | | | |
| NF00028 | 830 kb | 10 | inherited |
| NF00234 | 870 kb | 6 | <i>de novo</i> |
| NF00398 | 1.0 Mb | 9 | inherited |
| NF00358 | 1.2 Mb | 15 | inherited |
| DIE | 1.2 Mb | 10 | inherited |
| N806 | 5.5 Mb | ~50 | <i>de novo</i> |
| DUB | 7.6 MB | ~100 | <i>de novo</i> |

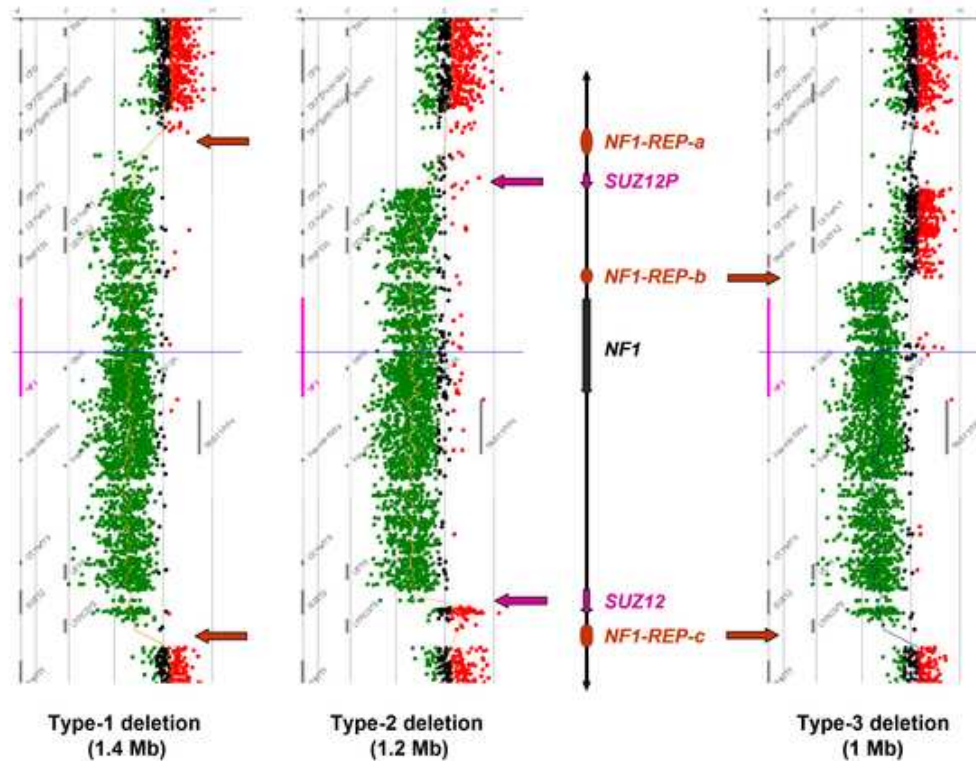


Figure 1. Array-CGH profiles of the three types of recurrent (typical) deletions (including type-1, type-2, and the new type-3) and *NF1* locus schematic representation.

Genotype-phenotype correlations

From the 70 microdeleted NF1 patients, 12 individuals were excluded from the statistical analysis of genotype-phenotype correlations: two had the largest atypical microdeletions (DUB: 7.6 Mb and N806: 5.5 Mb), and 10 patients had incomplete phenotype data. In the 58 remaining patients (27 men [46%] and 31 women [53%], mean age at examination = 20.5 ± 17.6 years), there were 44 type-1, 6 type-2, 3 type-3, and 5 atypical microdeletions. The reference group of non-deleted NF1 patients with intragenic truncating mutations ($n=389$) consisted of 169 men (43%) and 220 women (57%) with a mean age at examination of 33.2 ± 13.9 years.

The clinical characteristics associated with the non-deleted, the microdeleted, and the only type-1 microdeleted NF1 patients, are provided in Table 2.

Table 2. Non-deleted and microdeleted NF1 patients clinical characteristics comparison.

