ELSEVIER

Early Onset of Gastric Carcinoma and Constitutional Deletion of 18p

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ABSTRACT: *We report on the association of a gastric carcinoma and a constitutional deletion of the short arm of chromosome 18 in a 14-year-old patient. The phenotype of the patient, including microcephaly, ptosis, micrognathia, tetralogy of Fallot, and mental retardation, fits well with previously reported cases of del(18p); she also showed a positive serology against* Helicobacter pylori. *The comparison of the alleles of polymorphic loci located on the short arm of chromosome 18 between the patient and her parents showed a maternal origin of the abnormal chromosome. Loss of heterozygosity (LOH) for loci located in the long arm of chromosome 18 is a frequent event in gastric carcinomas; it was observed in the tumoral mass of our patient and again, the alleles lost were of maternal origin. We postulate that the constitutional chromosomal abnormality may have favored the loss of the abnormal chromosome in some cells and that the loss of the deleted chromosome 18 (demonstrated by LOH for this chromosome in the tumoral mass) has been an early step in the pathogenesis of the gastric carcinoma of our patient with* Helicobacter pylori *infection acting as a cofactor.*

INTRODUCTION

Deletion of the short arm of chromosome 18 with breakpoints at p11,del(18p) is a common cytogenetic abnormality, and at least 136 cases have been described to date. Its phenotype is well recognizable, and extensive data are available on the different malformations and associated dysmorphism [1].

To the best of our knowledge, no case of neoplastic disease has been reported in association with constitutional del(18p), while the same deletion has been observed as an acquired anomaly in breast cancer, ependymomas, and acute myeloid leukemia [2, 3]. Loss of heterozygosity (LOH) for the short arm of chromosome 18 has been reported by Hahn et al. [4] in pancreatic adenocarcinomas, while LOH for loci located in the long arm of chromosome 18 has been reported in at least 60% of gastric carcinomas [5].

Gastric adenocarcinoma is rare under the age of 20 years and only about 20 cases have been described [6]; we report on the occurrence of gastric adenocarcinoma in a 14-year-old patient carrying a constitutional deletion of the short arm of chromosome 18.

CASE REPORT

A female first child of healthy unrelated parents, was born at term after an uneventful pregnancy, with a birth weight of 1900 g (<3rd centile) and a length of 48 cm, (<10th centile). In the family history, we recorded a gastric cancer in the paternal grandfather diagnosed at 64 years, and a sarcoma localized in the lower leg in the maternal grandmother (at 74 years), but we have been unable to obtain more detailed information or pathology reports. Physical examination at birth revealed microcephaly, ptosis, downturned corner of the mouth, micrognathia, and widely spaced nipples. Abdominal ultrasonography demonstrated a situs viscerum inversus.

A heart murmur of 4/6 was also recorded; cyanosis was present when crying. Further cardiologic investigations

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disclosed a tetralogy of Fallot and patent ductus arteriosus. The patient underwent cardiac surgery at the age of 2.5 years, and subsequent development was uneventful, without any increase in number of infectious disorders.

Physical growth has always been at the 3rd centile; developmental milestones were delayed, and mild mental retardation was soon evident. At the age of 14, she complained of abdominal pain and jaundice became apparent.

Physical examination confirmed the dysmorphic features present at birth, with the addition of large, anteverted prominent ears (Fig. 1), scoliosis, bilateral clinodactyly of the 5th finger, and numerous dental caries. Weight (29 kg) and height (136 cm) were below the 3rd centile. An abdominal rock-hard, unpainful, epigastric mass was palpable.

Routine laboratory analyses were normal, except for direct bilirubin (5.6 mg/dL n.v. <1), GOT (370 UI, n.v. <40), GPT (459 UI n.v. <40), and γGT (203 n.v. <10). Antibodies against *Helicobacter pylori* were 75 U/mL (n.v. <2 U/mL).

