Sensitivity and predictive value of criteria for p53 germline mutation screening

Editor—The history of Li-Fraumeni syndrome (LFS) is a good illustration of the delineation of dominantly inherited family cancer syndromes. The identification of this syndrome is the result of the combination of two kinds of evidence, firstly, a number of reports on particular familial aggregations1 2 and, secondly, systematic family studies of childhood sarcomas.3-6 Among these studies, the decisive contribution came from Li and Fraumeni7 who were the first to publish the results of a family study on 641 children with rhabdomyosarcoma which led to the identification of four families in which a sib or a cousin was affected by rhabdomyosarcoma or another soft tissue sarcoma (STS). These families also had several members who were affected by diverse types of malignant tumours, in particular sarcomas and breast cancer at a very young age. This prompted the authors to propose the existence of a new familial syndrome.8 A prospective study on these families over 12 years provided evidence of a strong predisposition to cancer with a strikingly high frequency of multiple tumours.8 The term “Li-Fraumeni syndrome” was used for the first time in 19822 and the criteria, which subsequently became the classical definition of the syndrome, were proposed by Li and Fraumeni in 1988.9 These were a proband with a sarcoma before 45 years of age, a first degree relative with cancer before this age, and another close (first or second degree) relative in the lineage with either cancer before this age or a sarcoma at any age. These criteria led to the selection of 24 families which exhibited a wide variety of tumours including bone sarcomas, STS, breast cancer, brain tumours, leukaemia, adrenocortical carcinoma, lymphoma, lung, stomach, pancreas, and prostate cancer, but only the first six types were significantly in excess of the expected proportion among subjects affected by cancer before 45 years in the American population. The follow up of these families confirmed an unusually high predisposition to cancer.10 Other studies have indicated that a number of other cancers may occur in these families, the most notable being melanoma, germ cell tumours, gastric carcinoma, and Wilms tumour.11-16

The definition of the syndrome shifted from clinical and familial criteria to molecular criteria after Malkin et al19 described the involvement of germ line p53 mutations. The mutations initially found were all missense mutations of exon 7, but further studies, extensively reported by Varley et al20 showed that other regions might also be involved. Studies on families with the classical LFS criteria showed that 50 to 70% of these families displayed a p53 mutation,19-23 indicating that mutation screening may have overlooked alterations that affect regulatory regions and not p53 coding sequences or that germ line mutation of other gene(s) may be responsible for LFS. Indeed, the study recently published by Bell et al24 showed that heterozygous germline mutations in hCHK2 occur in LFS. The proportions of p53 mutations are somewhat lower when less stringent criteria are applied.25 21

After ascribing LFS to germline p53 mutations, different studies were conducted on series of patients with tumours typifying LFS, but not selected on family history, to determine the proportion of gene carriers among them. The studies on patients with bone sarcoma or STS25-29 showed that up to a third of the group with early onset, an unusual family history, or multiple primary tumours may be carriers. Children with adrenocortical carcinoma were found to have the highest rate (50-80%).30 31 The frequency of mutations among patients with multiple primary tumours was estimated to be between 7 and 20%.32-34 Far lower rates were found for patients with brain tumours,35-37 or early onset/familial breast cancer,40-41 although the breast cancer risk was clearly high in p53 mutation carriers. In some of these studies, a selection bias on family history may be suspected. Indeed, a significant proportion of mutations were found among cases with a strong positive family history, the frequency of which appeared to be unusually high.

None of these studies allowed an estimation of cancer risk in mutation carriers, although unaffected carrier relatives are found in family studies. Indeed, LFS selection criteria are so stringent that it is impossible to correct for selection bias. Even looser criteria, such as Li-Fraumeni-like (LFL) or Li-Fraumeni incomplete (LFI) do not allow correction for ascertainment bias. This was the reason that we undertook a study at the Institut Gustave Roussy with very loose criteria which offered two advantages: (1) they did not imply the existence of highly penetrant susceptibility genes and therefore potentially allowed the detection of mutations associated with a low cancer risk; (2) correction for selection bias was possible for the estimation of cancer risks in individual subjects. Our main conclusions are: (1) that cancer risks are very high, (2) although unaffected carriers may be observed, there is no evidence for the existence of mutations with particularly low penetrance, and (3) the proportion of de novo mutations is probably substantial.48

While the above mentioned were gradually defining with ever greater accuracy the relationship between constitutional mutations and cancer types and risks, an international multidisciplinary group was trying to establish recommendations for predictive testing.44 46-50 For such testing, it was essential, as a first step, to evaluate individual and familial criteria to undertake the initial search in a family, in terms of sensitivity and predictive value. We report here the results obtained from our study on childhood cancer at the Institut Gustave Roussy and on a study of breast cancer in very young women performed at the Institut Curie in France.

The family history of cancer in children under 18 years treated for all types of solid malignant tumours in the Department of Paediatric Oncology at the Institut Gustave Roussy in Villejuif (France) was investigated between January 1991 and May 1997. Information was collected through a direct interview with a trained counsellor for families of patients treated in the department during the study period. Information was obtained via a mailed questionnaire and completed in most cases by a telephone interview for patients treated before that period and no longer followed up or who had died. To minimise possible biases owing to genetic and environmental heterogeneity, only white children were included in the study.

Family data were collected through the proband’s parents. They included information on each of the proband’s first and second degree relatives and first cousins. When necessary, additional family members,
previously informed by the proband's parents, were contacted for a telephone interview. Information on relatives included general characteristics (sex, date of birth, malformations, diet and cause of death) and the occurrence of any cancer. When cancers had occurred, confirmation of the diagnosis and age at onset were sought in medical and pathology records. Only invasive cancers were considered, excluding non-melanoma skin cancer and in situ carcinoma.

A subgroup of children in whom the frequency of cancer susceptibility genes would be potentially increased was selected on the basis of the occurrence of either of the following criteria: (1) at least one cancer case affecting a first or second degree relative before the age of 46 (familial cases) or (2) multiple primary cancers in the proband regardless of his/her family history (multiple tumour cases). In the original protocol, the family was also included if cancer had occurred only in first cousins. This criterion had to be removed since it dramatically increased the proportion of chance aggregation in the selected sample.

*p53* was genotyped in peripheral lymphocytes isolated from fresh blood samples. Direct sequencing was used for the first set of 100 samples. Genomic DNA was amplified as three fragments including respectively exons 2-4, exons 5-8, and exons 9-11 which were fully sequenced. Genotyping was subsequently carried out with a functional assay in yeast (FASAY), as described by Ishioka *et al* and modified by Flaman *et al* when this test became available. Vent DNA polymerase (New England Biolabs) was used to amplify *p53* reverse transcripts before transfection in yeast. Yeast colonies carrying a *p53* mutant allele were identified either as His-auxotrophs or as red colonies. *p53* cDNA was extracted from mutant colonies and sequenced. The FASAY has been reported to show over 90% of *p53* mutant alleles as does direct sequencing of amplified *p53* exon scores in our hands.

Women suffering from invasive breast cancer before 36 years, which was diagnosed between January 1990 and August 1995 and followed up at the Institut Curie, were interviewed about their family history and were requested to give a blood sample for the study of genes involved in breast cancer predisposition. Among the 275 women fulfilling these criteria, 119 were interviewed between January 1993 and August 1995 and 116 gave their informed consent for DNA analysis.

The pedigrees were constructed by taking into account first to third degree relatives of the proband on both parental sides. Information concerning the family history of tumours and age at onset of the tumours was verified when possible in medical and pathology records.

Screening for the presence of mutations was performed by analysis of PCR products from genomic DNA with denaturing gradient gel electrophoresis (DGGE). Exons 4, 5, 6, 7, 8, 9, 10, and 11 and respective flanking regions were studied (unpublished data). PCR products exhibiting a variant electrophoretic pattern were directly sequenced on both strands. In order to confirm the loss of biological function of missense mutations detected, a functional assay in yeast was performed according to Flaman *et al*. Any mutation identified was confirmed on a second independent blood sample.

The objectives of defining criteria for recommending *p53* mutation screening are triple: (1) to look for a mutation in situations in which it is likely to be found; (2) to miss as few mutations as possible; (3) not to select subjects who are not carriers. The first objective needs a high positive predictive value, which is the probability that a mutation will be found given the mutation is found. The second objective needs a high sensitivity, which is the probability that the criteria will be fulfilled, given that the mutation is found. The third objective needs a high specificity, which is the probability that a mutation will not be found given that the criteria are not fulfilled. The positive predictive value can be estimated by the proportion of subjects carrying a germline *p53* mutation among those tested using given criteria. The estimation of sensitivity and specificity requires reference criteria that would allow the ascertainment of carriers and non-carriers from an unselected population. These parameters therefore cannot really be estimated. However, it is possible to estimate the relative sensitivity by the ratio between the number of mutations detected when given criteria are applied and the number of mutations detected in the whole sample. Besides, since a negative result is of no value at this point, specificity is not particularly interesting. At this point, the importance of wording should be emphasised. The sentence “a mutation will be found” is used instead of “a mutation exists”, because this would also depend on the sensitivity of the method used to detect mutations, which is not the subject of the present study. The positive predictive value and the relative sensitivity are estimated in relation to the whole sample when more and more stringent criteria are applied on: (1) the number and age of affected relatives, (2) the tumour spectrum (proband and relatives), and (3) the existence of multiple primary tumours.

Of the 2691 children eligible for the family study on 1 January 1998, 239 fulfilled the selection criteria and consented to give a blood sample. Among these 239 children, 211 had at least one first- or second-degree relative affected by cancer before 46 years of age, 16 had at least two primary malignant tumours, and 12 fulfilled both familial and multiple tumour criteria. Among these cases, 15 mutations were detected, nine in the first group (4.3%), one in the second (a de novo mutation in a child with rhabdomyosarcoma and adrenocortical carcinoma (6.2%), and five in the third group (4.2%). The complete descriptions of families with mutations are published elsewhere.

Among the 223 children (211 + 12) fulfilling the familial criteria, four levels of nested criteria were defined according to the number and tumour type in the affected relative(s) and are listed in Table 1: very loose criteria (223 children), at least one first or second-degree relative affected by any cancer; loose criteria (141 children), the tumour type in the affected relative(s) is restricted to sarcoma, brain tumours, breast cancer, adrenocortical carcinoma, haematological malignancies, stomach cancer, melanoma, and germ cell tumours, which are the most prevalent tumours described in LFS; stringent criteria (81 children), the tumour spectrum in relative(s) is restricted to unquestioned tumours, that is, sarcomas, brain tumours, breast cancer, and adrenocortical carcinoma (narrow spectrum); very stringent criteria (21 children), a new criterion is added to the previous ones, at least another first or second relative affected by cancer before 46 years or a sarcoma at any age.

<table>
<thead>
<tr>
<th>Criteria on relatives</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very loose</td>
<td>At least one first or second degree relative affected by any cancer</td>
</tr>
<tr>
<td>Loose</td>
<td>Tumour type in the affected relative(s) restricted to sarcoma, brain tumours, breast cancer, adrenocortical carcinoma, haematological malignancies, stomach cancer, melanoma, and germ cell tumours</td>
</tr>
<tr>
<td>Stringent</td>
<td>Tumour spectrum in relative(s) restricted to unquestioned tumours, ie sarcomas, brain tumours, breast cancer, and adrenocortical carcinoma (narrow spectrum)</td>
</tr>
<tr>
<td>Very stringent</td>
<td>New criterion added to the previous ones: at least another first or second degree relative affected by cancer before 46 years or a sarcoma at any age</td>
</tr>
</tbody>
</table>

Table 1. Definition of the four levels of nested criteria according to the number and tumour type in the affected relative(s) in the study on childhood cancer
ond degree relative affected by cancer before 46 years or a sarcoma at any age. Criteria were also defined by stratification on the tumour type in the proband with two levels, a narrow spectrum tumour (102 children) or any tumour. The results of the combination of criteria for relatives and probands among the 223 familial cases are given in table 2. They show that the positive predictive values for the criterion “any tumour” in the proband are quite low (less than 15%) except in the category “very stringent” criteria in relatives. It is significantly higher when the tumour type in the proband is restricted to the narrow spectrum and attains 23% when stringent criteria are fulfilled among relatives.

The parameters estimated among the 28 (16 + 12) multiple tumour cases (excluding bilateral tumours of paired organs) are shown in table 3. The positive predictive values are much higher than in the group selected on a familial basis. However, this criterion overlaps markedly with the previous ones; in the six carriers of a germ line mutation, five fulfil the “narrow spectrum” for the first tumour of the proband, of which four also fulfil the “stringent” criteria on relatives. Thus, adding the criterion “multiple tumours” in the proband to the combination of narrow spectrum in the proband’s tumour and stringent criteria for relatives yields 24 new cases, two of which have a germ line mutation. This results in an increase in relative sensitivity from 73% (11/15) to 87% (13/15) and a decrease in the predictive value from 23% (11/47) to 18% (13/71). If both tumours (or at least two tumours) are restricted to the narrow spectrum, then only six families are added, one of which carries a mutation, resulting in a relative sensitivity of 80% (12/15) and a predictive value of 23% (12/53). There are very few multiple tumours among relatives (excluding bilateral tumours of paired organs): six families among which three fulfil the stringent criteria in relatives and four fulfil the narrow spectrum in the proband. There are two p53 mutations, two of which belong to the group defined by narrow spectrum and multiple tumours in the proband and stringent criteria in relatives. Consequently, the predictive value and the relative sensitivity are not modified when multiple tumours are added to the narrow LFS spectrum in relatives (data not shown).

Among the 116 breast cancer cases fully analysed, a total of three germline p53 mutations (2.5%) were detected. Two of them are missense mutations (Leu130Phe, Arg175Gly) and one is an in frame deletion (GluAla346del3Asp). The deleterious effects of both missense mutations have been confirmed with FASAY. One mutation (Leu130Phe) was found in a woman who was affected at 31 years and had no family history of cancer and in particular five unaffected sisters aged from 34 to 49 years. The second mutation (GluAla346del3Asp) concerned a case of bilateral breast carcinoma at 29 and 30 years whose family history was clearly indicative of Li-Fraumeni syndrome, including chondrosarcoma at 16 years, leukaemia at 26 years, breast cancer at 20 years, and renal tumour at 36 years (unconfirmed) in the sibship, and the father affected with a soft tissue tumour of an unknown histological nature at 64 years. The third mutation (Arg175Gly) was detected in a woman suffering from osteosarcoma at 18 years and bilateral breast cancer at 27 and 29 years. Her father had developed a rectal carcinoma at 39 years, meningioma at 54 years, and pancreatic carcinoma at 55 years, and her paternal uncle had developed a germ cell tumour at 45 years.

Because of the small number of mutations found, we had to consider a smaller number of criteria than in the previous section, and only two levels of nested criteria were defined: loose criteria, at least one first or second degree relative affected by any cancer before 46 years of age or a proband with multiple primary malignant tumours; stringent criteria, the tumour spectrum in relative(s) (or in the proband in case of multiple tumours) is restricted to the narrow spectrum. However, since breast cancer is common in the general population, familial aggregation of breast cancers may be either because of chance or germ line BRCA1/2 mutations. Therefore, two situations were considered, the narrow spectrum tumour is breast cancer (situation A) or another tumour (situation B).

Thirty three cases fulfilled the loose criteria (two mutations), 21 cases the stringent criteria A (no mutation), and two the stringent criteria B (two mutations). The positive predictive values are presented in table 4, but not the relative sensitivities which would be meaningless with only three mutations.

