LETTERS TO THE EDITOR

Epidermal mosaicism and Blaschko's lines

I read with great interest the recent publication by Moss *et al* on epidermal mosaicism and Blaschko's lines.¹ In their paper, the authors describe the cytogenetic studies that were carried out in their patients. It is noteworthy that in their patient 1, mosaicism was found in only a small proportion of the cells analysed: the abnormal karyotype 46,XX,13,+mar was observed in 1/71 cells from a fibroblast culture of the dark skin area and in 2/15 cells of the light skin area, and in four karyotypes from cultured keratinocytes of the light skin area. In patient 2, trisomy 7 was present in 96% of cultured keratinocytes from a light skin area. In patient 3, 4-8% of fibroblasts from the dark skin area had a missing Y chromosome. This is a male with a 46,XY karyotype; therefore the eponym "Turner's syndrome" would not be appropriate.

The question remains whether very low levels of mosaicism are of significance in their possible association with Blaschko's lines or if they may be the result of artefacts of cell culture. We reported in 1982² a patient with Blaschko's lines and chromosomal mosaicism who presented with facial and body asymmetry and linear hyperpigmented areas with precise limitation at the midline. In this patient, chromosome studies showed pure trisomy 18 in fibroblasts grown from skin biopsies taken from the lines of Blaschko, whereas in normal areas of the skin the karyotype was normal. The comprehensive review on pigmentary anomalies with chromosomal mosaicism by Thomas *et al*³ showed that the majority of chromosomal abnormalities were structural defects present in varying proportions both in lymphocytes and in skin fibroblasts from either dark or light skin areas. The most constant chromosomal finding was i(12p). This is of particular interest since pigmentary skin changes are one of the characteristics of the Killian-Tischler-Nicola syndrome, and it is known that not all cases of pigmentary anomalies associated with chromosomal mosaicism are hypomelanosis of Ito.

These studies and the report of our patient seem to indicate that chromosomal structural anomalies or a non-disjunctional event in a euploid cell or chromatid loss in an aneuploid melanoblast may be associated with a mutational event at the level of melanoblast morphogenetic or mesodermal regulation. Great care should be taken in choosing the site of the skin biopsy to minimise the risk of an admixture of cell types, and it is also recommended that chromosomes from as large a number of cells as possible should be analysed.

J CHEMKE
Clinical Genetics Unit,
Kaplan Hospital,
Rehovot 76100,
Israel.

1 Moss C, Larkins S, Stacey M, Blight A, Farndon PA, Davison EV. Epidermal mosaicism and Blaschko's lines. *J Med Genet* 1993;30:752-5.
2 Chemke J, Rappaport S, Etrog R. Aberrant melanoblast migration associated with trisomy 18 mosaicism. *J Med Genet* 1983;20:135-7.
3 Thomas IT, Frias JL, Cantu ES, Lafer CZ, Flannery DB, Graham JG Jr. Association of pigmentary anomalies with chromosomal and genetic mosaicism and chimerism. *Am J Hum Genet* 1989;45:193-205.

Homozygosity at the dopamine D3 receptor locus is not associated with schizophrenia

Recently, Crocq *et al*¹ found that schizophrenia correlates with homozygosity at the D3/MscI locus. This locus is characterised by a point mutation causing the substitution of a Ser residue with a Gly residue in the extracellular N-terminal domain of D3.

We studied 76 unrelated schizophrenic patients (mean age 42±12.3, 55 males and 21 females), including 17 familial cases, and 86 unrelated controls (mean age 48±8.3, 50 males and 36 females). Diagnosis was made according to DSM III criteria. The controls had no family links with the patients and were free from psychiatric disorders and somatic illness. All subjects were white and from Normandy, France.

Genomic DNA was amplified by PCR according to Lannfelt *et al*.² Digestion with MscI yielded two bands of 111 and 47 bp in all subjects. Subjects with a 304 bp band were classified 1-1, those with 206 bp and 98 bp bands 2-2, and those with all five bands 1-2.

The allelic distributions in the patients and control groups were not significantly different. ($\chi^2_1=0.081$, $p>0.05$) (table). Data were analysed by the method of Woolf.³ There was no significant difference between genotype frequencies among patients and controls ($\chi^2_2=0.17$, $p=0.95$). Hardy-Weinberg equilibrium was conserved in both groups (schizophrenic patients $\chi^2_1=0.043$, $p>0.05$, controls $\chi^2_1=0.18$, $p>0.05$).

