High frequencies of ICF syndrome-like pericentromeric heterochromatin decondensation and breakage in chromosome 1 in a chorionic villus sample

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EDITOR—The immunodeficiency, centromeric region instability, and facial anomalies syndrome (ICF) usually involves mutations affecting the catalytic domain in *DNMT3B,* one of the three human genes known to encode DNA methyltransferases.[1–3] ICF always results in defective immunity, a high frequency of chromosomal abnormalities in the vicinity of the centromere (pericentromeric region) of chromosome 1 and/or chromosome 16 in mitogen stimulated lymphocytes, and hypomethylation of a small portion of the genome.[4–6] ICF symptoms are manifested often from infancy and this syndrome can cause early childhood death from infections. The DNA sequences targeted for undermethylation in ICF include the heterochromatin adjacent to the centromeres of chromosomes 1 and 16 (1qh and 16qh), where a high incidence of chromatin decondensation, chromosome and chromatid breaks, and rearrangements to form multiradial chromosomes are characteristically seen in mitogen stimulated ICF blood cultures and in ICF lymphoblastoid cell lines.[6–9] These aberrations are more common in chromosome 1 than in chromosome 16 and only infrequently observed in chromosome 9.[5 8 9] We describe an unusual primary culture from a chorionic villus (CV) biopsy in which a high frequency of ICF-like chromosomal abnormalities was observed. However, follow up indicated that the infant did not have ICF.

A 30 year old, gravida 4, para 0, ab 3 white female was referred for genetic counselling and CV sampling because of a previous pregnancy with trisomy 13. Both the patient and her husband were phenotypically normal and healthy apart from their reproductive history. The patient's first two pregnancies ended in spontaneous abortion at 15 and 10 weeks. No fetal studies had been performed; parental chromosomes were analysed and reported to be normal. The third pregnancy was found to be affected with trisomy 13 (47,XY,+13) and was then terminated. During the fourth pregnancy, the patient was offered and accepted CV sampling for prenatal diagnosis of trisomy conditions.

In this CV sample, routine cytogenetic analysis of 20 metaphases from an eight day culture in Chang B medium with Chang C supplement (Irvine Scientific) showed four cells with pericentromeric breaks in chromosome 1, one with a deletion of the long arm of chromosome 1, and seven with decondensation of 1qh (fig 1B, C). The high frequency of chromosome 1 abnormalities led to the examination of an additional 100 metaphases from the eight day culture. Forty-one percent of the 120 cells examined had abnormalities in the pericentromeric region of chromosome 1. Decondensation of the pericentromeric heterochromatin of chromosomes 1, 16, and 9 was seen in 38, 5, and 0.8% (one cell) of the metaphases, respectively (fig 1). Eleven cells (9%) had chromosome breaks in the pericentromeric heterochromatin of chromosome 1 such that both arms were present but widely separated, one cell (0.8%) had a similar break at 16qh, and one (0.8%) had a pericentromeric break of only one chromosome 1 chromatid. Three cells (2.5%) had a deletion of 1q. In addition, a triradial(1)(p,q,q) was observed (fig 1D).

We passaged these CV cells three times at 1:5 splits after the clinical analysis and examined 100 additional metaphases. The frequency of 16qh decondensation increased from 5% to 26% and decondensation of 1qh increased from 38% to 49% at passage 3 compared to the initial eight day primary culture. At passage 3, there were 11 metaphases with a deletion of 1q, six with a chromosome break at 1qh, two with a multiradial chromosome (a triradial(1)(p,p,q) in one and a quadriradial(1)(p,p,q,q) in the other), one with an isochromosome composed of two pericentromerically fused 1q arms, and one with a deletion of 16q.