| Clinical features | NF1 non-deleted patients ($n=389$) ^a | All NF1 microdeleted patients ($n=58$) | $P^{b,c}$ | NF1 type-1 microdeleted patients ($n=44$) | $P^{b,d}$ |
|--------------------------------------|---|--|--------------------|---|--------------------|
| Cutaneous neurofibromas | | | NS | | NS |
| 0 | 22.1% | 49.0% | | 51.3% | |
| 1-9 | 20.1% | 17.6% | | 15.4% | |
| 10-99 | 34.4% | 17.6% | | 17.9% | |
| ≥ 100 | 23.4% | 15.7% | | 15.4% | |
| Subcutaneous neurofibromas | | | NS | | NS |
| 0 | 41.8% | 54.4% | | 58.1% | |
| 1-9 | 40.5% | 38.6% | | 37.2% | |
| 10-99 | 15.2% | 5.3% | | 2.3% | |
| ≥ 100 | 2.6% | 1.8% | | 2.3% | |
| Plexiform neurofibromas ^e | 0.7 ± 1.0 | 0.6 ± 1.1 | NS | 0.6 ± 1.1 | NS |
| CAL spots ^e | 24.2 ± 16.6 | 19.3 ± 13.4 | NS | 20.8 ± 14.5 | NS |
| Skin-fold freckling | 92.0% | 87.7% | NS | 86.4% | NS |
| Blue-red macules | 16.2% | 4.0% | NS | 2.4% | NS |
| Lisch nodules | 64.2% | 43.2% | NS | 40.0% | NS |
| Macrocephaly | 13.1% | 20.6% | NS | 11.5% | NS |
| Childhood overgrowth ^f | 6.3% | 30.4% | 0.005 | 22.2% | NS |
| Facial dysmorphism | 10.8% | 51.9% | <0.0001* | 54.8% | <0.0001* |
| Scoliosis | 38.3% | 31.5% | NS | 31.0% | NS |
| Learning disabilities | 51.9% | 80.0% | 0.001* | 85.7% | 0.0006* |
| Optic gliomas | 14.9% | 16.0% | NS | 15.0% | NS |
| MPNSTs | 3.1% | 5.6% | NS | 7.1% | NS |

NS: non significant. ^a Patients with intragenic NF1 mutations leading to a truncated protein (nonsense or frameshift mutations).

^b P values from multiple logistic regression analyses, using sex and age at examination as covariates. Only P values < 0.05 are shown. ^c P values resulting from the comparison of non-deleted NF1 patients with all NF1 microdeleted patients. ^d P values resulting from the comparison of NF1 non-deleted patients with type-1 NF1 microdeleted patients. ^e Data are expressed as mean \pm standard deviation. ^f Only individuals under the age of 18 were evaluated for this trait (63 NF1 non deleted patients, 23 NF1 microdeleted patients, 18 NF1 type-1 microdeleted patients). * Significant after Bonferroni correction for multiple testing.

The prevalence of the different clinical features in non-deleted patients is comparable to previously reported features for the general NF1 population (Huson and Huges, 1994; Friedman and Birch, 1997; North, 2000). When compared to this reference group, the microdeleted patients as a whole ($n = 58$), had a significantly higher incidence of dysmorphic features (52% vs. 11%, $P < 0.0001$, OR = 5.3 [2.7-10.4]) and learning disabilities (80% vs. 52%, $P = 0.001$, OR = 3.3 [1.6-6.7]) at the Bonferroni-corrected significance threshold of 0.004 (0.05 divided by 14 for 14 traits tested individually), after adjusting for age and sex (Table 2). Similar results were obtained when only the type-1 microdeleted patients were evaluated ($n = 44$), with OR = 5.3 [2.5-11.2] and OR = 5.0 [2.0-12.6] for facial dysmorphism and learning disabilities, respectively. Suggestive evidence for association with

childhood overgrowth was observed when the microdeleted patients were considered as a whole, with this characteristic found to be overrepresented in microdeleted patients compared to non-deleted cases (30% vs. 6%, $P = 0.005$, OR = 8.3 [1.9-36.4]). This association was however not statistically significant after applying the conservative Bonferroni correction for multiple comparisons. No significant associations were observed for any other clinical features. The apparently higher incidence of cutaneous neurofibromas in non-deleted patients, as compared to microdeleted patients (78% vs. 51%, respectively), is easily explainable by the greater proportion of prepubertal children (< 10 years old) in the latter group (36% vs. 6%). The association of this trait with the presence of an *NF1* microdeletion was indeed not significant ($P = 0.57$) when the effect of age was properly taken into account in the multiple regression analysis by using age as a covariate.

The proportion of *de novo* cases (which may contain some cases of mosaicism) was significantly higher in microdeleted patients compared to patients with intragenic mutations (79% vs. 43%, Chi Square test, P value < 0.0001). Therefore, somatic mosaicism has to be taken into account in the evaluation of genotype-phenotype correlations as it could explain some of the observed differences between the two groups of patients. However, when the nature (inherited or sporadic) of the *NF1* mutation was introduced in the multiple regression models as a covariate, along with sex and age at examination, the associations of facial dysmorphism and learning disabilities with *NF1* microdeletions (all pooled together) were still significant ($P < 0.0001$, OR = 4.8 [2.3-10.0] for facial dysmorphism, and $P = 0.002$, OR = 3.4 [1.5-7.3] for learning disabilities). Significant results were also observed when only the type-1 microdeleted patients were considered ($P = 0.0001$, OR = 4.9 [2.2-11.1] for facial dysmorphism, and $P = 0.001$, OR = 5.6 [2.0-15.7] for learning disabilities). Therefore, the association of facial dysmorphism and learning disabilities with *NF1* microdeletions is unlikely to result from the presence of unknown cases of mosaicism in our group of *NF1* patients.

