UAB
Universitat Autònoma de Barcelona

Dipòsit digital de documents de la UAB

This is the **published version** of the journal article:

Bonache, Sandra [et al.]. «Neurofibromatosis type 1 families with first-degree relatives harbouring distinct NF1 pathogenic variants. Genetic counselling and familial diagnosis : what should be offered?». *Journal of medical genetics*, Vol. 59, Num. 10 (2022), p. 1017-1023 DOI 10.1136/jmedgenet-2021-108301

This version is available at https://ddd.uab.cat/record/325231

under the terms of the IN COPYRIGHT license.

# Neurofibromatosis type 1 families with first-degree relatives harboring distinct *NF1* pathogenic variants. Genetic counseling and familial diagnosis: what should be offered?

Belén García[ł1,2,13], Núria Catasús[ł2], Andrea Ros[1,2,13], Inma Rosas[2,3], Alejandro Negro[2,3], Mercedes Guerrero-Murillo[2,3], Ana Valero[12], Anna Duat-Rodriguez [4], Juan Luís Becerra[5,13], Sandra Bonache[2,3], Conxi Lázaro[6,7,13], Carmina Comas[8], Isabel Bielsa[9,13], Eduard Serra[7,10,13], Concepción Hernández-Chico[11,12], Yolanda Martin[11,12], Elisabeth Castellanos*[2,3,13] and Ignacio Blanco*[1,2,13].

ł equally contributed

* Corresponding author / equally contributed

1) Genetic Counseling Unit, Clinical Genetics Service, Northern Metropolitan Clinical Laboratory, Germans Trias i Pujol University Hospital (HGTP), Can Ruti Campus, Badalona, Barcelona, 08916; Spain

2) Clinical Genomics Research Unit, Germans Trias i Pujol Research Institute (IGTP); Can Ruti Campus, Badalona, Barcelona, 08916; Spain

3) Clinical Genomics Unit, Clinical Genetics Service, Northern Metropolitan Clinical Laboratory, Germans Trias i Pujol University Hospital (HGTP), Can Ruti Campus, Badalona, Barcelona, 08916; Spain

4) Neurology Service, Hospital Infantil Universitario Niño Jesús. Madrid, Spain

5) Department of Neurology, Germans Trias i Pujol University Hospital (HGTP), Can Ruti Campus, Badalona, Barcelona, Spain.

6) Hereditary Cancer Program, Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL, Catalan Institute of Oncology, Hospitalet de Llobregat, Spain

7) Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Spain

8) Department of Obstetrics, Germans Trias i Pujol University Hospital (HGTP), Can Ruti Campus, Badalona, Barcelona, Spain.

9) Department of Dermatology, Germans Trias i Pujol University Hospital (HGTP), Can Ruti Campus, Badalona, Barcelona, 08916; Spain

10) Hereditary Cancer Group, Germans Trias i Pujol Research Institute (IGTP-PMPPC); Can Ruti Campus, Badalona, Barcelona, 08916; Spain

11) IRYCIS. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

12) Servicio de Genética, Hospital Universitario Ramón y Cajal, Madrid, Spain.

13) Spanish National Reference Center (CSUR) of Phakomatoses (HGTP-ICO-IGTP)

Corresponding author:

Dr. Elisabeth Castellanos

Email: ecastellanos@igtp.cat

## ABSTRACT

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by pathogenic variants in *NF1*. Recently, *NF1* testing has been included as a clinical criterion for NF1 diagnosis. Additionally, preconception genetic counseling in NF1 patients focuses on a 50% risk of transmitting the familial variant as the risk of having a sporadic NF1 is considered the same as the general population.

Methods: 829 individuals, 583 NF1 sporadic cases and 246 NF1 patients with documented family history, underwent genetic testing for NF1. Genotyping and segregation analysis of *NF1* familial variants was determined by microsatellite analysis and *NF1* sequencing.

Results: The mutational analysis of NF1 in 154 families with two or more affected cases studied showed the co-occurrence of two different *NF1* germline pathogenic variants in four families. The estimated mutation rate in those families was $3.89 \times 10^{-3}$, twenty times higher than the *NF1* mutation rate ($\sim 2 \times 10^{-4}$) (p-value=0.0008). Furthermore, the co-occurrence of two different *NF1* germline pathogenic variants in these families was 1:39, sixty times the frequency of sporadic NF1 (1:2500) (p-value=0.003). In all cases, the *de novo NF1* pathogenic variant was present in a descendant of an affected male. In two cases variants were detected in the inherited paternal wild-type allele.

Conclusions: Our results, together with previous cases reported, suggest that the offspring of NF1 male patients could have an increased risk of experiencing *de novo NF1* pathogenic variants. This observation, if confirmed in additional cohorts, could have relevant implications for NF1 genetic counseling, family planning, and *NF1* genetic testing.

## 1. Introduction

Neurofibromatosis type 1 (NF1; MIM #162200) is a neurocutaneous genetic disorder that affects 1:2500-1:3000 live births [1]. The hallmarks of the disease are café-au-lait macules (CALMs), skin-fold freckling, cutaneous neurofibromas (NFs) and Lisch nodules. NF1 presents with complete penetrance in adults [2,3], although there is notable variability in the clinical expression [4–6]. Other tumors of the central and peripheral nervous system are also common among NF1 patients, like optic pathway gliomas and plexiform neurofibromas (PNFs), the latter may occasionally transform to malignant peripheral nerve sheath tumors (MPNSTs). In addition, NF1 patients can also show skeletal abnormalities such as scoliosis, tibial pseudarthrosis and sphenoid wing dysplasia. Learning difficulties, social and behavioral problems are also present in some individuals as well as attention-deficit/hyperactivity disorder (ADHD) traits [7]. Therefore, NF1 syndrome is a classical example of a chronic and complex genetically determined condition requiring a life-long multidisciplinary healthcare approach.