Because the ICF syndrome, in which these specific chromosomal anomalies are found, is always accompanied by hypomethylation of limited portions of the genome, including the major DNA sequence in 1qh and 16qh, satellite 2 (Sat2), we examined methylation of chromosome 1 Sat2 DNA sequences in the

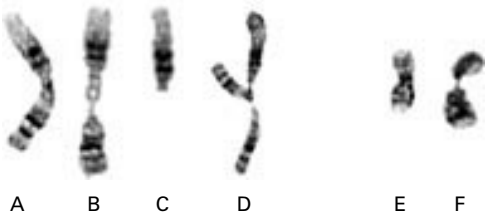

Figure 1 Examples of the different chromosome 1 and 16 abnormalities found in the proband's CV sample after an eight day culture. (A) Normal chromosome 1, (B) decondensation of chromosome 1 at the qh region, (C) whole arm deletion of 1q, (D) triradial(1)(p,q,q), (E) normal chromosome 16, (F) decondensation of 16qh.

J Med Genet 2001;**38**:882–884

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patient's CV sample. This was done as previously described[6] by Southern blot analysis of *Bst*BI digests of DNA from these cell cultures at passage 3 with a Sat2 probe specific for chromosome 1 (fig 2). We found that DNA from the patient's CV sample was hypomethylated in this 1qh sequence compared to normal postnatal somatic tissues. However, the patient's CV DNA was no more hypomethylated than randomly chosen CV samples (fig 2). The undermethylation of Sat2 DNA in these CV samples was the same as that seen in term placenta.[10] The differences in DNA methylation between normal postnatal tissues and CV samples or placenta are consistent with their different cell lineages. Term placenta (from which the embryonic membranes have been removed) and its chorionic villi are mostly derived from extraembryonic cells (see below) as compared to postnatal somatic tissues, which are derived from epiblast cells of the embryoblast (inner cell mass).

One case of ICF had been diagnosed from an amniotic fluid culture in a family known to be at risk because of a previously affected child.[12] The other was diagnosed from a blood sample of a 20 week old fetus who was the sib of an ICF patient.[4] There have been no reports of prenatal diagnosis of ICF by CV sampling, although by linkage analysis ICF was excluded (90% probability) in a CV sample from a family with an affected child.[13]

The present patient was counselled that the cytogenetic findings on the CV sample could reflect a culture artefact, a fetus affected with ICF, or other unknown aetiology. Given the high frequency of ICF-like chromosomal anomalies in the patient's CV cells, a follow up study of amniotic fluid at 16 weeks was done and showed no cytogenetic anomalies. At 39 weeks of gestation, the patient delivered a healthy male infant, weighing 3856 g, with no features of the ICF syndrome. The patient declined a peripheral blood study on the infant but at the current age of 23 months, the child is healthy and thriving.

Sat2 methylation analysis was done on the patient's amniocytes as described above for DNA from the CV cultures. Amniocytes, like postnatal somatic tissues, are derived from epiblast cells of the embryoblast. A high level of methylation of this sequence was seen in DNA from the patient's amniocyte culture as well as in random amniocyte samples (data not shown). These results are incompatible with the patient harbouring ICF type *DNMT3B* mutations because all tested ICF cell populations from diverse tissues or cultured cell types display Sat2 hypomethylation.[4–6 11]

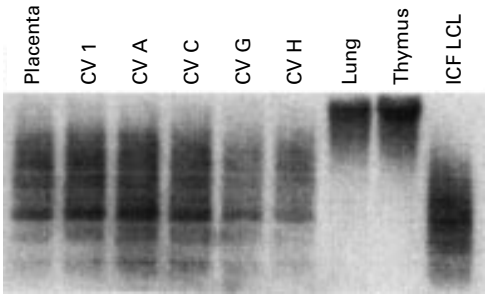

Figure 2 Southern blot analysis showing hypomethylation of Sat2 DNA from chromosome 1 in term placenta, CV samples, and ICF cells but not in postnatal somatic tissues. BstBI digested DNA from the following samples was hybridised to a chromosome 1 Sat2 specific probe and autoradiographed: term placenta; the patient's CV(CV1) culture from passage 3; four random CV samples from passage 3 (CV A, CV C, CV G, and CV H); lung and thymus from normal trauma victims; and a lymphoblastoid cell line (LCL) from an ICF patient.

