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ORIGINAL PAPER

Fine mapping of Noonan/cardio-facio cutaneous syndrome in a large family

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Noonan syndrome (NS) is an autosomal dominant condition with facial dysmorphy, congenital cardiac defects and short stature. A gene for NS has previously been linked to a 14 cM region in 12q24.[2] We performed linkage analysis in a four generation Belgian family with NS in some individuals and cardio-facio-cutaneous (CFC) syndrome in others. Clinical data and linkage data in this family indicate that NS and CFC syndrome result from a variable expression of the same genetic defect. We report a maximum lod score of 4.43 at zero recombination for marker D12S84 in 12q24. A crossover in this pedigree narrows the candidate gene region for NS to a 5 cM interval between markers D12S84 and D12S1341.

Keywords: Noonan syndrome; cardio-facio cutaneous syndrome; CFC syndrome; linkage analysis; chromosome 12

Introduction

Noonan syndrome (NS) is an autosomal dominant condition characterised by a congenital heart defect, typical facial dysmorphy and short stature.[1] Linkage of NS to chromosome 12 markers has been reported previously. Approximately 50% of cases are sporadic, and only one large family is reported in the literature with proven linkage to 12q24.[2] Some smaller nuclear families did not show linkage to this region, indicating genetic heterogeneity. In small families with NS and without proven linkage to 12q24 markers, crossovers cannot be interpreted, and therefore analysing large families is very important. Cardio-facio-cutaneous syndrome (CFC) is also characterised by heart defects, facial dysmorphy and short stature. Additional features however are present such as skin abnormalities (hyperkeratosis, ichthyosis), hypotrichosis, retinal abnormalities and a moderate mental retardation or developmental delay.[3] Most CFC cases are sporadic. There has been a lot of debate in the literature regarding NS and CFC syndrome. Some clinicians favour the hypothesis that NS and CFC syndrome are two genetically different conditions.[4] Other papers suggest that CFC syndrome and NS are allelic variants,[5] or contiguous gene syndromes.[6,7]

We performed linkage analysis in a very large four-generation family, previously reported as an example of CFC syndrome[8] to investigate possible linkage to 12q24 markers.

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Subjects, Materials and Methods

Subjects

We sampled 10 affected individuals, seven unaffected relatives, and three spouses from a large Belgian family after informed consent was obtained (Figure 1). Several individuals in this pedigree have been published previously.[8] All affected individuals have facial dysmorphy and short stature as in Noonan syndrome, and mild to moderate mental retardation. Individuals III7 and III12 are more retarded than their siblings and have IQs between 50 and 60. III12 lives in an institution for mentally retarded adults and he has a mild valvular pulmonic stenosis. Individual III2 was operated on

