

Original Article

Linkage disequilibrium and founder effect analysis of the *NF1* gene in French Canadians from the Quebec population

Li Juan Fang ^a, Wentian Li ^{b,1}, Nader Chalhoub ^a, Josué Feingold ^c, June Ortenberg ^d,
Jean-Paul Thirion ^{a,*}

^aDépartement de microbiologie et d'infectiologie, faculté de médecine, Université de Sherbrooke, Sherbrooke, PQ J1H 5N4, Canada

^bLaboratory of Statistical Genetics, Rockefeller University, Box 192, 1230 York Avenue, New York, NY 10021, USA

^cINSERM-393-Hôpital Necker, 75015 Paris, France

^d2300 Tupper Street, Department of Genetics, The McGill University-Montreal Children's Hospital, Montreal, PQ H3H 1P3, Canada

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Abstract

We genotyped 19 neurofibromatosis type 1 (NF1) families from French Canadians of the Quebec population with four intragenic microsatellites (IVS26-2.3, IVS27AC28.4, IVS27AC33.1, and IVS38GT53.0). Linkage analysis of the four microsatellite markers among the 19 NF1 families indicates that the four microsatellites are strongly linked with NF1 disease (LOD = 2.76–3.64). The four markers are associated ($P = 0-0.077$) except marker pair IVS26-2.3/IVS27AC33.1 ($P = 0.18$ or 0.17). However, perhaps due to the high mutation rate of the *NF1* gene, no founder effect for NF1 was detected in the Quebec French Canadians. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Neurofibromatosis type 1 (NF1); Microsatellite; Linkage disequilibrium; French Canadians; Founder effect

1. Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder which afflicts about one in 3500 individuals. NF1 is characterized by clinical features of café-au-lait spots, Lisch nodules and fibromatous skin tumours. Patients also display assorted medical complications including axillary or inguinal freckling, mental retardation, learning disabilities, or increased risk of malignancy. The disease exhibits pleiotropic clinical manifestations even among family members who carry the same mutation. The *NF1* gene spans about 350 kb at 17q11.2 with 60 exons and has a mutation rate, 1×10^{-4} /gamete/generation which is about 100-fold higher than the usual rate for a single locus. Approximately 50% of cases are the results of de novo mutations [4,11,24,26,29,35].

An estimated 8000–10 000 French migrants colonised the province of Quebec during 1608–1760. The geographic and cultural isolation of the French settlers and their descendants, coupled with early marriages and a high birth-rate favoured endogamy and contributed to the founder effect [2,6]. At least a dozen hereditary disorders in the French Canadian population show evidence of founder effects [3,5,7,13,16,19,22,25,31,34,38]. An isolated population itself does not guarantee a detectable founder effect. If the disease is not a single-gene trait, is caused by mutations at different locations within the gene, or has a high spontaneous mutation rate, we may observe various disease-bearing haplotypes, not just one or a few transmitted from the founder.

Linkage disequilibrium (LD) analysis examines whether a certain marker allele, or a certain haplotype, is abundant in affected individuals in a homogeneous population. A small number of disease-bearing haplotypes is an indication of a founder effect. Since we know the *NF1* gene is linked to the disease, previous studies did not show that a specific haplotype was associated with the NF1 disease [12]. Attempts were made in this study to examine the LD/founder

* Corresponding author.

E-mail address: jpthir@courrier.usherb.ca (J.P. Thirion).

¹ Present address: Division of Biology and Human Genetics, North Shore University Hospital, Research Building, 350 Community Drive, Manhasset, NY 11030, USA

in French Canadians from the Quebec population by a family-based association analysis program GASSOC, and other non-family-based programs. The failed test would indicate a lack of LD/founder effect. Meanwhile, we examined the LD or association between nearby markers [10,14,39]. LD among polymorphism markers in the *NF1* region has been previously reported [12,21,23,36]. The analysis of LD/association between markers is completely unrelated to the founder effect analysis. The LD/association between markers concerns the correlation between alleles at two nearby markers, while the LD analysis for founder effect is the correlation between a marker allele and the affection status.

