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 SIWARSKI
JENNIFER KIM
LENA DIAW
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

1 Marcu KB, Patel AJ, Yang Y. Differential regulation of the *c-MYC* P1 and P2 promoters in the absence of functional tumor suppressors: implications for mechanisms of deregulated MYC transcription. *Curr Topics Micro Immunol* 1997;**224**:47-66.
2 Obaya AJ, Mateyak MK, Sedivy JM. Mysterious liaisons: the relationship between *c-MYC* and the cell cycle. *Oncogene* 1999;**18**:2934-41.
3 Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. *Oncogene* 1999;**18**:3004-16.
4 Bentley DL, Groudine M. A block to elongation is largely responsible for decreased transcription of *c-MYC* in differentiated HL-60 cells. *Nature* 1986;**321**:702-6.
5 Eick D, Bornkamm GW. Transcriptional arrest within the first exon is a fast control mechanism in *c-MYC* gene expression. *Nucleic Acids Res* 1986;**14**:8331-46.
6 Nepveu A, Marcu, KB. Intragenic pausing and anti-sense transcription within the murine *c-MYC* locus. *EMBO J* 1986;**5**:2859-65.
7 Strobl LJ, Kohlhuber F, Mautner J, Polack A, Eick D. Absence of a paused transcription complex from the *c-MYC* P2 promoter of the translocation chromosome in Burkitt's lymphoma cells: implication for the *c-MYC* P1/P2 promoter shift. *Oncogene* 1993;**8**:1437-47.
8 Huppi K, Duncan R, Potter M. MYC-1 is centromeric to the linkage group Ly-Sis-Gdc-1 on mouse chromosome 15. *Immunogenetics* 1988;**27**:215-19.
9 Rothberg PG, Otto YM. A polymorphic variant of human c-Myc: Asn11-Ser. *Mamm Genome* 1995;**6**:209-11.
10 Huppi K, Siwarski D, Kim J, Diaw L, Mainhart C, Rabkin C, Leitman S, Chanock S. The frequency of variant alleles of the human *c-MYC* gene in healthy control and cancer populations. (Submitted.)
11 Bhatia K, Huppi K, Spangler G, Siwarski D, Iyer R, Magrath I. Point mutations in the *c-MYC* transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. *Nat Genet* 1993;**5**:56-61.
12 Yano T, Sander CA, Clark HM, Dolezal MV, Jaffe ES, Raffeld M. Clustered mutations in the second exon of the *MYC* gene in sporadic Burkitt's lymphoma. *Oncogene* 1993;**8**:2741-8.
13 Clark HM, Yano T, Otsuki T, Jaffe ES, Shibata D, Raffeld M. Mutations in the coding region of *c-MYC* in AIDS-associated and other aggressive lymphomas. *Cancer Res* 1994;**54**:3383-6.
14 Bhatia K, Spangler G, Gaidano G, Hamdy N, Dalla-Favera R, Magrath I. Mutations in the coding region of *c-MYC* occur frequently in acquired immunodeficiency syndrome-associated lymphomas. *Blood* 1994;**84**:883-8.
15 Gu W, Bhatia K, Magrath IT, Dang CV, Dalla-Favera R. Binding and suppression of the Myc transcriptional activation domain by p107. *Science* 1994;**264**:251-4.
16 Beijersbergen RL, Hijmans EM, Zhu L, Bernards R. Interaction of c-Myc with the pRb-related protein p107 results in inhibition of c-Myc-mediated transactivation. *EMBO J* 1994;**13**:4080-6.
17 Frykberg L., Graf T, Vennström B. The transforming activity of the chicken *c-MYC* gene can be potentiated by mutations. *Oncogene* 1987;**1**:415-21.
18 Riggs KJ, Saleque S, Wong KK, Merrell KT, Lee JS, Shi Y, Calame K. Yin-Yang-1 activates the c-Myc promoter. *Mol Cell Biol* 1993;**13**:7487-95.
19 Shrivastava A, Yu J, Artandi S, Calame K. YY1 and c-Myc associate in vivo in a manner that depends on c-Myc levels. *Proc Natl Acad Sci USA* 1996; **93**:10638-41.
20 Shrivastava A, Saleque S, Kalpana GV, Artandi S, Golf SP, Calame K. Inhibition of transcriptional regulator Yin-Yang-1 by association with c-Myc. *Science* 1993;**262**:1889-92.
21 Crossen PE, Morrison MJ, Colls BM. Increased frequency of the S allele of the L-*MYC* oncogene in non-Hodgkin's lymphoma. *Br J Cancer* 1994;**69**: 759-61.
22 Fernandez T, Saranth D, Advani SH, Deo MG, Soman CS, Restriction fragment length polymorphism of the L-*MYC* oncogene in non-Hogkin's lymphoma patients from India. *Cancer Lett* 1998;**125**:165-9.