NF1 is caused by loss of function variants in the *NF1* tumor suppressor gene (MIM #613113), located on chromosome 17q11.2, which encodes for neurofibromin, a negative regulator of the Ras GTPase [8]. Diagnostic criteria for NF1 have been recently revised [9]. Currently, sporadic NF1 patients are diagnosed when presenting two or more of the most characteristic clinical NF1 features or with only one for children, usually CALMs, and a *NF1* pathogenic variant. For familial cases, the presence of one clinical NF1 feature and an affected parent would be sufficient to clinically diagnose the patient, although the *NF1* test is recommended to confirm the clinical diagnosis.

*NF1* is one of the genes with the highest mutation rate reported in humans. The germline mutation rate for *NF1* was first estimated as $1\times10^{-4}$ per gamete per generation [10] but later it was defined as $3\text{-}6\times10^{-5}$, 10 to 100-fold higher than the average mutation rate for hereditary conditions [11–13]. Hence, approximately 50% of NF1 patients show no family history, with the disorder arising from *de novo* loss-of-function variants in the *NF1* gene [14].

Paternal age effect has been well described as a risk factor for NF1, similarly as it is for other genetic disorders, so that the risk of *de novo* occurrence of NF1 in offspring increases with increasing paternal age although statistical demographic demonstration remains to be shown on large cohorts [15,16]. In addition, a paternal age effect of mutated gametes has been observed for specific genes, some of them components of the RAS–MAPK pathway, which are positively selected in spermatogonia lineages [17–19], This effect might positively select and clonally expand *NF1* affected spermatogonia lineages. Furthermore, the type of pathogenic variant correlates with its parental origin: whole gene deletion occurs preferentially in oogenesis, while other types of small mutations appear to account for most of the paternally derived inherited mutations [18,20–22].

As NF1 is inherited following an autosomal dominant pattern, the risk of an affected offspring for an NF1 individual is 50% in every pregnancy. To avoid transmission of the disorder, there are several reproductive options that are discussed with a couple throughout the process of genetic counselling, such as prenatal diagnostic testing or preimplantation genetic testing for monogenic disorders (PGT-M), amongst others. In both cases, it is necessary that the NF1-causing variant had been determined in the affected parent [29]. In familial cases, it is usually assumed that members of a family harbor an identical molecular etiology. However, some studies have reported affected members of the same NF1 family harboring different *NF1* germline variants [5,27,30–32]. These data might suggest that offspring of NF1 individuals could be at an increased risk of having a *de novo* mutation in the *NF1* gene, higher than that estimated in the general population, which would pose a new challenge for genetic counseling of NF1 families, specifically for prenatal and PGT-M testing. In addition, the current diagnosis of NF1 is established in any child with a parent with NF1 when the child presents with one or more of the NF1 criteria. Since the *NF1* genetic test has been included in the revised clinical criteria [9] as a new criterion, affected children could be misdiagnosed as healthy if they did not share the same NF1 disease-causing variant as their affected progenitor.

This study presents four families from two independent cohorts, in which two different *NF1* germline pathogenic variants were identified in the affected members, from a total of 154 families. In all cases, the

family member harboring the new *NF1* pathogenic variant was a descendant of an affected male individual, and in two of them we were able to demonstrate that the new *NF1* variant occurred in the father's wild-type NF1 allele. If confirmed in other larger cohorts, these results would support the possibility that descendants of NF1 males could have a higher risk than previously expected of inheriting a *de novo NF1* variant, which would affect preconception genetic counseling. In addition, this should also be considered when genetic testing is performed to confirm NF1 in familial cases, as the absence of the familial *NF1* variant could erroneously rule out its *de novo* presence.

## 2. Materials and Methods

**Patients and samples**: Patients and their relatives were attended in two Spanish public hospitals, the National Reference Center (CSUR) of Phakomatoses (HGTP-ICO-IGTP, Hospital Universitari Germans Trias i Pujol, Badalona) and the Hospital Universitario Ramón y Cajal, (HRYC) Madrid. In both hospitals, anamnesis, genetic counseling and genetic testing are performed in-house according to institutional ethical standards and complying with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all individuals included in the study.

**NF1 genetic testing**: DNA and RNA was obtained from blood lymphocytes using standard protocols. Each center applied a distinct protocol for mutation detection, both previously described [33] [34]. Variants were classified following ACMG guidelines with minor modifications [35]. *NF1* reference sequences: NM_000267.3; NG_009018.1; LRG_214.

**Microsatellite analysis**: Several microsatellite markers located at chromosome 17 were studied to determine the parental origin of mutated NF1 allele. Families 1 and 2 were studied using MMPA [36] while families 3 and 4 as described in [34].

***De novo* NF1 frequency estimation in NF1 families**: the estimated frequency of *de novo* NF1 cases within NF1 families was calculated considering the observed frequency of families with two affected members harboring different NF1 pathogenic variants among all families attended. Statistical significance was established by comparison with *NF1 de novo* expected frequency (1:2500).