We genotyped 19 *NF1* families from the Quebec population and investigated linkage, LD/association between markers, LD/founder effect by using four microsatellites (IVS26-2.3, IVS27AC28.4, IVS27AC33.1, and IVS38GT53.0). These four microsatellites are distributed along approximately 65 kb of the gene [27]. The IVS26-2.3 polymorphism is at intron 27a and approximately 40 kb apart from IVS27AC28.4, while IVS27AC28.4, IVS27AC33.1 and IVS38GT53.0 are 5 kb and 20 kb apart, respectively. We first examined whether the gene is indeed responsible for the *NF1* disease in these 19 families by linkage analysis. We then examined association between these markers. The detection of the founder effect was carried out by associating particular alleles or haplotypes with the affection status.

2. Materials and pedigree data

2.1. Families and patients

Patients met National Institute of Health *NF1* diagnostic criteria. A total of 15 family cases and four sporadic cases (45 *NF1* patients and 40 normal individuals) from Quebec were investigated. Informed consent was obtained from all individuals. Sixteen families are two-generations and three are three-generations. Genomic DNA was extracted by the standard phenol–chloroform method [27]. One family bore deletion with chromosomes of the mother and son which did not contribute marker information [9].

2.2. Detection of polymorphisms

The four microsatellites loci are at intron 27a (IVS26-2.3 [11]), intron 27b (IVS27AC28.4 [17], IVS27AC33.1 [11]) and intron 38 (IVS38GT53.0 [18]). The size of each fragment was determined by a ladder constructed with a pUC18 sequence. The PCR was performed in 20 μ l total volume with 10 pmol of each primer, standard PCR buffer, 100 ng genomic DNA and 0.5–1 U of Taq polymerase, and labelled with [α -³²P]dCTP. The reaction was performed for 30 cycles with denaturation at 94 °C for 30 s, extension at 72 °C for 30 s at an annealing temperature as described

[1,17,18]. The products were subject to electrophoresis on 8% acrylamide–urea denaturing gels.

3. Analysis methods and results

3.1. Linkage analysis

The allele sizes and the frequency distribution of alleles (Table 1) on disease-bearing and non-disease-bearing chromosomes are analysed across all 19 pedigrees. The analysis used alleles only from parents and married-ins. Children do not provide independent samples, since they derive their alleles from their parents. A total of 43 individuals were parents and married-ins (*NF1* patients and normal individuals), which gave a total of 86 haplotypes. Seven haplotypes were not informative, thus a total of 79 haplotypes from parents and married-ins were used in the analysis. For the *NF1* haplotypes, we sampled 14 affected parents and married-ins from 14 families (families 1–4, 6, 7, 9–12, 14, 15, 18, 19). Parents and married-ins from the other five families (families 5, 8, 13, 16, 17) were either ambiguous in their affection status (sporadic cases) or uninformative. Because family 6 bore deletion, the mother was typed only for one haplotype. Therefore, we had 27 *NF1* haplotypes ($28 - 1 = 27$) in our analysis. In total, 26 normal parents and married-ins were taken as unaffected controls, which gave 52 unaffected haplotypes for the normal allele frequency. Some individuals, for example parents in sporadic families, who were ambiguous in their affection status, were not used as normal controls.

Linkage analysis between the disease and markers was carried out using the programs LINKAGE [33] and GENEHUNTER [15] to ensure that the regions being focused on were indeed related to the disease. Haplotypes were constructed with the GENEHUNTER program [15] assuming a minimum number of recombinations. A strong linkage signal was observed by the LINKAGE program (with the LOD scores of the four markers being 3.29, 2.97, 3.64 and 2.76, respectively, at recombination fraction $\theta = 0.01$) and the GENEHUNTER program (with a LOD score of 4.35), using a dominant model.

