SONOGRAPHIC ASSESSMENT OF NORMAL AND ABNORMAL FETAL DEVELOPMENT; EARLY AND LATE ASPECTS

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SONOGRAPHIC ASSESSMENT OF NORMAL AND ABNORMAL FETAL DEVELOPMENT; EARLY AND LATE ASPECTS

Echoscopische beoordeling van de normale en abnormale foetale ontwikkeling; vroege en late aspecten

Proefschrift

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Ter nagedachtenis aan mijn grootvader,
J.H. Kramer:
'AGE QUOD AGIS',

en daarom aan mijn kinderen:
Kasper, Roman en Fenna.

CONTENTS

Part two
Late fetal anomaly scanning:
second and third trimester of pregnancy

This thesis consists of two parts.

The first part deals with an increasingly important area of first and early second trimester (<14 weeks) normal and abnormal fetal sonoanatomy and its validity and impact in the early diagnosis of fetal anomalies in a high-risk population.

The second part of the thesis focuses on the clinical genetic aspects of fetal anomaly scanning with emphasis on the second half of gestation. Single fetal anomalies diagnosed by ultrasound may be associated with other and often minor anomalies, which pre- or postnatally may be identified as exogenic or genetic syndromes with specific recurrence risks. This will have important implications for genetic counseling.

PART ONE

SONOGRAPHIC ASSESSMENT OF EARLY NORMAL AND ABNORMAL FETAL DEVELOPMENT (< 14 weeks)

CHAPTER 1
INTRODUCTION

1.1 Early normal and abnormal fetal sonoanatomy (<14 weeks)

With the introduction of high-resolution two-dimensional ultrasound technology and the possibility of transvaginal scanning, there has been increasing interest in normal and abnormal fetal development as early as 6-14 weeks of gestation[1-24]. Considerable knowledge has been acquired, both regarding early fetal anatomy and biometry and early fetal hemodynamics as a result of the introduction of high-resolution transvaginal and transabdominal 2D real-time and colour-coded Doppler techniques and power angiography[25-33]. Lately, some reports appeared on three-dimensional ultrasonography and fetal anatomy as early as the late first trimester of pregnancy[32-36].

Sonographic identification of abnormal fetal anatomy is determined by a number of factors such as detailed knowledge of embryology and normal fetal sonoanatomy, extensive scanning experience both in transabdominal and transvaginal sonography and the availability of high-resolution ultrasound equipment.

Fetal anomalies may be caused by an exogenic, multifactorial, chromosomal or monogenic (autosomal recessive, autosomal dominant, X-linked) disorder. Some autosomal recessive malformation syndromes may show severe abnormalities enabling an early sonographic diagnosis as in some cases of Meckel syndrome. Variability of expression of a syndrome may preclude early diagnosis. Insight into the phenotypic variability of fetal malformation or dysmorphology syndromes is essential for making an accurate diagnosis during early fetal anomaly scanning and to be able to inform and counsel the parents correctly.

The introduction of routine scanning at 11-14 weeks of gestation in The Netherlands is currently subject to debate. This does not only include fetal dating and the establishment of chorionicity in twin pregnancies but also fetal nuchal translucency. Increased fetal nuchal translucency thickness at 11-14 weeks of gestation, is a common phenotypic expression of fetal trisomies[37], but is also associated with a wide range of fetal defects including genetic syndromes[38,39]. Alternatively, early fetal anomaly scanning would also allow detection of gross anomalies not associated with increased nuchal translucency[22].

The introduction of an 11-14 week fetal scan, including the nuchal translucency measurement, could also be seen in a wider perspective, i.e. the

clinical efficacy and public acceptance of maternal biochemical screening for Down syndrome as early as 9-14 weeks of gestation[40-44].

Similar to the 18-21 week fetal anomaly scan, a reliable early sonographic diagnosis will, amongst others, depend on the availability of detailed dysmorphological and post mortem findings of a previously affected pregnancy or diagnostic evaluation of a living index patient and information on the family history.

Termination of pregnancy may be requested by the patient in the presence of a severe fetal anomaly. Termination before 14 weeks of gestation is usually carried out through aspirotomy and thus may pose a problem as to the suitability of fetal material for post mortem examination. Availability of the intact fetal specimen will not only be essential for confirmation of the sonographic findings, but will also allow additional examinations to further establish the diagnosis, if necessary. It will depend on the skills of the gynaecologist and the availability and expertise of the fetal pathologist whether the quality of the fetal material acquired will permit a proper examination. However, with the increasing availability of cytogenetic and DNA methodologies for confirmation of a sonographic-dysmorphological diagnosis, there will probably rarely be a need to choose for a prostaglandin-induced termination in the future. A firm diagnosis will be important when counseling regarding the recurrence risk and diagnostic options in a future pregnancy is requested. Sonographic findings may be invaluable when post mortem examination is not permitted by the parents or is technically not possible in case of aspirotomy or maceration.