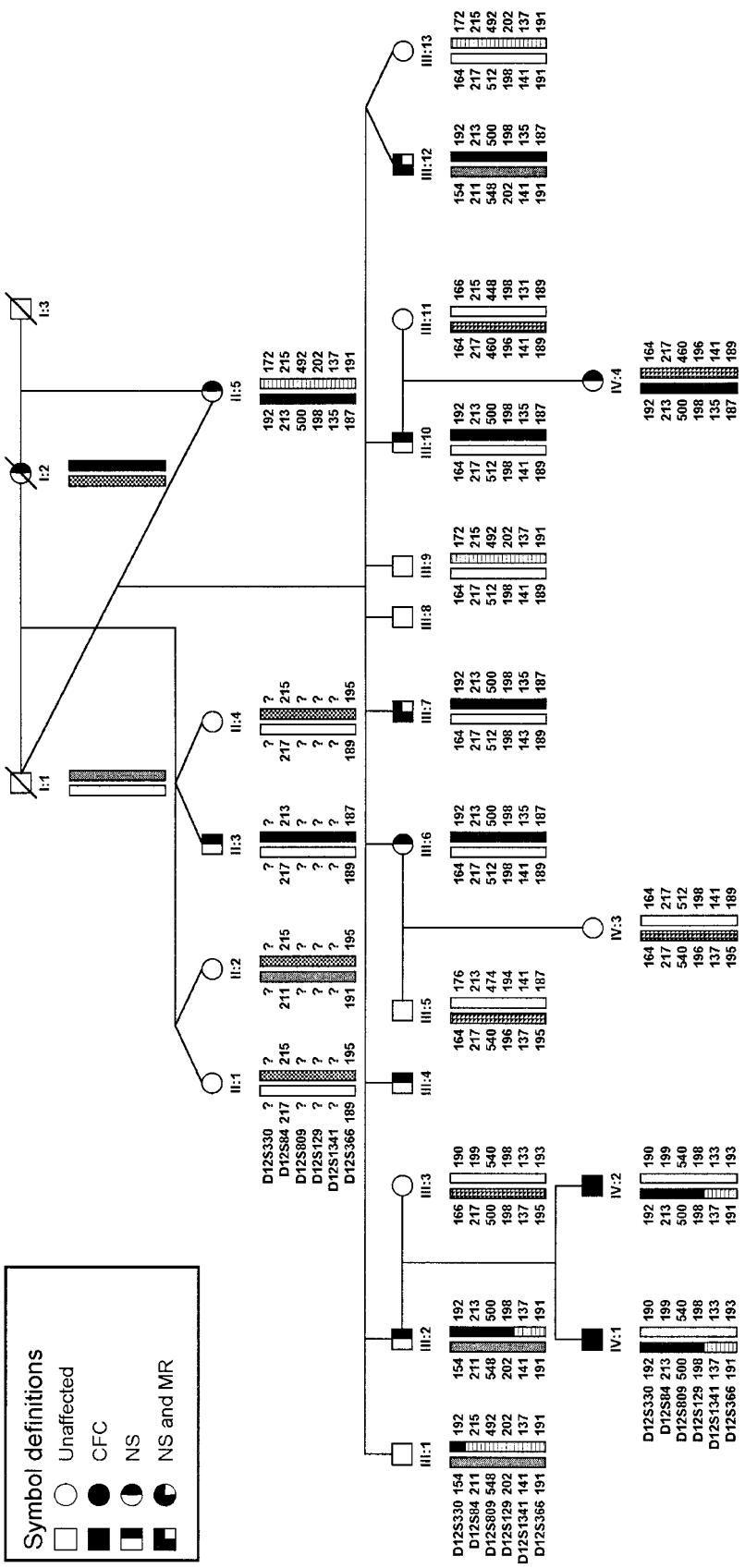

Figure 1 *Pedigree of the four-generation family with NS/CFC.*
Only the available individuals in the first two generations are shown. After I2 died, individual I1 married his stepdaughter II5. Females I2 and II5 were both affected. Only data for markers D12S330, D12S84, D12S809, D12S129, D12S1341, D12S366 are shown. The observed haplotypes are represented by patterned bars. A crossover occurred in III2 on the haplotype linked with the disorder, and in III1 on the haplotype not segregating with the disorder. A question mark (?) is used when the marker was not analyzed in that individual.

for a large ventricular septal defect and he received a valvulotomy for a pulmonic stenosis. His two sons IV1 and IV2 are also mildly retarded and their phenotype was compatible with the CFC syndrome. They have the same facial dysmorphy and growth retardation as in NS but show in addition a dry scaly skin with hyperkeratotic palms, thin sparse scalp hair and scanty eyebrows (Figures 2 and 3). Other affected individuals have a phenotype more typical of NS and are less severely affected.[8] Individual IV4 underwent balloon dilatation for severe pulmonic stenosis with a dysplastic valve.

Genotyping and Linkage Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures. Twelve dinucleotide repeat markers in the 12q24 region were PCR amplified from genomic DNA (Table 1). Of each PCR primer pair, one primer is fluorescently labelled (FITC, fluorescein-isothiocyanate). The resulting PCR fragments were sized on a Long Ranger™ gel in an ALF DNA Sequencer™ (Pharmacia Biotech, Uppsala, Sweden) using the ALF Fragment Manager™ 1.0 software (Pharmacia Biotech, Uppsala, Sweden). Allele sizes were assigned by comparison with a fluorescently labelled DNA molecular-size standard and with the CEPH reference sample,[9] if CEPH data for that particular marker were available.