J Med Genet 2001;**38**:49–52

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 populations[2–7] and this dominantly inherited familial cancer syndrome has been designated a hereditary diffuse gastric cancer (HDGC).[4] 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.[8]

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 (HNPCC), familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome, or

Table 1 Features of the families studied

Family	*No of gastric cancer cases*	*Age of onset and histological subtype (^{d}diffuse, ^{i}intestinal, ^{u}unknown)*	*Other cancer types*
1	7	33^{d}, 39^{u}, 40^{d}, 55^{d}, 66^{d}, 68^{d}, 75^{d}	Br*, Bla + Ov, Pro+Bas, Un
2	2	40^{d}, 42^{d}	Ski + Pan, Lu
3	3	37^{d}, 55^{d}, 57^{i}	Br, Mel, Sar, Un
4	2	36^{d}, 63^{u}	—
5	2	39^{d}, 67^{d}	Bas, CRC
6	6	41^{u}, 42^{d}, 50^{u}, 71^{u}, 71^{i}, 75^{u}	Br, Br, Leu† , Pan, Sar, Liv
7	1	56^{u}	Thy, Pan, Br, CRC
8	1	42^{d}	Lu, Kid
9	2	67^{d}, 68^{d}	Kid, Ut, Bas + Pro, Lu, Ov, Pan+Lu, Leu, Skin, Liv
10	2	27^{d}, 54^{d}	—
11	3	32^{u}, 64^{d}, Un^{u}	CRC‡, Lip
12	3	61^{u}, 70^{u}, 76^{d}	CRC, Pan
13	7	45^{i}, 53^{i}, 53^{i}, 55^{i}, 56^{u}, 65^{d}, 68^{u}	Bas, Lip§, Lu, Un, Un

*Gastric and breast cancer; †gastric cancer and leukaemia; ‡gastric and colon cancer; §gastric and lip cancer.
Bas, basalioma; Bla, bladder cancer; Br, breast cancer; CRC, colorectal cancer; Kid, kidney cancer; Leu, leukaemia; Lip, lip cancer; Liv, liver cancer; Lu, lung cancer; Mel, melanoma; Ov, ovarian cancer; Pan, pancreatic cancer; Pro, prostate cancer; Sar, sarcoma; Ski, skin cancer; Thy, thyroid cancer; Un, unknown; Ut, uterine cancer.