In conclusion, the potential of late first trimester and early second trimester fetal anomaly scanning is dependent on the intricate relationship between high quality ultrasound scanning and expertise, detailed knowledge of exogenic and genetic syndromes (congenital anomalies) and associated inheritance patterns, and the availability of a well-qualified fetal pathology unit.
In the first part of this thesis, the following questions will be addressed:

1. What is the aspect of normal fetal sonoanatomy at 11-14 weeks of gestation;
2. To what extent can early ultrasonography contribute to the identification of abnormal fetal anatomy;
3. What is the validity and impact of early fetal anomaly scanning in a high-risk population.

1.2 A literature review of early normal and abnormal fetal sonoanatomy (<14 weeks)

The most striking advances in human development occur in the first eight weeks following conception. The 23 developmental stages (Carnegie stages) of the human embryo have been described extensively[45]. The embryonic period is of particular importance because most congenital anomalies appear during that time. The transition from the embryonic period to the fetal period is arbitrary. In embryological literature the fetal period starts at nine weeks following conception, which is at 11 weeks of gestation based on the first day of the last menstrual period.

Ultrasonography is a method to investigate the *living* human embryo and fetus. The developmental stages of the human embryo and fetus as recognized by embryologists are not based on living embryos. To be able to evaluate the abnormally developing early pregnancy, knowledge of the normal human embryologic development *in vivo* is essential. The first systematic descriptive study on transvaginal sonographic assessment of normal embryonic and fetal development at 8-14 weeks of gestation was published in 1988[2]. This was followed by a practical method of determining the correct gestational age by transvaginal ultrasound at 4-12 weeks of gestation by the sequential appearance and disappearance of certain structures[3]. The growth of the human embryo was studied by several workers[7-9,16] but only once longitudinally[16]. As the brain dominates the sonographic appearance of the embryo, this was subject to several studies[4,11,12]. Other structures that have been studied in detail in early normally developing pregnancies are the anterior abdominal wall[1,13], the stomach[13], the heart[5,13], the skeleton[10,15,17] and the urinary tract[14]. Blaas and Eik-Nes[18] published the chronologic development of the human embryo based on the embryological literature together with studies using transvaginal sonography.

Based on these data, the following structures or functions are detectable by *high-frequency* (transvaginal) ultrasound at a given gestational age in the majority of normally developing embryos and fetuses:

- 4^{0-6} weeks: gestational sac
- 5^{0-6} weeks: yolk sac, embryonic pole, heart beat (100 beats per minute (bpm))
- 6^{0-6} weeks: heart beat always identifiable (105-130 bpm), rhombencephalic cavity in the cranial pole of the embryo
- 7^{0-6} weeks: brain cavities of which the rhombencephalic cavity on top of the embryo is the largest, separation of the cerebral hemispheres, limb buds, heart beat 130-160 bpm
- 8^{0-6} weeks: brain cavities (diencephalon, mesencephalon, rhombencephalon), spine (two small parallel echogenic lines), slight movements of the body and limbs

- 9^{0-6} weeks: falx, lateral ventricles, choroid plexus, facial profile, mandible, maxilla, ventral body wall, midgut herniation, fingers and toes, genital tubercle
- 10^{0-6} weeks: midgut herniation, elongation of limbs, heart: atrioventricular valves, stomach
- 11^{0-6} weeks: ossification of the skull and spine, cerebellum, cardiac anatomy, kidneys and bladder are visible, herniated bowel returns to abdominal cavity

An increasing number of structures can be visualized by sonography with advancing gestational age. The optimal gestational age for a *complete* anatomical survey is at 13 weeks[5,6,19,46]. This is also considered the optimal gestational age for nuchal translucency measurement[46].

Although transvaginal sonography provides better images of the embryo or fetus than transabdominal sonography, the two methods are complementary. Transvaginal sonography has a limitation of scanning planes and sometimes anatomical or positional limitations (anteverted uterus, position of placenta or vertex position of the fetus)[47,48].

A vast amount of case reports on abnormal development in early pregnancy has been published in the past years, which will not be reviewed here. The majority of the studies on abnormal development have been carried-out at a gestational age (10-14 weeks) which is more advanced than that in studies on normal anatomy/development.

