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MAPPING A GENE FOR NS - Nature Genetics

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Mapping a gene for Noonan Syndrome to the long arm of chromosome 12

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Noonan syndrome is characterized by typical facies, short stature and congenital cardiac defects. Approximately half of all cases are sporadic, but autosomal dominant inheritance with variable expression is well established. We have performed a genome-wide linkage analysis in a large Dutch kindred with autosomal dominant Noonan Syndrome, and localized the Noonan Syndrome gene to chromosome 12 (Z_{max}=4.04 at θ=0.0). Linkage analysis using chromosome 12 markers in 20 smaller, two-generation families gave Z_{max}=2.89 at θ=0.07, but haplotype analysis showed non-linkage in one family. These data imply that a gene for Noonan Syndrome is located on chromosome 12q, between D12S84 and D12S366.

Noonan Syndrome (NS) is a well known autosomal dominant condition, first described by Jacqueline Noonan in 1963 (ref. 1). NS patients have a typical face with hypertelorism, ptosis, down slanting palpebral fissures, low set posteriorly rotated ears and short neck. A congenital heart defect, which is most often a pulmonary valve stenosis or hypertrophic cardiomyopathy (HOCM), is often present. Other characteristic findings are short stature, a pectus deformity and cryptorchidism in males. The incidence of NS is estimated to be between 1:1,000 and 1:5,000 (ref. 2). Approximately half of all cases are familial. As yet, nothing is known about the etiology of this congenital malformation syndrome.

A condition with a strikingly similar phenotype is Turner Syndrome, one of the most common chromosomal disorders, associated with a 45,X karyotype. However, consistent chromosome aberrations including sex chromosome rearrangements have not been described in patients with NS. Reported chromosome rearrangements in four sporadic patients with a Noonan facial phenotype, but without typical NS, are t(5;7)(p15;q22) (ref. 3); del(13)(q21.32;q22.30) (ref. 4), t(3;22)(p21;q13) (ref. 5) and dup(11)(q13.3;q14.2) (ref. 6). Based on the occurrence of families in which NS co-segregates with neurofibromatosis type 1 (NF1), the locus for NF1 on chromosome 17 has been considered as a candidate for locus for NS. Wilson *et al.*[7] reported a patient with DiGeorge syndrome and NS, who

had a deletion in chromosome 22q11 suggesting the presence of a NS gene in that area. Both of these loci, however, have been excluded as the site for a NS gene by linkage analysis[8-10].

Here, we report the results of linkage analysis of a three-generation family with eight affected persons (Fig. 1)[10] and 20 smaller two generation families from Great Britain and the Netherlands. Our results localize a gene for NS to chromosome 12q22-qter.

Insert Fig. 1

Fig. 1 Pedigree and haplotypes of Family 1. The markers used for haplotype construction are shown. The relative order of *D12S84* and *D12S105*, which were mapped to the same locus[22], follows from a recombination observed in the affected individual III.2. the cross-hatched bars represent haplotypes, which co-segregate with the disorder.

Clinical assessment

112 persons from 21 families were physically examined by one of the authors, and ECGs and clinical photographs were obtained. NS was diagnosed based on the presence of the following major characteristics: typical face, pulmonary valve stenosis or HOCM plus abnormal ECG pattern, pectus carinatus superiorly and excavatum inferiorly, height below the 3rd centile and cryptorchidism in males. Persons with a typical face had to have at least one other major characteristic of the syndrome, whereas persons with suggestive facial findings had to have at least two other major characteristics in order to be diagnosed as a NS patient.

Linkage to chromosome 12q

To localize the gene for NS in Family 1 (the large Dutch kindred, Fig. 1), a search for genetic linkage was performed with highly polymorphic markers randomly distributed across the human genome. After exclusion of more than half of the genome with 325 CA-repeat markers, linkage was detected between NS and D12S105(Z_{max}=4.04at0=0.0). The same lod score was obtained with markers D12S354, D12S369 and D12S79, which are located slightly distal to D12S105 (Fig. 2; Table 1). Linkage was not observed for markers proximal to D12S105(D12S84, D12S101, D12S58, IGF1, D12S78) or distal to D12S79(D12S366, D12S349, D12S86). Haplotype analysis positioned the NS gene in this family within a 14 cM segment of 12q22-qter, defined by D12S84 and D12S366 (Fig. 1).

Insert Fig. 2

Fig. 2 A genetic map of the relevant region of chromosome 12q. Distances are given in centiMorgans. The relative order of D12S84 and D12S105 is based on recombination observed in Family 1 (see Fig. 1 legend). The order of D12S369 and D12S79, which were mapped to the same locus[22], is determined by a recombination event in one of the smaller families. The distance between these markers is estimated to be 0.2 cM.

Insert Table 1

To determine whether this region of chromosome 12 is also involved in other NS families, linkage analysis with the same set of markers was performed in 20 unrelated two-generation families. This resulted in a maximum lod score of Z=+2.89 at 0=0.07 for marker D12S369 (Table 2), arguing for the presence of a significant locus for NS on chromosome 12q22-qter. Varying the estimate for penetrance of the NS gene from 95% to 50% did not affect these results (for D12S369, Z_{max}=2.83 at 0=0.04 with 50% penetrance).