**Estimated *de novo NF1* mutation rate in NF1 families:** To estimate the frequency of *de novo* mutations in *NF1* that occur per gamete per generation we counted the number of *de novo* mutant alleles transmitted from NF1 patients to their offspring in relation to all NF1 offspring (*de novo* mutant / total offspring from NF1 familial cases x2)[12,37]. Statistical significance was established by comparison with *NF1* mutation rate ($1\times10^{-4}$ and $3\text{-}6\times10^{-5}$) by Fisher's Exact Test.

**Determination of allele-carrying *de novo NF1* variant:** We identified single-nucleotide polymorphisms located close to the *de novo* mutations in the coding sequence of the *NF1* gene. A fragment of the cDNA coding sequence containing both the *de novo* variant and the identified polymorphisms, were cloned using the Gateway® Gene Cloning system (Invitrogen) and analyzed by Sanger sequencing to determine the paternal origin of the allele in which the *de novo* mutation originated. Primers used were (5' to 3'): (Family 1: GGGGACAAGTTTGTACAAAAAAGCAGGCTGCTCAGTAAAGTAAACATATAGCTGTG and GGGGACCACTTTGTACAAGAAAGCTGGGTCATATGTGAATGCTTATTATACAGTC;
Family 2: GGGGACAAGTTTGTACAAAAAAGCAGGCTGTGCCGTCGAAGGAAAAAGC and GGGGACCACTTTGTACAAGAAAGCTGGGTAAGCAAGAGCTTTGGATCTGC.

**Statistical analysis:** The different groups were compared using the Chi squared -test or Fisher's exact test for binary data. The level of statistical significance was $p<0.05$. All statistical analyses were performed using R software (v3.6.1).

## 3. Results

### Genetic characterization of the Spanish cohort

A total of 829 individuals who had confirmed or suspected Neurofibromatosis Type 1 were diagnosed and given genetic counseling. Of these, 583 (66%) were sporadic NF1 cases, while 246 (34%) were index cases of NF1 families. We were able to genetically test two or more affected individuals in 154 out of the 246 NF1 families, including 154 index cases and 375 affected relatives (n=529) (Table 1). There were no significant differences in age and sex between the different groups of patients: the sex ratio among both cohorts was very similar: 47% of men and 53% of women in sporadic cases and 46% of men and 54% of women in familial index cases (Table 1, Chi square, $p>0.05$). Unexpectedly, familial cases were under-represented in both cohorts. However, the frequencies of the different types of *NF1*-causing variants identified agreed with the previously reported (expected) distribution [38] with nearly 90% of the cases due to point mutations, 4% single or multiple exon deletions and 6% whole *NF1* gene deletions (Supplementary Table 1)).

### Families harboring two *NF1* pathogenic variants

Among the 154 families with two or more affected individuals genetically analyzed, four families harboring two different *NF1* disease-causing variants were detected (Figure 1).

Family 1. A 35-year-old male (Fig 1A, II.3) was diagnosed with NF1 during childhood due to the presence of café-au-lait macules (CALMs) and a family history of NF1. The genetic test identified the pathogenic variant c.6792C>A (r.[6757_6858del,6792c>a], p.[Ala2253_Lys2286del,Tyr2264*])) in the *NF1* gene. The patient consulted when his partner was pregnant and they declined prenatal genetic testing after genetic counseling was provided. On the birth of a daughter (Fig 1A, III.2), the familial c.6792C>A variant was studied and NF1 was discarded due to the negative genetic test results. Some months later, in routine pediatric follow-ups, more than six CALMs were identified, alerting the pediatrician to the possibility of NF1. After genetic counseling, a comprehensive *NF1* gene test was performed for the daughter (III.2) identifying a different *NF1* pathogenic variant (c.2990G>A; r.2851_2990del; p.Leu952Cysfs*22). In addition, we determined that III.2 inherited the wild-type NF1 allele from her father.

Family 2. A 43-year-old male (Fig 1B, II.2) was clinically diagnosed with NF1. His father (I.1), sister (II.3) and niece and nephew (III.2 and III.3) were also affected. The genetic test identified the pathogenic genetic variant c.1466A>G (p.Tyr489*) in the *NF1* gene in the index case (II.2). His niece (III.2) got pregnant and she asked for prenatal diagnosis. After genetic counselling, the genetic study showed that she did not carry the variant detected in the index case (c.1466A>G), but a different pathogenic variant was identified through a comprehensive *NF1* study (c.5839C>T; p.(Arg1947*)). After prenatal testing, the pregnancy was interrupted as the same pathogenic variant was identified in the fetus. The other affected members of the family (I.1, II.3 and III.3) also carried the variant c.5839C>T, indicating it as the familial variant. The study of microsatellites showed that the index case (II.2) inherited the wild-type NF1 paternal allele, contrary to the rest of affected individuals of the family.

Family 3: A 20-year-old female (Fig1C, III.1) with family history of NF1, diagnosed with NF1 during childhood and presenting a Noonan-like phenotype was referred for genetic testing along with her parents. The genetic test identified a *de novo* pathogenic genetic variant in the *NF1* gene, c.3587T>G (p.Leu1196Arg). Her father (II.3), clinically diagnosed of NF1 did not harbor the same variant. Thus, a comprehensive *NF1* gene test was performed on the family. Her father (II.3) and paternal uncle (II.2) were carrying a different pathogenic genetic variant c.1756_1759del (p.Thr586Valfs*18) in the *NF1* gene, showing that in this case, the patient

(III.1) was carrying a *de novo* pathogenic *NF1* mutation. The study of microsatellites in this family did not allow us to determine which paternal allele III.1 had inherited.