Most of the studies on germline p53 mutations conducted to date and quoted in the introduction did not permit evaluation of different selection criteria. Some of them concerned families ascertained on the basis of strong familial aggregation (corresponding roughly to our very stringent criteria) and the relevance of looser criteria could not be assessed. Other studies concerned series of tumours with very limited information on family history, so that it was impossible to evaluate criteria. The most well documented studies are by the group of Jillian Birch, based on the Manchester Children’s Tumour Registry. All the authors showed the relatively high predictive value (4/18=22%) of the so called Li-Fraumeni-like criteria, that is, a proband with any childhood cancer or sarcoma, brain tumour, or adrenal cortical tumour diagnosed under the age of 45 years with one first or second degree relative with a typical LFS cancer at any age, plus a first or second degree relative in the same lineage with any cancer under the age of 60 years. There is some degree of overlap

| Table 2 | Positive predictive value and relative sensitivity of criteria on probands and relatives for predictive p53 screening in childhood cancer |
| --- | --- | --- |
| Criteria on proband | Predictive value | Relative sensitivity |
| **Very loose** | **Loose** | **Stringent** | **Very stringent** |
| Any tumour | 6% (14/233) | 93% (14/15) | 9% (13/141) | 3% (9/293) |
| Narrow spectrum | 12% (12/102) | 80% (12/15) | 16% (11/67) | 73% (13/18) |
| | 15% (12/81) | 80% (12/15) | 73% (13/18) | 60% (9/15) |
| | 41% (9/23) | 60% (9/15) | 53% (8/15) | 53% (8/15) |

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Positive predictive value and relative sensitivity in families ascertained on the existence of multiple tumours in the proband child</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criteria on relatives</td>
<td>Predictive value</td>
</tr>
<tr>
<td>None</td>
<td>21% (6/28)</td>
</tr>
<tr>
<td>Very loose</td>
<td>42% (5/12)</td>
</tr>
<tr>
<td>Loose</td>
<td>63% (5/8)</td>
</tr>
<tr>
<td>Stringent</td>
<td>71% (5/7)</td>
</tr>
<tr>
<td>Very stringent</td>
<td>100% (3/3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Positive predictive value in families of women with breast cancer before 36 years according to criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criteria</td>
<td>Predictive value</td>
</tr>
<tr>
<td>None</td>
<td>3% (3/116)</td>
</tr>
<tr>
<td>Loose</td>
<td>7% (2/29)</td>
</tr>
<tr>
<td>Stringent A</td>
<td>0% (0/21)</td>
</tr>
<tr>
<td>B</td>
<td>100% (2/2)</td>
</tr>
<tr>
<td></td>
<td>9% (2/23)</td>
</tr>
</tbody>
</table>
between these criteria and ours; 18 families conform to this
definition in our sample, four of which exhibit a p53 mutation,
which is exactly the same number and proportions as
those found by these authors. Note that all positive families
fulfil our stringent criteria in relatives and narrow spectrum
tumour in the proband.

In the present study, we have quantified various criteria
which can be a very useful basis for establishing
recommendations for conducting p53 screening.

The functional assay used in the study on childhood
cancer detects about 90% of mutations in p53.\(^1\,\(^2\)\) Mutations
in exons 2 and 3 are very rare and were not studied in the
study on breast cancer. A small number of mutations may
thus have been missed and the positive predictive value of
criteria may thus be slightly higher, which should not
modify our conclusions.

At present, searching for p53 germline mutations is still
a cumbersome task and laboratories which perform such
screening in France are in favour of criteria yielding a posi-
tive predictive value of at least 20%. In our study on child-
hood cancer, this value is achieved when the following cri-
tera are used: a proband with a narrow spectrum tumour
and at least one first or second degree relative affected
before 46 years by a narrow spectrum cancer or multiple
tumours or a proband affected by multiple primary tumours whatever the family history. It should be
noted that when very stringent criteria are used, that is, that
are very close to those used in previous studies to define
LFS, very similar results are obtained with a positive
predictive value of 53%.

For cancer occurring in adulthood, our data concern
only very early onset breast cancer, which is the most fre-
quent tumour in p53 carriers (80% of tumours occurring
among female carriers after 16 years of age\(^3\)). As it is also
common in the general population, we have presented the
results separately when breast cancer is the narrow spectrum tumour in the relative who was the determinant
factor in the inclusion of the family (criterion A) and when
it is another cancer (criterion B). Although this distinction
results in loss of precision of estimations owing to a
decrease in sample size, it should be noted that no
mutation among 21 cases was detected when criterion A
was found, whereas both cases with criterion B were p53
carriers (p=0.02, exact test). Consequently, as in childhood
cancer, it seems reasonable to use stringent criteria and to
add this restriction on tumour type, that is, exclude breast
cancer from the tumour spectrum in relatives.

Although the results obtained by ascertaining early onset
breast cancer probands cannot necessarily be extrapolated
to all tumours of the narrow spectrum, we expect that the
predictive value for the other tumours will be higher than
that obtained for breast cancer, given the high frequency of
this tumour in the population.

In addition, although we have very few data on adreno-
cortical carcinoma not selected on family history, it seems
logical to add this criterion considering the results of pub-
lished studies quoted in the introduction.

Finally, it should be noted that although leukaemia is
usually reported as belonging to the LFS, this was not
included among narrow spectrum tumours in the present
study for two reasons. (1) It is absent in our childhood
probands because the Department of Paediatries at the
Institut Gustave Roussy treats only solid tumours. (2) Leukaemia is frequent in the general population and the
inclusion of families with one relative affected by this can-
cer would dramatically increase the probability of chance
aggregation.

With our knowledge of the interest of the criteria
evaluated in this study and considering the state of the art for
p53 mutation screening, the French LFS group decided to

recommend a search for p53 mutation in families fulfilling
the following criteria: (1) a proband affected by a narrow
spectrum cancer before 36 years and at least one first or
second degree relative affected by a narrow spectrum tumour (other than breast cancer if the proband is affected
by breast cancer) before 46 years or multiple primary
tumours; (2) a proband with multiple primary tumours two
of which belong to the narrow spectrum and the first of which
occurred before 36 years, whatever the family history;
(3) a proband with adrenocortical carcinoma whatever the
age of onset and family history. Using such criteria, we
expect to find a mutation in about 20% of cases and to miss
about 20% (3/15) of the mutations which would be detected
using the loosest criteria. These criteria could of course be
modified in the near future with more efficient laboratory
detection methods and re-evaluated prospectively.

We are indebted to Thierry Frébourg for functional assays and helpful sugges-
tions and to Céline Delouis for her help in p53 testing. We are grateful to Marion
Gauthier-Villars for her help in data management and to Lorna Saint-Angé for
editing the manuscript. We acknowledge the financial support of the Fondation
de France, Fondation A et P Sommer, the Association pour la Recherche sur le
Cancer, the Ligue Nationale Contre le Cancer, the Caisse Nationale d’Assurance
Maladie, the Institut Electricité Santé, the Association ISIS, the Institut Gustave
Roussy, Le Ministère de la Recherche et de l’Enseignement Supérieur,
SNECMA, INSERM, and CNRNS.

AGNÈS CHOMPRET†
ANNE ABEL†
DOMINIQUE STOPPA-LYONNET*
LAURENCE BREUCHER‡
DOMINIQUE STOPPA-LYONNET‡
JEAN FEUNTEUN\*  
CATHERINE BOISSET-PELLIE\*  
Y

Correspondence to: Dr Bonatti-Pellie, bonatti@igr.fr

References

2 Lynch HT, Mitalavy GM, Hruban RH, Lynch JF, Futreal PA. Unix: the fruit
pathologic findings in a kindred with hereditary sarcoma, breast cancer,
brain tumours, leukemia, lung, laryngeal, and adrenal cortical carcinoma.
Cancer 1978;41:2053-64.
3 Li FP, Fraumeni JF. Rhabdomyosarcoma in children: epidemiologic study
of identification of a cancer family syndrome. J Natl Cancer Inst 1969;43:
1365-73.
4 Birch JM, Hartley AL, Marsden HB, Harris M, Swindell R. Excess risk of
breast cancer in the mothers of children with soft tissue sarcomas. Br J
5 Strong LC, Stine M, Norris TL. Cancer in survivors of childhood soft tissue
6 Birch JM, Hartley AL, Blair V, Kelsey AH, Harris M, Teare MD, Morris
Jones PH. Cancer in families of children with soft tissue sarcomas.
7 Li FP, Fraumeni JF. Soft tissue sarcomas, breast cancer and other neoplasms:
8 Li FP, Fraumeni JF. Prospective study of a cancer family syndrome. JAMA
9 Pearson ADJ, Craft AW, Ratcliffe JM, Birch JM, Morris-Jones PH, Roberts
DF. Two families with the Li-Fraumeni cancer family syndrome. J Med
10 Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA,
Maller RW. A cancer family syndrome in twenty-four kindreds. Cancer
11 Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF, Li FP. 
Follow-up study of twenty-four families with Li-Fraumeni syndrome.
12 Hartley AL, Birch JM, Marsden HB, Harris M. Malignant melanoma in
families of children with osteosarcoma, chondrosarcoma and adrenal cor-
13 Hartley AL, Marsden HB, Harris M, Teare MD. Are germ cell tumours
14 Hartley AL, Birch JM, Tricker K, Wallace SA, Kelsey AM, Harris M, Morris
Jones PH. Wilms’ tumor in the Li-Fraumeni cancer family syndrome.
15 Varley JM, McGowan G, Thorncroft M, Sanibange-Korek MF, Houlston RS,
Martin J, Birch JM, Evans DGR. An extended Li-Fraumeni kindred with
gastric carcinoma and a codon 175 mutation in TP53. J Med Genet 1995;
32:946-50.
16 Varley JM, McGowan G, Thorncroft M, Sanibange-Korek MF, Kelsey AM,
Tricker KJ, Evans DGR, Birch JM. Germline mutations of TP53 in
Li-Fraumeni families: an extended study of 39 families. J Med Genet
1997;34:3245-52.
Identification of a transcriptionally compromised allele of c-MYC in a North American family

EDITOR—Chromosomal translocations that target c-MYC at 8q24 are found in all Burkitt’s lymphomas (BL), AIDS-related non-Hodgkin’s lymphoma (AIDS-NHL), mouse plasmacytomas (PCTs), in many examples of diffuse large cell lymphoma (DLCL), and in multiple myeloma (MM). Indications are that c-MYC is under strict control and when deregulated results in unchecked cellular proliferation and hyperplasia. Non-random chromosomal translocations foci from primary BL at 8q24 (8p22;8q24), or t(2;8) (2p16;8q24) in these lymphoid neoplasias places c-MYC under the control of strong immunoglobulin enhancers, which leads to overexpression. In addition, c-MYC is amplified in many tumours including breast, prostate, gastrointestinal, ovarian, MM, myeloid leukaemia, and melanoma suggesting that the overall transcriptional level is probably a key transcribing element associated with c-MYC. Besides genetic lesions, other epigenetic factors such as activation of growth factor receptors may also lead to constitutive expression of c-MYC. Thus, considerable efforts have been made systematically to identify the c-MYC transcriptional apparatus (promoters and enhancers) in an effort to control c-MYC expression. While c-MYC transcription potentially initiates from one of three promoters, P0, P1, and P2 which reside in the 10q11 region, the P2 promoter normally accounts for 75-90% of cytoplasmic c-MYC RNAs. To date, more than 20 transcription factors have been found to reside in the proximity of exon 1 of c-MYC. Actually, c-MYC was one of the first genes to exhibit transcriptional blockage. DNA polymerase II initiation complexes were shown to pause on P2 before activation. Upon chromosomal translocation the insertion of IG enhancer elements renders a shift in promoter usage from P2 towards P1 and loss of transcriptional blockage. In concert with the intensely regulated transcriptional machinery, the sequences surrounding c-MYC appear to be strictly conserved across species boundaries and little in the way of sequence variation or allelic polymorphisms has

22. Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR, Reid R. A signature of mutations found such as t(8;14), t(8;22), or t(2;8) in these tumours including breast, prostate, gastrointestinal, ovarian, MM, myeloid leukaemia, and melanoma suggesting that the overall transcriptional level is probably a key transcribing element associated with c-MYC. Besides genetic lesions, other epigenetic factors such as activation of growth factor receptors may also lead to constitutive expression of c-MYC. Thus, considerable efforts have been made systematically to identify the c-MYC transcriptional apparatus (promoters and enhancers) in an effort to control c-MYC expression. While c-MYC transcription potentially initiates from one of three promoters, P0, P1, and P2 which reside in the 10q11 region, the P2 promoter normally accounts for 75-90% of cytoplasmic c-MYC RNAs. To date, more than 20 transcription factors have been found to reside in the proximity of exon 1 of c-MYC. Actually, c-MYC was one of the first genes to exhibit transcriptional blockage. DNA polymerase II initiation complexes were shown to pause on P2 before activation. Upon chromosomal translocation the insertion of IG enhancer elements renders a shift in promoter usage from P2 towards P1 and loss of transcriptional blockage. In concert with the intensely regulated transcriptional machinery, the sequences surrounding c-MYC appear to be strictly conserved across species boundaries and little in the way of sequence variation or allelic polymorphisms has
been identified. In fact, attempts to position c-MYC in the mouse by genetic recombination techniques were ultimately achieved only when wild mice were backcrossed to inbred mice. Recently, we and others have identified several alleles of human c-MYC (S11N, CAA-33) through single nucleotide polymorphism (SNP) analysis of the coding region among a large random panel of normal healthy subjects of African-American and white descent. Comparisons of the S11N and CAA-33 alleles to wild type alleles at the RNA level showed that the CAA-33 allele is transcribed less efficiently in peripheral blood leucocytes. Although the nature of this difference remains to be elucidated, the finding that CAA-33 is transcriptionally compromised and is found almost exclusively in people of African descent underlines the importance of the genetic background in studies on the control of c-MYC expression. Thus, studies of allelic differences and transcription of human c-MYC will provide useful paradigms in the attempt to control or regulate c-MYC expression in normal and disease conditions.

We have considered the possibility that sequence differences that exist in the coding region of c-MYC could result in feedback inhibition of transcription. For example, somatic point mutations have been found in both BL and AIDS-NHL to be clustered in c-MYC exon 2 in the region responsible for binding of P107 or other factors to the transactivation domain (TAD). This suggests that disruption of binding in this region might lead to a functional inactivation of c-MYC. Indeed, a substitution at residue Thr-58 in the TAD of v-Myc is known to bind in the proximity of this region. An alternative hypothesis is that S288K carries additional sequence differences in the 5' untranslated region which affect transcription. We cannot distinguish between these alternatives until more detailed cloning and sequencing is achieved. Nevertheless, S288K represents the second instance of an allele of c-MYC (in addition to CAA-33) which is transcribed less efficiently in peripheral blood leucocytes. Although the nature of this difference remains to be elucidated, the finding that CAA-33 is transcriptionally compromised and is found almost exclusively in people of African descent underlines the importance of the genetic background in studies on the control of c-MYC expression. Thus, studies of allelic differences and transcription of human c-MYC will provide useful paradigms in the attempt to control or regulate c-MYC expression in normal and disease conditions.

We wish to report the discovery of a unique sequence (S288K AGC→AAC) in the coding region of c-MYC which we have recently found in a North American family (fig 1). The S288K substitution resides just distal to the acidic domain and proximal to the nuclear localisation signal and was detected by PCR amplification and sequencing of the exon 3 region of human c-MYC. Subsequently, we developed a single stranded conformational polymorphism (SSCP) assay for S288K which we have used to survey panels of normal, healthy, white (around 200), African-American (around 200), Hispanic (two) and Asian-Pacific (two) subjects. We were unable to find further evidence of the S288K allele among these subjects or among disease panels of AIDS-NHL (around 200), BL (around 40), MM (around 20), small cell lung carcinoma (around 25), or neuroblastoma/neurocytoma (around 60). Thus, S288K appears to be the lowest frequency MYC variant allele identified to date in the North American population.