The allele frequencies were consistent with those previously reported.^{1,4} Moreover, as in the study of Crocq *et al*,¹ there was no allelic association between schizophrenia and the D3 MscI polymorphism. However, in contrast to Crocq *et al*¹ we did not find an association between schizophrenia and homozygosity at the D3/MscI locus.

Crocq *et al*¹ analysed British and eastern French groups of schizophrenic patients and their matched controls. The incidence of homozygosity was high in both samples of patients. However, close examination of the French data shows that the departure from Hardy-Weinberg equilibrium was significant not for schizophrenic patients but for the controls. Thus, differences in genotype frequencies between patients and controls in the French group were because of frequent heterozygosity in the controls rather than frequent homozygosity among the patients. Indeed, the frequency of homozygosity among the French patients was not significantly higher than among the UK controls ($\chi^2_1=2.98$, $p=0.091$). This rather puzzling finding strongly suggests that only the UK patient group has a high frequency of homozygosity and that the French controls described by Crocq *et al*¹ were, by chance, not representative of the general population. There was no significant difference between the genotype distributions of our controls and the UK controls ($\chi^2_2=0.71$, $p=0.72$), the French controls described by Crocq *et al*¹ and our controls ($\chi^2_2=2.45$, $p=0.29$), our patients and the UK patients ($\chi^2_2=5.23$, $p=0.08$), or the French patients described by Crocq *et al* and our patients ($\chi^2_2=1.05$, $p=0.61$).

When combining our data with those of

Crocq *et al*,¹ the genotype frequencies in controls and patients are still significantly different ($\chi^2_2=11.15$, $p=0.004$) with a significantly higher frequency of homozygosity in patients ($\chi^2_1=10.98$, $p=0.0011$). If the French controls of Crocq *et al*¹ are excluded, these differences are no longer significant ($\chi^2_2=5.90$, $p=0.052$). The high homozygosity in patients remains ($\chi^2_1=5.90$, $p=0.018$) although with a reduced statistical significance. Since the statistical significance of these results is entirely based on the groups reported by Crocq *et al*,¹ further studies including more subjects are needed before any definitive conclusion can be drawn concerning the association between schizophrenia and homozygosity at the D3/MscI locus.

We are grateful to Dr Guy Gorochov for advice. This work received financial support from the Centre National de la Recherche Scientifique, the Association Française contre les Myopathies, and Rhône Poulenc Rorer. CL was supported by a grant from the Association Claude Bernard.

1 Crocq MA, Mant R, Asherson P, *et al*. Association between schizophrenia and homozygosity at the D3 receptor gene. *J Med Genet* 1992;29:101-3.
2 Lannfelt L, Sokoloff P, Martres MP, *et al*. Amino acid substitution in the dopamine D3 receptor as a useful polymorphism for investigating psychiatric disorders. *Psychiatr Genet* 1992;2:249-56.
3 Woolf B. On estimating the relation between blood and disease. *Ann Hum Genet* 1955;19:251-3.
4 Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterisation of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* 1990;347:146-51.

C LAURENT
C SAVOYE
D SAMOLYK
R MELONI
J MALLET

Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs, Bat 32, CNRS, 1 avenue de la Terrasse, 91198 Gif-sur-Yvette, France
D CAMPION
M MARTINEZ
Unité de Génétique Epidémiologique, INSERM U 155, Château de Longchamp, 75016 Paris, France
T D'AMATO
SHU de Psychiatrie Adulte, Hôpital du Vinatier, 69677 Lyon-Bron, France
C BASTARD
CTS, 76230 Boisguillaume, France
S DOLLFUS
CHSR, 76300 Sotteville les Rouen, France

Clinical and molecular studies in fragile X patients with a Prader-Willi-like phenotype

We have read with great interest the recent paper by de Vries *et al*¹ who describe an extended phenotype in fragile X patients. They state that the typical fragile X phenotype, which is characterised by mental retardation, long face with large, everted ears, and megalotestes, is seen in the majority of adult patients. The clinical spectrum in young children is broad and not well delineated.

The eight patients described by de Vries *et al*¹ have truncal obesity and mental retardation.