DISCUSSION

The extreme variability of clinical expression in *NF1* makes difficult genotype-phenotype correlation, although they are of crucial importance for patients and their families. Hence, solid evidence for the existence of a microdeletion syndrome has awaited a comprehensive phenotype and genotype evaluation of a cohort of *NF1* microdeletion subjects ascertained in an unbiased manner and compared with an appropriate reference population phenotypically evaluated in the same standardized way as microdeleted patients.

The 70 newly characterized *NF1* microdeletion patients described here, the largest cohort ever reported, with the exception of meta-analysis from the published literature (Venturin et al., 2004b), has been both genotyped with an *ad hoc* array-CGH platform, and clinically phenotyped utilising a standardized questionnaire. The microdeletion patients were compared to 389 patients with *NF1* diagnosed according to the NIH clinical criteria and presenting intragenic *NF1* truncating mutations. *NF1* patients with *NF1* missense mutations, with no *NF1* mutations, and with *SPRED1* mutations were excluded as the NIH criteria cannot reliably distinguish *NF1* from *NF1*-like syndrome caused by *SPRED1* mutations (NFLS; MIM# 611431) (Pasmant et al. 2009b; Spurlock et al., 2009; Messiaen et al., 2009). This selection allowed comparing clinical phenotype consequences of *NF1* locus microdeletions versus the sole *NF1* gene haploinsufficiency (intragenic truncating mutations).

In contrast to previous molecular or cytogenetic techniques, such as multiplex ligation-dependent probe amplification or fluorescence in situ hybridization, our customised array-CGH platform was able to unambiguously differentiate and characterize *NF1* microdeletion types (De Luca et al., 2007; Pasmant et al., 2009a). Notably, the high-resolution of the array-CGH has allowed identification of a new recurrent *NF1* microdeletion type, designated as type-3, with breakpoints in the paralogous regions middle *NF1-REP-b* and distal *NF1-REP-c* (Figure 1). The most common type-1 microdeletion involved two paralogous sequences flanking the *NF1* gene: *NF1* proximal and distal LCR (*NF1-REP-a* and *NF1-REP-c*). A middle LCR (namely *NF1-REP-b*) has been previously identified between *NF1-REP-a* and the *NF1* gene (Jenne et al., 2003; Forbes et al., 2004; De Raedt et al., 2004, 2006) (Supp. Figure S1). These three LCR blocks (*NF1-REP-a*, b, and c) belong to a complex group of paralogs with three components on 17q11.2 (i.e. in the *NF1* microdeletion region: *NF1-REP-a*, b, and c), six at 17q21 (*NF1-REP-d*, e, f, g, h, and i), one at 17q24 (*NF1-REP-j*), and another on 19p13 (De Raedt et al., 2004). Interestingly, a genomic microdeletion caused by NAHR between the *NF1-REP-a*, and *NF1-REP-b* repeats and which included *RNF135* and five other genes, but not the *NF1* gene, has been described in an individual with an overgrowth syndrome (Douglas et al., 2007). For the first time, we describe a recurrent type-3 microdeletion in three unrelated *NF1* patients (GUE, OLI, and N2603), with breakpoints located in the paralogous sequences *NF1-*

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REP-b (located between *RNF135* and *NF1*) and *NF1-REP-c* (Figure 1), consistent with NAHR between these *NF1* medial and distal LCRs. This type-3 microdeletion is ~1 Mb in size and removed the entire *NF1* gene and at least eight other genes: *OMG*, *EVI2B*, *EVI2A*, *RAB11FIP4*, *CR17orf79*, *UTP6*, *SUZ12*, and *LRR37B*, but not *LRR37B2*, *CRLF3*, *ATAD5*, *CENTA2*, and *RNF135* (Table 1; Supp. Table S1). It is worth noting that the three type-3 *NF1* microdeletion patients showed facial dysmorphism. Two of them (GUE and OLI) had subcutaneous and plexiform neurofibromas. One of them (GUE) presented learning disabilities while this information was unavailable for N2603 and OLI who were 4 and 3 years old at the examination time, respectively.