To learn more about the origin of the S288K allele, we obtained peripheral blood samples from the North American family of the proband and we have concluded that the father (No 557) and a daughter (No 554) are heterozygous carriers and present with no apparent phenotypic abnormalities. The family is white with a largely western European background and no apparent predisposition to the development of cancer or other metabolic diseases. We have compared expression of the S288K allele to the wild type allele by SSCP and RT-PCR amplification of RNA made from peripheral blood (fig 1). We find that S288K is expressed at extremely low levels or not at all in either subject and this result has been confirmed by sequencing individual subclones (13 in total) of RT-PCR amplified RNA from No 554 (a ratio of 12:1 subclones for codon 288 K:S).

We present two hypotheses to explain the compromised expression of S288K in comparison to wild type. The conformational change associated with a serine to lysine change at codon 288 could abrogate binding of a transcription factor and lead to repression of c-MYC. In fact, the transcription factor YY1 which acts to down regulate c-MYC expression through both direct and indirect effects is known to bind in the proximity of this region. An alternative hypothesis is that S288K carries additional sequence differences in the 5' untranslated region which affect transcription. We cannot distinguish between these alternatives until more detailed cloning and sequencing is accomplished. Nevertheless, S288K represents the second instance of an allele of c-MYC (in addition to CAA-33) in which transcription is less robust in comparison to wild type. Numerous reports of an L-MYC polymorphism have been linked to disease susceptibility in soft tissue sarcomas, oral cancers, colorectal cancers, NHL, breast carcinoma, and non-SCLC, whereas the same alleles seem to be associated with resistance to hepatocellular carcinoma. The reason for this apparent paradox can be attributed to a basic lack of quantitative expression data for L-MYC alleles at the RNA or protein level in tumour versus normal tissue. Understanding gene expression today has progressed from studies of the 5' untranslated/promoter regions to include large constructs of enhancers, matrix attachment regions, locus control regions, and methylation.
sites. Even through the use of large YAC constructs, we know that not all the components necessary for regulation of transcription have been identified, nor will they be found in proximity to the coding region for the gene of interest. c-MYC is no exception in that deregulation can occur in conjunction with chromosomal translocations located as far downstream as the PVT locus (260 kb distant to c-MYC). Thus, we have attempted to identify alleles of c-MYC and to compare rates of transcription in a search for controlling regulatory elements in c-MYC. Through the identification of CAA-33, S11N, and S288K alleles, we can begin the process of systematic classification of c-MYC expression and predisposition to disease.

DAVID SPIWASRI
JENNIFER KIM
LENA DEW
KONRAD HUPPI

Laboratory of Genetics, National Cancer Institute/NIH, Building 37, Room 2B-21, Bethesda, Maryland 20892, USA

Correspondence to: Dr Huppi, huppi@helix.nih.gov


E-cadherin is not frequently mutated in hereditary gastric cancer

EDITOR—Inherited mutations in the E-cadherin gene (CDH1) were first described in three Maori kindreds with early onset, diffuse, familial gastric cancer.1 More recently, this finding has been confirmed in other populations2-5 and this dominantly inherited familial cancer syndrome has been designated a hereditary diffuse gastric cancer (HDGC).6 So far, no germline mutations have been identified in site-specific intestinal type gastric cancer. Based on the guidelines of the First Workshop of the International Gastric Cancer Linkage Consortium (IGCLC), the following criteria were introduced: (1) two or more documented cases of diffuse gastric cancer in first/second degree relatives, with at least one diagnosed before the age of 50 or (2) three or more cases of documented diffuse gastric cancer in first/second degree relatives, independently of age of onset. In addition, criteria for familial intestinal gastric cancer (FIGC) were defined.7

In the present study, we analysed 11 Finnish gastric cancer patients with a family history of disease and two sporadic cases with germline E-cadherin gene mutations (table 1, fig 1). None of these families completely fulfilled the criteria for other inherited cancer syndromes with predisposition to gastric cancer, for example, hereditary non-polyposis colorectal syndrome (HNPPC), familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome, or

Table 1 Features of the families studied

<table>
<thead>
<tr>
<th>Family</th>
<th>No of gastric cancer cases</th>
<th>Age of onset and histological subtype**</th>
<th>Other cancer types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>33°, 39°, 40°, 55°, 66°, 68°, 75°</td>
<td>Br*, Bla + Or, Pro+Bas, Un</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>37°, 55°, 57°</td>
<td>Sk, Pan + Lu</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>36°, 65°</td>
<td>Br, Mel, Sar, Un</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>39°, 67°</td>
<td>Bas, CRC</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>41°, 42°, 50°, 71°, 71°, 75°</td>
<td>Br, Br, Leu*, Pan, Sar, Liv</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>56°</td>
<td>Thy, Pan, Br, CRC</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>42°</td>
<td>Lu, Kid</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>67°, 68°</td>
<td>Kid, Bas + Pro, Lu, Or, Pan+Lu, Leu, Skin, Liv</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>27°, 54°</td>
<td>CRC3, Lip</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>32°, 64°, Un</td>
<td>CRC, Pan</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>61°, 70°, 76°</td>
<td>Bas, Lip, Lu, Un, Un</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>45°, 53, 59, 55, 56°, 65°, 68°</td>
<td>-</td>
</tr>
</tbody>
</table>

**Gastric breast cancer; ¶gastric cancer and leukemia; ¶¶gastric and colon cancer; ¶¶¶gastric and lip cancer.
Bas, basaloma; Bla, bladder cancer; Br, breast cancer; CRC, colorectal cancer; Kid, kidney cancer; Leu, leukemia; Lip, lip cancer; Liv, liver cancer; Lu, lung cancer; Mel, melanoma; Or, ovarian cancer; Pan, pancreatic cancer; Pro, prostate cancer; Sar, sarcoma; Ski, skin cancer; Thy, thyroid cancer; Un, unknown; Us, uterine cancer.
Li-Fraumeni syndrome (LFS). Five of the families studied fulfilled the criteria for HDGC syndrome (table 1, fig1, Nos 1-3, 5, and 10) and five families included two or more cases of gastric cancer (one of which was confirmed to be of diffuse type) (table 1, fig 1, Nos 4, 6, 9, 11, and 12). Family 13 included four intestinal type gastric cancer cases and therefore seems to belong to FIGC. However, one of the patients in this family had diffuse type carcinoma. In addition to gastric cancer, 11 families also displayed other cancer types.

E-cadherin mutation analysis was performed by genomic sequencing of the 16 coding exons including exon/intron boundaries. DNA from one patient with gastric cancer from each of the families was isolated according to standard procedures. Exons were amplified using primers described by Berx et al. except exons 4 and 5, which were amplified as described in Gayther et al.2 The reactions were carried out in a 50 µl reaction volume containing 100 ng of genomic DNA, PCR buffer (PE/ABI, Foster City, CA), 200 µmol/l each dNTP (Finnzymes, Espoo, Finland), 0.6 µmol/l each primer, and 1 unit AmpliTaq GOLD polymerase (PE/ABI). The concentrations of MgCl₂ in the reaction mixture were as described by Berx et al.10 except that for exon 6 the concentration of MgCl₂ was 1.5 mmol/l and for exon 1 DMSO (5%) was included in the reaction mixture. PCR reactions were carried out as described in Berx et al.10

![Pedigrees of gastric cancer families. Patients analysed in this study are marked by an asterisk. An arrow depicts the person carrying the P172R change (family 1). Bas, basalioma; Bla, bladder cancer; Br, breast cancer; CRC, colorectal cancer; Ga, gastric cancer; Kid, kidney cancer; Leu, leukaemia; Lip, lip cancer; Liv, liver cancer; Lu, lung cancer; Mel, melanoma; Or, ovarian cancer; Pan, pancreas cancer; Pro, prostate cancer; Sar, sarcoma; Ski, skin cancer; Thy, thyroid cancer; Un, unknown; Ut, uterine cancer. The age at diagnosis, when known, is shown in parentheses.](http://jmg.bmj.com/firstpublishedas/10.1136/jmg.38.1.43)
reported. A C to G change was found before the start codon of the P172R change, was detected in three of 13 (23%) gastric cancer patients. A C to T change in codon 751, resulting in aspartate substitution by asparagine, was also absent in 212 control samples from cancer-free subjects, as screened by SSCP analysis (fig 2B). The change was also absent in 212 control samples from cancer-free subjects, as screened by SSCP analysis. However, neither of them carried the P172R change. One of the patients studied was the mother of the mutation carrier. The father of this patient died at the age of 94 years and was cancer free. This change was also absent in 212 control samples from cancer-free subjects, as screened by SSCP analysis (fig 2B). The change appears to be a rare polymorphism.

Four additional polymorphisms of the E-cadherin gene were found in this series of gastric cancer patients. A C to T silent change in codon 692 (from alanine to alanine) occurred in eight of 13 (61.5%) gastric cancer patients. A C to T change in codon 751, resulting in aspartate substitution by asparagine, was detected in three of 13 (23%) patients. These two polymorphisms have been previously reported. 10 A C to G change was found before the start codon (−71 bp) in the non-coding region in one of 13 (7.7%) gastric cancer patients and in two of 51 (3.9%) cancer-free controls. A T to C change at position +6 in intron 1 occurred in five of 13 (38%) gastric cancer patients and in 18 of 51 (35%) cancer-free controls.

So far, altogether 14 truncating E-cadherin germline mutations have been detected in gastric cancer patients. A few putative missense germline mutations have been reported but their functional significance has not been tested. 11 In the sporadic type of cancer there seems to be a cluster of mutations between exons 7 and 9 whereas germline mutations are more evenly distributed. 12,13 A novel missense type change, P172R, found in this study is located in exon 4 which encodes a large extracellular domain with Ca2+ binding motifs (exons 4–13). 14 Based on the segregation of the mutation in affected cases in this particular family, it seems that this change is not a pathogenic mutation. It seems to be a very rare polymorphism because none of the 212 cancer-free controls carries this change. This finding is interesting because altogether seven gastric cancer cases were found in this family. Caldas et al. 15 have suggested that E-cadherin should account for 25% of the families fulfilling the established criteria for HDGC. However, PCR-based screening methods used in this study do not allow detection of all mutation types, for example, large deletions.

Our results support the notion that germline mutations in the E-cadherin gene are responsible for only a subset of gastric cancer patients with a family history of the disease. In our study, no mutations were found in 13 gastric cancer probands. Five of the families studied fulfilled the criteria for HDGC and one for FIGC. Our data suggest that for the purpose of efficient E-cadherin mutation detection, there may be a need for more stringent criteria for HDGC, such as requiring three affected subjects as is common in research on familial breast and colon cancer. However, our data set is limited. Loose inclusion criteria should encourage collection of gastric cancer families. This is important, because further work is necessary to identify predisposing gene(s) for a subset of HDGC families, as well as families segregating intestinal gastric cancer.

We thank Inga-Lill Svedberg, Tiula Lehtinen, Sinikka Lindh, Annika Lahti, and Kirs Laukkanen for technical assistance.

Egle Avizienyte*
Virpi Launonen†
Reijo Salovaara‡
Tuula Kiviluoto‡
Lauri A Aaltonen*

*Department of Medical Genetics, Haartman Institute, PO Box 21, FIN-00014 University of Helsinki, Finland
†Department of Pathology, Haartman Institute, PO Box 21, FIN-00014 University of Helsinki, Finland
‡Second Department of Surgery, FIN-00029 HUCH, Helsinki University Central Hospital, Helsinki, Finland

Correspondence to: Dr Aaltonen, lauri.aaltonen@helsinki.fi

www.jmedgenet.com
The spectrum and evolution of phenotypic findings in PTEN mutation positive cases of Bannayan-Riley-Ruvalcaba syndrome

EDITOR—Bannayan-Riley-Ruvalcaba syndrome (BRRS) is an autosomal dominant condition which includes the features of macrocephaly, hyperpigmented penile macules, and hamartomatous tumours, including lipomas, haemangiomas, and gastrointestinal polyps. In 1996, it was recognised that BRRS shared features with Cowden syndrome, another autosomal dominant condition with multiple hamartomas. Cowden syndrome is characterised by trichilemmomas (small, benign hair follicle tumours), oral papillomas, intestinal polyps, and increased frequency of breast and thyroid cancers in affected subjects. Germ-line mutations in the PTEN gene (phosphatase and tensin homologue deleted on chromosome 10), a gene associated with some of these features of macrocephaly, haemangiomas, or intestinal polyposes, were identified in several BRRS families, providing evidence that these conditions are allelic. Identical mutations have been identified in some families with Cowden syndrome and in others with BRRS. In addition, families whose members have overlapping features of both conditions have been identified. However, publications on BRRS provide little clinical information on the natural history and progression of this condition. Here we review our experience following 10 subjects in three families with BRRS and PTEN mutations, using the criteria for ascertainment were at least one affected person in a family with at least two of the three features of macrocephaly, haemangiomas (including at least one lipoma, haemangioma, or intestinal polyp), and penile macules in males. Affected subjects were found to have germ-line PTEN mutations by DNA analysis. The pedigrees of the families are presented in fig 1 and their clinical features are summarised in table 1.

Family 1 is of Native American descent and has been followed for six years (fig 1A). The father and four of his five children are affected. The proband, III.2, was born at 37 weeks’ gestation after an uneventful pregnancy with birth length and OFC between the 90th and 97th centiles. He has been a healthy child but had markedly delayed cognitive development. He sat at 8 months, walked at 16 months, and had only one word at 4 years of age. He has exhibited autistic behaviour consisting of arm flapping, head banging, and repetitive and self-stimulatory mannerisms. Hyperpigmented macules of the penile shaft were first detected at the age of 7 years 3 months. They had not been present at 4½ years during a previous evaluation when the diagnosis of BRRS had been considered based on the presence of macrocephaly, developmental delay, and a lipoma on the back. Laboratory evaluations included fragile X testing and karyotype analysis, both of which were normal. At his most recent evaluation, at the age of 8 years 10 months, he had height and weight measurements on the 95th centile. His OFC was 58.8 cm, on the 98th centile for an adult male. He had obvious mental retardation, with very few words and markedly autistic behaviour. His palpebral fissures were downward slanting.

The proband’s older sister, III.1 in family 1, was 5 years 9 months old at the time this family was first seen. She was born at ~37 weeks’ gestation after an uncomplicated pregnancy with birth weight 4000 g (>97th centile) and length 50.8 cm (97th centile). She had an isolated, small, left groin lipoma at the initial evaluation. She sat at 7 months, walked at 15 months, and exhibited delayed speech although to a much lesser degree than her brother. At her most recent evaluation at the age of 9 years 10 months, her height was between the 75th and 90th centiles, her weight was on the 75th centile, and her OFC was 57 cm, just less than the 98th centile for an adult female. She had downward slanting palpebral fissures, joint hypermobility, and a high arched palate.

III.3 in family 1 was first evaluated at 3 years. Her birth history was unavailable. She walked at 18 months and used a few single words at 3 years. When last evaluated, at the age of 7 years 1 month, she had height on the 95th centile, weight greater than the 95th centile, and head circumference of 58 cm, greater than the 98th centile for an adult female. She exhibited mild mental retardation, most notably speech delay, and had joint hypermobility and a high arched palate, but did not have any cutaneous manifestations of BRRS.

