Germline selection of NF1 mutant alleles drives transmission ratio distortion in NF1 carriers

Non-Mendelian inheritance of NF1 mutations through germline selection

Germline selection drives excess transmission of NF1 mutations to offspring

Short title: Transmission ratio distortion (TRD) in NF1.

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Abstract (250 words max)

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant tumor-predisposition syndromes (~1:3,000 worldwide), caused by pathogenic variants in the NF1 gene. NF1 encodes neurofibromin, a negative regulator of RAS-MAPK signaling, and behaves as a classic tumor suppressor, with tumorigenesis requiring biallelic inactivation. NF1 is clinically diverse, involving pigmentary, skeletal, and neurodevelopmental features, alongside a lifelong risk of benign and malignant tumors.

Here, we analyzed transmission patterns in 322 NF1 families across four well-characterized cohorts, applying strict inclusion criteria to minimize ascertainment bias and avoid potential mosaic cases. Among 701 offspring, 61.1% were diagnosed with NF1 ($p = 3 \times 10^{-8}$), a significant excess beyond the 50% expected under Mendelian inheritance. Transmission ratio distortion (TRD) was observed in both female (62.8%) and male (58.5%) transmitters. To test whether cohort size or other confounders explained this bias, we performed sub-sampling and large-scale random down-sampling analyses, which confirmed robust TRD independent of parental sex or sample size.

We propose that germline TRD in NF1 reflects a mechanism analogous to its somatic biology, involving preferential selection of NF1 homozygous alleles in germ cells, following loss of heterozygosity (LOH), uniparental disomy (UPD) or compound heterozygosity during early embryogenesis. Germline selection may represent a common phenomenon across cancer predisposition syndromes. These findings uncover a novel facet of NF1 biology with direct implications in clinical practice and reproductive counseling.

Significance statement (75 words):

Neurofibromatosis type 1 (NF1) is a common hereditary cancer syndrome also affecting neurodevelopment. Analysis of transmission patterns in a large NF1 cohort reveals a consistent transmission bias in favor of mutant alleles, violating Mendelian expectations. We propose that germline selection mechanisms, analogous to somatic processes in tumorigenesis, underlie this distortion. These findings reveal an unanticipated connection between cancer biology and inheritance, with direct implications for reproductive counseling and for other hereditary cancer predisposition syndromes.

Introduction

Although rare human disorders are inherently difficult to study, they often provide critical insights into fundamental biology. Among these, Neurofibromatosis type 1 (NF1, OMIM 162200), a well-characterized, dominantly inherited tumor predisposition syndrome affecting ~1:3,000 individuals worldwide(1), has provided a wealth of new conceptual advances in genetics. NF1 has informed both tumorigenesis and neurodevelopmental biology, providing a rare conceptual link between cancer mechanisms and cognitive/behavioral disorders. It also exemplifies how tissue and developmental context influence the clinical manifestations of genetic mutations.

NF1 is a large gene on chromosome 17q11.2, spanning ~283 kilobases, with multiple isoforms encoding Neurofibromin (UNIPROT P21359), a 2839-residue protein that negatively regulates the RAS-MAPK signaling pathway. While clinical presentation can be variable, even in families with the same mutation, heterozygous germline *NF1* mutations are typically associated with café-au-lait macules, skinfold freckling, and iris hamartomas (Lisch nodules). Additional features include learning disability, autism or ADHD traits, skeletal abnormalities (macrocephaly, short stature, scoliosis, pseudoarthrosis), and tumors. Cutaneous peripheral nerve sheath tumors (neurofibromas) are almost universal in adults with *NF1*, and 10-15% of plexiform neurofibromas transform into malignant peripheral nerve sheath tumors (MPNSTs), a major cause of morbidity and mortality. At the cellular level, NF1 acts as a classic tumor suppressor gene tumor formation follows the “two-hit” model, with heterozygous carriers requiring biallelic inactivation via loss-of-heterozygosity (LOH) or loss-of-function (LoF) mutations. Consistently, most neurofibromas harbor distinct, independent second-site NF1 mutations (2, 3).