Two-point lod scores were calculated using the FASTLINK version of the MLINK and ILINK computer programs,[10] with penetrance set at 1.0 for heterozygotes and a gene frequency of 0.0002 for CFC/NS. Allele frequencies for the different markers in Caucasian populations were obtained from GDB.

Results

Linkage analysis in this NS/CFC family, without inclusion of the two individuals diagnosed with CFC syndrome, results in a maximum lod score of 3.832 at zero recombination for marker D12S84 (Table 1). This means that the NS phenotype in this family links to 12q24.

Linkage analysis including the two individuals with CFC syndrome showed convincing evidence for linkage between NS/CFC and the 12q24 dinucleotide repeat markers. The dinucleotide repeat markers in Table 1 are

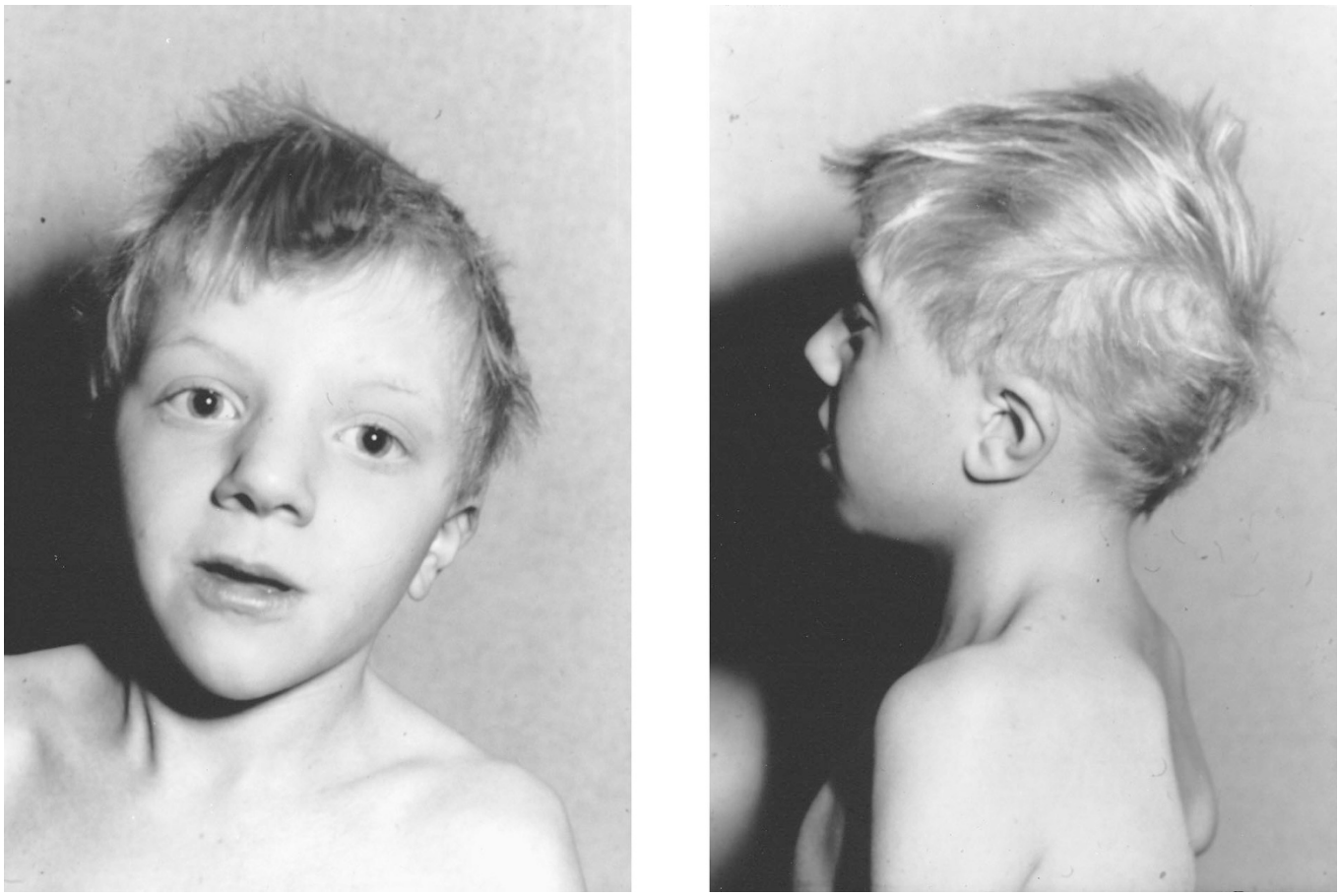

Figure 2 *Face and profile of individual IV1 diagnosed with CFC syndrome. Note the sparse scalp hair, scanty eyebrows and dry eczematous skin*