III.4 in family 1 (fig 2A) was born at 37 weeks’ gestation after an uncomplicated pregnancy with a birth weight of 3490 g. Other birth parameters were unavailable. He walked at 21 months and had three to four words at his first genetic evaluation at 22 months of age. At his next evaluation, aged 4 years 3 months, he had two small hydropigmented macules involving the penis. He developed seizures related to hypoglycaemia at the age of 12 months, and had an extensive metabolic workup that was not informative. Normal laboratory studies have included electrolytes, thyroid function tests, insulin, growth hormone, cortisol,
lactate, carnitine (urine and plasma), urine organic acids, and plasma amino acids. He has a history of ketonuria associated with hypoglycaemia. A muscle biopsy was normal, without evidence of lipid myopathy, although the muscle carnitine levels were somewhat low and he is being treated with oral carnitine. A cranial MRI performed at 3 years 8 months and repeated two years later showed normal ventricles and patchy increased T2 signal in the deep and subcortical white matter of both occipital lobes with prominent perivascular spaces. His EEG was abnormal, with diffuse slowing and epileptiform discharges over both occipital lobes. When last evaluated at the age of 7 years 2 months, he had height and weight both above the 95th centile and an OFC of 58.2 cm (98th centile for an adult male). He had four visible macules on the shaft of the penis. His back was hirsute. He continued to exhibit global developmental delay and had joint hypermobility and a high arched palate.

The proband’s father, II.5 in family 1, has had learning difficulties and macrocephaly (fig 2B). He was reportedly a large infant. He has multiple hyperpigmented macules on his penis. At the age of 28, he had a thyroidectomy for goitre. Pathological examination showed adenomatous nodular hyperplasia without evidence of carcinoma. At the last evaluation aged 29 years, his height was on the 50th centile, and OFC was 64.2 cm, much greater than the 98th centile. He was hirsute and had a high arched palate.

III.5, the youngest child in family 1, is normocephalic, without hamartomas or penile macules. When last evaluated at the age of 3 years 9 months, his height and weight were between the 75th and 90th centiles and his OFC was 52 cm (80th centile). He had mild joint hypermobility and a normal palate. He has had normal development. In this family, there has not been any documented breast or thyroid cancer, nor gastrointestinal polyps, although formal endoscopy has not been performed. The father’s sibs and parents were unavailable for evaluation.

Family 2 is of Dutch and other European extraction (fig 1B). The proband, III.1, has been followed for three years. He was initially evaluated for macrocephaly, speech delay, and a family history of Cowden syndrome. Born at term, his birth weight was on the 90th centile and birth length was between the 10th and 50th centiles. Because of a large head and a birthmark along the spine, he had a head CT scan which showed megalencephaly without hydrocephalus, and an MRI of the lumbar spine was normal. When evaluated at the age of 10 months, he had normal development, macrocephaly, and a lipomatous vascular malformation in the lumbar spine region. When evaluated at 3 years 2 months, he had a broad forehead, a fleshy vascular mal-
Table 1 Clinical features of BRRS patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female ratio</td>
<td>3:2</td>
<td>2:0</td>
<td>2:1</td>
<td>7:3</td>
</tr>
<tr>
<td>Primary selection criteria for BRRS</td>
<td>5/5</td>
<td>2/2</td>
<td>3/3</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>3/3 males</td>
<td>0/1 males</td>
<td>0/2 males</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>Penile macules*</td>
<td>2/5</td>
<td>2/2</td>
<td>0/2</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>Hamartoma*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipomas</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Haemangiomas</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Other findings

<table>
<thead>
<tr>
<th>Feature</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High arched palate*</td>
<td>5/5</td>
<td>1/1</td>
<td>2/2</td>
<td>8/8 (100)</td>
</tr>
<tr>
<td>Overgrowth</td>
<td>5/5</td>
<td>1/2</td>
<td>2/2</td>
<td>8/9 (89)</td>
</tr>
<tr>
<td>Preterm*</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Postnatal</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Joint hypermobility*</td>
<td>5/5</td>
<td>0/1</td>
<td>2/2</td>
<td>7/8 (88)</td>
</tr>
<tr>
<td>Hypothyroid*</td>
<td>2/5</td>
<td>1/1</td>
<td>2/2</td>
<td>5/8 (63)</td>
</tr>
<tr>
<td>Frontal bossing*</td>
<td>0/5</td>
<td>1/1</td>
<td>2/2</td>
<td>3/8 (38)</td>
</tr>
<tr>
<td>Hypoglycaemic episodes*</td>
<td>1/5</td>
<td>0/1</td>
<td>2/3</td>
<td>3/9 (33)</td>
</tr>
<tr>
<td>Seizures</td>
<td>1/5</td>
<td>0/2</td>
<td>2/3</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>Downpalp fissions*</td>
<td>2/5</td>
<td>0/1</td>
<td>0/2</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Broad thumbs/big toes*</td>
<td>0/5</td>
<td>1/1</td>
<td>2/2</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Café au lait macules*</td>
<td>1/5</td>
<td>1/1</td>
<td>1/2</td>
<td>3/8 (38)</td>
</tr>
</tbody>
</table>

MR/DD = mental retardation/developmental delay.
Downpalp fissions = downward slanting palpebral fissures.

*An affected parent(s) from families 1, 2, and/or 3 was unavailable for assessment of these features and is not included in the total.
†Birth weight or length greater than 95th centile; birth parameters not available for several members of family 1.

Figure 2 Features of BRRS in family 1. (A) Macrocephaly but otherwise normal cranial configuration of III.4. (B) The father (II.5) also has macrocephaly.

Family 1 had several members of family 1.†Birth weight or length greater than 95th centile; birth parameters not available for evaluation of these features and is not included in the total.

Female 3 is of predominantly European extraction, and the proband’s paternal grandfather. He did not have thyroid or learning problems.

Family 3 is of predominantly European extraction and has been followed in the genetics clinic for three years (fig 1C). The proband, III.1 (fig 4A, B), was born at 36 weeks’ gestation by caesarean section for fetal macrocephaly with birth weight 3940 g (97th centile) and length 53.5 cm (just greater than the 97th centile). OFC was not available. He had a normal newborn course but was evaluated at 6 months for hypotonia and motor delay. At 71/2 months, he was noted to have height and weight on the 50th centile but OFC measuring 51 cm (>98th centile). During genetic evaluation at 10 months, he had macrocephaly, frontal bossing, midface hypoplasia, hypotonia, and joint laxity. Diffuse hyperpigmentation of the penis was disorder, with normal EEG and MRI as an infant (except for macrocephaly), episodic ketogenic hypoglycaemia, and a history of hypotonia. Motor milestones were moderately delayed with crawling at 17 months and walking at 18 months, and he had language impairment with more severe expressive than receptive delays. When first evaluated in the genetics clinic aged 2 years 4 months, he was noted to have macrocephaly with mild frontal bossing but no dysmorphic facial features. His skin examination was notable for three café au lait macules on his trunk, only one measuring greater than 0.5 cm in diameter, as well as a left preauricular telangiectasia from an involved capillary haemangioma. No lipomas or penile macules were identified. At his most recent evaluation, aged 5 years 2 months, his height was between the 50th and 75th centiles, weight between the 90th and 95th centiles, and OFC measured 60.2 cm (>98th centile for an adult male). Other findings included a high arched palate, joint hypermobility, and broad thumbs and big toes, but no penile hyperpigmentation. He had speech articulation problems. A karyotype study was normal.

The proband’s maternal half brother, III.2 in family 3 (fig 4A, B), whose father is of probable African-American extraction, was born at 37.5 weeks’ gestation by repeat caesarean section. Prenatally detected macrocephaly prompted amniocentesis, which showed a normal karyotype. Birth weight was 3940 g (97th centile) and length was 53.5 cm (just greater than the 97th centile). OFC was not available. He had a normal newborn course but was evaluated at 6 months for hypotonia and motor delay. At 71/2 months, he was noted to have height and weight on the 50th centile but OFC measuring 51 cm (>98th centile). During genetic evaluation at 10 months, he had macrocephaly, frontal bossing, midface hypoplasia, hypotonia, and joint laxity. Diffuse hyperpigmentation of the penis was
noted. At his most recent evaluation, at 3 years 7 months, his growth parameters included height on the 50th-75th centile, weight on the 90th centile, and OFC of 59.8 cm (>98th for adult male). Four discrete hyperpigmented macules were identified on the penile shaft and two on the scrotum. He had mild hypotonia and joint hypermobility, as well as a high arched palate. In spite of mild motor delay, he has had normal language acquisition and has not qualified for special education programmes.

The biological mother of the two boys (II.6 in family 3) has not been available for evaluation and has not been their caregiver owing to concerns of neglect. She is reported to have an OFC of 63 cm (much greater than the 98th centile for an adult female) and a history of childhood seizures, hypoglycaemic episodes, and developmental disabilities. Of her four full brothers, two are reported to have macrocephaly, one with hydrocephalus and an Arnold-Chiari malformation (II.3). The proband’s maternal grandfather (I.1) reportedly had macrocephaly and diabetes and died aged 36 in a car accident. There are no reports of breast or thyroid cancer or gastrointestinal polyps in this family, although endoscopy has not been performed.

Molecular analysis was performed on genomic DNA extracted from peripheral blood leucocytes as previously described. In family 1, a germline heterozygous nonsense mutation in the PTEN gene was identified at codon 130 leading to premature termination of the protein (R130X) within the highly conserved phosphatase domain. The father and four affected children carried the R130X mutation, which was not present in the youngest unaffected child, III.5. In family 2, blood was obtained for PTEN mutational analysis on the proband and his unaffected mother, and a mutation was identified in the proband at position 5 in intron 6 (IVS6+5G→T). His mother did not share this allele. In family 3, only the proband and his half brother were available for testing. A mutation in the PTEN gene at position 5 in intron 6 (IVS6+5G→A) was detected in one allele from each of the two boys. This specific mutation has not been previously described. The mutations in families 2 and 3 are likely to lead to aberrant RNA splicing and a truncated protein product.

In identifying our families with BRRS, we focused on subjects possessing at least two of the three features of...
macrocephaly, hamartomas, and penile macules, criteria which have been used to ascertain BRRS patients for molecular studies.\textsuperscript{11,13} It has been suggested that the mere presence of macrocephaly/macrosomia and developmental delay, without hamartoma or penile macules, should warrant further evaluation for \textit{PTEN} mutations.\textsuperscript{14} In fact, macrocephaly has been the most consistent finding in BRRS in numerous reviews\textsuperscript{17,20} and was present in all of our affected subjects. The macrocephaly consists of megalencephaly without ventricular dilatation and is generally present at birth.\textsuperscript{20} We were surprised by the degree of macrocephaly in all affected members of family 1, with head circumference measurements greater than the 98th centile for age, in the absence of facial dysmorphism or other distinguishing cranial features such as frontal bossing. Presumably, the overall large body size masked the disproportionately large head size in these subjects. Families 2 and 3 also exhibited marked macrocephaly, and the affected children had a broad, prominent forehead that has been previously described in BRRS.\textsuperscript{21}

The broad spectrum of hamartoma is illustrated by these families. Although family 1 was first brought to medical attention because of familial macrocephaly and developmental delay, the diagnosis of BRRS was not suspected until single lipomas were identified in two of the four affected children. As the children have grown, these lipomas have become less visible. None of the members in family 1 has been evaluated for gastrointestinal polyposis and symptoms such as intestinal bleeding or chronic abdominal pain have not been reported. The father in family 2 had several classical tumours of BRRS, including multiple lipomas evident by his teens and intestinal polyps. His young son had only a vascular malformation along the lumbar spine. In contrast, the affected boys in family 3 have had no obvious hamartoma. These cases illustrate the importance of a thorough skin examination at each evaluation of a child with macrocephaly, although the absence of dermatological abnormalities does not exclude the diagnosis.

Penile macules are perhaps the most distinctive and potentially valuable diagnostic feature of this syndrome. This is shown by family 1, in which penile macules were important in establishing the diagnosis and were not identified in the oldest son (III.2) at the age of 4½ years but were seen at 7 years 3 months. His younger brother had two very small hyperpigmented macules identified at the age of 4½ years and four small penile macules by 7 years 2 months. Only one of the two affected half brothers in family 3 had penile macules, which were first visualised between the ages of 2½ and 3½ years, although generalised penile hyperpigmentation was seen in infancy. He is of mixed racial heritage, with an African-American father, which may increase the likelihood of pigmentation, since his fully white older brother with the identical mutation did not have penile macules. The penile macules detected in all of the children were very small pigmented changes that might be missed during a cursory examination and were far less obvious than many published photographs.\textsuperscript{5,10} We conclude that speckling of the penis is more likely to occur in later childhood, and its absence in infants and toddlers should not exclude consideration of the diagnosis of BRRS.

Mental retardation has been a feature in case reports of BRRS\textsuperscript{7} and has been reported in 15-20% of affected subjects in two previous surveys.\textsuperscript{7,20} More common are motor and speech delays occurring in childhood in approximately 50% of patients. These delays are reported to improve with age in many cases,\textsuperscript{14} and adults are often described as having motor dysfunction with normal IQ.\textsuperscript{20} In our families, all affected subjects had some degree of learning impairment. In family 1, the degree of cognitive disability was highly variable, with the father exhibiting the mildest learning problems. All of his affected children were in special education programmes, and the two affected sons had the greatest impairment, with the oldest son at the age of 9 years showing autistic behaviour and minimal expressive language. To our knowledge, autism has not been described previously in this condition. The second family had two affected males with learning problems; the father had borderline intelligence on repeated testing and his son had significant global delay and behavioural problems. The affected children in both of these families appeared to have more severe cognitive impairment than their parents, a phenomenon which has been described in other families with BRRS or Cowden syndrome.\textsuperscript{14,21,22} In family 3, both children exhibited motor and speech delay, although the oldest son was more severely affected; their mother was reported to have developmental disabilities as well. The true prevalence of mental retardation in this disorder remains to be established, but for those children suspected of carrying this diagnosis, developmental assessments and appropriate therapeutic interventions are important aspects of care.

Based on the clinical features in these affected subjects, summarised in table 1, we suggest that some “soft” clinical signs may aid in diagnosis when a young child exhibits macrocephaly and learning delay but may not have developed hamartoma or, if male, penile macules. One of these features is a high arched palate, which has been identified in 56-70% of BRRS patients in other surveys\textsuperscript{17,20,25} and was identified in all of our patients. Overgrowth, of either prenatal or postnatal onset, is a feature exhibited by almost 90% of our cohort. Approximately 50% of newborns with BRRS have been reported to have large birth weight and length, with subsequent postnatal growth deceleration resulting in normal growth parameters by adulthood.\textsuperscript{18,20} As newborns, five of the patients were macrosomic, with either birth weight or length greater than the 95th centile. Three of the affected children in family 1 have exhibited postnatal overgrowth, as has the affected boy in family 2. The father and oldest daughter in family 1 now have normal height and weight, suggesting that the overgrowth resolves by adulthood and perhaps puberty. Since the disturbance in growth velocity appears to be age dependent, it may not be appreciated without following growth curves over time. Other findings with greater than 50% incidence in our cohort include joint hypermobility and hypotonia. Hypertensibility of joints has been reported in approximately 50% of patients with BRRS,\textsuperscript{17,20,25} and hypotonia in approximately 20%.\textsuperscript{17,20} These reports also identified downward slanting palpebral fissures in a majority of their patients, a finding which was less common in our cohort. Frontal bossing, hypoglycaemic episodes, seizures, and café au lait macules were all identified in approximately 1/3 of our patients. The presence of these less specific findings may support the diagnosis of BRRS.

