

CASE REPORT

First trimester diagnosis of iniencephaly associated with fetal malformations and trisomy 18: Report of a new case and gene analysis on folate metabolism in parents

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ABSTRACT Iniencephaly is a rare congenital malformation consisting of a complex alteration of the embryonic development occurring around the third post-fertilization week and characterized by a hyper-retroflexion of the cephalic pole. We report a case of iniencephaly associated with acrania-encephalocele, spina bifida and abnormal ductus venosus in a fetus with trisomy 18 diagnosed at 12 week's gestation in a 41-year-old woman. A co-occurrence between aneuploidy and iniencephaly was documented and polymorphisms on folate metabolism-related genes were investigated in the parents to assess possible etiologic factors and recurrence risk for neural tube defects (NTD). An homozygous state for the MTRR polymorphism was diagnosed in the mother, identifying a clinical risk for NTD. Once iniencephaly or any other NTD are suspected, genetic analysis, second level ultrasound and fetal karyotype are recommended. Autopsy should also be performed in all cases of early ultrasound-based diagnosis of fetal malformations.

Key Words: autopsy, chromosomal anomalies, iniencephaly, neural tube defects, prenatal ultrasound

INTRODUCTION

Iniencephaly is a rare congenital malformation classified as a neural tube defect (NTD) and characterized by a hyper-retroflexion of the cephalic pole (Gupta *et al.* 1996; Casbay *et al.* 1998). Iniencephaly consists of a complex alteration of the embryonic development occurring around the third post-fertilization week. Its incidence varies from 0.1 to 10/10,000, but it could be higher due to possible misdiagnosis (Lewis 1987). The recurrence risk may be estimated in 1–4% of cases, as for other NTD. Iniencephaly affects female fetuses in 90% of cases. The disease is usually seen in association with other anomalies such as hydrocephaly, Dandy-Walker malformation, encephalo-meningocele, holoprosencephaly, cardiac and renal malformations, diaphragmatic hernia, omphalocele and gastroschisis (Romero *et al.* 1988; Dogan *et al.* 1996). The diagnostic criteria of iniencephaly, suggested by Morocz *et al.* (1986) are: (a) variable defects of the occipital bones resulting in an enlarged foramen magnum; (b) partial or total absence of cervical and thoracic vertebrae with an irregular fusion of those present, accompa-

nied by incomplete closure of the vertebral arches and/or bodies; (c) significant shortening of the spinal column due to the marked lordosis and hyperextension of the malformed cervical–thoracic spine; and (d) upward turned and absence of neck, so that the face and mandibular skin are tightly and directly connected with the chest. The association between aneuploidy and iniencephaly has been under-investigated in the past and few reports can be identified in the medical literature (Coerd *et al.* 1997; Phadke & Thakur 2002; Seller *et al.* 2004; Halder *et al.* 2005). A first-trimester diagnosis of iniencephaly associated with fetal malformations and abnormal karyotype is reported. Analysis of polymorphisms in the folate metabolism-related genes has also been conducted in the parents to identify possible etiologic factors for NTD.

CASE REPORT

A 41-year-old woman, para 3, with regular menses and known last menstrual period, underwent a first-trimester Down's screening test at 12 week's gestation according to the Fetal Medicine Foundation guidelines (Nicolaidis 2004). The ultrasound examination revealed a fetus of 38 mm crown-rump length (CRL) with exacerbated retroflexion of the head, a flat profile and a 'bulging mass' at lumbo-sacral level (Fig. 1). A 'targeted' transvaginal scan (TVS) was then performed and confirmed the finding of acrania-encephalocele, a flat face with absent nasal bones, a kyphoscoliotic spine with large rachischisis and reduced lower limb movements (Fig. 2). Micromelia of the left forearm (Fig. 3), bilateral club foot and ductus venosus with abnormal Doppler A-wave (atrial contraction) profile (Fig. 4) were additional findings. The amniotic fluid was qualitatively increased. A presumptive, early prenatal diagnosis of iniencephaly was made at that time and, following informed consent, fetal karyotype was achieved by a combination of chorionic villous sampling (CVS) plus amniocentesis, using a single needle procedure.

Cytogenetic analysis revealed trisomy 18 (47,XX,+18) (Fig. 5) and elevated amniotic fluid α -fetoprotein (>100 000 ng/mL) levels.

After extensive counselling, the patient opted for termination of pregnancy, achieved by vaginal administration of prostaglandin PGE1 (Cervidil).

Necropsy and postmortem X-ray confirmed the ultrasound finding of acrania, flat face with absent nasal bones, micromelia of the left forearm, club foot and a large spinal defect extending from occiput to sacrum (Fig. 6). No signs of thoraco-abdominal malformations were noted.

Parents underwent phenotypic analysis in order to exclude or confirm a differential diagnosis with Adams-Oliver syndrome (%100300 OMIM) (congenital scalp defects with distal limb reduction anomalies, autosomal dominant (Adams & Oliver, 1945).

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Fig. 1 Transvaginal scan at 12th week of gestation showing acrania with associated encephalocele (ENC) and spinal bifida (SB) (white arrows).