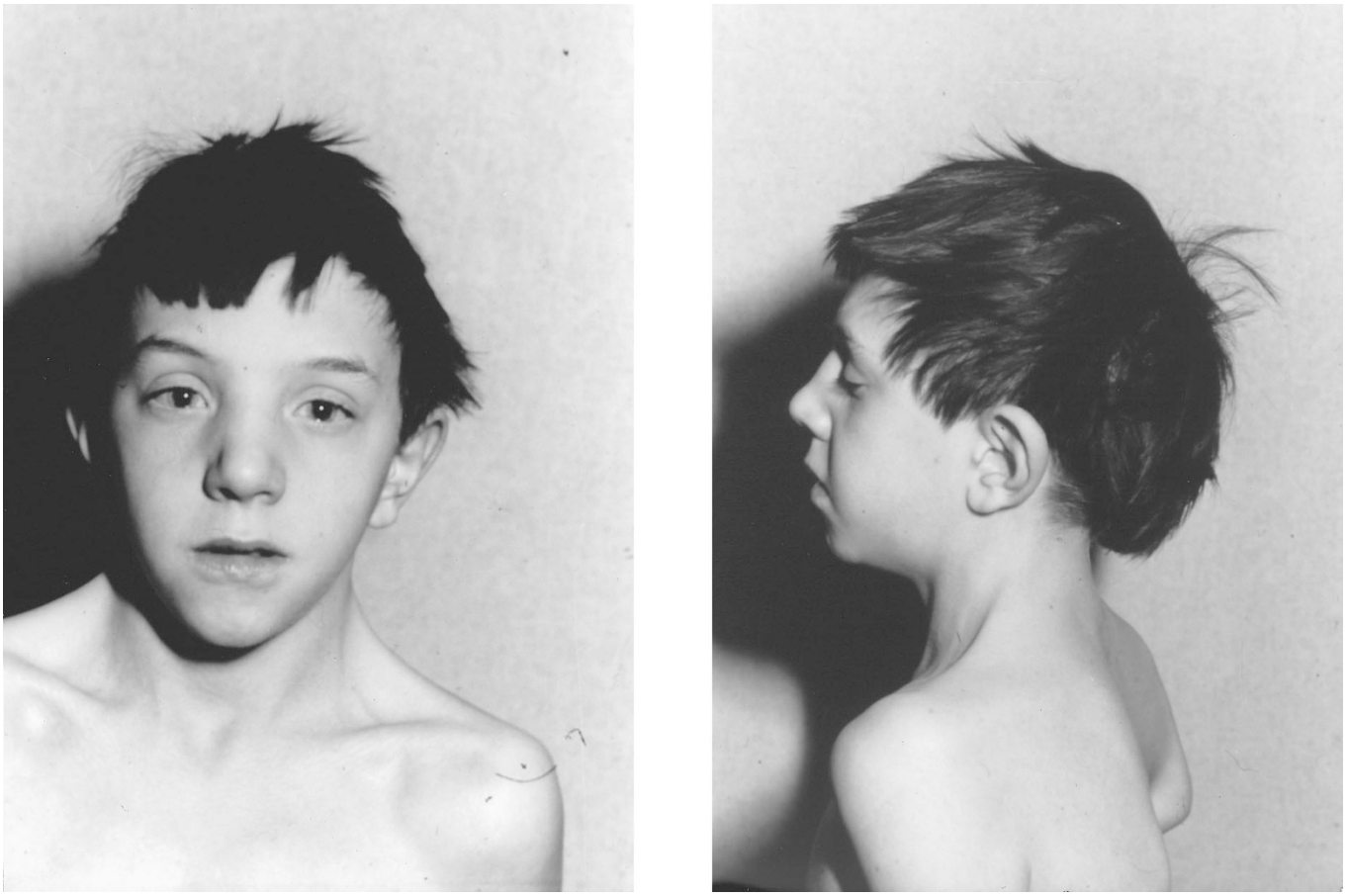

Figure 3 *Face and profile of individual IV2, diagnosed with CFC syndrome. Note the same facial characteristics as his brother (Figure 2). This individual also shows a marked ptosis of the eyelids and a webbed neck.*