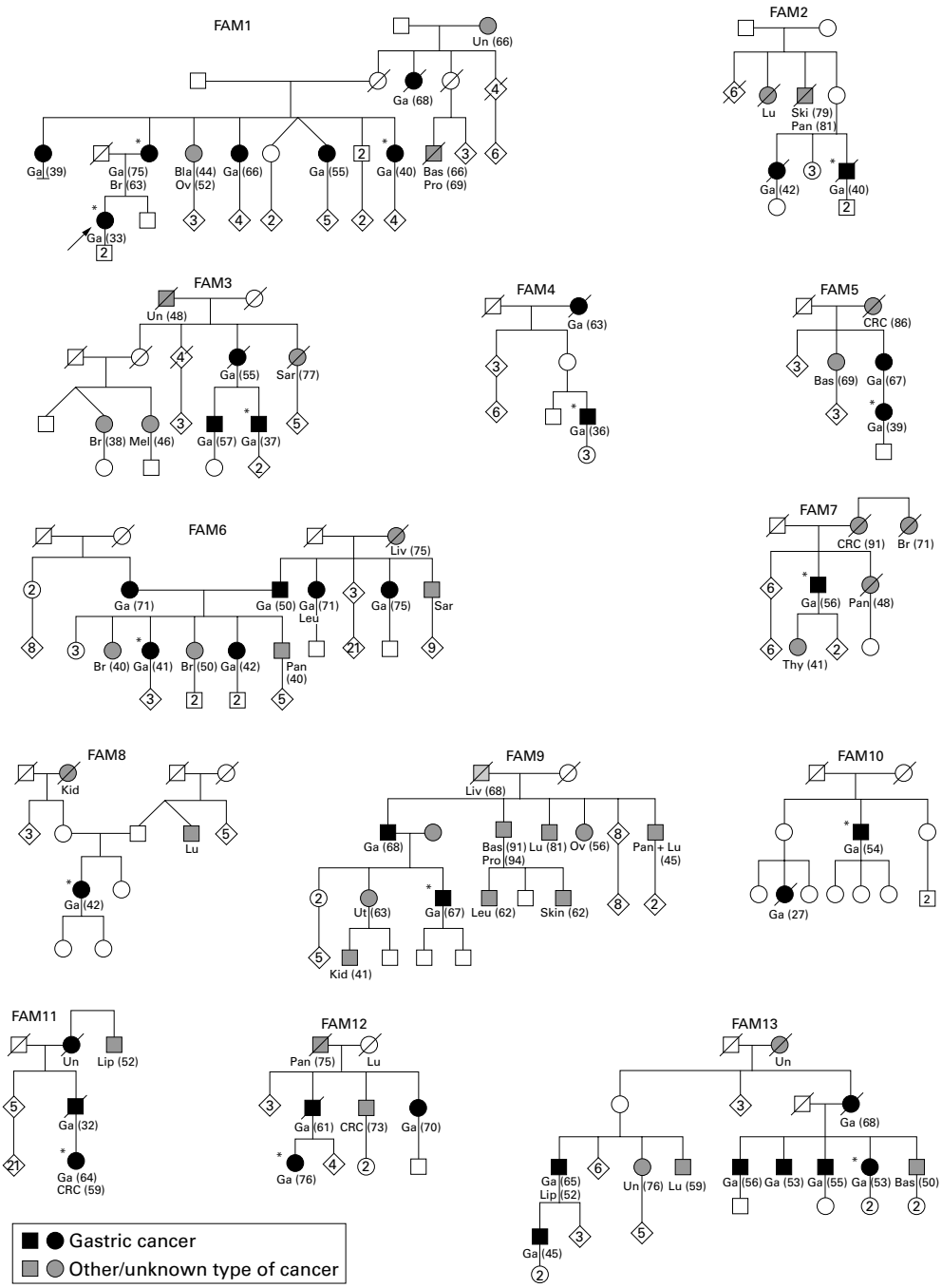

Figure 1 Pedigrees of gastric cancer families. Patients analysed in this study are marked by an asterisk. An arrow depicts the person carrying the P172R change (family 1). Bas, basalioma; Bla, bladder cancer; Br, breast cancer; CRC, colorectal cancer; Ga, gastric cancer; Kid, kidney cancer; Leu, leukaemia; Lip, lip cancer; Liv, liver cancer; Lu, lung cancer; Mel, melanoma; Ov, 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.