3.2. Family-based association analysis

Linkage signal can be enhanced by considering possible association between the disease and particular alleles. One example of this type of linkage analysis is the family-based association which considers the parents–affected-child triads. The unaffected (normal) haplotype is not sampled from the population, but rather from the parental and married-ins' haplotypes not transmitted to that affected child. The unaffected haplotype is used as the control in a case-control study. We used the program GASSOC [28], which is slightly different from the popular transmission/

Table 1
Alleles Sizes, Frequency Distributions (Overall, Normal and NF1), and Observed Heterozygosity for Each Microsatellite

Microsatellites	Alleles	Size (bp)	Allelenumber	Overall	Normal (total = 52)	NF1 (total = 27)	Observed heterozygosity
IVS26-2.3	1	226	8	0.1013	0.0962	0.1111	0.86
	2	222	11	0.1392	0.0962	0.2222	
	3	218	4	0.0506	0.0385	0.0741	
	4	216	4	0.0506	0.0769	0.0000	
	5	214	5	0.0633	0.0577	0.0741	
	6	212	10	0.1266	0.0962	0.1852	
	7	210	20	0.2532	0.2885	0.1852	
	8	208	7	0.0886	0.0962	0.0741	
	9	206	9	0.1139	0.1346	0.0741	
	10	204	1	0.0127	0.0192	0.0000	
IVS27AC28.4	1	219	2	0.0253	0.0192	0.0370	0.81
	2	217	12	0.1519	0.1346	0.1852	
	3	215	12	0.1519	0.1346	0.1852	
	4	213	1	0.0127	0.0192	0.0000	
	5	211	7	0.0886	0.0577	0.1481	
	6	209	24	0.3038	0.3077	0.2963	
	7	207	18	0.2278	0.2692	0.1481	
	8	205	3	0.0380	0.0577	0.0000	
IVS27AC33.1	1	116	18	0.2278	0.2115	0.2593	0.75
	2	114	8	0.1013	0.1346	0.0370	
	3	112	1	0.0127	0.0192	0.0000	
	4	110	5	0.0633	0.0385	0.1111	
	5	108	30	0.3797	0.3846	0.3704	
	6	106	16	0.2025	0.1923	0.2222	
	7	104	1	0.0127	0.0192	0.0000	
IVS38GT53.0	1	189	5	0.0633	0.0577	0.0741	0.82
	2	187	17	0.2125	0.2308	0.1852	
	3	185	24	0.3038	0.2500	0.4074	
	4	183	7	0.0886	0.1154	0.0370	
	5	181	10	0.1266	0.1154	0.1481	
	6	177	3	0.0380	0.0385	0.0370	
	7	175	3	0.0380	0.0385	0.0370	
	8	173	7	0.0886	0.0962	0.0741	
	9	171	3	0.0380	0.0577	0.0000	

disequilibrium test (TDT) [30] in that a disease model (e.g. dominant model) can be used.

The GASSOC program does not show any significant results on our data set. The P -values for a generalised TDT statistic (GTDT in GASSOC) are 0.16, 0.11, 0.18, 0.61, and the P -values for the TDT-like test under a dominant disease model (GDOM in GASSOC) are 0.18, 0.15, 0.25, 0.60, respectively. None of these P -values are significant. The lack of a family-based association and the presence of linkage point out that there is no association between the disease and the markers.

3.3. LD/Founder effect analysis

We used the program HAL (currently renamed TRIM-HAP) [20] to examine whether there is evidence for a founder haplotype. The null hypothesis that a founder haplotype does not exist was tested, and the test result was presented by a P -value. The DISEQ program [32] was also used to test the assumption that an allele is abundant by a likelihood ratio test and the standard χ^2 test. Again, a

P -value for the null hypothesis of no association between the disease and a marker is obtained. Association can be established only when the P -value is small. To run HAL, haplotypes from the affected individuals were prepared manually because the four markers are very close to each other, and there is no recombination between them. We also checked these haplotypes independently by running the GENEHUNTER program.

