EDITOR—Noonan syndrome (NS) is an autosomal dominant developmental disorder in which the cardinal features include short stature, typical facies with hypertelorism, ptosis, downward slanting palpebral fissures, and low set, posteriorly rotated ears. In addition, there is a notable cardiac involvement seen in these patients, principally pulmonary valve stenosis and hypertrophic obstructive cardiomyopathy.\(^1,2\) The frequency of NS has been estimated to be between 1:1000–1:2500 live births.\(^3,4\)

Using linkage analysis in a large three generation pedigree, we have previously mapped a gene for NS to an interval of more than 6 cM on 12q24 flanked by the markers D12S1637 and NOS1.\(^5,6\) A similar analysis in smaller two generation families showed genetic heterogeneity for this disorder.\(^7\) Despite the relatively high incidence of NS, there appears to be a distinct lack of large families suitable for linkage analysis, possibly resulting from an increase of infertility in males.\(^8\) However, the location of the NS gene has recently been further refined to a 5 cM interval through the identification of additional recombinants in one additional large NS family.\(^9\) No chromosome rearrangements associated with the disease have so far been discovered. In view of this, one approach currently being used to identify the underlying gene responsible for this disorder is examination of candidate genes from within this large region of chromosome 12. We present below the examination of candidate genes from within this large interval of more than 6 cM on 12q24 flanked by the markers D12S1637 and NOS1.\(^10\) However, our FISH analysis localised the gene to chromosome 12p13, confirming the localisation of each gene was derived from sequences determined by one of the authors. The subchromosomal localisation of each gene was determined by hybridisation of fluorescently labelled PCR products to metaphase chromosome spreads.\(^9\) PCR products for DCN, EPS8, and MYL2 (exon 4 product) are shown in table 1. Thermocycling parameters were 96°C for five minutes, 35 cycles of 96°C for 30 seconds, 55°C (DCN) or 50°C (EPS8) for 30 seconds, and 72°C for 30 seconds, using 1.5 mmol/l MgCl\(_2\). The primers for DCN, EPS8, and MYL2 were derived from database sequences. Those for RPL6 were derived from sequences determined by one of the authors.

The positive clones obtained also hybridised with probe for FISH. Primers used were GACAATACACGACATCCACG (DCN-F), GGATCTCTACTTGCCCTAGGA (DCN-R), CTTCTGGTGTGTTTTTCTTTG (EPS8-F), and CTCGAGAGTGCACTTTGA (EPS8-R). The primers used for SSCP analysis of the MYL2 and RPL6 genes, and for the FISH of MYL2 (exon 4 product) are shown in table 1. Thermocycling conditions were optimised for each primer set and are available upon request. Amplified fragments were analysed for SSCP on a 30 × 40 cm gel containing 5% acrylamide, 0.25% bisacrylamide, with and without 10% glycerol in 9 TBE (100 mmol/l Tris, 100 mmol/l boric acid, 2 mmol/l Na\(_2\) EDTA, pH 8.3). Electrophoresis was performed at 30 W and 4°C.

EPS8 is highly conserved between species,\(^10\) is widely expressed during mouse development,\(^11\) and had previously been assigned to 12p13.\(^12\) However, our FISH analysis localised the gene to chromosome 12p13.2 (fig 1). To confirm this localisation, the EPS8 cDNA was used to screen a chromosome 12 specific cosmid library (Lawrence Livermore National Laboratory, kindly provided by Dr Sue Chamberlain). The positive clones obtained also hybridised to chromosome 12p13, confirming the localisation and exclusion of this gene (fig 1).

Through its ability to bind extracellular matrix constituents and growth factors, DCN is thought to play an impor-
tiant role in the remodelling and maintenance of extracellular matrices. Two previous studies, both using radiolabelled in situ hybridisation, suggested different localisations for the DCN gene on chromosome 12 at bands 12q21-q22 and 12q23. In view of its proposed function, DCN would be an excellent candidate for NS if it mapped within the interval. FISH clearly showed that the DCN gene maps at 12q13.2q proximal to both of the previous locations, and once again can be excluded as a candidate for NS.

While the genes described above were shown to be located outside the NS locus, this was not the case for the MYL2 gene. MYL2 has previously been assigned to chromosome 12q23-q24.3 by in situ hybridisation. Although the precise function of the protein is not understood, MYL2 is known to be critical for the correct regulation of myosin ATPase activity in smooth muscle. The non-muscle myosin II-B is known to be required for normal development of the mouse heart and an increase in ventricular MYL2 has been observed during myocardial hypertrophy in patients with valvular stenosis. In addition, missense mutations within the MYL2 gene have been identified in patients with a rare variant of cardiac hypertrophy, an intriguing observation in view of the cardiac anomalies associated with NS. As a result of its position and putative function, MYL2 was regarded as a strong candidate gene for NS.

Using a labelled MYL2 gene fragment in conjunction with genomic clones that flank the NS critical interval, we were able to show that the MYL2 gene overlaps the assign-
Hall-Riggs syndrome: a possible second affected family?

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She has had feeding problems, failure to thrive, and severe developmental retardation. She walked unassisted at 6 years and she never achieved any language. Metabolic screening, and lysosomal and peroxisomal enzymes has been negative.

Sialotransferrin, cholesterol, and 7-dehydrocholesterol were within normal limits. The EEG showed moderate multifocal irritative anomalies, without evidence of clinical seizures. MRI of the brain showed the presence of a large cyst in the septum pellucidum and a cavum vergae. The high resolution karyotype was normal, 46,XX.

Physical examination at 11½ years showed height 120 cm, weight 23 kg, and head circumference 47 cm (all <3rd centile). She has severe microcephaly, hypothalamic, a flat nasal bridge, a large nose with a large nasal tip, severe micrognathia, a small jaw, and everted, giving a coarse appearance to the lower part of the face (fig 1). The permanent teeth have not yet erupted and its mapping to chromosome 12q23-24. Oncogene 1995;4:3057-61.


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ymi Members of the Noonan Syndrome Collaborative Group.


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Physical examination at 11½ years showed height 120 cm, weight 23 kg, and head circumference 47 cm (all <3rd centile). She has severe microcephaly, hypoplasia, a flat nasal bridge, a large nose with a large nasal tip, and interverted nostrils. The mouth is wide and hooded. Both the upper and lower lips are thick and everted, giving a coarse appearance to the lower part of the face (fig 1). The permanent teeth have not yet erupted and the gums are large.