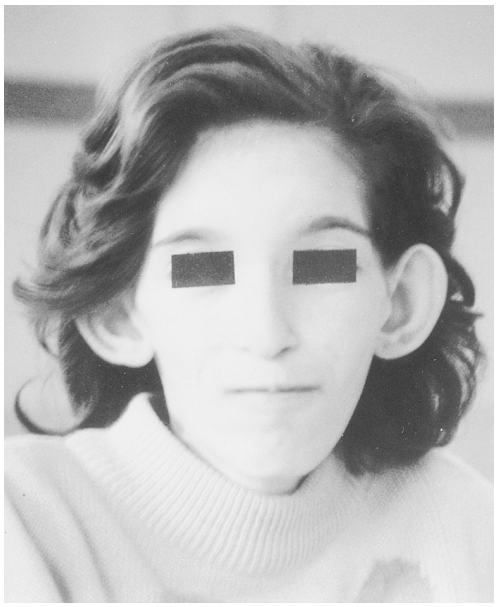
At surgery, a large mass arising in the gastric antrum, extending toward and incorporating the pancreas, biliary ducts, and surrounding tissues was observed. Metastatic dissemination to perigastric nodes and omentum was also evident. Partial resection of the mass with gastrojejunostomy and colecistojejunostomy was performed. The patient responded poorly to chemotherapy, and died 4 months later.

METHODS

Histopathology

Specimens were fixed in 10% buffered formal saline and paraffin; immunohistochemical studies were performed on representative sections of tumor by means of an immunoperoxidase technique. The following panel of gastric, intestinal, and pancreatic differentiation markers was applied: BD5 reacting with epithelial cells of normal and neoplastic human intestine, but not with normal gastric mucosa; DU-PAN-2 marking pancreatic ductal epithelium and related tumors; M1 and Cathepsin E reacting with columnar mucus cells of surface gastric epithelium; Pepsinogen II (or C) (PGII) expressed by pyloric and oxyntic gastric gland cells [7].

Figure 1 The patient at 14 years of age.



Cytogenetic

Chromosome preparations were obtained with routine techniques from stimulated lymphocytes, and stained for QFQ and GTG bands.

Molecular Biology

Autoptic material (tumor mass, kidney, normal nodes) from the patient and peripheral blood from the parents were used for DNA extraction with routine techniques. Genotyping of short tandem repeat polymorphisms (Research Genetics) was performed on the patient and her parents by use of standard procedures. Polymerase chain reaction (PCR) amplifications were performed in 8 μl reaction mixtures containing 20 ng genomic DNA; 330 nM of each primer; 200 μM of dCTP, dGTP, and dTTP; 25 μM of dATP; 1 μCi (α^{35}S) dATP; 50 mM KCI; 10 mM TRIS pH 9; 1.5 mM $MgCI_2$; 0.1% Triton X-100; 0.01% gelatine, and 0.2 U of *Taq* Polymerase. The PCR conditions consisted of an initial denaturation step followed by 30 cycles of 94° C for 40 seconds, 57° C for 40 seconds, 72° C for 40 seconds, and a final extension at 72° C for 5 minutes, using a PTC-100 thermal cycler (MJ Research). Four microliters of each PCR product were resolved by electrophoresis on denaturing (7 M urea) 6% polyacrylamide gels for 3–5 hours at 50 W. Gels were fixed in 10% methanol, 10% acetic acid, and dried and exposed to x-ray film at room temperature.

Informative results were obtained at the following loci: D18S63 and D18S1370 located on the short arm of chromosome 18, and D18S363 and GATA06 located in 18q12–21. Three other loci, D1S549 (located on 1q35-qter), D5S820 (5q), and D17S796 (17p13) where chosen to study regions frequently involved in LOH in gastric cancer [8].

RESULTS

Histopathology

Routine examination of hematoxylin & eosin (H & E) stained sections allowed a diagnosis of poorly differentiated intestinal-type gastric carcinoma according to Lauren [9] which infiltrated the gastric wall transmurally (Fig. 2).

M1, PGII, BD5, and DP2 antibodies gave consistently negative results, while Cathepsin E antiserum marked about 80% of cancer cells (data not shown).

Cytogenetic

Chromosome analysis on peripheral blood lymphocytes from the patient demonstrated a deletion of the whole short arm of chromosome 18 in all 30 mitoses examined (Fig. 3d) with a karyotype 46,XX,del(18)p11). The parents' karyotypes were normal.