Table 1 Two-point lod score table of NS/CFC syndrome versus 12q24 markers

	Lod score at θ								
Locus	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*	*0.40*	*Zmax*	θ*max*
D12S1613	–∞	1.263	1.765	1.808	1.546	1.087	0.510	1.816	0.085
D12S84	4.434	4.360	4.055	3.656	2.787	1.807	0.734	4.434	0.000
D12S84–CFC	3.832	3.767	3.498	3.146	2.379	1.515	0.576	3.832	0.000
D12S105	0.440	0.438	0.429	0.410	0.353	0.268	0.152	0.440	0.000
D12S1583	2.709	2.666	2.486	2.252	1.740	1.161	0.507	2.709	0.000
D12S1339	3.311	3.259	3.044	2.762	2.148	1.453	0.666	3.311	0.000
D12S809	3.311	3.259	3.044	2.762	2.148	1.453	0.666	3.311	0.000
D12S129	1.505	1.475	1.349	1.185	0.834	0.462	0.133	1.505	0.000
D12S1341	–∞	1.263	1.765	1.808	1.546	1.087	0.510	1.816	0.085
D12S354	0.602	0.593	0.558	0.511	0.408	0.292	0.158	0.602	0.000
D12S1023	1.204	1.187	1.115	1.021	0.816	0.585	0.317	1.204	0.000
D12S369	–∞	1.263	1.765	1.808	1.546	1.087	0.510	1.816	0.085
D12S79	–∞	2.747	3.159	3.084	2.567	1.821	0.923	3.165	0.060
D12S366	–∞	2.153	2.602	2.574	2.159	1.529	0.764	2.618	0.066

Two-point lod score results obtained in the reported family, at different recombination frequencies (θ) and with inclusion of the two individuals with CFC syndrome. Data in row D12S84-CFC refer to the results obtained with marker D12S84 without including the two individuals with CFC syndrome.

ordered from the centromere to the telomere according to data from the Third International Workshop on Human Chromosome 12 Mapping 1995.[11] Three markers (D12S84, D12S1339 and D12S809) showed a maximum lod score of more than 3 at no recombination and 1 (D12S79) at 6% recombination. The highest two-point lod score was obtained with marker D12S84 (4.434; $\theta = 0$) (Table 1).

A crossover in individual III2 between NS/CFC and markers D12S1341, D12S369, D12S79 and D12S366 was observed. Markers D12S354 and D12S1023 were not informative in individual III2. This crossover indicates that the *NS/CFC* locus is proximal to D12S1341 (Figure 1 and 4). Markers D12S809 and D12S129, proximal to the crossover, and marker D12S1341, distal to the crossover have been mapped to the same yeast artificial chromosome (YAC) (920g2).[12] The exact position of D12S129 in relation to D12S809 is not known.[11] No other markers were available for a more precise mapping of the crossover.

Another crossover was observed in an unaffected individual (III1) between the *NS/CFC* locus and markers D12S78, D12S330 and D12S1613. This indicates that the *NS/CFC* in this family is localized between marker D12S1613 proximally and marker D12S1341 distally (Figures 1 and 4). A multipoint linkage analysis using markers D12S330, D12S84 and D12S1341 showed a maximum lod score of 4.43 at D12S84. This family shows linkage to exactly the same region on the long arm of chromosome 12 as NS.[2]

A remarkable finding is the presence of three pairs of dizygotic twins in the offspring of two affected females. In the previously reported NS family (2) there is also a dizygotic twin in the offspring of an affected female. In these two families four dizygous twins are present on a total of 27 full term pregnancies in seven affected females. The frequency of dizygotic twinning in these families is markedly higher than expected[13] for this region of Europe (RR = 27.4; Fisher exact test: $p < 0.001$).