Family 4: A 6-year-old male (Fig 1D, III.2) was clinically diagnosed with NF1 with a Noonan-like phenotype. His father (II.2), brother (III.1), aunt (II.3) and cousins (III.3 and III.4) were also affected. A comprehensive *NF1* genetic test was performed on patient II.3 identifying the pathogenic variant c.7996_7997delAG (p.Ser2666Cysfs*5). When the test was extended to other members of the family, the genetic test showed that the individual III.2 diagnosed with NF1 did not carry the familial variant, but a *de novo* mutation was identified through a comprehensive *NF1* study (c.2543G>A; p.Gly848Glu). In addition, we determined III.2 inherited the wild-type NF1 allele from his father.

In all four cases, paternity was confirmed by microsatellite analysis and the *de novo NF1* variants detected in their offspring in a proportion of 50%. In addition, these *de novo* variants not detected in blood in affected fathers, although germline mosaicism could not be confirmed.

**Parental origin of new *NF1* mutated alleles**

It is known that parental age and gender influence both mutation type and *de novo* occurrence of *NF1* pathogenic variants. Thus, we studied the parental origin of the *de novo* mutations in these four families. In all of them, the *de novo* NF1 cases occurred in the offspring of an affected male (Figure 1). Microsatellite analysis confirmed paternity and in three families, *de novo* NF1 individuals inherited the wild-type allele from the affected father (Family 1, 2 and 4), although the analysis was not informative in Family 3. Furthermore, in Families 1 and 2, we were able to analyze the *NF1* coding region of parents and affected children to determine the differential polymorphisms surrounding familial and *de novo NF1* pathogenic variants. We then cloned and sequenced the *NF1* coding region harboring both the *NF1* variants and the closest polymorphisms, allowing us to determine the allele carrying the *de novo NF1* variant. In both cases we demonstrated that the *de novo NF1* pathogenic variants were present in the wild-type *NF1* allele inherited from the affected father, thus discarding a *de novo* sporadic mutation in the maternal allele (Figure 2).

**Incidence of the novo *NF1* mutations in familial cases**

In 154 out of 246 families in our cohort, we were able to analyze whether the pathogenic *NF1* variant co-segregated in more than one affected individual in the same family. Additionally, the 154 families analyzed had a total of 514 offspring, of which 164 individuals were diagnosed as healthy due to the absence of any clinical NF1 manifestation and by *NF1* DNA testing, 346 were diagnosed with NF1 and carrying the familial *NF1* pathogenic variant, and 4 affected NF1 individuals were cases in which a different *NF1* variant was identified (Table 2). In the light of these transmission results, we estimated that the *de novo* mutation rate per gamete and per generation was $3.89 \times 10^{-3}$, around twenty times the first *NF1* mutation rate to be described ($\sim 2 \times 10^{-4}$) [10] and 100 times higher considering the most recent *NF1* mutation rate estimates of $3–6 \times 10^{-5}$ [11–13]. In both scenarios, this difference is statistically significant. Similarly, if we considered the 246 families present in our cohort and assuming that all not tested NF1 individuals (n=100) harbor the familial NF1-causig variant, the estimated NF1 mutation rate per gamete and generation was $2{,}81 \times 10^{-3}$, 100 times higher considering the lowest *NF1* mutation rate and fourteen times considering the highest *NF1* mutation rate described ($\sim 2 \times 10^{-4}$) (Table 2).

Furthermore, given that the increased risk of transmission in individuals with NF1, due to their higher *de novo* mutation rate, could be appreciated as negligible considering the 50% risk of inheriting the familial variant, we estimated the co-occurrence of *de novo* NF1 cases in NF1 families to quantify the possible risk for these families of presenting a similar situation. In our cohort we identified 4 families where the *NF1* pathogenic variant detected in the index case did not co-segregate within the family. These results correspond to a frequency of 1:39 of familial cases with two or more genetically tested individuals (4 of 154), considerably higher than the expected *de novo* cases by chance assumed to be similar to those in the general population (~1:2500) about sixty times the frequency of sporadic NF1 (p-value <0.05).

## 4. Discussion

Our results highlight the fact that *de novo NF1* pathogenic variants may appear more frequently than expected in NF1 affected families. 154 families of our cohort with familial NF1 in which two or more individuals were genetically tested were attended and in four of the families, two independent pathogenic variants in NF1 gene have been described. Several evidences have been published considering a possible higher mutation rate in *NF1* in NF1 individuals and, to date, 11 NF1 families with two or more independent mutations in the *NF1* gene have already been reported [5,30–32]. More specifically, considering NF1 offspring data from our cohort, the estimated mutation rate of *NF1* gene within NF1 families could be at least fourteen times higher the highest *NF1* mutation rate previously described ($\sim 2 \times 10^{-4}$). However, this data could be biased due to the small cohorts studied and it needs to be confirmed in larger cohorts.

Further evidence is needed to consider an elevated *NF1* mutation rate in NF1 families compared to the general population. A possible explanation was that the presence of an inheritable defect in DNA repair segregating in the family, which is unlinked to the *NF1* gene, could be responsible for the occurrence of independent *NF1* pathogenic variants within a family [31]. The results obtained from the patients attended at the HGTP-ICO CSUR show that the *de novo* mutation occurs in the healthy allele of the affected progenitor and we can discard alterations in the most common genes related to DNA repair in the families studied there, since the NGS panel used to genetically diagnose them also contains more than 130 genes involved in other cancer predisposition syndromes [33] many caused by defects in DNA repair genes. Thus, our data do not support the hypothesis of a DNA repair defect unlinked to *NF1* gene as the molecular cause of this increased *NF1* mutation rate.