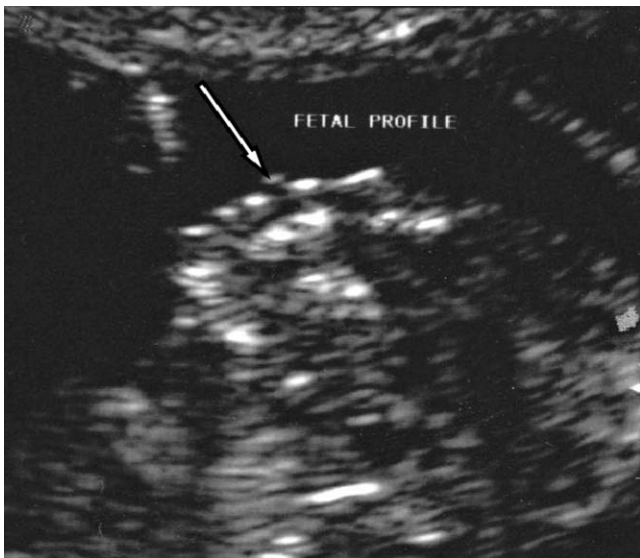


Fig. 2 Transvaginal scan at 12th week of gestation showing the flat face with absent nasal bones (white arrows).

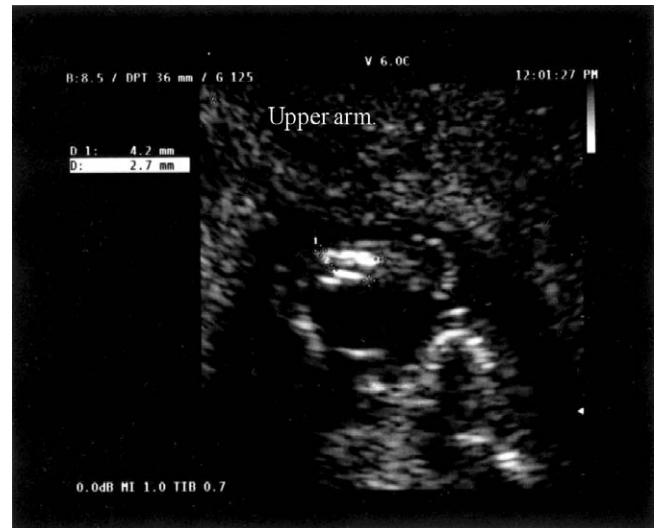


Fig. 3 Transvaginal scan at 12th week of gestation showing micromelia of the left forearm (radius and ulna).

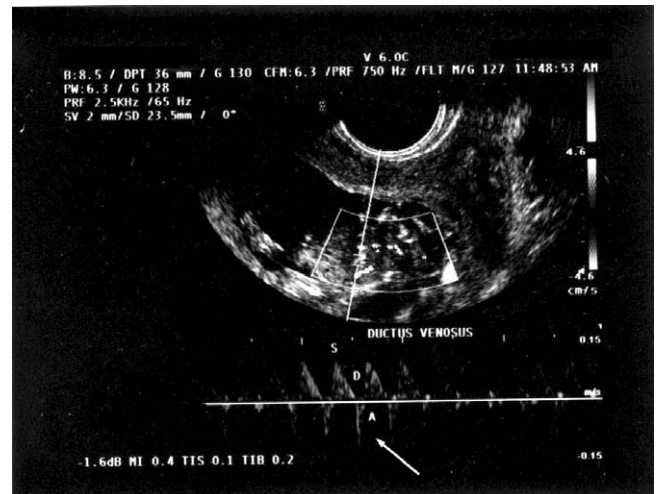


Fig. 4 Transvaginal Doppler waveform analysis at the level of ductus venosus: abnormal signal identified by negative A wave (atrial contraction) (white arrow).

Genes related to folate metabolism (MTHFR, MTRR, CBS) were studied in both parents by investigating the 677 C-T and A1298C polymorphisms transition of the MTHFR, the 66 A-G transition polymorphism of MTRR and the T833C transition and 844Ins68 insertion of CBS gene.

The mother showed a wild type homozygous pattern in the MTHFR polymorphism, a mutated homozygous state for the MTRR polymorphism and a heterozygous state for the CBS 844ins68 polymorphism. Both 677 C-T and A1298C polymorphisms were detected in heterozygous state in the father, while MTRR genotyping showed an homozygous 66 A-G polymorphism. Only wild type CBS alleles were found.

DISCUSSION

Iniencephaly is a rare NTD resulting from a defective neuro-developmental process during the embryonic period. It has been shown experimentally that the onset of iniencephaly (dysraphia or first phase of development of anencephaly) takes place before 28 days post-fertilization (Aleksic *et al.* 1983) and before 18–20 days post-fertilization in cases of anencephaly (O'Rahilly & Muller 1991). Cerebral dysraphia arises probably as a mesenchymal defect before fusion of the neural folds begins and as early as Carnegie stages 8 or 9 because elevation of the folds depends on the production of sufficient mesenchyme.