Another unusual feature of the NF1 biology, is its unusually high spontaneous germline mutation rate, reported to be significantly higher than that of most other disease genes, with some studies suggesting a 10-fold increase in NF1 mutation rate (4, 5). Consequently, ~50% of *NF1* germline mutations occur sporadically as *de novo* mutations to unaffected couples. In addition, segmental NF1 caused by post-zygotic mosaicism is

also commonly reported, while somatic *NF1* mutations are frequently found in tumors, contributing to tumor evolution, especially in leukemia, lung cancer and other solid tumors. A likely, but not often acknowledged, explanation for the high NF1 germline spontaneous mutation rate is the contribution of selfish spermatogonial selection (6), a process by which mutations occurring spontaneously in the adult paternal germline are progressively enriched over time because they provide a selective advantage to mutant spermatogonial stem cells that outcompete their wild-type neighbors, thus increasing the proportion of mutant sperm with age. Studies of this process that manifests in so-called Paternal Age Effect (PAE) disorders have mainly implicated gain-of-function (GoF) mutations in components of the RTK/RAS/MAPK pathway (6, 7), with best documented examples in *FGFR2* (i.e. Apert and Crouzon syndromes)*, FGFR3* (achondroplasia and thanatophoric dysplasia), *HRAS* (Costello syndrome) and *PTPN11* (Noonan syndrome). As a negative regulator of the RAS/MAPK signaling, loss-of-function (LoF) mutations in NF1 are anticipated to show the same effect and result in small clonal expansions in the testes of all men as they age.(8) (9) However, whether selfish selection of *NF1* LoF mutations requires loss of one or both alleles in the testes of ageing men remains unclear.

As an autosomal dominant disorder, NF1 confers a 50% risk of transmitting the mutant allele to each offspring. Yet, reports have suggested the possibility of transmission ratio distortion (TRD) in NF1, whereby one allele is preferentially inherited by offspring of NF1 carriers, raising questions about potential deviations from Mendelian expectations. To date, these epidemiological studies have generally been small in scale and inconclusive, likely because of methodological and/or interpretive issues such as inconsistent application of the strict NF1 diagnostic criteria established in 1988 (10, 11), potential ascertainment bias in family studies (4, 12), and/or inclusion of *de novo* NF1 cases, some of whom may have been germline mosaic and thus at reduced risk of transmitting the pathogenic variant.

While preimplantation genetic testing (PGT) offers an alternative means of assessing TRD, results were again inconsistent. Merker et al. (2015) analyzed 1060 embryo biopsies from 77 couples with one NF1-affected partner and observed a 54.4%

transmission of the mutant allele, representing a significant deviation from the expected 50:50 ratio (two-sided exact binomial p = 0.00426) (12). In a similar approach, Vernimmen et al. (2023) analyzed 746 biopsied embryos from 82 couples in the Netherlands and found a 50.4% transmission rate, not significantly different from Mendelian expectation (13). Of note, only 30 couples (37%) in this latter cohort had familial NF1 mutations, while the majority were sporadic (56%) or presumed de novo cases (7%), again raising the possibility that parental germline mosaicism diluted the strength of the TRD.

Given the inconsistency of these reports and the unusual biology of NF1, we sought to assess the possibility of TRD by analyzing a large, combined cohort of NF1 families, applying strict criteria to minimize ascertainment bias. In the present report, we analyzed 322 familial NF1 cases and identified a statistically significant excess of affected offspring, consistent with transmission ratio distortion. To account for potential confounding factors, we applied different statistical approaches including stratification and down-sampling, which allowed us to systematically evaluate and exclude alternative explanations for the observed distortion. We then explore the most plausible biological mechanisms underlying the NF1 transmission distortion and consider its clinical implications for the counseling of NF1 families.

Materials and Methods

Collection of NF1 transmission data from archival clinical pedigrees was performed following strict criteria to ensure that only familial transmissions from NF1 heterozygous individuals were considered. A ‘family’ unit or sibship was defined as two parents and their offspring, with the transmitting parent being an affected child of an NF1 parent (i.e., second or later generation cases. Families in which both parents had NF1 were excluded, and only multi-generation pedigrees were included. In some pedigrees, longitudinal data allowed inclusion of additional sibships from lower branches. Families were included only if NF1 status of parents and all siblings unequivocally met clinical diagnostic criteria, in some cases confirmed by molecular testing (14). Further, to minimize ascertainment bias and avoid over-representation of cases, the following approaches were used: (1) pedigrees specifically ascertained for linkage or pedigree studies were excluded, except for sibships in branches other than that of the study’s proband (i.e. proband’s generation and below); (2) the proband’s own sibship was excluded and (3) when counting sibships in prior generations, the transmitting parent was not included. Only sibships in which the affected parent was a familial case (i.e., also had an affected parent) were retained.