On the other hand, these *de novo* NF1 variants could be associated with both paternal origin and paternal age in addition to some intrinsic effect of NF1. In our cohort, four families showed *de novo* pathogenic variants in the offspring of NF1-affected males, all of whom were older than 30. In addition, in two of these families, the *de novo* mutation was found to be in the wild-type male allele. Studies of *de novo* mutations in humans have estimated the mutation rate of single-nucleotide variants to be $\sim 10^{-8}$ mutations per generation, giving rise to 45–60 *de novo* mutations per genome, mostly from paternal origin and very variable within families [23,24]. It is established that the cumulative number of *de novo* variants transmitted by 20 year-old parents is around 45 and it increases by a factor of 1.3 per year, reaching around 90 at a parental age of 45 [25]. In the case of NF1, similar evidences have been established: point *de novo* pathogenic variants have been previously associated to a paternal origin [22,26], and the risk of *de novo* occurrence of NF1 in offspring increases with increasing paternal age [15,16,20,27,28]. In addition to this genome-wide paternal age effect, an additional paternal age effect has been observed for specific genes (as for example components of the RAS–MAPK pathway), which are positively selected in spermatogonia lineages [17–19]. As a consequence, the effective germline mutation rate in these genes is up to four orders of magnitude higher than the genome-wide rate [18]. In these cases, the association of these mutations with paternal age is stronger than the linear genome-wide paternal age effect. Therefore, the increased mutation rate in NF1 families could be affected by both gender and age of affected parent.

Regardless of the underlying cause of this higher *NF1* mutation rate, the data reported suggest that NF1 families are at an increased risk of experiencing a *de novo* mutation in *NF1* (1:39; 1,64%) compared to the general population (1:2500; 0.04%), especially in families in which the father is the affected member (p-value=0.0034). This observation, if confirmed in larger cohorts, would have relevant implications in genetic counseling and family planning, and also for genetic studies offered to NF1 relatives. Therefore, a negative result for a familial variant genetic test would not ensure the absence of the disease in ante-natal tests. Furthermore, this should also be considered when genetic testing is performed to confirm NF1 in familial cases, as the absence of the familial *NF1* variant could erroneously rule out its *de novo* presence. Hence, without additional data, it is very important to reinforce the message during genetic counseling that all members of an affected family may not harbor the same variant, especially when considering family planning. In this context, the current approach to ante-natal testing for the familial variant should be reconsidered, particularly if the father is the affected parent. There is the need to assess whether it could be more

advantageous to propose to the family that they undertake a prenatal comprehensive study of the *NF1* gene (NGS panel and copy-number alterations detection) or genotyping of the sperm of fathers, as proposed by Pasmant and Pacot [39], while considering all the implications, such us family stress, response time, incidental findings, technical limitation and cost-effectiveness. In the era of next generation sequencing, the sequencing of a gene takes on average three weeks, and consequently could be done in the context of prenatal diagnosis.

## 5. Conclusion

In summary, an unexpected number of NF1 families with two independent pathogenic variants in the *NF1* gene have been identified in our cohort, which comprises families from two expert NF centers. Moreover, 11 families with two different NF1-causing variants have been described in the literature. These findings suggest that NF1 individuals could present a *NF1* mutation rate of $3.89 \times 10^{-3}$, twenty times the highest *NF1* mutation rate described in the general population ($\sim 2 \times 10^{-4}$), and could be at risk of 1:39 of experiencing *de novo* mutations in the *NF1* gene. The knowledge of the estimated NF1 recurrence risk, together with a gender and age effect, is an essential component in genetic counseling when deciding on ante-natal and familial NF1 testing strategies. Therefore, if this observation could be confirmed in other cohorts, would have relevant implications in *NF1* testing, genetic counseling and family planning for families affected with NF1.

## Table 1:

| Table 1. NF1 patient cohort | | | | |
|---|---|---|---|---|
| | **A Series (H. Ramón y Cajal)** | **B Series (CSUR HGTP_ICO)** | **Total** | **% women / men** |
| NF1 individuals | 590 | 239 | 829 | 53% / 47% |
| Sporadic NF1 | 426 | 157 | 583 | 53% / 47% |
| NF1 Families (index cases) | 166 | 80 | 246 | 54% / 46% |
| NF1 families (index cases) with >1 affected individual tested | 126 | 28 | 154 | 54% / 46% |

* Men proportion Chi square, p=0.07, 95% CI: 0.437 - 0.503

## Table 2:

| Table 2: Estimation of the *de novo* NF1 mutation rate in NF1 families | | | | | |
|---|---|---|---|---|---|
| | **NF1 offspring** | | | | |
| | Healthy ł | Familial NF1 variant | New NF1 variant | Total | ***de novo NF1* mutation rate in NF1 families** |
| **Familial NF1** (n=246) | 260 | 446* | 4 | 710 | Observed: 2,81 $x10^{-3}$<br>Expected: $2x10^{-4}$; p-value = $6.55x10^{-3}$<br>Expected: $3x10^{-5}$; p-value = $1.9x10^{-5}$ |
| **NF1 families with >1 affected individuals tested** (n=154) | 164 | 346 | 4 | 514 | Observed: 3,89 $x10^{-3}$<br>Expected: $2x10^{-4}$; p-value = $8.88x10^{-4}$<br>Expected: $3x10^{-5}$; p-value = $3,31x10^{-7}$ |

ł Paediatric cases, clinically determined by the absence of NF1 features; * 100 patients without genetic testing. Assumed to harbour the familial variant. *n* indicates the total number of families studied

## STATEMENTS:


### FUNDING:

This work has been supported by: The Children's Tumor Foundation (CTF-2019-05-005), the Spanish Association of NF Affected (AANF), Fundación Proyecto Neurofibromatosis, the Catalan NF Association (AcNeFi), the Ministry Science and Innovation (PI20 / 00215); Fundació La Marató de TV3 (126/C/2020) and the Generalitat of Catalonia (2017 SGR 496).