We compared the frequency of ICF-like pericentromeric chromosome 1 anomalies in this patient's CV sample to others. Retrospective examination of 2250 metaphases from 26 clinical CV samples (50-100 metaphases each) from eight day random CV cultures, which had been interpreted as normal, showed that only 47 of the metaphases (2%) had a pericentromeric abnormality of chromosome 1 or 16. However, the majority of the CV samples (58%) displayed low levels of these anomalies with 1-8% (median 2%) of their metaphases showing these chromosome 1 or chromosome 16 aberrations. Ninety-four percent of these anomalies were decondensation of 1qh or 16qh, with 3.4-fold more 1qh than 16qh decondensation. The only pericentromeric rearrangements seen were two whole arm deletions of 1q and one break at 1qh resulting in separated 1p and 1q arms. No clonal abnormalities except for the chromosome 1 and chromosome 16 aberrations were observed in these metaphases from randomly chosen CV samples. These CV samples were obtained over several years and included samples tested at the same time as the patient's sample and all were analysed by the same method. Also, no change in the lot of medium or fetal calf serum can explain the different results obtained from the patient's sample and the random samples.

Anecdotal observations of these types of pericentromeric chromosome 1 and 16 anomalies in normal CV metaphases are common although, to our knowledge, they have been described by only one group in detail[14] and mentioned by another.[13] In the latter case, Bjorck *et al*[13] stated without elaboration that "heterochromatic decondensation can occasionally be seen in both amniocytes and cultured chorionic villi without any pathological significance." The former group, Miguez *et al*,[14] found in a study of 244 24 hour CV cultures that about 9% of the metaphases displayed chromosomal lesions, usually breaks or gaps at various fragile sites. The most common site was at 1qh (1q12) or 1q21.1 although the quality of the chromosomes allowed only 36% of the preparations to be "successfully banded" and the frequency of cases with this anomaly was not given. In another report, this group described decondensation in 1qh, 9qh, 16qh, or Yqh in 2.4, 3.6, and 0.3, and 0.2%, respectively, of the 5820 examined metaphases, with 47% of 339 24 hour CV cultures displaying such condensation in at least one metaphase.[15] In contrast to those investigators who observed decondensation in 9qh to be most frequent, we found

only a single metaphase exhibiting decondensation of 9qh in this study and that was in the CV sample with the high frequency of 1qh decondensation. This difference between their results and ours could be because of the use of one day rather than eight day cultures. The longer culture time affords better quality metaphase chromosomes but selects for extraembryonic mesoderm cells (which are derived from hypoblast cells of the embryoblast) in the CV samples as opposed to mostly cytotrophoblast metaphases (which are trophoblast derivatives) in the one day CV cultures.[16] Cell type specific differences in the frequencies of chromosome anomalies could also explain why we did not observe chromosomal aberrations in the eight day amniocyte culture from the patient who gave such a high percentage of chromosome 1 anomalies in the corresponding CV culture.

The chromosome 1 (and chromosome 16) pericentromeric aberrations observed in this clinical CV sample may have formed during culture in vitro. However, there was nothing remarkable about the CV tissue used for cell culture or in this culture's hypomethylation in the 1qh region that could explain why it yielded such a high frequency of chromosome 1 anomalies. Whatever the cause of the anomalies in this patient's sample, our analysis of CV samples indicates that ICF-like chromosomal abnormalities are part of the normal spectrum for CV chromosomes and need not indicate any clinical condition. Furthermore, we conclude that CV sampling should not be attempted to prenatally diagnose ICF by the chromosome abnormalities used to diagnose this syndrome in mitogen treated blood samples from immunodeficient patients. In families at risk for ICF, prenatal analysis for *DNMT3B* mutations[13] would be preferable.

This work was partially supported by NIH grant CA 81506 (to ME).

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