The present study confirms in an unbiased manner the existence of a contiguous gene syndrome associated with specific clinical signs in *NF1* microdeletion patients. Two *NF1* clinical features, learning disabilities and facial dysmorphism show significant association with *NF1* microdeletions, after adjusting for multiple comparisons. While this finding is consistent with most previous *NF1* microdeletion studies, facial dysmorphism appeared to be much less prevalent in the microdeleted patients herein described than previously reported (52% in this study versus 78% in a meta-analysis of the published literature on the *NF1* microdeletions in 78 patients) (Venturin et al., 2004b). It is noteworthy that the concomitant presence of both learning disabilities and facial dysmorphism was considerably overrepresented in the *NF1* microdeleted patients compared to the non-deleted *NF1* cases (45% vs. 7%, $P < 0.0001$, OR = 11.0 [5.6-21.4]). Nevertheless, while patients with large deletions of the *NF1* locus do have a much greater probability to have learning disabilities and facial dysmorphism, it is still impossible to predict the presence of a *NF1* microdeletion based solely on these clinical symptoms, as previously claimed (Tonsgard et al., 1997). No association between these two clinical features was observed in our group of microdeleted patients ($P = 0.45$, after adjusting for age and sex), suggesting that different genetic factors may be involved in the determination of each of these traits. Remarkably, haploinsufficiency of *OMG* gene, mapping in the typical *NF1* microdeletion interval, has been proposed to be involved in the learning disabilities found in *NF1* microdeletion patients (Venturin et al., 2006).

Our study also suggests some evidence of an association of childhood overgrowth with *NF1* microdeletion, although this was not significant at the Bonferroni-corrected threshold. This observation confirms results published by Spiegel et al. who proposed that overgrowth was indeed a component of the phenotypic spectrum of the *NF1* microdeletion syndrome (Spiegel et al., 2005). *RNF135* haploinsufficiency was proposed to be responsible for the overgrowth often observed in individuals with *NF1* microdeletions (Douglas et al., 2007). Interestingly, none of the four microdeleted patients with no *RNF135* deletion (i.e. *RNF135* not included in the microdeletion interval), NF00234, and three type-3 microdeletion patients (GUE, OLI, and N2603), presented childhood overgrowth. It is also of note that no *RNF135* point mutation or deletion has been found in patients with *NF1* intragenic truncating mutation and with overgrowth, ruling out a putative double heterozygosity for mutations in both the *NF1* and *RNF135* genes in these patients (data not shown). Our cohort of *NF1* microdeleted patients had twice as many MPNSTs as did the non-deleted patients (6% vs. 3%), in agreement with previous published results (De Raedt et al., 2003). This finding is however not significant, because of the lack of power from small sample size and the low incidence of this trait. Hence, these two last findings (childhood overgrowth and possibly increased incidence of MPNSTs) warrant be replicated in an independent sample before any conclusions can be drawn.

This study confirms that custom high-resolution array-CGH is a sensitive approach, suitable for accurate characterization of *NF1* microdeletions and able to unambiguously differentiate between the types of microdeletions. Further studies are necessary to identify which gene(s) among the 14 genes deleted in *NF1* microdeletion syndrome, are responsible for learning disabilities and facial dysmorphism. Our study demonstrates however that *NF1* microdeletion patients present a more severe phenotype than that observed in classical *NF1* patients, particularly in respect to the presence of learning disabilities. These data must now to be taken into account in *NF1* patients' follow-up care.

REFERENCES

- Box GEP, Cox DR. An analysis of transformations. J. R. Stat. Soc. B 1964; 26: 211-252.
- Carey JC, Baty BJ, Johnson JP, Morrison T, Skolnick M, Kivlin J. 1986. The genetic aspects of neurofibromatosis. Ann N Y Acad Sci 486:45-56.
- Castle B, Baser ME, Huson SM, Cooper DN, Upadhyaya M. 2003. Evaluation of genotype-phenotype correlations in neurofibromatosis type 1. J Med Genet 40:e109.