### ACKNOWLEDGEMENTS

We thank the Hospital Ramón y Cajal and HGTP Clinical Services and staff for their collaboration in generating and collecting patient clinical data and the whole Hereditary Cancer Group at IGTP for their help in improving this work. We would like to acknowledge the constant support of the different NF lay associations: Asociación de Afectados de Neurofibromatosis, Chromo22 and ACNefi.


### COMPETING INTERESTS

The authors declare no conflicts of interests.

### ETHICS APPROVAL STATEMENT

All procedures performed were in accordance with the ethical standards of the IGTP Institutional Review Board (PI-17-524), who approved this study and with the 1964 Helsinki declaration and its later amendments.

### CONTRIBUTORSHIP STATEMENT (SPECIFIC CONTRIBUTION OF AUTHORS IN THE PAPER IF APPLICABLE)

E.Castellanos and I.Blanco designed the study and wrote the manuscript that was revised, corrected, and improved by all authors. B.Garcia , N.Catasús and Yolanda Martin performed most of the experimental work and analyzed the data. M Guerrero-Murillo performed statistical analysis. I.Rosas, A.Negro and A. Valero performed genetic analysis. A.Ros, A.Duat, JL.Becerra, C.Comas, I.Bielsa contributed with clinical data collection. S. Bonache, C Hernández-Chico, C.Lazaro and E.Serra provided scientific input. N.Catasús generated the figures for the paper and contributed in writing the manuscript. All authors approved the final version of the manuscript.

## References


1 Evans DG, Howard E, Giblin C, Clancy T, Spencer H, Huson SM LF. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. *Am J Med Genet A* 2010;**152A**:327–32. doi:10.1002/AJMG.A.33139

2 Boyd KP, Korf BR TA. Neurofibromatosis type 1. *J Am Acad Dermatol* 2009;**61**:1–14. doi:10.1016/J.JAAD.2008.12.051

3 DeBella K, Szudek J, Friedman JM. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. *Pediatrics* 2000;**105**:608–14. doi:10.1542/peds.105.3.608

4 Szudek J, Joe H, Friedman JM. Analysis of intrafamilial phenotypic variation in neurofibromatosis 1 (NF1). *Genet Epidemiol* 2002;**23**:150–64. doi:10.1002/gepi.1129

5 Sabbagh A, Pasmant E, Imbard A, Luscan A, Soares M, Blanché H, Laurendeau I, Ferkal S, Vidaud M, Pinson S, Bellanné-Chantelot C, Vidaud D, Parfait B, Wolkenstein P. NF1 Molecular Characterization and Neurofibromatosis Type I Genotype-Phenotype Correlation: The French Experience. *Hum Mutat* 2013;:1–9. doi:10.1002/humu.22392

6 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./pmc/articles/PMC1682337/?report=abstract (accessed 19 Oct 2021).

7 Ferner RE. Neurofi bromatosis 1 and neurofi bromatosis 2 : a twenty fi rst century perspective. 2007;**6**.

8 Ratner N, Miller SJ. A RASopathy gene commonly mutated in cancer: the neurofibromatosis type 1 tumour suppressor. *Nat Rev Cancer* Published Online First: 2015. doi:10.1038/nrc3911

9 Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, Blakeley J, Babovic-Vuksanovic D, Cunha KS, Ferner R, Fisher MJ, Friedman JM, Gutmann DH, Kehrer-Sawatzki H, Korf BR, Mautner V-FF, Peltonen S, Rauen KA, Riccardi V, Schorry E, Stemmer-Rachamimov A, Stevenson DA, Tadini G, Ullrich NJ, Viskochil D, Wimmer K, Yohay K, Gomes A, Jordan JT, Mautner V-FF, Merker VL, Smith MJ, Stevenson DA, Anten M, Aylsworth A, Baralle D, Barbarot S, Barker F, Ben-Shachar S, Bergner A, Bessis D, Blanco I, Cassiman C, Ciavarelli P, Clementi M, Frébourg T, Giovannini M, Halliday D, Hammond C, Hanemann CO, Hanson H, Heiberg A, Joly P, Kalamarides M, Karajannis M, Kroshinsky D, Larralde M, Lázaro C, Le L, Link M, Listernick R, MacCollin M, Mallucci C, Moertel C, Mueller A, Ngeow J, Oostenbrink R, Packer R, Papi L, Parry A, Peltonen J, Pichard D, Poppe B, Rezende N, Rodrigues LO, Rosser T, Ruggieri M, Serra E, Steinke-Lange V, Stivaros SM, Taylor A, Toelen J, Tonsgard J, Trevisson E, Upadhyaya M, Varan A, Wilson M, Wu H, Zadeh G, Huson SM, Evans DG, Plotkin SR, International Consensus Group on Neurofibromatosis Diagnostic Criteria (I-NF-DC), Huson SM, Evans DG, Plotkin SR. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genet Med* 2021;**23**:1–8. doi:10.1038/s41436-021-01170-5

10 Crowe FW, Schull WJ NJ. A clinical, pathological and genetic study of multiple neurofibromatosis. *Springfield, Illinois Charles C Thomas, 1956* 1956.