In total, we obtained existing data from independent cohorts previously collected by four medical centers for clinical or non-linkage research purposes over ~40 years (denoted as M [USA1], S [Spain], and U [UK], V [USA2]). We analyzed transmission of NF1 from each affected parent to their offspring. For each family unit, five primary data records were recorded: (i) a unique family ID and the proband (first ascertained case), (ii) sex of the transmitting parent, (iii) number of affected offspring per sibship, (iv) total number of offspring per sibship and (v) number of affected female offspring per sibship. Three additional data points were derived from these records, as follows: (6) number of affected male offspring = number of affected offspring per sibship − number of affected female offspring per sibship; (7) number of unaffected offspring per sibship = total number of offspring per sibship − number of affected offspring per sibship; and (8) rate of transmission (mutant) = number of affected offspring per sibship / total number of offspring per sibship (Supplementary Table S1).

The estimated transmission rate is the pooled transmission rate derived as follow:

$$rat\,e_{pooled} = \sum_{i=1}^{n_{families}} i_{affected_{offsprings}} / \sum_{i=1}^{n_{families}} i_{total_{offsprings}}$$

All analyses were conducted using R Statistical Software (v4.4.1; R Core Team, 2024). Given an expected transmission rate of 50%, a one-sided proportion test (using the prop.test function from R stats v4.4.1) was performed with the null hypothesis that the transmission rate is ≤50%. This test was applied to the aggregated cohort as well as seven sub-cohorts (Supplementary Table S2) including:

1. Cohort excluding one-child families.
2. Male transmitter families.
3. Female transmitter families.
4. Male transmitter families with an even number of offspring.
5. Female transmitter families with an even number of offspring.
6. Male transmitter families excluding one-child families.
7. Female transmitter families excluding one-child families.

To evaluate the impact of cohort size on male and female transmitter cohorts, we performed random down-sampling followed by a one-sided proportion test Supplementary Table S3). Specifically, 500 random subsets of the female transmitter cohort were down-sampled were generated to match the size of male transmitter cohort (119 families), followed by another 500 subsets to match the male cohort with an even number of offspring (51 families).

Lastly, random down-sampling was performed at varying sample sizes for 5,000 trials for the whole cohort, as well as separately for the male and female transmitter sub-cohorts (Supplementary Table S4). Specifically, the respective cohort was randomly sampled with size ranging from 1 to 322 (all families), 1 to 203 (female transmitters), and 1 to 119 (male transmitters) in each trial. A one-sided one sample proportion test was carried out in each down sample trial to determine if the proportion of affected offspring as significantly greater than the expected 50%. The results were visualized by plotting the

proportion, i.e. estimated transmission rate, from each trial against the corresponding cohort size, i.e. total number of offsprings. Note that the observed transmission rate is the mean of individual familial transmission rates derived as follow:

$$rate_{mean} = \frac{1}{n_{families}} \sum_{i=1}^{n_{families}} \frac{i_{affected_{offsprings}}}{i_{total_{offsprings}}}$$

Results

We collected a total of 322 families with demonstrable transmission of familial NF1 from a total of four study cohort groups from the USA (cohorts M/V), Spain (cohort S) and the UK (cohort U). Strict inclusion criteria were applied to avoid ascertainment bias and exclude potential mosaic cases (see Methods). Overall, eligible sibships included: (i) offspring of second-generation probands, (ii) siblings from prior generations other than the direct transmitting ancestor, (iii) offspring of affected aunts or uncles of the proband, and (iv) any subsequent generations from affected individuals in those sibships. Each sibship could therefore include no affected individuals, and only families in which the NF1 status was unambiguously known for all offspring were included in the study.