It has been suggested that BRRS shows a male preponderance, and that this reflects the overall increased incidence of macrocephaly in males.\textsuperscript{19,26} In previous surveys, even before the recognition of penile macules as a clinical feature of the disorder, –70% of identified patients were male.\textsuperscript{17,20} In our three families, seven out of 10 subjects at risk for BRRS were male, with six of these males mutation positive. However, both at risk females in family 1 were also affected, so we do not have adequate numbers to draw conclusions regarding this male preponderance. Since our inclusion of penile macules as a diagnostic criterion may lead to bias in recognising males with this disorder, further studies incorporating mutation analysis are warranted to confirm the observation that more males are affected with BRRS. The opposite sex ratio may exist for...
Cowden syndrome, which has been reported to exhibit a female preponderance. However, breast cancer is much more prevalent in females in general, and women may be more likely to report facial papules because of cosmetic concerns, so females with Cowden syndrome may be more readily identified than affected males. As a consequence of these sex specific clinical features, the reported ratios in each disorder may merely reflect ascertainment bias.

**PTEN** mutations have now been identified in up to 80% of patients with Cowden syndrome and in up to 60% of those with BRRS, indicating that they are allelic disorders. In several cases, the same **PTEN** mutation has been identified in families with a diagnosis of either Cowden syndrome or BRRS, and in other cases family members carrying the same **PTEN** mutation have different diagnoses. There is significant overlap between Cowden syndrome and BRRS, and two of the features found in both conditions are macrocephaly and thyroid abnormalities.

The father in family 1 had thyroidectomy for adenomatous nodular changes although he and his children fit the description of BRRS better than Cowden syndrome. Family 2 also illustrates the overlap in phenotypic features of these conditions. The son had the BRRS findings of macrocephaly, a hemangioma, and developmental delay, while his father had macrocephaly, borderline intelligence, and features more consistent with Cowden syndrome, including GI polyposis, thyroid cancer, and facial papules. In contrast, family 3 has no distinctive features usually associated with Cowden syndrome, although assessment of all at risk subjects has not been performed and the boys are still quite young. Cowden syndrome may be a more likely diagnosis in adolescents or adults because the cardinal features of GI polyposis and thyroid and breast carcinomas are of later onset than the findings of macrocephaly and developmental delay identified in children diagnosed with BRRS. As we accumulate more data on these children with **PTEN** mutations and the diagnosis of BRRS, they may develop features classically associated with Cowden syndrome.

Correlations between genotype and phenotype are beginning to be elucidated for Cowden syndrome and BRRS, with resulting implications for genetic counselling for cancer and related health risks. An association between the presence of a **PTEN** mutation and the development of cancer or breast fibroadenomas has been observed in both BRRS and Cowden syndrome. Thus, affected females in particular may have an increased risk of breast cancer, and we have recommended breast cancer surveillance beginning at puberty for the daughters in family 1. Mutations in the core phosphatase domain are common, and this domain appears to be a crucial region for the function of the tumour suppressor. The **PTEN** mutation identified in family 1, R130X, is present in this core motif, and has been described in other families with either Cowden syndrome, BRRS, or features that overlap both of these conditions. The **PTEN** mutation in family 2 has also been identified as a somatic mutation in a tumour (C Eng, unpublished data) and probably results in abnormal RNA processing with a truncated protein product. Since the father with overlapping BRRS/Cowden syndrome features had GI polyposis as well as thyroid cancer in his mid-20s, we have recommended that the son has an annual manual thyroid examination as well as thyroid function studies, and that he seek medical attention for the development of any breast or neck masses or rectal bleeding. The mutation in family 3 is at the same intronic position as that in family 2, although the symptoms appear to be more subtle in the two half brothers, who are still less than 6 years old. We have recommended that the same evaluations as those for family 2 be offered to these brothers and their other at risk family members.

In summary, our three families with **PTEN** mutation confirmed BRRS illustrate the phenotypic variability within family members with this condition and the time course for the development of some of the manifestations. Macrocephaly appears to be the most consistent feature in BRRS, but may not be obvious and requires measurement and documentation. The natural history of this disorder suggests that the distinctive finding of penile macules in males may not appear until mid childhood, and that cognitive impairment, in addition to macrocephaly, may be a prominent feature of BRRS in many families. We suggest that postnatal overgrowth during childhood may be common in this condition, and other features such as high arched palate, joint hypermobility, and hypotonia may aid in diagnosis. With the availability of **PTEN** molecular analysis, genotype-phenotype correlations may be feasible. Our cases confirm the observation that the clinical features of BRRS and Cowden syndrome show significant overlap, and suggest that until these conditions are better understood, genetic counselling should include suggestion about the risk of developing thyroid and breast cancers and gastrointestinal polyps for anyone with a documented **PTEN** mutation.
A frameshift mitochondrial complex I gene mutation in a patient with dystonia and cataracts: is the mutation pathogenic?

EDITORS—Mitochondrial DNA (mtDNA) is highly polymorphic. Each person is estimated to differ from another on average at about 25 base pairs among the 16 569 that comprise the mitochondrial genome. Thus, only a small fraction of mtDNA variants are likely to be of pathogenic significance. Criteria used for determining the likelihood that a missense mutation is pathogenic include heteroplasmacy (the percentage of mtDNA molecules in a cell or tissue harbouring a mutation), evolutionary conservation of the altered amino acid, a maternal inheritance pattern, absence of the mutation in controls, clinical features commonly linked to known pathogenic mtDNA mutations, and defects in mitochondrial morphologies and enzyme activities. However, these criteria are inadequate for several reasons. Many mitochondrial missense mutations are homoplasmic. Pathogenic mtDNA mutations are typically characterised by incomplete penetrance, even when homoplasmic, presumably reflecting interactions with environmental and genetic factors. As a result, inherited mtDNA mutations may manifest as “sporadic” disorders rather than with the classical maternal inheritance pattern. Biochemical assays may also be inconclusive, as the expression of a defect in mitochondrial function depends on the nuclear background and tissue type in which the mutation is studied. As a result, mtDNA mutations identified in rare families or subjects with a putative mitochondrial genetic disorder are often of uncertain pathogenic significance.

Over 100 point mutations have been identified in mitochondrial genes in association with human disease, at least 45 of which are missense mutations in protein encoding genes. However, frameshift mtDNA mutations are exceedingly rare. An acquired frameshift 4 bp deletion mutation was identified in the cytochrome b gene at nucleotide position (np) 14 787-14 790 in a patient with parkinsonism-MELAS overlap syndrome and somatic mutations including frameshift mutations have been found in human cancers. In contrast, inherited frameshift mutations in mtDNA have not previously been reported. We report the identification of an inherited frameshift mutation in a patient with dystonia and maternally inherited cataracts. The normal base pair (T) is replaced by AC at np 3308 (T3308AC) in the mitochondrial gene encoding the ND1 subunit of complex I. Dystonia and cataracts have each been linked previously to complex I dysfunction and to mtDNA mutations but, for the reasons outlined above, the pathogenicity of the T3308AC mutation remains uncertain.

DNA was isolated by standard proteinase K and SDS digestion followed by phenol and chloroform extractions. DNA was isolated from muscle (III.4), fibroblasts (II.8), or skin (IV.1) of affected family members and one control. Samples (10 µg) were loaded and run on a 1% agarose gel permeated with ethidium bromide. The DNA was isolated by standard proteinase K and SDS digestion followed by phenol and chloroform extractions. DNA was isolated from muscle (III.4), fibroblasts (II.8), or skin (IV.1) of affected family members and one control. Samples (10 µg) were loaded and run on a 1% agarose gel permeated with ethidium bromide. Membranes were thoroughly rinsed, then incubated with primary antibody (1:500), as described previously.
horseradish peroxidase conjugated secondary antibody. Secondary antibodies were detected by chemiluminescence (Amersham ECL, UK).

The proband (III.4, fig 1) developed a unilateral (right) cataract in her late teens. At the age of 30 she experienced several episodes lasting 30 minutes each of bilateral paraesthesias in her arms and legs with right hemifacial paraesthesias and paresis. Neurological examination showed sensory loss and weakness in the right face, as well as right arm weakness. The following year, examination showed a right facial dystonia and diffuse hyperreflexia with a positive Hoffman’s sign on the left, but full strength and normal sensation. Plantar responses were flexor. Her sister (III.1) developed cataracts in her early teens with a severe left and mild right cataract. This sister’s daughter (IV .1) died a few months after birth with a hypoplastic left heart, polycystic kidneys, and an ectopic pancreas. The maternal grandmother (I.1) developed a right cataract by the age of 40 years. There was no history of ocular trauma in any of the family members with cataracts.

An extensive evaluation of III.4 included normal brain computed tomography and magnetic resonance imaging. Electromyography and nerve conduction studies were normal. Muscle biopsy (right vastus lateralis) showed normal light and electron microscopic results. Cytochrome oxidase c staining was normal. No ragged red fibres were seen. Complex I activity, measured as rotenone sensitive NADH cytochrome c reductase activity normalised to citrate synthase, was normal. Cytochrome oxidase activity was normal. Citrate synthase activity was raised (11.08 µmol/min/g compared to 3.35 ± 1.1 for controls). Serum and cerebrospinal fluid lactates were normal. Serum ammonia was raised at 45 µmol/l (compared to normal of 9–33 µmol/l). Cerebrospinal fluid glucose and protein were normal with no cells or oligoclonal bands. Serum creatine kinase levels were normal.

Sequencing both the H and L strands of PCR amplified muscle derived DNA in III.4 showed a T to AC insertion/deletion at np 3308 (T3308AC) (fig 2). This converts an ATA (methionine) codon to AAC, creating a frameshift in the initiating methionine codon of the mtDNA gene encoding the ND1 subunit of complex I. The presence of a homoplasmic mutation was confirmed by the elimination of an MspI (New England Biolabs) restriction site in III.4 as well as in each of her maternal relatives from whom DNA was available for analysis (I.1, II.8, III.1, IV.1, IV.5, and IV.6). The mutation was absent in 108 control subjects including 29 with Parkinson’s disease and 23 with adult onset focal dystonia. An initiating methionine at this site is highly conserved evolutionarily.16

Sequencing of the entire mitochondrial genome in subject III.4 showed 17 known errors or consensus changes in the Cambridge sequence.17 Additional changes were observed as follows. Synonymous base pair changes: T6620C, C7028T, G11719A, G12007A, C12705T, and A14470G. Known polymorphisms in the non-coding D loop: C16223T, C16290T, G16319A, T16325C, T16362C, G64T, A73G, T146C, A153G, A235G, T310C, and C514CAC. Known polymorphisms in rRNA...
Consists of 318 amino acids. Such an abnormality seems likely given the relatively mild clinical and biochemical features associated with the mutation. Alternatively, translation may begin in frame at the next methionine codon, which occurs at np 3313-5, resulting in a truncation of the first two amino acids (methionine and proline) at the amino terminal end of the ND1 subunit, with preservation of a methionine at the amino terminal end. Except for the initiating methionine, amino acids at the amino terminal end are not highly conserved evolutionarily.

Mutations involving np 3308 have been reported in several patients with neurological abnormalities including two with dystonia. Campos et al reported a woman with transient ataxia and later seizures and marked generalised dystonia who harboured a missense point mutation at np 3308. Two other unrelated patients, each with multiple neurological deficits, also were found to harbour a missense point mutation at 3308. One additional family with maternally inherited hearing loss attributed to the T7511C mtDNA mutation also harboured a T3308C point mutation but it was not thought likely to be pathogenic as it was homoplasmic and had been reported previously in controls.

Mitochondrial complex I dysfunction 11–13 and neuropathy (LHON).19–22 Dystonia is commonly a prominent component of Leigh’s syndrome, which can be associated with mtDNA point mutations or deletions.23 Dystonia, along with other neurological deficits, was reported in a subset of affected members of a family harbouring the LHON associated 11 778 complex I mutation,24 and can be the presenting feature of the 3243 “MELAS” mutation.25 Cataracts have also been reported as a clinical feature associated with the mutation. Alternatively, translation may begin in frame at the next methionine codon, which occurs at np 3313-5, resulting in a truncation of the first two amino acids (methionine and proline) at the amino terminal end of the ND1 subunit, with preservation of a methionine at the amino terminal end. Except for the initiating methionine, amino acids at the amino terminal end are not highly conserved evolutionarily.

This is the first identification of an inherited frameshift mutation in a human mitochondrial protein coding gene. However, the pathogenic significance of this mutation remains uncertain. This case highlights the difficulties often encountered when interpreting associations between rare mtDNA variants and putative mitochondrial disorders.

This work was supported by grants from the National Institutes of Neurological Disorders and Stroke (K08NS01971, DKS), the National Eye Institute (RO1 EY10864, DRJ), and the National Institute on Deafness and Other Communication Disorders (RO1 DC03401, DRJ).

DAVID K SIMON*†
MARK A TARNOPOLSKY†
J TIMOTHY GREENAMRYE‡
DONALD R JOHNS§

*Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, 77 Avenue Louis Pasteur, Room HIM847, Boston, MA 02115, USA
†Department of Neurology, Emory University, Atlanta, GA, USA
‡Department of Neurology, McMaster University, Hamilton, Ontario, Canada
§Department of Ophthalmology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA

Correspondence to: Dr Simon, dsimon1@caregroup.harvard.edu
A common ancestor for COCH related cochlear vestibular (DFNA9) patients in Belgium and The Netherlands bearing the P51S mutation

Editor—Hearing impairment is extremely heterogeneous, both phenotypically and genetically. It is the most frequent form of sensory impairment in the western world, affecting approximately 1/1000 newborns and approximately half of the people above the age of 80.1 In all these cases hereditary factors are a prominent cause. So far, more than 60 loci for monogenic non-syndromal hearing impairment have been described and 14 responsible genes have been identified (Van Camp and Smith, Hereditary Hearing Loss 2000;55:1-117). Balance problems are also relatively frequent, but considerably less is known about the causes. Purely genetic forms of vestibular impairment are extremely rare and no genes have been identified. However, it is commonly known that many hearing impaired people also suffer from balance problems. Moreover, it is now recognised that many hearing impaired people also suffer from vestibular system dysfunction. This can be caused by a dysfunction of the vestibular system.1 The prevalence of vestibular dysfunction may be severely underestimated, as it often remains unnoticed until specialised vestibular tests are performed. Owing to the intimate relationship between the auditory and the vestibular systems, there are probably many genes with a function in both systems.
mutation at base pair 151 was found. This mutation leads to the substitution of a conserved proline for a serine at position 51 of the COCH protein (P51S). Just like the three previously described mutations, this is a missense mutation residing in the FCH domain, and the substituted amino acid is conserved between the human, mouse, and chicken COCH homologues. In this study, eight additional families (or at least one of their members) were shown to carry the P51S mutation in the COCH gene. Fine mapping of the markers from the COCH region and haplotype analysis of markers flanking the COCH gene strongly suggested a single common founder for at least nine of these families.

Details of the families are shown in Table 1. The clinical characteristics of family 1, family 5, family 7, and families 4-7 have been described previously. Symptoms similar to those described were observed in families 8, 9, 11, and 12, as well as in the isolated patients 10, 13, 14, and 15. In family 8, a genome search was performed by the Mammalian Genotyping Center (Marshfield, USA), which mapped the disease locus to chromosome 14q12-13 (data not shown).

As the P51S mutation creates an extra site for the restriction enzyme DdeI, the mutation was analysed by PCR amplification followed by restriction enzyme digestion and gel electrophoresis, as described previously. In normal controls, the 295 bp PCR product is digested into two bands of 247 and 48 bp, respectively. In heterozygous patients carrying the P51S mutation, two additional bands of 143 and 104 bp are visible on agarose gel electrophoresis.

The Whitehead database (http://www-genome.wi.mit.edu/) was screened with markers D14S262 and D14S1071 flanking the COCH region at 14q12-13, which yielded YAC contig WC14. YACs 746-F10, 732-A9, 855-C7, 814-E9, 857-D12, 905-B1, 925-C2, 952-D9, and 62...
949-A9 were obtained from the UK Human Genome Mapping Project (HGMP, Harwell, UK). The relative marker order of seven polymorphic Généthon markers, as well as the position of the COCH gene, were determined by STS content mapping through PCR analysis.