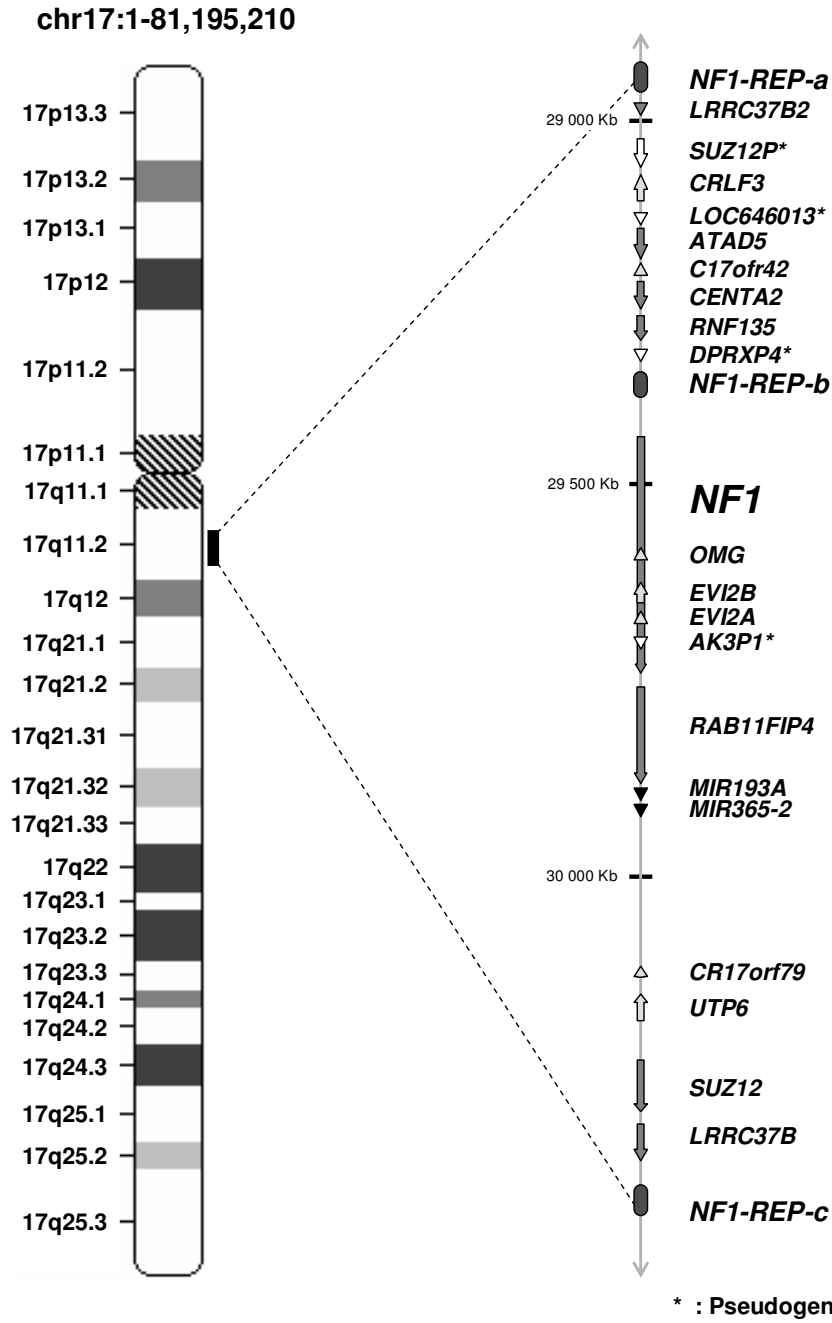
10 Pasmant et al.