Counts for each cohort are summarized in Table 1 and Figure 1A-B and families were stratified on the basis of the sex of the transmitting parent. For 203 families (63%), the mother was the transmitting parent and in only 119 families was the father the NF1 carrier. The aggregated cohort had a total of 701 offspring, corresponding to an average of 2.2 children per family (median = 2.0 children per family), with most families having 1-4 offsprings (Figure 1B). Male transmitters had a slightly larger number of offspring (p = 0.02539, unpaired t test), with an average of 2.4 children per family (median = 2.0), while female transmitters had an average of 2.1 children per family (median = 2.0).

Collating the transmission data from each of these 322 families, allowed us to estimate the transmission ratio for the aggregated cohort (Figure 1C). We found that 428/701 offsprings were diagnosed with NF1 (61.1%), a transmission ratio significantly higher than the expected 50% from a typical mendelian inheritance (p = 3E-8, one-sample

proportion test) (Figure 1C; Table 1). To further query this unexpected result, we stratified the data by considering the sex of the transmitting parent, in order to examine if there is a sex-specific difference on the observed transmission distortion. An increased transmission ratio above the expected 50% was also observed in male and female transmitter cohorts when considered independently. However, the families with a female transmitter (n = 203 families) had the higher transmission bias with 62.8% of affected offspring (282 affected out of a total 417 offspring) (One-sample proportion test; p = 1E-7), while male transmitter families (n = 119) showed 58.5% transmission bias for NF1 (166 affected out of 284 total offsprings from male carriers, p = 2.6E-3). While the elevated transmission was more pronounced in female transmitters compared to male transmitters, the difference was not statistically significant (Chi-Square test, X-squared = 0.18, df = 1, p-value = 0.276). Additionally, there is no significant sex bias among affected offspring with NF1 in our cohort (200 females, 212 males; two-sided binomial test with expected proportion of 50%, p = 0.588) (Supplementary Table S1).

As these transmission results deviated from Mendelian expectation, we sub-sampled our cohort further to ensure that the results were robust and that the observed effect was not an artifact of sample heterogeneity or statistical noise.

First, to avoid inflating results due to the large numbers of relatively small families, we repeated the analysis by excluding single-child families (n = 214 families after excluding 108 families with only one child). Single-child families tend to produce extreme transmission rates that are not always informative and may bias the data toward the extremes. As shown in Figure 1C (and Table 1 Supplementary Table S2), non-single-child families showed a 59.0% transmission rate (350 affected offsprings from a total of 593 children across 214 families), a bias that remained significantly higher than the expected 50% (p = 0. 0000067). This confirmed that removing extreme transmissions (i.e. single child families) did not reduce the significance of the observed TRD.

Second, to address potential biases associated with family structure, we excluded families with an odd number of offspring, as these families cannot produce an exact 50% transmission rate under the null hypothesis of random segregation, thereby potentially skewing the results. Analysis of the 145 families with an even number of offspring

revealed a transmission rate of 58.8%, which remained significantly higher than the expected 50% (p = 0.00048) (Figure 1C, Table 1, Supplementary Table S2). These results indicate that the inclusion of families with an odd number of offspring is not a major source of bias contributing to the observed transmission distortion. Additionally, when stratifying by the sex of the transmitting parent after excluding families with an odd number of offspring, we observed a transmission rate of 54.4% in families with male transmitters (n = 51) and 61.4% in families with female transmitters (n = 94). Notably, only female-transmitter families with an even number of offspring exhibited a transmission rate significantly higher than the expected 50% (p = 0.00037), whereas the deviation observed in male-transmitter families was not statistically significant (p = 0.173). However, it is important to note that the ‘male-even’ cohort was the smallest subgroup (n = 51 families, totaling 136 offspring), suggesting that the lack of statistical significance may reflect limited sample size rather than the absence of a true effect.

Because there were nearly twice as many female transmitters as male transmitters (203 vs. 119), we assessed the impact of sample size on the significance of the observed bias by randomly down-sampling the female-transmitter cohort to match the size of the male-transmitter cohort. As illustrated in Supplementary Figure 1, the results of the first 30 random down-sampling trials consistently showed a higher transmission rate in the female-transmitter cohort compared to the male-transmitter cohort, despite all comparisons being based on matched cohort sizes. Supplementary Table S3 further summarizes the outcomes of all 500 down-sampling trials, with only 5 trials showing a transmission rate in the female-transmitter cohort lower than that of the male-transmitter cohort. Although across trials, the female-transmitter cohort appeared to exhibit higher transmission rates than the male-transmitters, in individual size-matched female trials, these differences were not significant (Supplementary Figure S1; Supplementary Table S3), consistent with the cohort-wide Chi-Square test (p-value = 0.276).