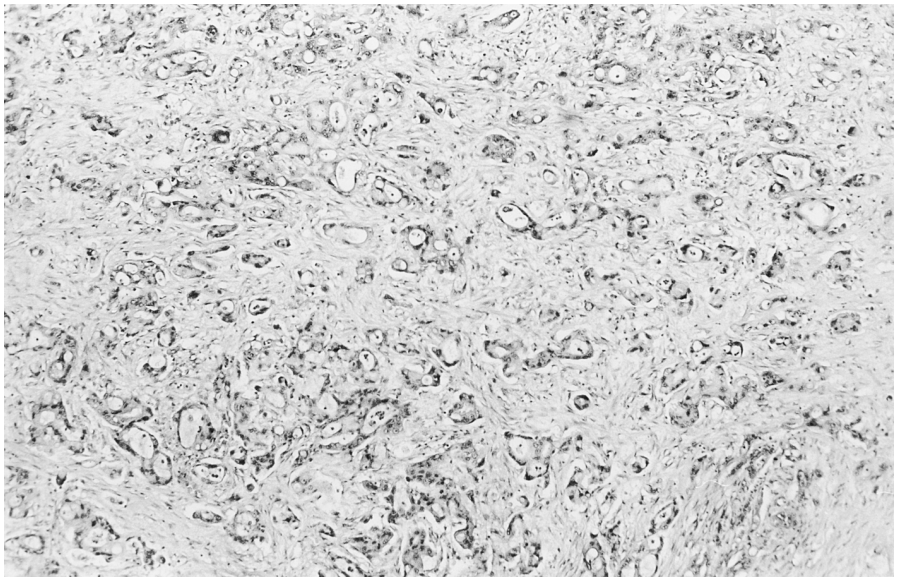


Figure 2 Histopathological aspects of gastric neoplasia: cancer cells arranged in irregular, poorly-differentiated gland structures, and in small clusters (H & E ×6.3).

Molecular Biology

The comparison of STR polymorphisms between the child and her parents at loci D18S63 and D18S1370, both located in short arm of chromosome 18, showed the presence of the paternal allele and the absence of the maternal one, both in normal tissues (kidney and normal nodes) and in the tumor mass of the patient, thus demonstrating that the deletion occurred on the maternal chromosome (Figs. 3a, 3b)

We also analyzed two other loci, D18S363 and GATA06, located in 18q12∼q21 (a region known to be frequently involved in LOH in gastric cancer) comparing tumor mass and normal tissues, and an asymmetric pattern of the alleles was observed in the tumor mass, but not in the normal tissues of the proposita or in the samples from her parents (Fig. 3c). This finding is interpreted as evidence of LOH for the loci tested, limited to the tumor mass.

No evidence of LOH was observed at D1S549, D5S820 and D17S796, but microsatellite instability was observed at D5S820 in the tumor mass (data not shown).

DISCUSSION

The phenotype of the patient fits well with previously reported cases of del(18p); in our patient we recorded 14 of the 34 clinical signs which occur in more than 10% of the 136 cases included in Schinzel's Human Cytogenetic Database [1].

Histopathology (Fig. 2) could be easily interpreted as adenocarcinoma, and the finding of 80% positive cells with Cathepsin E antibodies, coupled with a negative stain for DP2, is consistent with a gastric origin of the tumor [10].

The parental origin of the deleted chromosome has been studied in only two cases of del(18p) in which a paternal origin was demonstrated [11]; in our patient the origin of the anomaly was demonstrated to be in the maternal chromosome by molecular analyses (Figs. 3a, 3b), however, the number of observations is too limited to propose a random parental origin and to exclude a preferential paternal origin of the anomaly as observed for terminal deletions [11].

Gastric cancer is rarely observed in patients below 20 years; a recent correlation with *Helicobacter pylori* (HP) infection has been established [12] and confirmed also for early-onset gastric cancer by Craanen et al. [13]. In our patient, the presence of the infection from HP was demonstrated on the basis of an increased titer of antibodies.