Reviews[22,38] on fetal abnormalities diagnosed at 10-14 weeks of gestation establish the possibility of diagnosing anomalies of many organ systems: central nervous system/neural tube defects (anencephaly, exencephaly, iniencephaly, encephalocele, spina bifida, holoprosencephaly, etc), cardiac defects, diaphragmatic hernia, abdominal wall defects (omphalocele, gastro-schisis, body stalk anomaly), renal (bilateral renal agenesis, hydronephrosis, etc) and skeletal (achondrogenesis type II, thanatophoric dysplasia) anomalies. Next to this, several different syndromes, for example Meckel Gruber syndrome, Fryns syndrome, Roberts syndrome, Jarcho-Levin syndrome and Smith-Lemli-Opitz syndrome, have been diagnosed as early as the late first to early second trimester of pregnancy. Again, variable phenotypic expression of syndromes has to be taken into consideration in high-risk patients. One has to bear in mind that a single ultrasound scan will not detect all fetal anomalies as some anomalies develop as late as the second or third trimester of pregnancy.

The psychological implications of diagnosing fetal anomalies in early pregnancy have not yet been fully assessed. There is probably little difference in the psychological morbidity between late and early pregnancy terminations for fetal anomalies[24].

Conclusions:

- The optimal gestational age for a complete anatomical survey by ultrasound and for nuchal translucency measurement is 13 weeks.
- The majority of structural and chromosomal anomalies can be diagnosed during the late first to early second trimester of pregnancy.
- The potential of late first to early second trimester anomaly scanning is dependent of the expertise of the sonographer, the availability of high-resolution ultrasound equipment and detailed knowledge of congenital anomalies, their possible variable phenotypic expression and their inheritance patterns.
- A single ultrasound scan in pregnancy will not detect all fetal anomalies. Therefore, the standard second trimester scan should not be abandoned.

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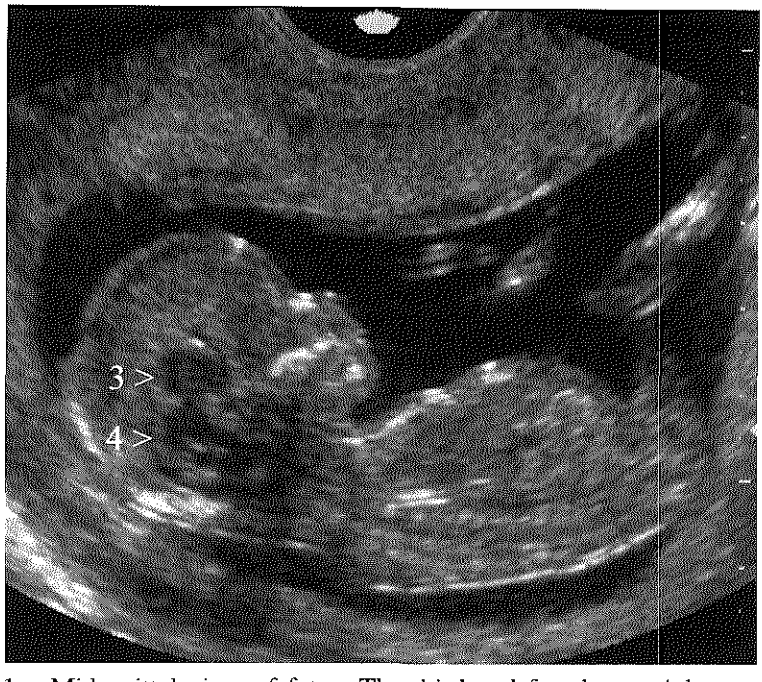
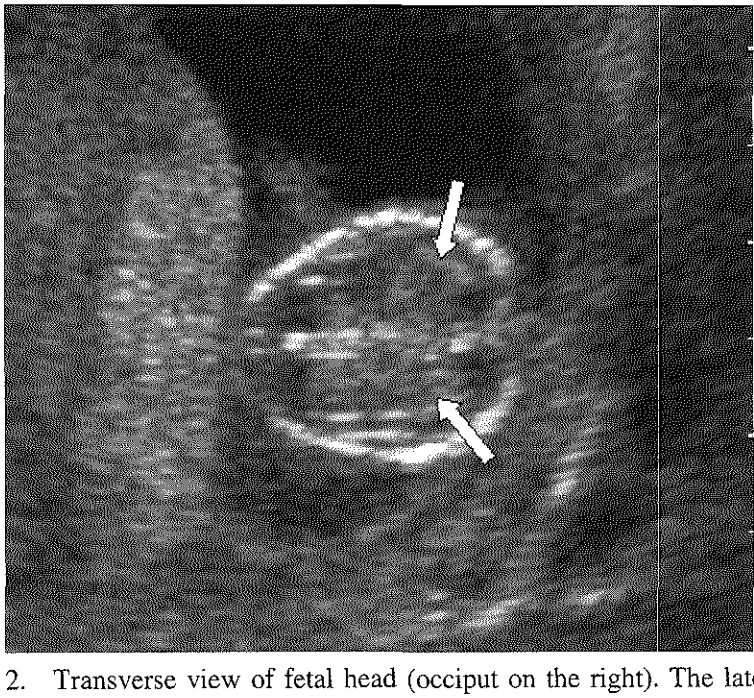
1.4 APPENDIX