Finally, to further evaluate the effect of sample size, we simulated data by performing random down-sampling across a range of sample sizes. This analysis aimed to illustrate how small sample size can hinder the detection of transmission distortion. The results

were visualized by plotting the mean transmission rate against the total number of offspring in each trial (Figure 2, Supplementary Table S4). As expected, trials with larger sample sizes exhibited less variability in transmission rate compared to those with smaller sample sizes. A clear trend emerged: including more families and families with larger numbers of offspring resulted in more precise estimates of the transmission rate, which increasingly converged toward the rate observed in the full cohort. These findings confirm that cohort size influences the stability of the estimated transmission rate, with larger cohorts yielding more accurate estimates of transmission bias.

Table 1: Summary of the aggregate cohort characteristics and the stratification strategies used to estimate NF1 transmission rates. See Supplementary Table 1 for the complete dataset and statistical results.

Condition	Families tested	Affected offspring	Total offspring	Transmission ratio	P Value*	CI low
All families	322	428	701	61.1%	3.0E-09	57.9%
All families, even number of offspring	145	214	364	58.8%	4.8E-04	54.4%
All families, excluding single child family	214	350	593	59.0%	6.7E-06	55.6%
Female transmitter only	203	262	417	62.8%	1.0E-07	58.7%
Female transmitter only, even number of offspring	94	140	228	61.4%	3.7E-04	55.8%
Female transmitter only, excluding single child family	130	211	344	61.3%	1.7E-05	56.8%
Male transmitter only	119	166	284	58.5%	2.6E-03	53.4%
Male transmitter only, even number of offspring	51	74	136	54.4%	1.7E-01	47.0%
Male transmitter only, excluding single child family	84	139	249	55.8%	3.8E-02	50.4%

*A one-tailed one-sample proportion test was used to assess whether the observed transmission rate significantly exceeded the expected 50% under the null hypothesis (Confidence Interval (CI)).

Figure 1. Characteristic features of the 322 NF1 families included in this study.

(A) Number of families/sibships collected for the four NF1 cohorts (M = USA1; S = Spain; U = UK; V= USA2).

(B) Distribution of family sizes per sibship count for each cohort and in aggregate (bottom), stratified by the sex of the transmitting parent: male carriers (blue) and female carriers (red).

(C) Forecast plot illustrating different data stratification approaches sorted by estimated NF1 transmission rate, including stratification by sex of transmitting parent to assess sex effects, excluding single-child families or families with an odd number of offspring to reduce extreme skews. P-value and lower confident interval (arrow) were derived from one-tailed one-sample proportion test. The dotted red line represents the expected 50% random Mendelian segregation; size of the cohort and level of significance are described according to circle and color key, as indicated on the chart.

Figure 2: Illustration of the impact of sample size on transmission rate: *for the whole cohort (left) or split by sex of the transmitting parent (i.e. female transmitter (middle) or male transmitter (right). A total of 5,000 random down-sampling trials were performed at varying sample sizes to systematically assessed the effect of sample size on the estimated transmission rate. For each trial, a one-tailed one-sample proportion test was conducted under the null hypothesis of 50% transmission rate (dotted red line). The Y-axis indicates the mean transmission rate across all trials of a given sample size, rather than the observed transmission of the entire cohort as shown in Table 1. Each point represents one trial, plotted against the total number of offspring in that trial. Points are colored by p-value (see key on figure), with trials showing no statistical significance ($p < 0.05$) greyed out. Results of each individual trial are given in Supplementary Table S3.*

Discussion

In this study, we showed that familial *NF1* heterozygous mutations are transmitted to offspring at a significantly higher than expected frequency, representing a clear deviation from Mendel's second law of random segregation of heterozygous alleles. Our analysis was based on data collected from 322 family sibships, each carefully ascertained and evaluated in order to minimize bias and control for potential confounders. From this data, we observed a clear transmission ratio distortion (TRD), with 61.1% of offspring inheriting the mutant NF1 allele.

