J Med Genet 2000;**37**:883–884

Attitudes towards termination of pregnancy in subjects who underwent presymptomatic testing for the *BRCA1*/*BRCA2* gene mutation in The Netherlands

EDITOR—The identification of the *BRCA1* and *BRCA2* gene mutations in 1994 and 1995 respectively[1 2] allowed detection of mutation carriers in families with autosomal dominant hereditary breast/ovarian cancer. Female mutation carriers have a risk of 56-87% of developing breast cancer and of 10-60% for ovarian cancer.[3] The options are either frequent surveillance or prophylactic surgery. For male mutation carriers, cancer risks are only slightly increased. The offspring of mutation carriers have a 50% chance of inheriting the gene mutation. The possibility of prenatal genetic diagnosis for "late onset diseases", such as hereditary breast/ovarian cancer, raises complex ethical questions.[4 5] The present study addresses the question to what extent physicians and policy makers working in genetics or oncology may expect requests for prenatal diagnosis and termination of pregnancy because of carriership for *BRCA1*/*BRCA2*.

A questionnaire assessing attitudes towards termination of pregnancy if the fetus was found to be a *BRCA1*/*BRCA2* female or a male mutation carrier was answered by 78 subjects (67 women and 11 men) who underwent presymptomatic DNA testing for hereditary breast/ovarian cancer, six months after receiving their test results. Subjects were asked to indicate to what extent they found termination of pregnancy acceptable for themselves. Subjects with and without a desire to have children were included in the study. There were 26 carriers of the *BRCA1*/*BRCA2* mutation (23 females/three males, mean age 36.5) and 52 non-mutation carriers (44 females/eight males, mean age 38.8). The latter group served as a reference group; they cannot transmit the mutation to their offspring, but are well informed about the implications of hereditary breast/ovarian cancer.

None of the 26 mutation carriers found termination of pregnancy in the case of a female or a male mutation carrier fetus as acceptable for themselves. A minority of the non-mutation carriers viewed termination of pregnancy as acceptable in the case of a female (14%) or a male mutation carrier fetus (10%, table 1). The differences between mutation and non-mutation carriers are significant ($p<0.05$, Pearson chi-square test, SPSS/PC, release 8.0). Five of the seven non-mutation carriers accepting termination of pregnancy thought this to be acceptable independent of the sex of the mutation carrier child. This is surprising, since the lifetime risk of developing cancer for males with a *BRCA1*/*BRCA2* mutation is not so high. However, the majority of the non-mutation carriers and all the mutation carriers in the present study rejected termination of pregnancy in the case of a child who (1) has a high risk of developing breast or ovarian cancer later in life (a girl) and/or (2) can transmit the gene to his/her offspring (boy or girl).

Table 1 Attitudes of BRCA1/BRCA2 mutation carriers and non-mutation carriers towards termination of pregnancy because of a fetus carrying a mutation

If there was a pregnancy in my family, I would find termination of pregnancy acceptable if the child was:	*Mutation carriers (n=26)*	*Non-mutation carriers (n=52)*
A female *BRCA1*/*BRCA2* mutation carrier	0%	13.5%
A male *BRCA1*/*BRCA2* mutation carrier	0%	9.6%

The stronger reluctance in mutation carriers than in non-mutation carriers towards terminating a pregnancy of a mutation carrier boy or girl may have several reasons. Firstly, mutation carriers may be more acutely aware of the burdensome emotional implications of terminating a pregnancy because of *BRCA1*/*BRCA2* carriership than non-mutation carriers. Secondly, they may perceive terminating the pregnancy of a mutation carrier child as incompatible with their own existence.

In subjects at risk for autosomal dominant Huntington's disease, the actual demand for prenatal diagnosis and termination of pregnancy is much lower than would be expected based on studies assessing attitudes towards these techniques.[6 7] Prenatal diagnosis and termination of pregnancy for late onset diseases, with decades of healthy life before onset of the disorder, are considered very difficult choices for parents. In our experience of 500 families at risk for hereditary breast/ovarian cancer seen during the past five years, two requests for prenatal diagnosis were made by recently identified mutation carriers, who wanted to have children in the near future. Considering the few actual requests for prenatal diagnosis for *BRCA1*/*BRCA2*, the emotional burden of such a decision, and the general reluctance to terminate a pregnancy of a mutation carrier child (this study), the demand for prenatal diagnosis in hereditary breast/ovarian cancer families is expected to remain low. Genetic counselling of couples considering these highly complex and burdensome options should focus on supporting parents in the decision making process. There are no general rules of wisdom or ethical desirability that could take priority over finding individual solutions and the need to support each couple.