- 1
2
3 De Luca A, Bottillo I, Dasdia M C, Morella A, Lanari V, Bernardini L, Divona L, Giustini S, Sinibaldi L, Novelli A, Torrente I,
4 Schirinzi A, Dallapiccola B. 2007. Deletions of NF1 gene and exons detected by multiplex ligation-dependent probe
5 amplification. *J Med Genet* 44:800-8.
- 6 De Raedt T, Brems H, Wolkenstein P, Vidaud D, Pilotti S, Perrone F, Mautner V, Frahm S, Sciort R, Legius E. 2003. Elevated
7 risk for MPNST in NF1 microdeletion patients. *Am J Hum Genet* 72:1288-1292.
- 8 De Raedt T, Brems H, López-Correa C, Vermeesch JR, Marynen P, Legius E. 2004. Genomic organization and evolution of the
9 NF1 microdeletion region. *Genomics* 84:346-60.
- 10 Descheemaeker MJ, Roelandts K, De Raedt T, Brems H, Fryns JP, Legius E. 2004. Intelligence in individuals with a
11 neurofibromatosis type 1 microdeletion. *Am J Med Genet A* 131:325-326.
- 12 Dorschner MO, Sybert VP, Weaver M, Pletcher BA, Stephens K. 2000. NF1 microdeletion breakpoints are clustered at
13 flanking repetitive sequences. *Hum Mol Genet* 9:35-46.
- 14 Douglas J, Cilliers D, Coleman K, Tatton-Brown K, Barker K, Bernhard B, Burn J, Huson S, Josifova D, Lacombe D, Malik M,
15 Mansour S, Reid E, Cormier-Daire V, Cole T; Childhood Overgrowth Collaboration, Rahman N. 2007. Mutations in
16 RNF135, a gene within the NF1 microdeletion region, cause phenotypic abnormalities including overgrowth. *Nat Genet*
17 39:963-5.
- 18 Edgar R, Domrachev M, Lash AE. 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data
19 repository. *Nucleic Acids Res* 30:207-10.
- 20 Evans DG, Baser ME, McGaughran J, Sharif S, Howard E, Moran A. 2002. Malignant peripheral nerve sheath tumours in
21 neurofibromatosis 1. *J Med Genet* 39:311-4.
- 22 Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, Upadhyaya M, Towers R, Gleeson M, Steiger C, Kirby A.
23 2007. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet* 44:81-8.
- 24 Forbes SH, Dorschner MO, Le R, Stephens K. 2004. Genomic context of paralogous recombination hotspots mediating
25 recurrent NF1 region microdeletion. *Genes Chromosomes Cancer* 41:12-25.
- 26 Friedman JM, Birch PH. 1997. Type 1 Neurofibromatosis: a descriptive analysis of the disorder in 1728 patients. *Am J Med*
27 *Genet* 70:138-43.
- 28 Friedman JM. 1999. Epidemiology of neurofibromatosis type 1. *Am J Med Genet* 89:1-6.
- 29 Huson SM, Huges RC. 1994. The neurofibromatosis: a clinical and pathogenetic overview. London: Chapman and Hall, Inc.
- 30
31 Jenne DE, Tinschert S, Reimann H, Lasinger W, Thiel G, Hameister H, Kehrer-Sawatzki H. 2001. Molecular characterization
32 and gene content of breakpoint boundaries in patients with neurofibromatosis type 1 with 17q11.2 microdeletions. *Am J*
33 *Hum Genet* 69:516-27.
- 34 Jenne DE, Tinschert S, Dorschner MO, Hameister H, Stephens K, Kehrer-Sawatzki H. 2003. Complete physical map and gene
35 content of the human NF1 tumor suppressor region in human and mouse. *Genes Chromosomes Cancer* 37:111-20.
- 36 Kayes LM, Riccardi VM, Burke W, Bennett RL, Stephens K. 1992. Large de novo DNA deletion in a patient with sporadic
37 neurofibromatosis 1, mental retardation, and dysmorphism. *J. Med. Genet* 29:686-690.
- 38 Kehrer-Sawatzki H, Tinschert S, Jenne DE. 2003. Heterogeneity of breakpoints in non-LCR-mediated large constitutional
39 deletions of the 17q11.2 NF1 tumour suppressor region. *J Med Genet* 40:e116.
- 40 Kehrer-Sawatzki H, Kluwe L, Sandig C, Kohn M, Wimmer K, Krammer U, Peyrl A, Jenne DE, Hansmann I, Mautner VF.
41 2004. High frequency of mosaicism among patients with neurofibromatosis type 1 (NF1) with microdeletions caused by
42 somatic recombination of the JJAZ1 gene. *Am J Hum Genet* 75:410-423.
- 43 Kehrer-Sawatzki H, Schmid E, Fünsterer C, Kluwe L, Mautner VF. 2008. Absence of cutaneous neurofibromas in an NF1
44 patient with an atypical deletion partially overlapping the common 1.4 Mb microdeletions region. *Am J Med Genet A*
45 146A:691-9.
- 46 Kluwe L, Siebert R, Gesk S, Friedrich RE, Tinschert S, Kehrer-Sawatzki H, Mautner VF. 2004. Screening 500 unselected
47 neurofibromatosis 1 patients for deletions of the NF1 gene. *Hum Mutat* 23:111-6.
- 48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Korf BR, Schneider G, Poussaint TY. 1999. Structural anomalies revealed by neuroimaging studies in the brains of patients with neurofibromatosis type 1 and large deletions. *Genet Med* 1:136-40.