In iniencephaly, the absence of the initial head deflection in the embryo and the abnormal persistent retroflexion of the head blocks



Fig. 5 Chromosomal map on chorionic villi showing trisomy 18.

the physiological closure of the neural groove in the cervical or superior thoracic areas of the spine.

Iniencephaly, as other NDT, recognizes a genetic heterogeneity pattern, and sporadic or familial cases have been documented. Direct evidence of a link between increased risk of NTD and Down's syndrome in the same family have been recently demonstrated by Barkai *et al.* (2003). In families at risk of NTD, there were a total of 11 pregnancies affected by Down's syndrome in 1492 at-risk pregnancies (compared with 1.87 expected on the basis of maternal age). In the families at risk of Down's syndrome, seven NTD pregnancies were observed in 1847 at risk, compared with 1.37 expected. A Medline search revealed that only a small number of manuscripts addressed or explained the association between NTD and chromosomal abnormalities (Coerd *et al.* 1997; Winsor *et al.* 1997; Hahm *et al.* 1999; Babcook *et al.* 2000; Luo *et al.* 2000; Phadke & Thakur 2002; Doray *et al.* 2003; Seller *et al.* 2004; Thangavelu *et al.* 2004; Halder *et al.* 2005).

Sepulveda *et al.* (2004) reported 144 fetuses with open NTD that underwent prenatal chromosome analysis between 12 and 37 weeks of gestation.

The prevalence of chromosomal abnormality varied according to the defect present in the fetus, with a 14% prevalence among those with cephalocele, 9% among those with spina bifida and 2% among those with lethal defects such as acrania, anencephaly or iniencephaly. Karyotype results revealed trisomy 18 in seven cases, trisomy 13 in two and mosaicism for a marker chromosome in one (Sepulveda *et al.* 2004). In the series reported by Coerd *et al.* (1997), five of the nine cases showed chromosomal abnormalities. Trisomy 18 and triploidy were associated with spina bifida in three cases, trisomy 7 with parieto-occipital encephalocele and monosomy X with spina bifida and iniencephaly in one case.

Furthermore, the C-677 mutation in the 5,10 MTHFR gene has also been reported as a risk factor for spina bifida as it leads to lower homocysteine levels with impaired metabolic pathways involved in embryonic neurodevelopment (Christensen *et al.* 1999; Lucock *et al.* 2000; De Marco *et al.* 2001; Relton *et al.* 2004).

In our case, the wild type MTHFR maternal genotype should not be considered a risk factor either for fetal NTD or trisomy 18. However, the coexistence of polymorphisms in other genes involved in the folate pathway such as MTRR and CBS has been demonstrated. The 68 base pairs insertion in the CBS gene, as well



(a)



(b)

Fig. 6 (a) Necropsy specimen showing acrania with kyphoscoliotic spine and a large, opened spina bifida extending from occiput to sacrum; (b) rear-view of the large spinal defects.

as the homozygous A66G transition in the MTRR gene, have been associated with impaired folate metabolism (Gaughan *et al.* 2001; Ulvik *et al.* 2007). This condition has been linked both to maternal non-disjunction and to NTD occurrence risk (Cavalli & Luongo 2005).

Therefore, even though it seems difficult to predict recurrence risk on the basis of MTHFR, MTRR and CBS genotyping, the possibility that a genetic testing strategy might reveal an impaired folate metabolism in the mother should be considered in prenatal counselling. In fact, these folate-related gene polymorphisms have been associated with impaired folate pathways, and folate metabolism abnormalities are involved both in abnormal meiotic segregation as well as increased NTD occurrence and recurrence risk (Chenet *et al.* 1998).

Al-Gazali *et al.* (2001) suggested that altered folate status plus homozygous mutation in the MTHFR gene in the mother could promote chromosomal instability and meiotic non-disjunction resulting in trisomy 21. Our case-report showed that trisomy 18 and

iniencephaly were found associated in the same pregnancy. As both maternal non-disjunction and NTD have been linked to reduced folate metabolism, an investigation of the folic acid pathway should be considered in trisomy pregnancies and/or in cases of NTD. The ultrasound findings were consistent with the diagnostic clusters for iniencephaly as previously described by Foderaro *et al.* (1987). Necropsy confirmed the anatomical lesions diagnosed by ultrasound and compared to sonography, was more precise in the definition of the severity of the spinal defect extending from occiput to sacrum. No signs of thoraco-abdominal malformations were noted and X-ray confirmed the skeletal malformations seen on ultrasound.

The study of the case and the revision of the literature indicate that: (i) gene testing for folate metabolism should be carried out in the mother/parents and/or fetuses when investigating for clinical risk factors for NTD; (ii) the application of this protocol led to the identification of the possible causative mechanism of iniencephaly; (iii) once iniencephaly is diagnosed, a second level scan and fetal karyotype should be carried out because the association between NTD and chromosomal anomalies have been under-investigated until now; (iv) although ultrasound has a high accuracy in identifying NTD or the presence of associated anomalies that can suggest an underlying chromosomal defect, an autopsy is recommended in all cases of early ultrasound-based diagnosis of fetal malformations.

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