Microsatellite markers were analysed using a standard radioactive PCR assay. Primer sequences and allele frequencies for all markers used in this study were retrieved from the Généthon database. The P51S mutation in the COCH gene has been found previously in seven families characterised by a combination of progressive sensorineural hearing impairment and vestibular dysfunction. We analysed the presence of this mutation in a total of 29 additional families or isolated patients with similar symptoms. All families originate from Belgium or the southern part of The Netherlands. In eight of them, the P51S mutation was present, bringing the total number of P51S bearing families to 15. In the remaining 21 families without the P51S mutation, no further mutation analysis has been performed, so it is possible that they have a different, as yet unidentified COCH mutation.

General data about the families included in this study are summarised in table 1. Although all P51S bearing families originate from the same region, we found no genealogical evidence for a common ancestor.

To investigate a possible founder effect, we reconstructed the disease haplotypes for polymorphic markers flanking the COCH locus. This was complicated by the fact that on the Généthon map the order of these flanking markers was not completely resolved (not shown). In addition, the exact position of the COCH gene with respect to these markers was unknown. Therefore, we constructed a YAC contig spanning the COCH region and determined the marker order through PCR analysis on this contig. As a result of this analysis, the marker order and the position of COCH could be unambiguously determined (fig 1). This marker order is different from the one on the Généthon map, which locates D14S1034 proximal to D14S1021, D14S1040, and D14S1071, and D14S975 distal to D14S257.

The results of the haplotype analysis of the markers flanking the COCH gene are shown in table 2. Families 1, 4, 7, 8, 9, and 12 show exactly the same haplotype for all seven markers examined: 1-1-2-2-1-6-1 (for allele lengths and frequencies, see table 3).

This common disease haplotype may also be present in family 11 (two brothers) and in patients 10, 13, and 15, but only partly in patient 14. In these cases, the disease haplotypes could not be unequivocally determined. Families 2, 5, and 6 have the same haplotype as families 1, 4, 7, 8, 9, and 12 for the four markers that most closely flank the COCH gene on both sides. Marker D14S1071 has a different haplotype and therefore the three most distal markers are not shared with the other families. In family 3, only marker D14S975 has the same haplotype as all the other families.

The P51S mutation in the COCH gene, which is responsible for progressive cochleovestibular impairment (DFNA9), had previously been found in seven families. In this study we have found the same mutation in eight novel families and isolated patients. This could indicate that either all 15 P51S bearing families share a common ancestor or that the P51S mutation represents a mutation hotspot that gave rise to several independent mutational events. Analysis of the disease associated haplotype of the polymorphic markers around COCH showed significant haplotype sharing in nine families and six of them (1, 4, 7, 8, 9, 12) shared haplotypes for all seven markers examined. The probability that six independent families would share a seven allele haplotype merely by chance is negligible (table 3). Therefore, families 1, 4, 7, 8, 9, and 12 must be related to and originate from a common ancestor.

Most probably, families 2, 5, and 6 also originate from this common affected ancestor. These latter families have four alleles in common with families 1, 4, 7, 8, 9, and 12, which are the ones that most closely flank the COCH gene (table 2). One ancestral recombination between D14S257 and D14S1071 can explain the different haplotypes of the distal markers D14S1071, D14S1040, and D14S1034. Taken together, a total of nine families have the identical

---

Table 3 Allele lengths of 14q12-13 markers*

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele No</th>
<th>Allele length</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>D14S257</td>
<td>1</td>
<td>182</td>
<td>0.426</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>184</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>180</td>
<td>0.037</td>
</tr>
<tr>
<td>D14S1040</td>
<td>1</td>
<td>211</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>227</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>229</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>231</td>
<td>0.446</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>233</td>
<td>0.107</td>
</tr>
<tr>
<td>D14S1034</td>
<td>1</td>
<td>172</td>
<td>0.304</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>182</td>
<td>0.036</td>
</tr>
<tr>
<td>D14S1071</td>
<td>1</td>
<td>277</td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>279</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>283</td>
<td>0.463</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>285</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>198</td>
<td>0.574</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>200</td>
<td>0.037</td>
</tr>
<tr>
<td>D14S262</td>
<td>1</td>
<td>260</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>266</td>
<td>0.441</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>272</td>
<td>0.429</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>276</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>268</td>
<td>0.071</td>
</tr>
<tr>
<td>D14S975</td>
<td>1</td>
<td>174</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>170</td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>168</td>
<td>0.125</td>
</tr>
</tbody>
</table>

*Data are based upon the Généthon database. Only the alleles mentioned in table 3 are given.
haplotype (1-1-2-2) for markers D14S262, D14S975, D14S1021, and D14S257 and it can be concluded that they all share a common ancestor.

Only family 3 has a haplotype that is distinct from the other families for the markers flanking COCH, with the exception of marker D14S975. However, as allele 1 is a very common allele for D14S975 (66%), sharing of it could be a coincidence. It cannot be excluded that the mutation in family 3 arose independently from the other families, but, on the other hand, this family may be more distantly related to the others and the shared interval around the COCH gene may be very narrow.

Families 10, 13, 14, and 15 represent isolated patients with the P51S mutation, whereas family 11 consists of two affected brothers. Here, the disease haplotype could not be unambiguously determined. However, with the exception of patient 15, who is homozygous for allele 4 of marker D14S1021, the haplotypes observed in these isolated patients indicate that it cannot be excluded that they, too, are a carrier of the 1-1-2-2-1-6-1 haplotype. This question can only be resolved if more family members become available.

The 10 families in which the exact disease associated haplotype could be determined may allow an estimate of the number of generations passed since the common ancestor. Formulæ to calculate this age rely on the probability of having recombinations in an interval with a known size. The accuracy of these calculations, however, are heavily dependent upon \( p \) and thus upon the accuracy of the genetic maps. However, the data obtained here indicate that the Génethon genetic map around the COCH locus is not reliable. Therefore, the intermarker distances around the COCH gene are unknown and even a rough estimation of the age of the mutation is not feasible. This question may be resolved in the future by an improvement of the map or by the sequencing of the COCH region as part of the Human Genome Project.

The geographical spreading of the P51S mutation may be another clue to the age of the mutation. The 15 families in which the P51S mutation was found contain more than 200 patients in Belgium and The Netherlands and there must be many more unidentified mutation carriers (table 1). This makes the P51S mutation a frequent cause of late onset hearing impairment combined with vestibulocochlear dysfunction, at least in the Dutch and the Belgian populations. A systematic mutation analysis in other populations would indicate whether this mutation is also present elsewhere. A more general occurrence of the P51S mutation would indicate an ancient origin of the mutation or a mutational hotspot. This would imply that COCH is a major gene for cochleovestibular impairment in many different populations harbouring a recurrent mutation that can easily be screened for in a diagnostic setup.

The authors wish to thank all the families who contributed to this study. Gerard J te Meeran is acknowledged for his critical remarks on the manuscript. This project was funded by a research grant from the Flemish Fund for Scientific Research (FWO-Vlaanderen) to GVC and PV, and a grant from the University of Antwerp to GVC and PVDH, and by a grant from The Netherlands Organization for Scientific Research to FPV. The clinical studies in the six Dutch (Nijmegen) DFNA9 families were funded by grants from the Heinsius Antwerp to GVC and PVdH, and by a grant from The Netherlands Research (FWO-Vlaanderen) to GVC and PVdH, a grant from the University of Antwerp to GVC and PVdH, and by a grant from The Netherlands to GVC.

Correspondence to: Professor Van Camp, gvcamp@uia.ua.ac.be

The spastic paraplegia SPG10 locus: narrowing of critical region and exclusion of sodium channel gene SCN8A as a candidate

EDITOR—The hereditary spastic paraplegias (HSPs) are clinically characterised by progressive lower limb spasticity. The spasticity may occur in isolation (“pure”) or may be complicated by other major clinical features. Autosomal dominant, autosomal recessive, and X linked recessive inheritance patterns have been described for pure and complicated forms of HSP.1,2

There are loci for ADPHSP on chromosomes 2p (SPG4, MIM 182601),3 4q (SPG8, MIM 603563),5 14q (SPG3, MIM 182600),7 15q (SPG6, MIM 600363),6 and 19q (SPG12).8 In addition, we recently mapped an ADPHSP locus on chromosome 12q13 (SPG10, MIM 604187) in a large UK family, family 4.9 This locus was narrowed to a 9.2 cM region between markers D12S368 and D12S83.

Clinical features and diagnostic criteria for family 4 have previously been described.10 11 Briefly, subjects were classified as being possibly affected if they had lower limb hyperreflexia in at least one of the following: progressive spastic gait abnormality, bilateral extensor planter reflex, or bilateral sustained (≥5 beats) ankle or knee clonus. Subjects were classified as being possibly affected if lower limb hyperreflexia was present without other abnormal signs and as being normal if they had an entirely normal neurological examination. Thirteen members of family 4 are affected by ADPHSP and the family has a relatively young mean age at onset of 10.8 (SD 9.6) years (range 8–40).

We have now genotyped additional markers D12S803, D12S390, D12S270, and D12S355 for subjects from family 4, using previously described methods.10 Primer sequences for these markers are available from the Généthon microsatellite linkage map12 or from the Marshfield Medical Research Foundation website. Haplotypes were constructed for these and for previously genotyped markers (fig 1). Obligate recombination events in two affected subjects (III.5 and III.9) redefine the centromeric boundary of the SPG10 region at D12S270, and an obligate recombinant event in affected subject IV.1 redefines the telomeric boundary at D12S355. These recombination events narrow the SPG10 candidate region to 6.95 cM (Marshfield Medical Research Foundation website).

A database search (Online Mendelian Inheritance in Man) was carried out to identify candidate genes within the SPG10 critical region. The neuronal sodium channel gene SCN8A is located at chromosome 12q13, close to marker D12S368.13 It is expressed in neurones throughout

---

Figure 1 Family tree of ADPHSP family 4, showing haplotypes for markers around the SPG10 region. The marker order is shown alongside each generation. For clarity non-contributory haplotypes are represented by a uniform hatched shading pattern. Recombination events in affected subjects narrow the SPG10 critical region to a 6.95 cM region between D12S270 and D12S355. Subjects’ sexes have been anonymised for confidentiality reasons. Filled symbol=affected, half filled=possibly affected, quarter filled=clinically normal, ++=DNA available.
the central and peripheral nervous systems. Mice homozygous for null mutations in \( \text{SCN8A} \) (med and med\(^d\)) develop progressive hind limb paralysis and muscle atrophy, with death in the juvenile period. The progressive paralysis in mouse \( \text{SCN8A} \) null mutants is caused by functional denervation of skeletal muscle, with normal motor neurone number and diameter. Although ADPHSP is pathologically characterised by “dying back” of the terminal ends of corticospinal tract axons, we considered there to be sufficient overlap between the ADPHSP phenotype and the \( \text{SCN8A} \) mouse null mutant phenotypes to investigate this gene as a candidate for the disease.

The 26 exons (with flanking intronic sequence) of \( \text{SCN8A} \) were amplified by polymerase chain reaction, as previously described, using DNA from affected subject III.2. Each exon, with its flanking intronic sequence, was sequenced manually, as previously described, or with dye terminators (Perkin Elmer, Foster City) on an ABI377 automated sequencer (Perkin Elmer). Two previously identified single nucleotide polymorphisms were detected, a C to T in intron 19, position +27 from exon 20, and a C to T in exon 22, at position +90. Neither of these variants change the amino acid sequence. We also detected a T for C substitution in exon 14 at position +175 (fig 2A). This new variant results in an arginine to cysteine substitution in the \( \text{SCN8A} \) protein at amino acid 1026, within a conserved functional domain of the sodium channel. Further study will be required to determine whether this polymorphism has any functional effect on the \( \text{SCN8A} \) protein, or whether it confers any predisposition to polygenic diseases which may arise from ion channel disorders.

To date, four different classes of genes have been implicated in HSP. X linked spastic paraplegia is caused by mutations in the cell adhesion molecule \( \text{L1-CAM} \) and mutations in a myelin gene, the proteolipid protein gene. One form of autosomal recessive HSP is caused by mutations in a nuclear encoded mitochondrial gene (paraplegin) which has metalloprotease and chaperone function. Finally, a gene for the most common form of ADPHSP, \( \text{spastin} \), encodes a protein which may be involved in the structure of the nuclear proteosome. The diversity of HSP genes suggests that the neurodegeneration seen in HSPs may be a final common pathway for a number of processes, some of which may involve novel proteins. This makes selection of candidate genes problematical. Identification of additional SPG10 linked families will be crucial to narrow the candidate region and facilitate positional cloning of the SPG10 gene.


The first two authors contributed equally to this work. We thank the members of family 4 for taking part in this study. Marker genotyping and automated sequence analysis of \( \text{SCN8A} \) was carried out in the UK Medical Research Council HGMP Linkage Hotel. Patient assessment and sample collection was supported by the UK Medical Research Council. DCR is a Glaxo/Wellcome Research Fellow and ER is a Wellcome Research Training Fellow. ER is supported by the US National Institutes of Health.

**E REID**
**A ESCAYO**
**A M DEARLOVE**
**M H MEISLER**
**D D LEE**
**E REID**
**A ESCAYO**
**A M DEARLOVE**
**M H MEISLER**
**D D LEE**
**E REID**
**A ESCAYO**
**A M DEARLOVE**
**M H MEISLER**
**D D LEE**
**E REID**
**A ESCAYO**
**A M DEARLOVE**
**M H MEISLER**
**D D LEE**

*Department of Medical Genetics, Level 4, Wellcome Trust Centre for the Study of Molecular Mechanisms in Disease, Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge CB2 2XY, UK
†Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA
‡Medical Research Council Human Genome Mapping Project Resource Centre, Cambridge, UK

Correspondence to: Dr Rubinsztein, dcr1000@cus.cam.ac.uk

www.jmedgenet.com
Cytogenetic and molecular study of a jumping translocation in a baby with Dandy-Walker malformation

EDITOR—Jumping translocations are rare chromosomal events in which a donor chromosome segment is translocated to various recipient chromosome sites. Jumping translocations were initially described in constitutional chromosome syndromes, but the majority of published cases have been observed in haematological malignancies, where their presence has been related to poor prognosis.

Most jumping translocations involve acrocentric chromosomes and a characteristic feature is that breakpoints usually concern areas of repetitive DNA (telomeric, centromeric, or heterochromatic regions). Recipient chromosome involvement seems to be randomly distributed but with a preferential involvement of telomeric segments. This has led to the hypothesis that repetitive telomeric sequences could be implicated in the occurrence of jumping translocations, as suggested by FISH studies which have linked the presence of interstitial telomeric sequences at the junction sites of jumping translocations.

However, the molecular basis underlying these complex chromosomal rearrangements is not well understood.

Constitutional jumping translocations are extremely rare and are usually associated with various phenotypic abnormalities. We report the finding of a jumping translocation in a baby with Dandy-Walker malformation. Dandy-Walker malformation consists of the triad (1) hypoplasia or absence of the vermis, (2) upward displacement of the falx, lateral sinuses, and torcular, and (3) a large, thin walled retrocerebellar cyst formed by the roof of the fourth ventricle. Most Dandy-Walker malformations are sporadic. The Possum database reports six autosomal dominant syndromes and 37 autosomal recessive syndromes which can be associated with Dandy-Walker malformation. However, in these disorders, Dandy-Walker malformation is always one feature of a larger spectrum of anomalies. Various cytogenetic abnormalities have been reported, but to date no consistent chromosomal aberration has been recognised.

The present study combines molecular and cytogenetic investigations, including FISH and PRINS labelling procedures, to characterise a constitutional jumping translocation.