- Leppig KA, Kaplan P, Viskochil D, Weaver M, Ortenberg J, Stephens K. 1997. Familial neurofibromatosis 1 microdeletions: cosegregation with distinct facial phenotype and early onset of cutaneous neurofibromata. *Am J Med Genet* 73:197-204.
- López Correa C, Brems H, Lázaro C, Estivill X, Clementi M, Mason S, Rutkowski JL, Marynen P, Legius E. 1999. Molecular studies in 20 submicroscopic neurofibromatosis type 1 gene deletions. *Hum Mutat* 14:387-93.
- López Correa C, Brems H, Lázaro C, Marynen P, Legius E. 2000. Unequal meiotic crossover: a frequent cause of NF1 microdeletions. *Am J Hum Genet* 66:1969-74.
- Mantripragada KK, Thuresson AC, Piotrowski A, Díaz de Stáhl T, Menzel U, Grigelionis G, Ferner RE, Griffiths S, Bolund L, Mautner V, Nordling M, Legius E, Vetrie D, Dahl N, Messiaen L, Upadhyaya M, Bruder CE, Dumanski JP. 2006. Identification of novel deletion breakpoints bordered by segmental duplications in the NF1 locus using high resolution array-CGH. *J Med Genet* 43: 28-38.
- Mensink KA, Ketterling RP, Flynn HC, Knudson RA, Lindor NM, Heese BA, Spinner RJ, Babovic-Vuksanovic D. 2006. Connective tissue dysplasia in five new patients with NF1 microdeletions: further expansion of phenotype and review of the literature. *J Med Genet* 43:e8.
- Messiaen L, Yao S, Brems H, Callens T, Sathienkijanchai A, Denayer E, Spencer E, Arn P, Babovic-Vuksanovic D, Bay C, Bobele G, Cohen BH, Escobar L, Eunpu D, Grebe T, Greenstein R, Hachen R, Irons M, Kronn D, Lemire E, Leppig K, Lim C, McDonald M, Narayanan V, Pearn A, Pedersen R, Powell B, Shapiro LR, Skidmore D, Tegay D, Thiese H, Zackai EH, Vijzelaar R, Taniguchi K, Ayada T, Okamoto F, Yoshimura A, Parret A, Korf B, Legius E. Clinical and mutational spectrum of neurofibromatosis type 1-like syndrome. *JAMA*. 2009;302:2111-8.
- North K. 2000. Neurofibromatosis type 1. *Am J Med Genet* 97:119-27.
- Pasmant E, de Saint-Trivier A, Laurendeau I, Dieux-Coeslier A, Parfait B, Vidaud M, Vidaud D, Bièche I. 2008. Characterization of a 7.6-Mb germline deletion encompassing the NF1 locus and about a hundred genes in an NF1 contiguous gene syndrome patient. *Eur J Hum Genet* 16:1459-66.
- Pasmant E, Sabbagh A, Masliah-Planchon J, Haddad V, Hamel MJ, Laurendeau I, Soulier J, Parfait B, Wolkenstein P, Bièche I, Vidaud M, Vidaud D. 2009a. Detection and characterization of NF1 microdeletions by custom high resolution array CGH. *J Mol Diagn* 11:524-9.
- Pasmant E, Sabbagh A, Hanna N, Masliah-Planchon J, Jolly E, Goussard P, Ballerini P, Cartault F, Barbarot S, Landman-Parker J, Soufir N, Parfait B, Vidaud M, Wolkenstein P, Vidaud D, France RN. 2009b. SPRED1 germline mutations caused a neurofibromatosis type 1 overlapping phenotype. *J Med Genet*. 46:425-30.
- Petek E, Jenne DE, Smolle J, Binder B, Lasinger W, Windpassinger C, Wagner K, Kroisel PM, Kehrer-Sawatzki H. 2003. Mitotic recombination mediated by the JJAZF1 (KIAA0160) gene causing somatic mosaicism and a new type of constitutional NF1 microdeletion in two children of a mosaic female with only few manifestations. *J Med Genet* 40:520-5.
- Raedt TD, Stephens M, Heyns I, Brems H, Thijs D, Messiaen L, Stephens K, Lázaro C, Wimmer K, Kehrer-Sawatzki H, Vidaud D, Kluwe L, Marynen P, Legius E. 2006. Conservation of hotspots for recombination in low-copy repeats associated with the NF1 microdeletion. *Nat Genet* 38:1419-23.
- Riva P, Corrado L, Natacci F, Castorina P, Wu BL, Schneider GH, Clementi M, Tenconi R, Korf BR, Larizza L. 2000. NF1 microdeletion syndrome: refined FISH characterization of sporadic and familial deletions with locus-specific probes. *Am J Hum Genet* 66:100-9.
- Roehl AC, Cooper DN, Kluwe L, Helbrich A, Wimmer K, Hogel J, Mautner VF, Kehrer-Sawatzki H. 2010. Extended runs of homozygosity at 17q11.2: an association with type-2 NF1 deletions? *Hum Mutat* [Epub ahead of print].
- Sabbagh A, Pasmant E, Laurendeau I, Parfait B, Barbarot S, Guillot B, Combemale P, Ferkal S, Vidaud M, Aubourg P, Vidaud D, Wolkenstein P; members of the NF France Network. 2009. Unravelling the genetic basis of variable clinical expression in neurofibromatosis 1. *Hum Mol Genet* 18:2768-78.
- Spiegel M, Oexle K, Hom D, Windt E, Buske A, Albrecht B, Prott EC, Seemanová E, Seidel J, Rosenbaum T, Jenne D, Kehrer-Sawatzki H, Tinschert S. 2005. Childhood overgrowth in patients with common NF1 microdeletions. *Eur J Hum Genet* 13:883-8.