Given the unexpected nature of this result, we conducted further analyses to determine whether the observed TRD could be confounded by the sex of the transmitting parent or the structure of the family pedigrees. We showed that none of these factors were likely to account for the distortion, which remained consistent across all stratification conditions tested. Importantly TRD was observed from both male and female transmitters, although female transmitters exhibited a stronger TRD (62.8% affected offsprings) than male transmitters (58.8% offsprings), this difference was not significant. While incomplete family records, small family sizes, and other limitations inherent to retrospective clinical data could contribute to apparent TRD, we have attempted to mitigate these effects through strict inclusion criteria, stratification, down-sampling and data simulation. Given the large size and the consistent findings between the different sub-cohorts, major sources of artifacts are unlikely to affect our conclusions.

While segregation distortion is a well-recognized phenomenon in genetics, the biological mechanisms involved are not always fully understood (15). Notably, most data on TRD derive from studies on animal and plant models, with comparatively few documented in human disease contexts (16-19). Below we briefly review the most common mechanisms previously shown to account for TRDs (15) and evaluate their potential relevance to the transmission distortion observed in our NF1 cohort.

The best-known form of TRD is probably meiotic drive, in which alleles bias their own transmission through mechanisms such as unequal chromosome segregation and/or gamete killing. Such systems typically exploit sex-specific features of gametogenesis to

favor the inheritance of the driver allele. In females, driver alleles can hijack the inherent asymmetry of meiosis: for example, alleles associated with expanded centromeres are preferentially retained in the oocyte, while the competing wild type allele is relegated to a polar body and ultimately lost. This process involves altered centromere architecture, changes in kinetochore dynamics, and regulation by aurora kinases (20, 21). In this context it may be relevant to note that *NF1* is a peri-centromeric gene which encodes neurofibromin, a protein that has itself been implicated in spindle organization and chromosome segregation (22). By contrast, male meiotic drivers lack the opportunity to exploit asymmetric meiosis, and often act through post-gametogenic mechanisms such as gamete killing. A well-studied example is the “t” haplotype in mice, which contains two major selfish genetic elements: distorter genes that target and disable functions on the wild-type haplotype; and a series of structural changes and inversions that suppress recombination and preserve linkage of the driver complex (23). After meiosis, the driver exploits syncytial cytoplasmic bridges to transfer the distorter gene products into neighboring wild-type gametes (23, 24). Rather than outright “killing”, these factors impair the motility of wild-type sperm, reducing their fertilization success and thereby increasing the relative transmission of the t-bearing gametes.

Overall, meiotic drive is an unlikely mechanism to account for the observed pattern in our NF1 cohort for several reasons. Firstly, we detected elevated NF1 transmission in both male and female carriers, with no statistical significance difference between the sexes. This observation would require either a universal, sex-independent mechanism, or sex-specific drivers that would nevertheless converge on the same transmission distortion. This seems improbable, as most known meiotic drivers in animals operate through sex-restricted mechanisms (23, 25). Of note, a small number of gamete killers in plants have been reported to act in both male and female gametes (26-28). The alternative explanation, in which males and females achieve the same transmission distortion through distinct meiotic drive mechanisms cannot be categorically ruled out, but it risks overcomplicating the picture. Moreover, although meiotic drivers and gamete killers are well documented across a wide range of eukaryotic species (29), neither process has yet been described in humans so far.

Epigenetic factors, including allele-specific methylation, may also result in TRD (19). However, NF1 is not an imprinted locus (30), and to the best of our knowledge, no parent-of-origin effects have been documented. Similarly, chromosomal abnormalities, such as Robertsonian translocations, can result in biased chromosomal segregation (31, 32). But, chromosomal abnormalities are rare events, making them unlikely to account for the pronounced and consistent distortion observed in our cohort.