The simultaneous presence of a chromosomal anomaly and a neoplastic disorder in the same patient may, of course, be a random event. However, as both the type of chromosomal anomaly and the type of tumor are quite rare, the alternative hypothesis of a causal relationship should also be taken into account.

Loss of heterozygosity on the long arm of chromosome 18 is frequent in gastric carcinomas and is considered an early event with involvement of the *DCC* gene [5]; in our case, LOH was indeed observed in the tumor mass for loci of the long arm of chromosome 18 (Fig. 3c), and the allele lost was from the maternal chromosome.

The loss of a structurally abnormal chromosome, eventually followed by duplication of the normal chromosome, has been described in somatic cells [14], and will result in LOH. As in our case, the constitutionally abnormal chromosome is of maternal origin, and the alleles lost in tumoral mass are again of maternal origin; we postulate that the presence of a constitutional structural anomaly (the deleted maternal chromosome 18) has favored the loss of the abnormal chromosome in some gastric cell, and may have been an early step in the pathogenesis of the gastric cancer, with HP infection acting as a cofactor.

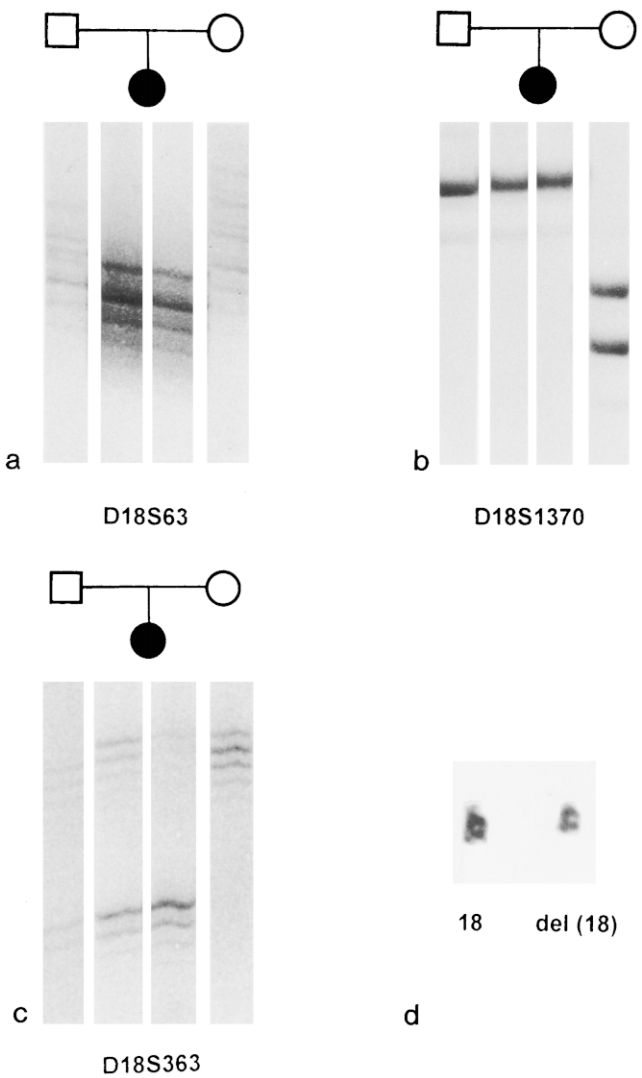


Figure 3 (a, b) The two central lanes refer to normal (left) and tumoral (right) tissue from the patient; for both loci studied, only the paternal alleles are present. (c) LOH at locus D18S363 limited to the tumoral mass (right central lane) of the patient; the allele showing reduced intensity is the maternal one. (d) Partial karyotype of the patient with normal (left) and deleted (right) chromosome 18.

In addition, the short arm of chromosome 18 may harbor gene(s) relevant in the control of cell proliferation, as suggested by the involvement of this region in breast and pancreatic cancers, ependymomas, and in myeloid leukemia [2–4] and confirmed by the recent identification of a repressor of *c-myc* on 18p [15]. It is thus worthwhile to acquire more data on the incidence of cancer in patients with deletions of the short arm of chromosome 18, to verify if they are in fact at increased risk to developing malignancies.

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