When HAL was applied to our data, no evidence for a founder haplotype was found. If we assume that the founder haplotype contains only one marker allele, the P -values are between 0.24 and 0.85, depending on the marker allele chosen to be that founder haplotype, and depending on the test statistic. If the founder haplotype is assumed to contain two, three, or four markers, the P -value ranges are 0.55–0.90, 0.64–0.82, and 0.32–0.82, respectively. Under the null hypothesis of no LD between the disease and a marker, another program, DISLAMB in the DISEQ package found that the P -values for all four markers are between 0.42 and 0.79 for the χ^2 test, and all close to 0.5 for the likelihood ratio test. In other words, there is no indication of

Table 2
The most frequent haplotypes

Haplotypes	Allele sizes (bp)	Frequencies	Percentage (%) in total 79 founder haplotypes
7-7-1-8	210-217-116-173	3 times	3.80
9-7-5-1	206-207-108-189	Twice	2.53
7-6-6-5	210-209-106-181	Twice	2.53
2-3-1-2	222-215-116-187	Twice	2.53
1-2-5-3	226-217-108-185	Twice	2.53

an LD/founder effect between the disease and any marker in the data set. This conclusion is consistent with the family-based association studies presented earlier.

3.4. LD/Association between markers

Linkage disequilibrium between markers was determined by a Monte Carlo version of the Fisher's exact test by constructing the joint count table of alleles at two markers from the haplotype data. Table 2 gives the most frequent haplotypes which are 7-7-1-8, 9-7-5-1, 7-6-6-5, 2-3-1-2 and 1-2-5-3. All other possible joint count tables with the same column and row margins can be in principle enumerated, and from the distribution of these tables, the P -value of any test statistic can be calculated. Rather than enumerating all tables, we randomly sampled 100,000 tables to determine an approximate P -value.

To avoid the bias probably caused by the NF1 alleles, we used only the unaffected (normal) alleles in the analysis of association/LD between microsatellites. For pairs of IVS26-2.3/IVS27AC28.4, IVS26-2.3/IVS27AC33.1 and IVS26-2.3/IVS38GT53.0, we used 48 from 52 unaffected haplotypes because the 48 haplotypes were accepted by the analysis, with the same situations for the other pairs. With the exception of between marker pair IVS26-2.3 and IVS27AC33.1, all other marker pairs show significant linkage disequilibrium (Table 3). Out of six marker pairs, four pairs are in complete LD ($P < 0.001$), and one pair exhibits reasonably strong LD/association ($P < 0.05$) (see Table 3). When the P -value is compared to the marker distance, no clear trend is observed. Such an observation is typical in marker LD/association studies, where the theoretical smooth curve of decaying of LD as a function of the marker distance is usually not realised in a real data set.

Table 3
The association between microsatellite markers

Marker pairs	Number of pairs used	P-value from a Fisher's test	99% interval of p -value	Physical distances (kb)
IVS26-2.3/IVS27AC28.4	48	0**		40
IVS26-2.3/IVS27AC33.1	48	0.18	(0.177-0.182)	45
IVS26-2.3/IVS38GT53.0	48	0.0002***	(0.0001-0.0003)	65
IVS27AC28.4/IVS27AC33.1	49	0.035*	(0.034-0.037)	5
IVS27AC28.4/IVS38GT53.0	49	0***		25
IVS27AC33.1/IVS38GT53.0	51	0***		20

* significant $0.01 < p < 0.05$

** highly significant $0.001 < p < 0.01$

*** very highly significant $p < 0.001$

4. Discussion

Our analyses have shown mainly three results. (1) No LD/founder effect was observed in this data set; (2) there are strong LD/associations between these four markers; and (3) there is a strong linkage between the four microsatellite markers and the NF1 disease.

Our observations are in agreement with previous reports [12,21,23,36] using other markers in an independent set of 17 NF1 families including Caucasian, Hispanic, and Chinese [12], in the Spanish population [36], and in African and Japanese [23]. However, as with any association study, LD/founder results are much more convincing when replicated in multiple populations, especially with those isolated and well-defined populations with well-characterized histories [12].