A different class of TRD mechanisms involves selection at the level of gametes or zygotes. In gametic selection, post-meiotic gametes with different genotypes have diverse competitive success, conferring advantages such as increased motility in sperm competition or enhanced pollen tube growth in plants (33). This mechanism could, in principle, explain elevated transmission from male carriers if mutant sperm were more competitive (i.e. fast swimmers). However, while gametic selection may be a contributing factor, it is unlikely to underlie the consistent TRD observed across both male and female NF1 transmitters, given the fundamental differences between spermatogenesis and oogenesis. Alternatively, TRD may arise from zygotic selection, whereby certain genotypes exhibit reduced viability or lethality. In such cases, the bias typically favors the wild-type allele, and can explain the apparent depletion of some pathogenic genotypes, particularly in recessive disorders (34). This again does not adequately explain the pattern of TRD observed in our NF1 cohort.

In contrast to the TRD mechanisms discussed above, we propose an alternative explanation that is conceptually similar to the process of tumor development in NF1, involving secondary loss of heterozygosity (LOH) followed by selection of homozygous mutant cells (Figure 3). In this model, an early recombination event, or a loss-of-function mutation in the second allele (i.e., compound heterozygosity), within germline tissue generate cells with bi-allelic *NF1* inactivation. Homozygosity for NF1 confer a selective advantage to mutant primordial germ cells (PGCs) over their heterozygous counterparts, enabling clonal expansion within the developing gonad. During subsequent meioses, these NF1-null clones generate exclusively mutant haploid gametes, thereby contributing a disproportionate share of mutant gametes and shifting the transmission ratio above the

Mendelian expectation of 50%. Importantly, to account for the observed sex-independent and pronounced transmission ratio distortion (~61%), such events must occur early in embryogenesis - at a developmental stage when only a small number of cells are present and before sex determination (35, 36), which takes place ~6 weeks of development in humans. Consistent with this scenario, *NF1* is expressed in both male and female adult (https://www.proteinatlas.org/ENSG00000196712-NF1/tissue and https://www.gtexportal.org/home/gene/NF1, last accessed: Aug 2025) (37, 38) and embryonic gonads (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-6592, last accessed Aug 2025) (39).

Of particular relevance in this context, is the process of copy-neutral LOH (cnLOH) or Uniparental Disomy (UPD) which arises when one parental allele is lost and the remaining allele is duplicated, resulting in homozygosity (i.e. isodisomy) without altering overall DNA copy number. cnLOH is well documented in tumors and is a common mechanism of pathogenesis in neurofibromas (3), and can occur during early embryogenesis. At this stage, rapid cellular proliferation makes developing tissues vulnerable to chromosome segregation errors, which can be rescued via UPD and/or mitotic recombination (40-42). Such cnLOH events occurring early within the gamete-producing lineages would generate a subpopulation of homozygous NF1 germ cells. Importantly, mitotic recombination relying on well-defined hotspots (43-45) in and around *NF1* is a known pathogenic mechanism in NF1-associated tumors, being observed in ~50% of neurofibromas (46-49) but also reported in normal CNS-derived tissue of a NF1 young patient (50).

Such a parsimonious model in which positive selection of homozygous NF1 cells occurs in the developing germline lineage is attractive, as it relies on a well-established somatic process in NF1 tumorigenesis to explain the pattern of consistent transmission bias observed in both male and female carriers in our cohort.

Figure 3: Proposed mechanism accounting for the TRD in familial NF1 cases. In this scenario, a random event of mitotic recombination between sister chromatids (top) or a deletion/ loss-of-function mutation affecting the second NF1-bearing chromosome 17 (yellow thunder) (i.e. compound heterozygosity) (bottom) within the developing germline tissue can give rise to bi-allelic NF1 inactivation in the primordial germ cell (PGC) lineage. The red box or star represents different NF1 mutations. Homozygosity for NF1 loss confers a selective advantage to mutant PGCs over their heterozygous counterparts, which clonally expands in the developing gonad (right). Subsequent gametogenesis and meiosis produce a higher-than-expected proportion of mutant gametes (>50%), driven by the increased prevalence of homozygous NF1 germ cells (oogonia or spermatogonia). To account for the observed sex-independent and pronounced transmission ratio distortion (~61%), such events must occur early in embryogenesis - at a developmental stage when only a small number of embryonic cells are present and before 6 weeks of development, the time at which sex determination occurs in humans. These mechanisms are analogous to that described in NF1-associated neoplasia, where NF1-null cells arise through cnLOH (at mitotic recombination hotspots), or alternatively via deletion of the wild-type allele or as compound heterozygotes following an acquired mutation on the second NF1 allele, i.e. a second hit (3, 48, 50).