This study is part of a larger study on psychosocial implications of the presymptomatic DNA test for HBOC, which is funded by the Dutch Cancer Society.

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J Med Genet 2000;**37**:884–886

Detailed mapping, mutation analysis, and intragenic polymorphism identification in candidate Noonan syndrome genes *MYL2*, *DCN*, *EPS8*, and *RPL6*

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.[2 3]

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*.[4 5] A similar analysis in smaller two generation families showed genetic heterogeneity for this disorder.[4] 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.[6] 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.[7] 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 four candidate genes, the precise localisation of three of which, epidermal growth factor receptor pathway substrate-8 (*EPS8*), decorin (*DCN*), and myosin light chain 2 (*MYL2*), had not previously been accurately determined. The fourth, ribosomal protein L6 (*RPL6*) was known to lie within the NS interval on 12q24.[8]

PCR was used to produce gene specific products for FISH (see below) and to produce exonic fragments for SSCP (see below). Sequence information from the cDNA clones of epidermal growth factor receptor pathway substrate-8 (*EPS8*) and decorin (*DCN*) were used to design primers for FISH. Primers used were GACAACTAACAGCATCCAGC (*DCN*-F), GGATTCCTACTTGCCTTGGA (*DCN*-R), CTTCCTTATTCTTGGTGT (*EPS8*-F), and CTCGAACTTGGGTCATTG (*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 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 produced from database sequences. Those for *RPL6* were 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) were labelled using the PCR Digoxigenin Probe Synthesis nick translation kit (Boehringer Mannheim). Conditions for hybridisation and immunofluorescent detection were performed according to the manufacturer's instructions.

Primers for SSCP analysis of genomic DNA were designed from intronic sequences such that the entire exon and flanking splice sites could be analysed (table 1). PCR 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 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 12q24.[10] 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-

Table 1 Oligonucleotide sequences flanking each of the exons of the MYL2 and RPL6 genes

	Forward 5'-3'	*Reverse 5'-3'*
MYL2		
Exon 1	CTCACCTATGACTGCCAAAAG	CCCTCGCTTGTAGTGGCTTC
Exon 2	CCCAGAGTAGGGGCCTGACCTAG	CCATCCAGGCGGATGATTCAATAG
Exon 3	CCAGGCTGAGCTGCCAATCAC	CATGCAGGGCTAGAGAGGGGT
Exon 4	CCCTGAGTGTGTGTTTCCTACCC	TTCTGCCAGCCCCCCCGAAGAA
Exon 5	CCCAGCCACCCCCAGTACATGT	CCCGAACGCTGCAGAGAAAGGA
Exon 6	GACACCAACACCTGCTTTCCTTTC	GGAGAACCAGGAGCTGGGTTAGAGG
Exon 7	CTTAGCACGTGTTGCTGGCTCA	CACTCTGCAAAGACGAGCCCA
RPL6		
Exon 1	CCGGCCTAGGATTTACTA	CTCAGTTAGCCTTGGACATG
Exon 2	TTGTTAGAGAGATGACTGGTG	CAATTAAGGTTAAGACATAATGG
Exon 3	CTTAATTGGCATTCTCTTACTG	TTCAAGCATAAACAGGAAATCC
Exon 4	GCTTCTAGTAATCTGAATGGC	GCAGCTGCAGTGAAGCGC
Exon 5	GATGCCTGTGATTTTATGAATTC	AAGTTTCACAGAACATCAC
Exon 6	CACCTAAATTGCAGGATGATG	CAGTGCTAACACAGGAGATG
Exon 7	AAGTAATTTGGTATGTGCCTG	AGTCAGCTATTTAATTAGGTTC