Distribution and frequencies of alleles and genotype counts for patients and controls

	Distribution and frequency of allele 1	Distribution and frequency of allele 2	Genotypes		
			1-1	1-2	2-2
Schizophrenic patients (n = 76)	103 0.68	49 0.32	35	33	8
Controls (n = 86)	114 0.66	58 0.34	37	40	9

These are non-specific features and are present in the Prader-Willi syndrome (PWS) and many other conditions. The authors themselves state that their patients lack the typical features of PWS, which are low birth weight, neonatal hypotonia, narrow bifrontal diameter, hypogonadism, short stature, and feeding problems during the first year of life followed by hyperphagia and obesity in early childhood. In contrast, patients 1 to 7 developed severe obesity between the ages of 5 and 10 years without a change in diet. None of the patients fulfils the diagnostic criteria described by Holm *et al.*²

Although we agree that obese and mentally retarded boys should be tested for the fragile X syndrome, we feel that this phenotype should not be described as "Prader-Willi-like". This description is misleading and confusing, because all of the typical features of PWS are absent in the patients described by de Vries *et al.*¹ Careful use of the terms "Prader-Willi syndrome" and "Prader-Willi-like" is important, because the syndrome is overdiagnosed by geneticists and paediatricians who are not familiar with the specific features of PWS. Although PWS can be rapidly tested for at the DNA level,³ careful clinical distinction of this syndrome from other conditions is necessary.

In conclusion, we suggest that obesity should be included as an important feature in the fragile X syndrome and the term "Prader-Willi-like" should be avoided.

GABRIELE GILLESSEN-KAESBACH
BERNHARD HORSTHEMKE
Institut für Humangenetik,
Universitätsklinikum Essen,
Hufelandstrasse 55,
D-45122 Essen,
Germany.

1 de Vries BBA, Fryns JP, Butler MG, *et al.* Clinical and molecular studies in fragile (X) patients with a Prader-Willi-like phenotype. *J Med Genet* 1993;30:761-6.
2 Holm VA, Cassidy SB, Butler MG, *et al.* Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics* 1993;91:398-402.
3 Dittrich B, Robinson W, Knoblauch H, *et al.* Molecular diagnosis of the Prader-Willi and Angelman syndromes by detection of parent-of-origin specific DNA methylation in 15q11-13. *Hum Genet* 1992;90:313-15.

Genetic studies of thymic carcinoids in multiple endocrine neoplasia type 1

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disease characterised by hyperplasia or neoplasia of the parathyroids, anterior pituitary, and the endocrine pancreas.¹ Other associated features, such as asymptomatic adrenal neoplasia, thyroid nodules, carcinoid tumours, lipomas, and pinealomas, have been reported at a much lower frequency.² The age of presentation can range from early teens to late fifties. To date, only a few MEN1 related thymic carcinoids have been described. In the largest reported MEN1 family,³ four affected males were found to have metastatic thymic carcinoids but none of the patients was

immediately related.⁴ One malignant and one benign case were reported in a kindred of German extraction⁵ and one case each in two kinships from Canada.⁶

The gene responsible for MEN1 was first mapped to chromosome 11q13⁷ and subsequently predictive testing using RFLP markers was developed.⁸ The two commonest MEN1 lesions, parathyroid and endocrine pancreatic neoplasia and their sporadic counterparts, have been shown to have loss of heterozygosity in the MEN1 region suggesting that the putative MEN1 gene is a tumour suppressor gene.⁷⁻¹¹ To date, only one bronchial carcinoid from a MEN1 patient has been studied but no loss of heterozygosity was found in the MEN1 genomic region.¹² Thymic carcinoids, on the other hand, whether sporadic or familial, have never been studied at the molecular level.

We report here five affected sibs from a Tasmanian MEN1 family (Tasman family 2), of whom two were found to have malignant thymic carcinoids. Despite exhaustive genealogy study extending back to the first generation of this kindred in Tasmania, no consanguineous link can be established between this family and the largest reported MEN1 family in Tasmania.³ Furthermore, the mother of five affected sibs, who died of metastatic glucagonoma, was found to be the oldest member affected (figure).

The aims of this study were to determine the region of genetic linkage in Tasman family 2 and thus the feasibility of using MEN1 linked markers for predictive testing in this family, and to elucidate the genetic defects of MEN1 related thymic carcinoids.