11 Sergeyev A. On the mutation rate of neurofibromatosis. *Humangenetik* 1975;**28**:129–38. doi:10.1007/BF00735745

12 Huson SM, Compston DA, Clark P HP, Huson SM, Compston DA, Clark P, Harper PS, SM H, DA C, P C, PS H. A genetic study of von Recklinghausen neurofibromatosis in south east Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. *J Med Genet* 1989;**26**:704–11. doi:10.1136/JMG.26.11.704

13 Clementi M, Barbujani G, Turolla L, Tenconi R, Medica G, Pediatria D, Giustiniani V, Padova U, Loredan V. Original investigations Neurofibromatosis-l : a m a x i m u m likelihood estimation of mutation rate. 1990;:116–8.

14 Upadhyaya M, Ruggieri M, Maynard J, Osborn M, Hartog C, Mudd S, Penttinen M, Cordeiro I, Ponder M, Ponder BA, Krawczak M CD. Gross deletions of the neurofibromatosis type 1 (NF1) gene are predominantly of maternal origin and commonly associated with a learning disability, dysmorphic features and developmental delay. *Hum Genet* 1998;**102**:591–7. doi:10.1007/S004390050746

15 Fahmideh MA, Tettamanti G, Lavebratt C, Talbäck M, Mathiesen T, Lannering B, Johnson KJ, Feychting M. Parental age and risk of genetic syndromes predisposing to nervous system tumors: nested case–control study. *Clin Epidemiol* 2018;**10**:729. doi:10.2147/CLEP.S159183

16 Bunin GR, Needle M, Riccardi VM. Paternal Age and Sporadic Neurofibromatosis 1: A Case-Control Study and Consideration of the Methodologic Issues. *Genet Epidemiol* 1997;**14**:507–16. doi:10.1002/(SICI)1098-2272(1997)14:5

17 Tartaglia M, Patton M a., Gelb BD, Cordeddu V, Kalidas K, Shaw A, Jeffery S, Sorcini M, van der Burgt I, Crosby A, Chang H. Paternal Germline Origin and Sex-Ratio Distortion in Transmission of PTPN11 Mutations in Noonan Syndrome. *Am J Hum Genet* 2004;**75**:492–7. doi:10.1086/423493

18 Goriely A, Wilkie AOM. Paternal Age Effect Mutations and Selfish Spermatogonial Selection: Causes and Consequences for Human Disease. *Am J Hum Genet* 2012;**90**:175–200. doi:10.1016/j.ajhg.2011.12.017

19 Penrose LS. PARENTAL AGE AND MUTATION. *Lancet* 1955;**266**:312–3. doi:10.1016/S0140-6736(55)92305-9

20 Riccardi VM, Dobson CE, Chakraborty R, Bontke C. The pathophysiology of neurofibromatosis: IX. Paternal age as a factor in the origin of new mutations. *Am J Med Genet* 1984;**18**:169–76. doi:10.1002/ajmg.1320180121

21 Lázaro C, Gaona A, Ainsworth P, Tenconi R, Vidaud D, Kruyer H, Ars E, Volpini V, Estivill X. Sex differences in mutational rate and mutational mechanism in the NF1 gene in neurofibromatosis type 1 patients. *Hum Genet* 1996;**98**:696–9. doi:10.1007/s004390050287

22 Jadayel D, Fain P, Upadhyaya M, Ponder MA, Huson SM, Carey J, Fryer A, Mathew CG, Barker DF PB. Paternal origin of new mutations in von Recklinghausen neurofibromatosis. *Nature* 1990;**343**:558–9. doi:10.1038/343558A0

23 Conrad DF, Keebler JEM, DePristo M a, Lindsay SJ, Zhang Y, Casals F, Idaghdour Y, Hartl CL, Torroja C, Garimella K V, Zilversmit M, Cartwright R, Rouleau G a, Daly M, Stone E a, Hurles ME, Awadalla P, 1000 Genomes Project. Variation in genome-wide mutation rates within and between human families. *Nat Genet* 2011;**43**:712–4. doi:10.1038/ng.862

24 Goldmann JM, Wong WSW, Pinelli M, Farrah T, Bodian D, Stittrich AB, Glusman G, Vissers LELM, Hoischen A, Roach JC, Vockley JG, Veltman JA, Solomon BD, Gilissen C, Niederhuber JE. Parent-of-origin-specific signatures of de novo mutations. *Nat Genet* 2016;**48**:935–9. doi:10.1038/ng.3597

25 Goldmann JM, Veltman JA, Gilissen C. De Novo Mutations Reflect Development and Aging of the Human Germline. *Trends Genet* 2019;**35**:828–39. doi:10.1016/j.tig.2019.08.005

26 Stephens K, Kayes L, Riccardi VM, Rising M, Sybert VP PR. Preferential mutation of the neurofibromatosis type 1 gene in paternally derived chromosomes. *Hum Genet* 1992;**88**:279–82. doi:10.1007/BF00197259