To ensure that our conclusion on the lack of LD/founder in the Quebec French Canadian is reliable, we used several methods and computer programs to tackle the problem. We present here a reliable demonstration to show the absence of founder effect at the *NF1* locus by using a well-characterized population and numerous statistical analyses.

It is often difficult to collect random individuals to represent the whole population. Various complicating factors such as migration and inter-marriage create heterogeneity within the population (subpopulations). Family-based association analysis was developed to eliminate this problem. We have included a family-based program GASSOC in our analysis to ensure the power of our approach. It has been shown that a significant result for family-based association requires both linkage and linkage disequilibrium [30]. Family-based association uses linkage disequilibrium to enhance the test for linkage. For this reason, it is ideal to apply family-based association to markers known to be

close to the disease gene from individuals in a population with a founder effect. Lack of a significant result in family-based association analysis indicates either a lack of linkage or a lack of founder effect. Since we know that the *NF1* gene is linked to the disease, a failed test for a family-based association would indicate a failed test for LD/founder effect.

A direct test for the existence of a founder haplotype (using the programs HAL and DISLAMB) also led to a negative result. This is in agreement with the conclusion simply by examining the haplotype in affected individuals in our data set manually. There is no evidence for one or a few common haplotypes.

Also one or a few common mutations, as one or a few common haplotypes, in a population is an indication for possible founder effect. In these 19 *NF1* Quebec French Canadian families, we have identified and characterised three mutations: one splice site mutation identified by a protein truncation test [9] and two deletions identified by the loss of heterozygosity analysis and Southern blotting [8]. The three mutations have nothing in common, which provides more evidence for the lack of founder effect in the population.

We attempted to explain why markers in the *NF1* gene region do not exhibit LD/founder effect in the Quebec population, even though it is very clear that the *NF1* gene is involved in the disease, through both the population (Quebec) and the disease (*NF1*).

As in the Finns and Ashkenazim [16], in the French Canadians, there are more than a dozen genetic diseases at elevated frequencies. Approximately two-thirds of the present French Canadians gene pool in Quebec is attributable to about 1500 men and 1000 women who came to Quebec from France between 1632 and 1680 and settled along the St. Lawrence River [34,37]. To date, most French Canadian genetic diseases described with a strong founder effect are believed to originate around 12 generations [2]. Thus, the French Canadians from the Quebec population is an isolated and well-defined population with well-characterized history.

The *NF1* disease has a high mutation rate with sporadic cases of 50% (21% in our study). The founder effect was not observed in the Quebec French Canadians. The founder effect might be interrupted by the high mutation rate.

The LD/association between nearby markers, in particular, has attracted more attention recently because the existence of such LD may provide a diagnostic opportunity for *NF1* disease. The LD between markers at the *NF1* locus have been observed previously. A strong LD was detected between five intragenic RFLPs which were revealed by AE25 (covering exons 9–38) with *Bgl*III, *Pvu*II and *Taq*I, *Evi*2A with *Bam*HI (at intron 27b), and *Evi*2B with *Eco*RI (at intron 27b) [21]. LD between the AAAT *Alu* repeats and the microsatellite markers ACI27.2 and IVS38GT53.0 were observed [36]. An LD with six polymorphic markers was also reported [23]. Six internal polymorphisms are all in

extremely tight LD with one another. In fact, five of the six markers are in complete LD, and a high degree of LD was maintained among the seven intragenic markers [12]. However, the observed LD between markers does not always follow the theoretical prediction: LD is higher among markers with shorter distances than those far apart. This is due to the fact that individuals in the data set for an association analysis may not always represent a random sampling of the population. For example, an LD between the AAAT *Alu* repeat and the IVS27AC28.4 marker was not observed previously [36]. Since our four markers are very close (< 65 kb), the LD among them is not surprising.

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