Li-Fraumeni syndrome (LFS).[8 9] Five of the families studied fulfilled the criteria for HDGC syndrome (table 1, fig 1, 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*,[10] 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 Ampli*Taq* GOLD polymerase (PE/ABI). The concentrations of $MgCl_2$ in the reaction mixture were as described by Berx *et al*,[10] except that for exon 6 the concentration of $MgCl_2$ 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]

with the following changes: annealing temperature for exon 2 was 54°C, for exon 6 56°C, for exon 8 54°C, and for exon 13 57°C. Sequencing reactions containing 40 ng of the PCR product with 3.2 pmol of the sequencing primer in a volume of 12 µl were performed using ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA) or ABI Big Dye Terminator Kit (Perkin-Elmer, Foster City, CA) according to the manufacturer's instructions. Sequencing reactions were electrophoresed either on 6% Long Ranger gels, containing 8 mol/l urea, or 5% Long Ranger gels, containing 6 mol/l urea, and analysed on an Applied Biosystems model 373A or 377 automated DNA sequencers, respectively.

Exons 1 and 4 were amplified as described above and analysed using SSCP from 84 and 212 cancer free controls, respectively. After PCR, 5 µl of each sample was mixed with 5 µl of denaturing loading buffer (95% formamide, 20 mmol/l EDTA, 0.05% bromphenol blue, 0.05% xylene cyanole FF), denaturated for five minutes at 94°C and loaded into a 0.4 mm × 30 cm × 45 cm gel. Electrophoresis was performed for exon 1 using gels containing 0.5 × MDE solution (AT Biochem, Malvern, PA) and 0.6 × TBE buffer and were run at 4 W for 20 hours. Exon 4 was analysed using 1 × MDE solution and 2.5 mol/l urea at 6 W for 14.5 hours. The gels were silver stained according to standard procedures.

We detected one potential missense mutation in the coding *E-cadherin* gene sequence (table 1, fig 1, No 1). A C to G change occurred in codon 172 in exon 4 resulting in substitution of proline by arginine (P172R) (fig 2A). This family contains seven gastric cancer cases in three different generations. Three of the affected subjects had gastric cancer under 50 years of age (33, 39, and 40 years). One of them also had ductal breast cancer. In addition, one patient with both bladder and ovarian cancer and another with prostate cancer and basalioma were found in this family. To investigate the segregation of this missense type change in the family, we screened two additional family members with gastric cancer (fig 1). DNA from paraffin embedded tissues was isolated according to standard procedures and mutation analysis was performed as described above. 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 11] 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.[8] A few putative missense germline mutations have been reported but their functional significance has not been tested.[1 6 7] 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.[8 11] A novel missense type change, P172R, found in this study is located in exon 4 which encodes a large extracellular domain with Ca^{2+} binding motifs (exons 4-13).[10] 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*[8] 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.

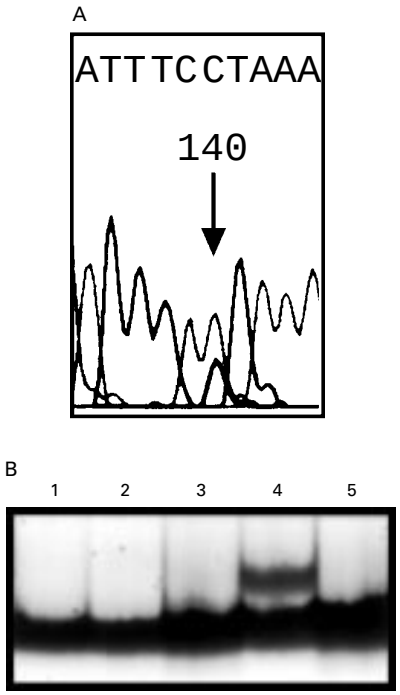

Figure 2 (A) Direct sequencing shows a heterozygous C→G change (P172R, see arrow). (B) SSCP analysis of the P172R change. A positive control (lane 4) was included in all SSCP runs.