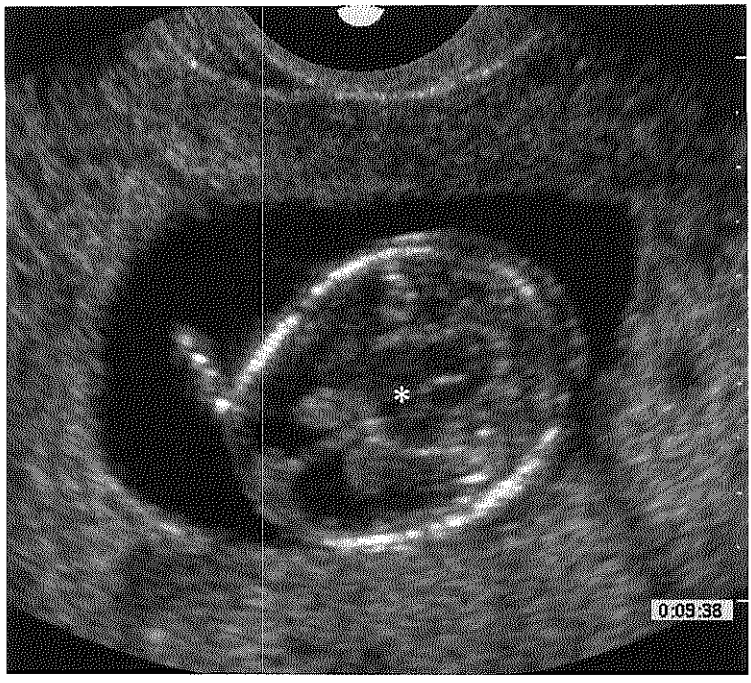
Various aspects of normal and abnormal fetal sonoanatomy; a display of images



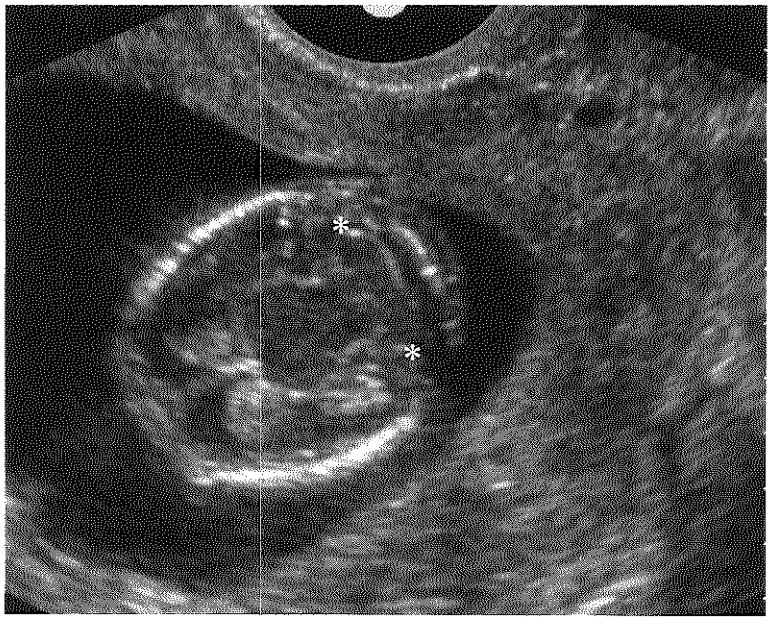
1. Midsagittal view of fetus. The third and fourth ventricles are marked; 13^{6} weeks of gestation.