Given that NF1 is a clinically significant genetic target, with many patients, often from large families, presenting to clinics, it will be important to consider the implications of our findings for both clinical practice and genetic counselling. A major challenge in managing NF1 individuals is the unpredictable phenotypic impact of NF1 mutations, which often complicates reproductive counseling and decision-making for affected couples. Our findings, which showed that the average risk of transmission is higher than 50%, suggest

that current reproductive guidelines require updating. For example, a heterozygous NF1 parent may be given a broader range of transmission ratio (i.e. 50%-60%, rather than the classical 50% risk). Even a modest increase in transmission risk, combined with the uncertainty in disease severity, could significantly influence reproductive decision-making. Available reproductive options for NF1 carriers exist and include for example in vitro fertilization with preimplantation genetic testing for monogenetic disorders (PGT-M), chorionic villus sampling (CVS), amniocentesis, and/or non-invasive cffDNA analysis (51). To further personalize transmission risk estimates, gamete testing could be offered to NF1 carriers, at least for males due to the ease of sperm sample collection (52). This approach would simply involve quantifying the ratio of sperm carrying the family-specific NF1 pathogenic variant prior to a pregnancy to estimate the individual transmission risk (52).

Another consideration important for clinical practice is to establish the precise process that give rise to NF1-null cells and whether they arise through copy-neutral loss of heterozygosity (via mitotic recombination/UPD), via compound heterozygosity (deletion or point mutation of the second allele (Figure 3) (3, 48, 50) or yet via other mechanisms. Indeed, if compound heterozygosity is involved, the second allele will carry a variant distinct from the original familial mutation and will likely escape detection during prenatal testing. Given the large size of the *NF1* gene and its high genetic heterogeneity with over 5000 known likely/pathogenic variants recorded in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), most testing strategies in familial cases involve linkage analysis and determination of the risk haplotype using microsatellite markers. Once linkage is established, a single diagnosis test can be performed through detection of the parental risk-haplotype, rather than needing a custom assay to detect the family-specific mutation itself. Hence, under the “second hit” compound heterozygosity hypothesis, parental haplotype testing is insufficient to detect the new *NF1* variant acquired on the second allele. Independent mutations in NF1 families, although infrequent, have been documented in several reports (53-57). In these cases, thorough examination of the whole *NF1* gene may be required via sequencing.

As highlighted by our sample size analysis, large-scale studies with rigorous diagnostic and inclusion criteria are needed to validate and extend our findings, also ensuring the inclusion of families with different mutation types and from different ethnic backgrounds. Diverse cohorts would improve statistical power, reduce bias, and allow more precise estimates of NF1 mutation transmission and phenotypic variability. Moreover, analysis of sperm samples from male NF1 carriers would also provide a more direct assessment of the frequency of TRD across NF1 individuals and reveal whether certain alleles (for example, point mutations vs. large deletions) are more prone to mosaic mitotic recombination or LoF in the germline. Such studies will help refining reproductive counselling guidelines, offer possible personalized interventions such as gamete testing prior to pregnancy to estimate individual risks and allow NF1 families to make informed reproductive decisions (52).

In conclusion, we have extended the well-documented behavior of *NF1* mutant cells in somatic tissues (i.e. LOH/UPD) to the germline, providing a simple mechanistic explanation for the segregation distortion observed in our large NF1 cohort and revealing a novel facet of the unusual biology of NF1. Given that TDRs have also been reported in other cancer predisposition syndromes, including Retinoblastoma (RB1)(18) and Li-Fraumeni syndrome (TP53)(16), it will be important to establish whether the distortion via LOH and selection in germ cells represents a more general phenomenon than previously recognized.

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Authors contribution

Conceived and supervised the study: MRW, VMR and AG

Provided patient cohort data: VMR, ECB, CLG, ECP, MU, MRW

Performed the data analysis: YP, AB, MRW and AG

Wrote the manuscript: YP and AG with input from MRW and MU. All authors read and approved the final version

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