12 Pasmant et al.

- 1
2
3 Spurlock G, Bennett E, Chuzhanova N, Thomas N, Jim HP, Side L, Davies S, Haan E, Kerr B, Huson SM, Upadhyaya M.
4 2009. SPRED1 mutations (Legius syndrome): another clinically useful genotype for dissecting the neurofibromatosis type 1
5 phenotype. *J Med Genet.* 46:431-7.
- 6
7 Steinmann K, Cooper DN, Kluwe L, Chuzhanova NA, Senger C, Serra E, Lazaro C, Gilaberte M, Wimmer K, Mautner VF,
8 Kehrer-Sawatzki H. 2007. Type 2 NF1 deletions are highly unusual by virtue of the absence of nonallelic homologous
9 recombination hotspots and an apparent preference for female mitotic recombination. *Am J Hum Genet* 81:1201-20.
- 10
11 Streubel B, Latta E, Kehrer-Sawatzki H, Hoffmann GF, Fonatsch C, Rehder H. 1999. Somatic mosaicism of a greater than 1.7-
12 Mb deletion of genomic DNA involving the entire NF1 gene as verified by FISH: further evidence for a contiguous gene
13 syndrome in 17q11.2. *Am J Med Genet* 87:12-6.
- 14
15 Tongsgard JH, Yelavarthi KK, Cushner S, Short MP, Lindgren V. 1997. Do NF1 gene deletions result in a characteristic
16 phenotype? *Am J Med Genet* 73:80-6.
- 17
18 Upadhyaya M, Ruggieri M, Maynard J, Osborn M, Hartog C, Mudd S, Penttinen M, Cordeiro I, Ponder M, Ponder BA,
19 Krawczak M, Cooper DN. 1998. Gross deletions of the neurofibromatosis type 1 (NF1) gene are predominantly of maternal
20 origin and commonly associated with a learning disability, dysmorphic features and developmental delay. *Hum Genet*
21 102:591-7.
- 22
23 Upadhyaya M, Spurlock G, Monem B, Thomas N, Friedrich RE, Kluwe L, Mautner V. 2008. Germline and somatic NF1 gene
24 mutations in plexiform neurofibromas. *Hum Mutat.* 29:E103-E111.
- 25
26 Venturin M, Gervasini C, Orzan F, Bentivegna A, Corrado L, Colapietro P, Friso A, Tenconi R, Upadhyaya M, Larizza L, Riva
27 P. 2004a. Evidence for non-homologous end joining and non-allelic homologous recombination in atypical NF1
28 microdeletions. *Hum Genet* 115:69-80.
- 29
30 Venturin M, Guarnieri P, Natacci F, Stabile M, Tenconi R, Clementi M, Hernandez C, Thompson P, Upadhyaya M, Larizza L,
31 Riva P. 2004b. Mental retardation and cardiovascular malformations in NF1 microdeleted patients point to candidate genes
32 in 17q11.2. *J Med Genet* 41:35-41.
- 33
34 Venturin M, Bentivegna A, Moroni R, Larizza L, Riva P. 2005. Evidence by expression analysis of candidate genes for
35 congenital heart defects in the NF1 microdeletion interval. *Ann Hum Genet* 69:508-16.
- 36
37 Venturin M, Moncini S, Villa V, Russo S, Bonati MT, Larizza L, Riva P. 2006. Mutations and novel polymorphisms in coding
38 regions and UTRs of CDK5R1 and OMG genes in patients with non-syndromic mental retardation. *Neurogenetics* 7:59-66.
- 39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Supp. Figure S1. Schematic representation of the 17q11.2 region. Type-1 1.4 Mb microdeletion encompasses the entire 350-kb NF1 gene, 14 flanking protein-coding genes, four pseudogenes, and two microRNAs.

14 Pasmant et al.

Supp. Table S1. Gene content of the three typical microdeletions and the < 2 Mb atypical microdeletions.

| Microdeletion | Type-1 | Type-2 | Type-3 | Atypical NF00028 | Atypical NF00234 | Atypical NF00398 | Atypical NF00358 | Atypical DIE | Atypical N806 | Atypical DUB |
|------------------|--------|--------|--------|---------------------|---------------------|---------------------|---------------------|-----------------|------------------|-----------------|
| Patient | 14 | 13 | 8 | 10 | 6 | 9 | 15 | 10 | -50 | -100 |
| Genes | | | | | | | | | | |
| <i>SLC6A4</i> | | | | | | | | | | |
| <i>BLMH</i> | | | | | | | | | | |
| <i>TMIGD1</i> | | | | | | | | | | |
| <i>CPD</i> | | | | | | | | | | |
| <i>GOSR1</i> | | | | | | | | | | |
| <i>TBC1D29</i> | | | | | | | | | | |
| <i>LRRC37B2</i> | | | | | | | | | | |
| <i>CRLF3</i> | | | | | | | | | | |
| <i>ATAD5</i> | | | | | | | | | | |
| <i>C17orf42</i> | | | | | | | | | | |
| <i>CENTA2</i> | | | | | | | | | | |
| <i>RNF135</i> | | | | | | | | | | |
| <i>NF1</i> | | | | | | | | | | |
| <i>OMG</i> | | | | | | | | | | |
| <i>EVI2B</i> | | | | | | | | | | |
| <i>EVI2A</i> | | | | | | | | | | |
| <i>RAB11FIP4</i> | | | | | | | | | | |
| <i>MIR193A</i> | | | | | | | | | | |
| <i>MIR365-2</i> | | | | | | | | | | |
| <i>C17orf79</i> | | | | | | | | | | |
| <i>UTP6</i> | | | | | | | | | | |
| <i>SUZ12</i> | | | | | | | | | | |
| <i>LRRC37B</i> | | | | | | | | | | |
| <i>RHOT1</i> | | | | | | | | | | |

For Peer Review