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 fulfil 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, Tuula Lehtinen, Sinikka Lindh, Annika Lahti, and Kirsi 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

1 Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. *Nature* 1998;**392**:402-5.
2 Gayther SA, Gorringe KL, Ramus SJ, Huntsman D, Roviello F, Grehan N, Machado JC, Pinto E, Seruca R, Halling K, MacLeod P, Powell SM, Jackson CE, Ponder BAJ, Caldas C. Identification of germline E-cadherin mutations in gastric cancer families of European origin. *Cancer Res* 1998;**58**:4086-9.
3 Richards FM, McKee SA, Rajpar MH, Cole TRP, Evans DGR, Jankowski JA, McKeown C, Sanders SA, Maher ER. Germline E-cadherin gene (CDH1) mutations predispose to familial gastric cancer and colorectal cancer. *Hum Mol Genet* 1999;**8**:607-10.
4 Guilford PJ, Hopkins JB, Grady WM, Markowitz SD, Willis J, Lynch H, Rajput A, Wiesner GL, Lindor NM, Burgart LJ, Toro TT, Lee D, Limacher JM, Shaw DW, Findlay MPN, Reeve AE. E-cadherin germline mutations define an inherited cancer syndrome dominated by diffuse gastric cancer. *Hum Mutat* 1999;**14**:249-55.
5 Keller G, Vogelsang H, Becker I, Hutter J, Ott K, Candidus S, Grundei T, Becker K-F, Mueller J, Siewert JR, Höfler H. Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation. *Am J Pathol* 1999;**155**:337-42.
6 Yoon KA, Ku JL, Yang HK, Kim WH, Park SY, Park JG. Germline mutations of E-cadherin gene in Korean familial gastric cancer patients. *Am J Hum Genet* 1999;**44**:177-80.
7 Shinmura K, Kohno T, Takahashi M, Sasaki A, Ochiai A, Guilford P, Hunter A, Reeve AE, Sugimura H, Yamaguchi N, Yokota J. Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations. *Carcinogenesis* 1999;**20**:1127-31.
8 Caldas C, Carneiro F, Lynch HT, Yokota J, Wiesner GL, Powell SM, Lewis FR, Huntsman DG, Pharoah PDP, Jankowski JA, MacLeod P, Vogelsang H, Keller G, Park KGM, Richards FM, Maher ER, Gayther SA, Oliveira C, Grehan N, Wight D, Seruca R, Roviello F, Ponder BAJ, Jacson CE. Familial gastric cancer: overview and guidelines for management. *J Med Genet* 1999;**36**:873-80.
9 Vogelstein B, Kinzler KW. *The genetic basis of human cancer*. New York: McGraw-Hill, 1998.
10 Berx G, Cleton-Jansen AM, Nollet F, de Leeuw WJF, van de Vijver MJ, Cornelisse C, van Roy F. E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. *EMBO J* 1995;**14**:6107-15.
11 Berx G, Becker K-F, Höfler H, van Roy F. Mutations of the human E-cadherin (CDH1) gene. *Hum Mutat* 1998;**12**:226-37.

J Med Genet 2001;**38**:52–58

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.[1–4] In 1996, it was recognised that BRRS shared features with Cowden syndrome, another autosomal dominant condition with multiple hamartomas.[5] 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.[6] Germline mutations in the *PTEN* gene (phosphatase and tensin homologue deleted on chromosome 10), a gene associated with somatic deletion in a number of cancer cell lines and some primary tumours, were identified in families with Cowden disease the following year.[7–10] At the same time, mutations in the *PTEN* gene were identified in several BRRS families,[11] providing evidence that these conditions are allelic. Identical mutations have been identified in some families with Cowden syndrome and in others with BRRS.[12] In addition, families whose members have overlapping features of both conditions have been identified.[13 14] 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. The criteria for ascertainment were at least one affected person in a family with at least two of the three features of macrocephaly, hamartomas (including at least one lipoma, haemangioma, or intestinal polyp), and penile macules in males. Affected subjects were found to have germline *PTEN* mutations by DNA analysis.[12 13 15] 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 hyperpigmented 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,