The patient is a baby boy, the second child of healthy, consanguineous parents (the parents are double third cousins). The family history is otherwise unremarkable. Consent was obtained from the parents for genetic studies. At the time of birth, the mother and father were 26 and 34 years old respectively. There was no history of maternal exposure to environmental hazards during pregnancy. The pregnancy was uneventful until sonographic examination at 20 weeks' gestation suggested partial agenesis of the left lateral ventricle. Fetal sampling for chromosome analysis was not undertaken. The baby was delivered at 40.5 weeks' gestation by caesarean section due to persistent vertex presentation. The Apgar scores were 8 at one minute and 9 at five minutes. The baby was born with a weight of 3.35 kg, a length of 51 cm, and head circumference of 32 cm, which are below the 10th centile. A left sided occipital bone defect was noted. Ultrasound examination of the brain showed an occipital bone x-ray showed an occipital bone defect. MRI showed (1) hypoplasia of the cerebellar vermis (only a part of the posterior and anterior segments are

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Total cells examined</th>
<th>t(12;25)</th>
<th>t(2;26)</th>
<th>t(2;12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>First sample</td>
<td>126</td>
<td>108</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Second sample</td>
<td>72</td>
<td>65</td>
<td>1</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>49</td>
<td>47</td>
<td>1</td>
</tr>
</tbody>
</table>
visible), (2) upward displacement of the cerebellar falx, and (3) a retrocerebellar cyst communicating with the fourth ventricle. These features led to a diagnosis of Dandy-Walker malformation. Neurological developmental milestones were normal at the age of 1 month. At the age of 5 years, neurological examination and psychomotor development were normal. The patient walked at 16 months, language skills were acquired normally, and school performance is normal for age.

Initial cytogenetic investigations in the patient were performed on peripheral blood lymphocytes and then subsequently repeated on a second blood sample and skin biopsy. Chromosomes were studied according to standard procedures for RHG banding.
Dual colour FISH labelling was performed with commercially available whole chromosome paints for chromosomes 1, 2, 5, 6, and 12, according to the suppliers’ instructions (Oncor, Gaithersburg, MD). Specific painting probes were combined as follows: biotinylated chromosome 2 specific paint with chromosome 1, 5, 6, or 12 specific paint labelled with digoxigenin. Biotinylated painting was visualised using fluorescein-avidin whereas detection of digoxigenin was done with antidigoxigenin-rhodamine. Specific labelling of subtelomeric regions 1p, 2q, 5q, 6q, and 12q was performed using subtelomeric specific probes. Probes for 2p, 5q, 6q, and 12q were direct fluorophore labelled probes provided by Vysis (Vysis Inc, Downers Grove, IL). The YAC probe 762B5, specific for the subtelomeric region 1p, was a gift from Dr Rocci (Barri, Italy). This probe was labelled by nick translation with digoxigenin-11-dUTP (Boehringer Mannheim, Meylan, France) and used under the same conditions as the purchased subtelomeric probes. The subtelomeric probes correspond to loci estimated to be within 300 kb of the end of the chromosomes.

Telomere repetitive sequences were labelled by PRINS using the telomeric consensus primer (CCTAA)7. PRINS experiments were performed as previously described.10 Fifty µl of a reaction mix composed of the telomeric consensus primer, a nucleotide mixture including fluorescein-dUTP, Taq DNA polymerase buffer, and 2 units of Taq DNA polymerase (Boehringer Mannheim) was used. PRINS reactions were performed on a programmable thermal cycler equipped with a flat plate block. The denatured slides were put on the plate block and the reaction mixes placed on the slides and overlaid with coverslips. The reaction cycle consisted of two programmed steps: 10 minutes at the specific annealing temperature of the primer (60°C) and 20 minutes at 72°C in order to allow the in situ nucleotide chain elongation. At the end of the reaction, slides were washed in 2 × SSC-0.5% Tween 20 and then directly counterstained with propidium iodide in Vectashield antifade solution (Vector Laboratories, Burlingame, CA).

Combined FISH and PRINS experiments were also performed according to the previously reported protocol.11 We used a centromeric repeat probe specific for chromosome 2 (Oncor) and the consensus telomeric primer (CCTAA)7. Fluorescent signals generated by FISH or PRINS were visualised by using a Leitz DMRD microscope equipped with appropriate filters. Combined FISH and PRINS labelling experiments were analysed by confocal microscopy. Optical sections were sampled with a confocal Nikon microscope. Images were transferred to a Power Macintosh computer and standard software tools (Adobe Photoshop 5.0) were used for image analysis.

The parental origin of chromosome 2 was investigated by PCR analysis of two microsatellite markers, D2S337 and D2S2232, located on both sides of the breakpoint 2p12 (respectively in 2p14 and 2p11). DNA was extracted from peripheral blood of the proband and his parents. Oligonucleotide primer sets were obtained from the Genethon. PCRs were performed in a total volume of 50 µl containing 200 ng genomic DNA, 5 µl of 1 × PCR buffer (10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl2, 0.01% (w/v) gelatin), 200 µmol/l each of dNTPs, 0.15 µl dUTP-R110, 0.2 µmol/l primers, and 1 U of Taq DNA polymerase. After an initial denaturation (96°C for 2½ minutes), the PCR was carried out for 30 cycles consisting of 20 seconds at 94°C, 20 seconds at 55°C, 40 seconds at 72°C, followed by a final extension step of 72°C for 10 minutes. One µl of each reaction product was mixed with 5 µl of 50% formamide, 0.1 μg/ml ethidium bromide, 10 mM Tris-HCl (pH 8.0), 1 mM MgCl2, and was loaded onto a 6% polyacrylamide gel. After electrophoresis, the gel was stained with ethidium bromide and photographed.
with 2 µl of formamide, 0.5 µl of molecular weight marker, and 0.5 µl of loading buffer. The mix was denatured for three minutes at 90°C and 1 µl was loaded onto a denaturing 4% polyacrylamide gel (36 cm × 20 cm × 0.2 mm thick). The electrophoresis conditions were one hour 40 seconds at 3000 V and 51°C. The analysis was carried out with the Genescan 2.1 software (Applied Biosystems) on an ABI 377.

Chromosome studies on the initial blood sample and control blood and fibroblast cultures showed four different cell lines: 46,XY,t(1;2)(p36;p12)/46,XY,t(2;5)(p12;q35)/46,XY,t(2;6)(p12;q27)/46,XY,t(2;12)(p12;q24). A summary of the cytogenetic data is given in table 1 and partial ideograms and RHG banded karyotypes are presented in fig 1. This constitutional mosaicism was consistent with a jumping translocation involving almost the entire short arm of chromosome 2 (donor chromosome) alternately transferred to the telomeric regions of four recipient chromosomes (chromosomes 1, 5, 6, and 12). The predominant cell line in both initial and control samples was t(1;2)(p36;p12) (table 1). The parents' chromosomes were normal with RHG banding. Derivative chromosomes resulting from the jumping translocation were confirmed in metaphase spreads by the use of whole chromosome

Figure 3 Results of combined in situ hybridisation of the subtelomeric specific probes for region 2p, labelled in green, and the four regions 1p (A), 5q (B), 6q (C), and 12q (D), labelled in red. In each case, lack of labelling of the der(2) can be seen.

Figure 4 Detection of telomeric sequences by the PRINS technique showing the presence of interstitial telomeric sequences (arrow) at the junction of chromosome arm 2p12-pter and the recipient chromosomes 1 (A) and 6 (B).
The possibility of uniparental disomy of disease in patients with apparently balanced chromosome in Dandy-Walker syndrome. As shown in a recent systemic analysis, the translocation breakpoint on chromosome 2 has not been observed. Thus, mosaicism cannot be totally excluded and it is also possible that the translocation does not seem to contribute to the phenotype; however, abnormality in our patient. Subtelomeric rearrangement does not seem to contribute to the phenotype; however, mosaicism cannot be totally excluded and it is also possible that the translocation breakpoint on chromosome 2 has disrupted the sequences of one (or more) genes implicated in Dandy-Walker syndrome. As shown in a recent systematic study, cryptic deletions may be an important cause of disease in patients with apparently balanced chromosome rearrangements. The possibility of uniparental disomy of chromosome 2, which might be related to the malformation of the proband, has been minimised by the microsatellite analysis.

At present, little is known about the origin of jumping translocations. In three other reports of constitutional jumping translocations, interstitial telomeric sequences have been observed. Interstitial telomeric sequences thus seem to be a common feature of constitutional jumping translocations occurring at telomeric recipient sites. The presence of interstitial telomeric sequences was not systematically observed in cases of jumping translocations associated with haematological disorders. Nevertheless, failure to observe interstitial telomeric sequences may be because of an inherent limitation of in situ labelling procedures for the detection of submicroscopic telomeric sequences.

The results of our classical cytogenetic investigations and whole chromosome painting suggested a simple transposition with fusion between the 2p12 segment and the telomeres of recipient chromosomes rather than a reciprocal exchange, since recipient chromosomes appeared cytogenetically intact. The observed subtelomeric labelling was in accord with this interpretation since no translocation of subtelomeric sequences was observed between chromosome 2 and the four recipient chromosomes. Nevertheless, this does not rule out the possibility of translocations occurring distal to subtelomeric sequences since the subtelomeric probes are usually located between 40 and 300 kb from the telomeres.

PRINS labelling showed interstitial telomeric sequences at the telomeric breakpoint of the recipient chromosomes and at the broken end of chromosome 2. These data may suggest that the telomeric repeat sequences of the recipient chromosomes were partially deleted and translocated onto the proximal end of the broken chromosome 2, which would be in favour of a translocation event. However, for the jumping translocation we report, this event would have to occur four times which seems unlikely.

An alternative explanation for the presence of telomeric sequences at the proximal end of der(2) is the de novo formation of telomeric sequence at the end of the der(2). This has been shown to occur through healing of broken chromosomal ends by seeding of telomeric sequences and requires the expression of telomerase in the cell. The stabilisation of broken chromosome ends by telomeric addition is a well documented phenomenon and appears to be controlled by developmental factors. This phenomenon occurs early in embryonic development, shortly after zygote formation when telomerase activity is still efficient.

This information contributes to an understanding of when our proband’s rearrangement occurred. Mosaicism was found in blood and skin cultures of the patient. For both tissues, there is one largely predominant cell line, involving chromosomes 1 and 2. No chromosomally normal cells were found. It seems likely that chromosome 2 is prone to breakage. Breakage could have occurred in four (or more) different cell lineage precursors and the broken chromosome 2 fragment translocated to the end of different chromosomes.

The presence of interstitial telomeric sequences is considered to represent a form of chromosome instability and, consequently, these sequences could play a role in generating the observed mosaicism. Although the telomeric repeat nucleotide sequences are similar in telomeres...
and intrachromosomal areas, their different locations (terminal or interstitial) could contribute to differential organisation of the chromatin in the two domains, as shown by the variable effect of exonuclease on terminal and interstitial telomeric sequences. In jumping translocations, the presence of interstitial telomeric sequences on a receptor chromosome makes this chromosome unstable and makes the transferred fragment susceptible to "jumping" from one telomere to another. An alternative suggestion is that preferential involvement of telomeric regions in jumping translocations could result from the stabilisation of chromosome breakage by a telomeric capture process. Our report provides further evidence that the jumping process and the presence of interstitial telomeric sequences could be related. The preferential involvement of interstitial telomere-like sequences in chromosome breakage, rearrangement, and instability could be the result of quantitative or qualitative differences in the constitution of proteins associated with telomeric repeat sequences according to their location. It would be interesting to determine whether specific telomeric proteins such as TRF1 and TRF2 are present in interstitial telomere sequence regions.

The elucidation of the underlying mechanism and the role of telomeres in the jumping translocation process will require studies combining cytogenetic and molecular technologies. In the future, the understanding of jumping translocation processes will greatly benefit by the molecular cloning of breakpoints as well as the search for specific genes or sequences involved in the occurrence of these rare chromosomal events.

Figure 6 Results of PCR analysis of chromosome 2 microsatellite markers D2S337 and D2S2232 on DNA extracted from peripheral blood of the proband and his parents. For each locus, the two parental alleles were found in the proband's DNA (A and B).
Clinical and cytogenetic characterisation of a patient with Down syndrome resulting from a 21q22.1→qter duplication

EDITOR—Trisomy of human chromosome 21 is one of the most frequent aneuploidies in humans and results in Down syndrome (DS), affecting approximately 1 in 700 live births.1 DS is a major cause of mental retardation and congenital heart disease in humans, but is also associated with other major features, such as characteristic facies, skeletal abnormalities, and an increased risk of leukemia and Alzheimer’s disease. In most cases (95%), the trisomy of chromosome 21 involves the whole chromosome through maternal (most frequent) or paternal non-disjunction, whereas in some cases (4%) Down syndrome is the result of an unbalanced translocation. Among the latter, a very small proportion of cases are the result of partial trisomy of chromosome 21 arising either from non-Robertsonian translocations or from intrachromosomal duplications.2 The DS phenotype is also found in cases (1%) of mosaicism of trisomy 21.3

During the last decade, considerable progress has been made towards discovering the gene content of chromosome 21, but the functions of most of these genes and their specific contributions to the final DS phenotype still remain unknown. The identification and characterisation of cases of partial trisomy of chromosome 21 has allowed a phenotypic map of this chromosome and DS to be constructed. Clinical and molecular studies of these cases have suggested that most of the phenotypic features of DS are the result of the triple dose of the genes contained in the region around marker D21S55. This region is called the Down syndrome critical region (DSCR).4,6 However, recent evidence suggests that genes outside this region may also contribute to the DS phenotype.7 To evaluate DS patients clinically, a checklist of 25 clinical traits was first reported by Jackson et al8 and later revised by Epstein et al.9 The accurate and exhaustive clinical evaluation of the patients, along with the characterisation of the extent of each trisomy, should help to establish genotype/phenotype correlations. Comparison of the clinical findings in DS patients with similar partial trisomies is now possible through the database created by J Delabar (http://www.infobiogen.fr/services/aneu21), which includes the

**Figure 1** Patient with a duplication 21q22-qter at the age of 2 years 11 months. Note the flat face, brachycephaly, palpebral fissures, epicanthic folds, and malformed and low set ears.

---

molecular characterisation and clinical findings of patients with aneuploidy of chromosome 21. FISH analysis has proven very useful for characterising the extent of partial trisomies of chromosome 21, since a contig of YACs covering the whole long arm of the chromosome is available\textsuperscript{10,11} and the sequenced clones are constantly released to the public databases.

We report here the clinical findings of a patient with a partial trisomy of chromosome 21 resulting from a dup(21q22.1→qter). The patient has some clear signs of DS but also lacks some of the characteristic features of the disorder. The extent of the duplication was characterised by FISH. It extends from marker D21S304 to the telomere and the centromeric breakpoint is localised in the chromosomal region encompassed by YAC 280b1, centromeric to the SOD1 gene.

The patient is a boy from São Paulo, Brazil. The family history is unknown and the patient is currently in a Shelter House of the Brazilian Government waiting for adoption. The patient was first seen at the age of 1 year 10 months.

Figure 2  Schematic representation of human chromosome 21, indicating the region involved in the partial trisomy of the patient with dup(21q22.1→qter). A panel of 18 YACs covering the proximal region involved in the duplication is shown. Circled YACs were used in FISH experiments. The markers near the duplication breakpoint (D21S304, D21S306, D21S310, and D21S305) are indicated in bold. Other markers outside the duplication breakpoint are shown as reference loci.
hybridised as described elsewhere. Twenty metaphases were hybridised (fig 2). Probes were labelled and described by Epstein et al.9 At this second examination, the patient was 2 years 11 months old (fig 1).