Insert Table 2

Genetic heterogeneity of NS

Based on the fact that the lod scores did not reach their maximum values at 0=0.0 for any of the genetic markers from chromosome 12, the possibility of genetic heterogeneity for NS in our sample of

20 smaller families was raised. Haplotype analysis demonstrated three crucial recombination events that involved only clinically affected individuals, and thus cannot be explained by non-penetrance of the NS gene (Fig. 3*a-c*). In two of these pedigrees, the recombination is consistent with a locus between D12S354 and D12S79 (Fig. 3*a* and *b*). In the third family, however, one out of three clinically affected siblings has inherited a different haplotype for the entire region between D12S84 and D12S366 (Fig. 3*c*). the findings in this single family suggest that NS is genetically heterogeneous, based on the clinical criteria used in this study.

Insert Fig. 3

Fig. 3 Haplotype analysis of three families with recombination events involving affected persons. In two families (*a* and *b*) partial sharing of haplotypes between affected sibs was found, whereas in the third family © affected sibs had different paternal haplotypes.

A heterogeneity analysis using HOMOG was carried out for the most informative marker, D12S369. For two uninformative families, which did not show recombination in the region studied, lod scores for D12S354 or D12S79 were used to increase the possibility of achieving significance.

Analyses were performed for all 21 families, and for all families without Family 1. For the first analysis, homogeneity versus heterogeneity gave an estimate of alpha (linked families) of 0.86, but the chi square was only 0.771 and p value was 0.19. The lod score for linkage versus heterogeneity was 8.18 compared to 8.01 for linkage versus homogeneity. With Family 1 removed from the analysis, $\log_n$ likelihoods for linkage versus heterogeneity and linkage versus homogeneity were identical, with the data giving no significance ($p=0.5$, $x^2=0.0$).

Discussion

Using linkage analysis of one large three-generation family (Fig. 1) and 20 smaller two-generation families, we have mapped a gene for Noonan Syndrome on the distal part of chromosome 12q (12q22-qter). Haplotype analysis of one of the smaller families shows the existence of genetic heterogeneity (Fig. 3*c*), although the degree of heterogeneity is still unclear.

The present localization is a prerequisite for the definition, and subsequent examination, of candidate NS genes. Identification of the NS gene should improve our understanding of valvular pulmonary stenosis and HOCM, both of which are characteristic NS features. Also, the resemblance of the facial features of Turner syndrome and NS is so striking that this may implicate a shared pathogenesis. The identification of a gene for NS could thus provide an important clue towards the identity of the putative Turner phenotype gene on the X chromosome[11].

So far, no obvious candidate gene for NS has been identified. It has recently been reported that elastin gene mutations cause supravalvular aortic stenosis in Williams syndrome[12]. This cardiac anomaly is clearly different from the valvular pulmonary stenosis of NS. Moreover, genes encoding components of the extracellular matrix have not been mapped to the relevant region of chromosome 12q.

Interestingly, a locus for Holt Oram syndrome (HOS) has recently been localized to chromosome 12q22-qter (refs 13-15). On clinical grounds NS and HOS are clearly different conditions, that are characterized by different congenital heart defects. Probably, the distal part of chromosome 12q contains at least two genes involved in cardiac development. Several genes have been localized which can cause HOCM[16-19], a condition which is frequently encountered in patients with NS. However, in our study all known loci for HOCM were excluded by linkage analysis. The present mapping of a gene for NS on chromosome 12q is an important step towards the heart malformations. Eventually,

the characterization of this gene will provide insight into the complex process of cardiac development and should explain the other phenotypic findings in NS.

Methodology

Genotyping. Venous blood was sampled from relevant persons and genomic DNA was isolated[20]. With regard to Family 1, DNA for the present study was available from eight affected and 12 non-affected members. A dense network of highly polymorphic microsatellite markers covering the complete genome with, on average, a genetic distance of less than 15 cM was selected using recent mapping data[21, 22]. Genotyping was performed either by a non-radioactive (London) or a radioactive (Nijmegen) Procedure. The non-radioactive analysis involved PCR amplification of 50 ng genomic DNA in 15 µl with 30 ng of each primer, 200 µm each dGTP, dCTP, dTTP, dATP, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 0.01% gelatin, 0.1% Triton X-100 and 0.1 U SuperTaq DNA polymerase (HT Biotechnology Ltd.). 30 cycles (1 min at 94 °C min at 55 °C and 2 min at 72 °C with a final 7 min elongation step) were carried out in an automated thermal cycler (Hybaid) and amplified products were separated by electrophoresis on 10% polyacrylamide gels. The gels were silver stained[23] and dried. Radioactive PCR was performed with a similar amount of genomic DNA and primers in the same volume under slightly different conditions (10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 m $MgCl_2$, 0.1% Triton X-100, 0.01% (w/v) gelatin) with 200 µM of each dATP, dGTP, dTTP and 2.5 µM dCTP, 0.6 µCi α^{32}P-dCTP (10 mCi ml^{-1}, 3000 Ci $mmol^{1}$). Amplification was carried out in a 96 wells thermal cycler (M.J. Research, Inc., Watertown) involving 30 cycles as mentioned above. Samples were analyzed on 6.6% denaturing polyacrylamide gels. After electrophoresis at 60 W for 3 h, gels were dried and exposed overnight to Kodak X-OMATS film to visualize the allelic bands.

Linkage analyses. Linkage analyses were performed using the ILINK and MLINK options of the LINKAGE program package, Ver. 5.10 (ref. 24) with an estimated disease gene frequency of 0.0002, 95% penetrance, mutation rate of 0.0001 for both males and females and a phenocopy frequency of 0.0001. Heterogeneity tests were carried out using the HOMOG program[25].

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