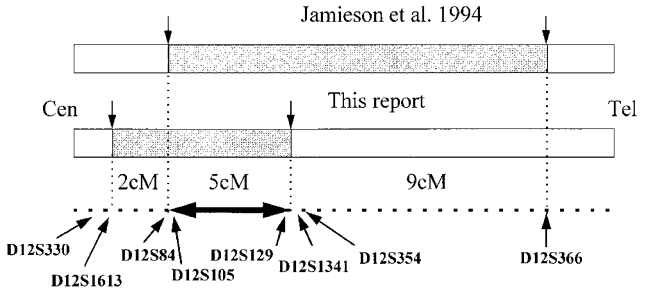

Figure 4 *Schematic of the genetic map showing the localization of several markers used in the linkage study. Crossovers in the present family and in the previous reported family with NS2 are indicated by vertical arrows (↓)*

Discussion

The family reported here is the largest family in the literature with NS/CFC. Linkage analysis unequivocally maps the NS/CFC phenotype in this family to the same region on chromosome 12q as NS.[2] Even if the two individuals with CFC syndrome are excluded from the analysis the maximum lod score remains higher than 3 for D12S84. This is the second family in the literature with proven linkage to 12q24. A crossover is described between NS/CFC and markers D12S1341, D12S369, D12S79 and D12S366, and with markers located proximally to D12S84 (D12S78, D12S330, D12S1613). The NS/CFC phenotype in this family is localized in a 7 cM region between markers D12S1613 proximally and D12S1341 distally (Figure 4). A three-generation NS family previously reported showed a recombination between *NS* and markers D12S84 proximally and D12S366 distally, mapping *NS* to a 14 cM region between these two markers[2] Assuming that NS and NS/CFC are caused by a defect in the same gene, we can narrow the candidate gene region for NS/CFC to a 5 cM interval between D12S84 and D12S1341 (Figure 4).

In the reported family the linkage data and the clinical features (*NS* in some, *CFC* syndrome in others) support the hypothesis that CFC syndrome is a variant of NS. Most CFC cases are sporadic, probably because these individuals are so severely affected that they do not reproduce. It is possible that in some families CFC syndrome is not related to a gene on 12q24, but this has not yet been documented. However, present and previous data indicate that in several cases CFC syndrome is a severe expression of NS.[5–7] We hypothesize that CFC syndrome is related to NS, either as an allelic variant or as a disorder with a similar molecular and cellular pathogenic mechanism.

Several reports suggested the possibility of CFC syndrome and NS being contiguous gene syndromes.[6,7] NS and CFC syndrome can result from different mutations or deletions in the same 5 cM chromosomal region in 12q24. In the reported family, however, the variable expression of the phenotype resulted in NS in some individuals and CFC syndrome in others. Moreover, a contiguous gene syndrome results from a deletion

of several genes but none of the 7 markers in the 5 cM candidate gene region showed any evidence for a deletion in this family. Nor did Kremer *et al.* observe any abnormalities in a systematic screen with 28 12q24 polymorphic markers in 10 unrelated CFC syndrome and 100 NS individuals.[14] A contiguous gene syndrome cannot be ruled out by these data, but it is less likely.

A remarkable finding in the reported family is the presence of three dizygotic twins in the offspring of two affected females (Figure 1). A dizygotic twin in the offspring of an affected female is also observed in the other large NS family with linkage to 12q24.[2] It is possible that an increased frequency of dizygotic twinning is associated with NS/CFC linked to 12q24. The fragile X syndrome is another example of a Mendelian disorder with an increased frequency of dizygotic twinning.[15,16] It is also possible that the association between NS/CFC and twinning is fortuitous because of the large number of offspring. However we believe the possible association merits further exploration.

Large families such as the one reported here are very helpful for fine mapping and cloning of the *NS/CFC* gene. In the presence of genetic heterogeneity, smaller families with *NS/CFC* are not very informative for linkage analysis, and their use in defining the candidate gene region is limited. However these smaller families might shed some light on the possible association with dizygotic twinning.

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