Subject II.1 was admitted for surgery for primary hyperparathyroidism. Preoperative chest x ray showed a shadow in the anterior mediastinum and CT scan identified a tumour mass arising in the thymus. An infiltrating mass of tumour and metastatic lymph node could not be dissected from the great vessels but were biopsied. Malignant thymic carcinoid was confirmed histologically. Patient II.5 had a history of insulinoma and multiple lipomata and was found to have hypercalcaemia. CT scan showed a tumour in the anterior mediastinum arising from the thymus. Again a mass of tumour and lymph node extending around and infiltrating the great vessels was inoperable but was biopsied and malignant thymic carcinoid was confirmed. The other three sibs (II.3, II.7, II.9) and four children of the next generation (III.2, III.4, III.9, III.12) were all found to have hyperplastic parathyroid glands and an insulinoma was removed in addition from III.2. Lymphoblastoid cell lines were established from 24 family members.

Eleven DNA probes previously shown to be linked to the MEN1 locus,⁸ spanning 14% meiotic recombination, were used. DNA from the cell lines and tumours was extracted, digested to completion with appropriate restriction enzymes, electrophoresed, blotted onto nylon membranes, and hybridised to radiolabelled probes as previously described.¹³ Linkage was analysed using the program LIPED with the criteria for scoring the disease state as described previously.⁸ Two malignant thymic carcinoids (II.1, II.5), five hyperplastic parathyroid glands (II.1, II.5, II.9, III.2, III.12), and one pancreatic tumour (III.2) were studied for loss of heterozygosity.

In linkage analysis, two markers, CL15 and CLGW4, were uninformative in the pedigree. Meiotic recombinants were detected for markers telomeric of D11S427 (INT2 and D11S97), so negative lod scores (-2.80 in both cases) were obtained for these markers. However, peak positive lod scores were obtained at a recombination fraction of 0 for each of the other markers, ranging from 0.21 (CD20) to 1.85 (D11S149/D11S288), thus supporting linkage of this family to the MEN1 locus at 11q13 (results not shown). Genotypes of the family members are shown in the figure. In the youngest generation, four are evidently affected but the other 11, despite negative findings in biochemical and radiological screenings, were labelled as "unknown" as all were below 35 years of age.⁷ One of these "unknown" cases (III.6) was found to have inherited the mutant (hatched) chromosome and thus requires repeated follow up to detect early signs of disease.

The insulinoma (III.2) and one hyperplastic parathyroid gland (II.9) showed loss of heterozygosity for all informative markers from D11S288/D11S149 to INT2 (results not shown). In all cases the loss involved the allele derived from the unaffected parent, that is, the putative wild type allele. The other four hyperplastic parathyroid glands and two malignant thymic carcinoids did not show any loss of heterozygosity in the MEN1 region. Although minute somatic deletions or point mutations, undetectable by the current method, cannot be excluded, this finding, together with the lower incidence of thymic carcinoids in MEN1 patients, suggests that the genetic trigger for their tumorigenesis might be different from that of the common MEN1 related tumours. Further studies in delineating specific genetic mutations in thymic tumours are required.

This work was supported by grants from the Tasmanian Cancer Committee, Queensland Cancer Fund, Australian Medical Association, Paul Bolton Foundation, Swedish Cancer Foundation, Swedish Medical Research Council, and the Magnus Bergwell Foundation. We would like to thank Dr John McArdle for performing the histopathology and all of the family members for their kind cooperation.

BIN T TEH*
NICHOLAS K HAYWARD
MARILYN K WALTERS,
Queensland Cancer Fund Research Unit,
Joint Experimental Oncology Program,
Queensland Institute of Medical Research,
Herston 4029, Australia.
JOSEPH J SHEPHERD
STEPHEN WILKINSON
Department of Surgery,
University of Tasmania,
Hobart 7001, Australia.
MAGNUS NORDENSKJÖLD
CATHARINA LARSSON
Department of Clinical Genetics,
Karolinska Institute,
S-10401 Stockholm, Sweden.

*Present address: Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Woollongabba 4102, Queensland, Australia.

1 Wermer P. Endocrine adenomatosis and peptic ulcer in a large kindred. *Am J Med* 1963;35:205-12.

Pedigree of Tasman family 2 showing segregation of chromosome haplotypes for the marker systems which are listed on the left. The chromosome carrying the mutant allele (hatched line) has been inherited by subject III.6, illustrating the usefulness of linkage studies in presymptomatic testing for MEN1. Meiotic crossovers, indicated by thin lines extending from the hatched lines, occurred in II.5, II.7, III.6, and III.9.