Chromosome analyses were performed on standard phytohaemagglutinin (PHA) stimulated lymphocyte cultures from peripheral blood. Some chromosome spreads were G banded and 5-bromo-2’-deoxyuridine (BrdU) was added to two cultures to perform R banding. We generated painting probes from chromosome 21 in order to elucidate the origin of the extra material. We used the hybrid (mouse-human) cell line WAV-17 that has chromosome 21 as its only human component.12 We extracted the DNA and selected Alu-PCR.13 To define the partial trisomy further, YACs from the long arm of chromosome 21 were added to two cultures to perform R banding. We generated painting probes from chromosome 21, showed that the patient had a duplication of the duplicated region. This approach is much more sensitive and accurate than metaphase banding techniques (fig 3).

The first examination showed some clinical features suggestive of DS, such as oblique eye fissures, low set ears, high and narrow palate, mild hypotonia, and protruding tongue, as well as delayed psychomotor milestones. A karyotype was performed then and showed 46,XY,21q+. This cytogenetic result prompted a further clinical examination of the patient according to the protocols described by Epstein et al.9 At this second examination, the patient was 2 years 11 months old (fig 1).

Phenotypic analyses were performed on standard phytohaemagglutinin (PHA) stimulated lymphocyte cultures from peripheral blood. Some chromosome spreads were G banded and 5-bromo-2’-deoxyuridine (BrdU) was added to two cultures to perform R banding. We generated painting probes from chromosome 21 in order to elucidate the origin of the extra material. We used the hybrid (mouse-human) cell line WAV-17 that has chromosome 21 as its only human component.12 We extracted the DNA and selected Alu-PCR.13 To define the partial trisomy further, YACs from the long arm of chromosome 21 were selected14 and also amplified by inter Alu-PCR.15 To define the partial trisomy further, YACs from the long arm of chromosome 21 were selected14 and also amplified by inter Alu-PCR. A total of 14 YACs were hybridised (fig 2). Probes were labelled and hybridised as described elsewhere.13 Twenty metaphases and 50 interphase nuclei were studied for each probe.

The clinical evaluation of the patient at the age of 2 years 11 months showed short stature, brachycephaly, flat facies, oblique palpebral fissures, epicanthic folds, flat nasal bridge, high palate, malformed and low set ears, short and broad hands with clinodactyly of the fifth finger, and moderate mental retardation. The patient also had delayed psychomotor development; he sat at the age of 8 months and walked at 16 months (table 1, fig 1). Dermatoglyphics were normal and an electrocardiogram showed that the patient had an incomplete block of the right branch (with no charge), but no major congenital heart defect. Abdominal scan and audiometry were normal. The cytogenetic study performed with both G and R banding showed that out of 45 metaphases analysed, 37 had extra material on chromosome 21q. A more detailed study of the case by FISH using a painting probe that covers the whole long arm of chromosome 21 showed that all the extra material attached to one chromosome 21 was derived from the same chromosome. FISH performed with YACs along the q arm of chromosome 21, showed that the patient had a duplication 21q22.1→qter. The contig of YACs used showed that the breakpoint was located just proximal to SOD1, within YAC 280b1, which contains markers DS21S304 to D21S306 (fig 2). All distances between the double signals on the dup(21) were identical for all YACs studied. All the metaphases analysed by FISH showed the duplication, discounting the possibility of mosaicism observed using banding techniques (fig 3).

We have identified a case of partial trisomy of chromosome 21 and a DS phenotype caused by duplication of 21q22.1→qter. Using FISH with YAC probes from the long arm of chromosome 21, we have identified the extent of this duplication as well as its orientation. The trisomic region spans marker D21S213 to the telomere, which covers about 13 Mb of the chromosome, as judged from the physical map.17 Comparison of the clinical features resulting from partial trisomy of chromosome 21 has provided the basis for construction of the DS phenotypic map. The coverage of chromosome 21 by a panel of YAC clones and the use of these YACs in FISH analyses allows us to combine the phenotypic information from our DS patient with a fairly accurate molecular definition of the duplicated region. This approach is much
Stable non-Robertsonian dicentric chromosomes: four new cases and a review

EDITOR—Dicentric autosomes are rarely encountered as stable constitutional chromosomes in humans, with the exception of Robertsonian translocations. The presence of two alpha satellite sequences on the same chromosome leads to a high risk of attachment of the same chromatid to opposite poles and to the formation of anaphase bridge during cell division. Therefore, breakage of the dicentric can occur with subsequent cell death. Stability can be achieved when the centromeres are very close together and form only one heterochromatin arm. When the two are far apart, dicentric chromosomes are expected to be formed by the latter. We estimated that the majority of the features of DS, as shown in table 1, the case reported here further confirms that the genes contained in the region from 21q22 to the telomere are responsible for the majority of the features of DS, as previously reported by Korenberg et al. Three other cases have been reported with a similar duplicated region. As shown in table 1, the case reported here further confirms that the majority of the phenotypic features of DS are contained in the region triplicated in the four cases. However, the penetrance of the majority of the clinical features of DS is not complete, so to establish the correlation only the presence (not the absence) of a given trait should be taken into account. When the three published cases of DS with a similar partial trisomy and the present case are compared, we can see that almost all the physical traits typical of DS are present, perhaps with the exception of the furrowed tongue which has an overall frequency of 55% in the DS population. When the three published cases of DS with a similar partial trisomy and the present case are compared, we can see that almost all the physical traits typical of DS are present, perhaps with the exception of the furrowed tongue which has an overall frequency of 55% in the DS population. One of the difficulties in the construction of a phenotypic map of DS, based on cases of partial trisomy, is that a large proportion of these cases have in addition other chromosomal abnormalities which may contribute to the clinical findings. Our patient presents no other chromosomal abnormality, so all his clinical traits can be assumed to be associated with the duplication of chromosome 21. However, we have established comparisons with a very small partial monosomy of another chromosome, which may have a very small contribution to the final phenotype of the patients. With the continued development of molecular cytogenetic tools and the availability of methods and probes for the accurate determination of the chromosomal rearrangements in each case, phenotype-genotype correlations can be obtained with a high degree of accuracy and diagnosis can be performed at the molecular level with a high degree of certainty.

The first two authors contributed equally to this work. We thank D Patterson for the gift of the human chromosome 21 somatic hybrid cell lines and D Cohen, I Chumakov, and D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113. The first two authors contributed equally to this work. We thank D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113. The first two authors contributed equally to this work. We thank D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113. The first two authors contributed equally to this work. We thank D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113. The first two authors contributed equally to this work. We thank D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113. The first two authors contributed equally to this work. We thank D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113. The first two authors contributed equally to this work. We thank D Patterson for YAC clones. This work was supported in part by the Servei Català de la Salut and the Generalitat de Catalunya CIRIT/1997/SGR/113.

MARGA NADAL* CESAR GUZMAN VIGO† MARIA ISABEL MEJABRAGNON JOYCE A ANDRADE‡ LUIS GARCIA ALONSO§
We present four new cases of non-homologous, non-Robertsonian, dicentric chromosomes. We have found 22 reported cases of cytogenetically recognisable dicentric chromosomes. Figure 1 shows the dicentric chromosomes of our cases. Panels A-D: chromosomes G banded at the 450-500 band level of resolution from cases 1-4, respectively. In all four panels, the dicentric chromosome is placed in between its normal monosomic homologues. 

Case 1 was ascertained through amniocentesis at 14 weeks’ gestation for advanced maternal age. The mother was a 41 year old gravida 3, para 2, aborta 0. The couple's family history was unremarkable. The GTG banded karyotype at a resolution of approximately 350 bands showed the presence of an apparently balanced translocation between the telomeric regions of 16q and 22p, creating a dicentric chromosome 16 (fig 1A). The dicentric showed only one primary constriction at the site of chromosome 18 centromere in all cells analysed. Karyotyping was performed according to the protocol provided by AL Technology.

Case 2 was ascertained through fetal tissues received for IUFD at 32 weeks of gestation. The mother was a 29 year old gravida 3, para 1, aborta 1. The second trimester screening ultrasound at 18 weeks’ gestation showed an isolated choroid plexus cyst. At 32 weeks, IUFD was diagnosed. Necropsy showed a growth retarded male fetus with a weight of 650 g and with crown-heel length of 32 cm. The phenotype was consistent with trisomy 18, including mild hirsutism, small mouth, overlapping fingers, rocker bottom feet, short hallux, nail hypoplasia, interventricular septal defect, and Meckel's diverticulum. The tissue cultures showed two abnormal and discordant G banded cell lines: 46,XY,+18,dic(14;18) (p11.2;p11.3)[12]/45,XX,dic(14;18)(p11.2;p11.3)[12]. One cell line was an unbalanced male karyotype composed of a dicentric chromosome formed by an apparently balanced translocation between the short arms of chromosomes 14 and 18, as well as two normal chromosomes 18, resulting in trisomy 18. The other cell line was female, carrying the same dicentric with a chromosome count of 45 as a result of the formation of the dicentric. This finding could be explained by the presence of a female resorbed twin or by maternal contamination. The latter was more likely since the phenotypically normal mother did indeed carry the dicentric (fig 1B). The grandmother's karyotype was normal. The paternal grandfather could not be reached. The dicentric showed only one primary constriction at the site of chromosome 18 centromere in all cells analysed from the fetus and the mother. C banding confirmed the presence of two blocks of centromeric heterochromatin (fig 1D).

Case 3 was born at 40 weeks of gestation after an uneventful pregnancy. The birth weight was 3960 g (75th centile), birth length was 54 cm (90th centile), and OFC was 36.5 cm (75th centile). Apgar scores were 8, 9, and 10 at one, five, and 10 minutes respectively. Karyotyping was requested at the age of 4 1/2 years because of psychomotor retardation. He was globally delayed with severe difficulties in language. At the age of 4 1/2 years, he could walk, hop, and ride a tricycle but had difficulty with his balance, could only speak 20 words with no sentences, and would not play with other children. Physical examination showed mild dysmorphic features, including a long face, bilateral epicanthic folds, bulbous nose, large and slightly anteverted ears, high arched and narrow palate, microretrognathia, pectus excavatum, and bilateral fifth finger clinodactyly. Thyroid evaluation was normal. The karyotype, 45,XY,dic(13;18)(p12;p11.2), showed an unbalanced translocation between the short arms of chromosomes 13 and 18 creating a dicentric chromosome with a deletion of the distal band of chromosome 18p, 18p11.3 (fig 1C). There was only one primary constriction corresponding to chromosome 18 centromere in all cells analysed. FISH with probes against the alpha satellites 13/21 (fig 1G) and 18 (fig 1H) confirmed the presence of two centromeres.

Case 4 was first evaluated at 20 months of age because of developmental delay and dysmorphic features. She was born at 37 weeks of gestation after an uneventful pregnancy. Her birth weight was 2565 g (5th-10th centile), birth length was 47 cm (10th centile), and OFC was 30.8 cm (<5th centile). At birth she was diagnosed with transposition of the great arteries and operated on as a neonate. At 3 months of age she suffered from a urinary tract infection and renal ultrasound showed mild bilateral hydronephrosis. Her psychomotor development was de-
Table 1 Summary of cases reported to carry non-homologous non-Robertsonian heterodicentric autosomes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dicentrics</th>
<th>Acrocentric</th>
<th>Primary constriction</th>
<th>Phenotype</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>(5;13)(p12;p12) +</td>
<td>Chrom 5 &amp; 13 (34%), chrom 5 (54%), chrom 13 (10%)</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>(12;14)(p13;p13) +</td>
<td>Chrom 12</td>
<td>Primary amenorrhea</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>(19;20)(p7;p9) +</td>
<td>NA</td>
<td>Abnormal</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>(7;13)(p21p11) +</td>
<td>Chrom 7</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>(13;18)(p11p11) +</td>
<td>NA</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(8;22)(p23;p13) +</td>
<td>Chrom 8</td>
<td>Normal</td>
<td>Familial</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>(15;18)(p12p11) +</td>
<td>NA</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(13;18)(p12p11.2) +</td>
<td>Chrom 18</td>
<td>Normal</td>
<td>Familial</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(9;22)(p22p11) +</td>
<td>Chrom 15</td>
<td>Abnormal</td>
<td>Familial</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(5;15)(p13p11) +</td>
<td>Chrom 5 &amp; 15 (85%), chrom 15 (8%), chrom 5 (7%)</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>(14;16)(p17q22) +</td>
<td>Chrom 14</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>(12;22)(p25p12) +</td>
<td>NA</td>
<td>Normal</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(19;23)(p10q13) +</td>
<td>NA</td>
<td>Normal</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(12;17)(p13q23) +</td>
<td>NA</td>
<td>Normal</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(13;18)(q23p22) +</td>
<td>NA</td>
<td>Normal</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(15;20)(p13q13) +</td>
<td>Chrom 19</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>(13;18)(p11p11.1)+</td>
<td>NA</td>
<td>Abnormal</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(13;20)(p12q13) +</td>
<td>Chrom 20</td>
<td>Primary amenorrhea</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>(15;20)(p12p12) +</td>
<td>Chrom 20 in lymphocytes</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>(4;21)(p16q22) +</td>
<td>Chrom 21</td>
<td>Normal ( miscarriage)</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>(16;22)(q24p11) +</td>
<td>Chrom 6</td>
<td>Normal</td>
<td>Familial</td>
<td></td>
</tr>
<tr>
<td>Case 2</td>
<td>(14;16)(p12p11.3) +</td>
<td>Chrom 18</td>
<td>Normal</td>
<td>Familial</td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>(13;18)(p12p11.2) +</td>
<td>Chrom 18</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
<tr>
<td>Case 4</td>
<td>(13;18)(p11p11.2) +</td>
<td>Chrom 18</td>
<td>Abnormal</td>
<td>De novo</td>
<td></td>
</tr>
</tbody>
</table>

NA: not available.
formation. However, when the centromeres are close to each other, the inactivation process can occur later, leading to mosaicism for centromere inactivation. Vig and Zinkowski observed centromere separation in dicentric chromosomes at the metaphase-anaphase point. In prometaphase, most dicentrics showed two primary constrictions. However, 18% already showed premature centromere separation of one centromere, suggesting the activity of only one centromere. There was consistency from cell to cell with respect to which centromere separated early. By metaphase, 95% of the dicentrics showed premature separation of one centromere.

After the acrocentrics, chromosome 18 is most frequently involved in non-Robertsonian heterodicentrics (10 cases). The high frequency of involvement of this chromosome may reside in the fact that both 18p− and 18q− are viable syndromes. Other chromosomes are involved more or less randomly (table 1).

In conclusion, our cases indicate further the predominance of acrocentric chromosomes in stable dicentric autosomes. Most of them will reach stability by inactivating one centromere and will be functionally monocentric. If an acrocentric is involved, its centromere is most often the inactivated one.

The excellent technical assistance of the staff of the Cytogenetics Laboratory of the Montreal Children’s Hospital is gratefully acknowledged. This work has been partially funded by a grant from the Fonds de la Recherche en Santé du Québec.

EMMANUELLE LEMYRE*‡
VAZKEN M DER KALOUSTIAN†‡
ALESSANDRA M V DUNCAN*‡

*Division of Cytogenetics, Department of Pathology, Montreal Children’s Hospital and McGill University, Montreal, Canada
†Department of Human Genetics, McGill University, Montreal, Quebec, Canada
‡F Clarke Fraser Clinical Genetics Unit, Department of Pediatrics, Montreal Children’s Hospital and McGill University, Montreal, Canada

Correspondence to: Dr Duncan, Cytogenetics Laboratory, Montreal Children’s Hospital, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3, alessandra.duncan@muhc.mcgill.ca


6 Drets ME, Therman E. Human telomeric 6;19 translocation chromosome with a tendency to break at the fusion point. Chromosoma 1983;88:139-44.