27 Dubov T, Constantini S, Toledano-Alhadef H, Ben-Shachar S, Bokstein F. The effect of parental age on the presence of de novo mutations - Lessons from neurofibromatosis type I. *Mol Genet Genomic Med* 2016;**4**:480–6. doi:10.1002/mgg3.222

28 Liu Q, Zoellner N, Gutmann DH JK. Parental age and Neurofibromatosis Type 1: a report from the NF1 Patient Registry Initiative. *Fam Cancer* 2015;**14**:317–24. doi:10.1007/S10689-014-9774-8

29 Brabbing-Goldstein D, Ben-Shachar S. Ante-natal counseling in phacomatoses. *Child's Nerv Syst* 2020;**36**:2269–77. doi:10.1007/s00381-020-04776-3

30 Klose A, Peters H, Hoffmeyer S, Buske A, Lüder A, Hess D, Lehmann R, Nürnberg P, Tinschert S. Two independent mutations in a family with neurofibromatosis type 1 (NF1). *Am J Med Genet* 1999;**83**:6–12.http://www.ncbi.nlm.nih.gov/pubmed/10076878

31 Upadhyaya M, Majounie E, Thompson P, Han S, Consoli C, Krawczak M, Cordeiro I, Cooper DN. Three different pathological lesions in the NF1 gene originating de novo in a family with neurofibromatosis type 1. *Hum Genet* 2003;**112**:12–7. doi:10.1007/s00439-002-0840-1

32 Pacot L, Burin des Roziers C, Laurendeau I, Briand-Suleau A, Coustier A, Mayard T, Tlemsani C, Faivre L, Thomas Q, Rodriguez D, Blesson S, Dollfus H, Muller Y-G, Parfait B, Vidaud M, Gilbert-Dussardier B, Yardin C, Dauriat B, Derancourt C, Vidaud D, Pasmant E. One NF1 Mutation may Conceal Another. *Genes (Basel)* 2019;**10**:633. doi:10.3390/genes10090633

33 Castellanos E, Gel B, Rosas I, Tornero E, Santín S, Pluvinet R, Velasco J, Sumoy L, Del Valle J, Perucho M, Blanco I, Navarro M, Brunet J, Pineda M, Feliubadaló L, Capellá G, Lázaro C, Serra E. A comprehensive custom panel design for routine hereditary cancer testing: preserving control, improving diagnostics and revealing a complex variation landscape. *Sci Rep* 2017;**7**:39348. doi:10.1038/srep39348

34 Valero MC, Martín Y, Hernández-Imaz E, Marina Hernández A, Meleán G, Valero AM, Javier Rodríguez-Álvarez F, Tellería D, Hernández-Chico C. A highly sensitive genetic protocol to detect NF1 mutations. *J Mol Diagn* 2011;**13**:113–22. doi:10.1016/j.jmoldx.2010.09.002

35 Castellanos E, Rosas I, Negro A, Gel B, Alibés A, Baena N, Pineda M, Pi G, Pintos G, Salvador H, Lázaro C, Blanco I, Vilageliu L, Brems H, Grinberg D, Legius E, Serra E. Mutational spectrum by phenotype: panel-based NGS testing of patients with clinical suspicion of RASopathy and children with multiple café-au-lait macules. *Clin Genet* 2020;**97**:264–75. doi:10.1111/cge.13649

36 Garcia-Linares C, Fernández-Rodríguez J, Terribas E, Mercadé J, Pros E, Benito L, Benavente Y, Capellà G, Ravella A, Blanco I, Kehrer-Sawatzki H, Lázaro C, Serra E. Dissecting loss of heterozygosity (LOH) in neurofibromatosis type 1-associated neurofibromas: Importance of copy neutral LOH. *Hum Mutat* 2011;**32**:78–90. doi:10.1002/humu.21387

37 Peter D Turnpenny SE. *Emery's Elements of Medical Genetics*. 15th Editi. Elsevier 1900. doi:10.1093/nq/s9-V.114.174-a

38 Messiaen LM, Wimmer K. NF1 mutational spectrum. *Neurofibromatosis Kaufmann D (ed)* 2008;**16**:63–77.

39 Pasmant E, Pacot L. Should we genotype the sperm of fathers from patients with 'de novo' mutations? *Eur J*

*Endocrinol* 2019;**182**:C1–3. doi:10.1530/EJE-19-0759

## FIGURE AND TABLE LEGENDS

**Table 1:** Number of sporadic and familial cases visited at Hospital Ramón y Cajal and at the Spanish National Reference Center (CSUR) of Phakomatoses. Sex distribution is also included.

**Table 2:** Estimation of the *de novo* NF1 pathogenic variants in NF1 families.

**Figure 1.** Pedigree of families 1-4. Families 1 and 2 were diagnosed at the Spanish National Reference Center (CSUR) of Phakomatoses while families 3 and 4 at Hospital Ramón y Cajal. Family members clinically diagnosed with NF1 are depicted with blackened symbols. Grey denotes possibly affected subjects referred by their relatives, and white denotes healthy individuals. Polymorphism analyses determine that in families 1,2 and 4 *de novo* mutation has originated in the paternal allele. Paternal and maternal ages at conception are indicated above the patient in blue and orange, respectively. *De novo* variant is showed in bold and highlighted in grey. Arrow indicates the index case in each family.

**Figure 2**: Diagram of the analyzed NF1 region in families 1 and 2 is shown. Polymorphism analyses determine that in both families *de novo* mutation has originated in the paternal allele.

**Supplementary Table 1:** Type of NF1 pathogenic variant detected in both familial and sporadic NF1 cases studies.