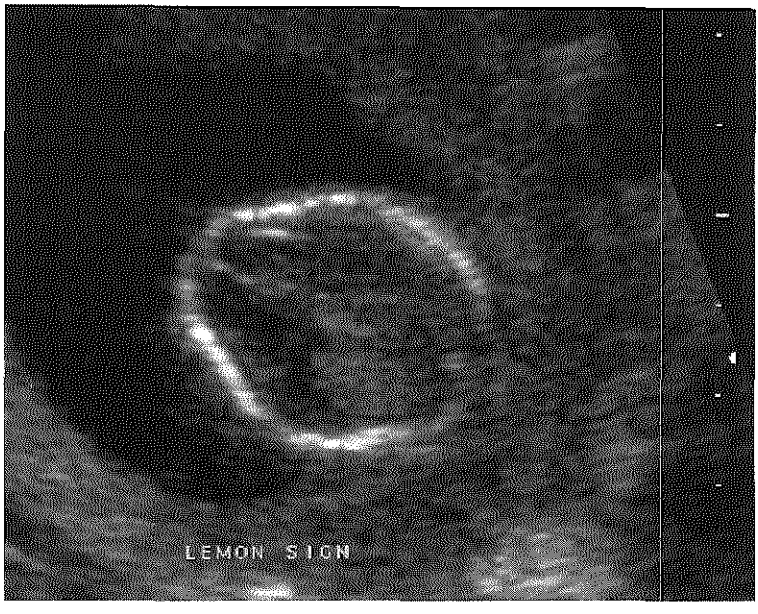
2. Transverse view of fetal head (occiput on the right). The lateral ventricles are completely filled with choroid plexus (arrows); 13 weeks of gestation.



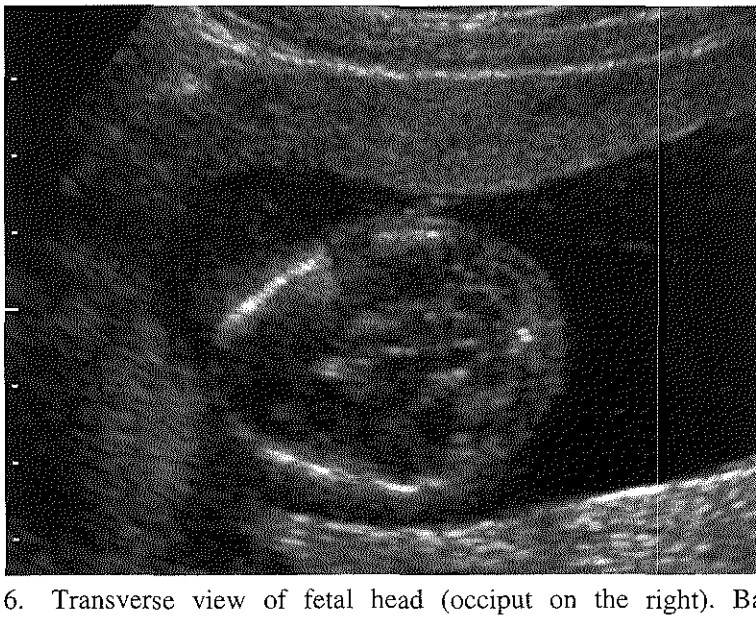
3. Transverse view of fetal head (occiput on the right). Thalamus (*); 13^{6} weeks of gestation.



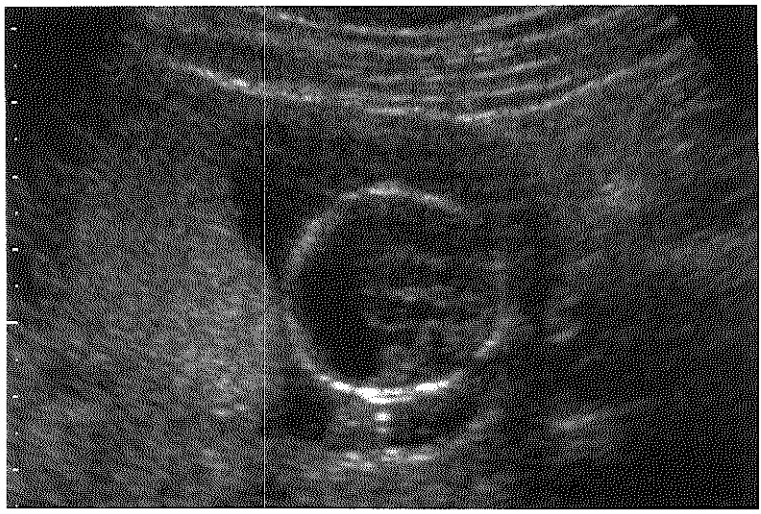
4. Transverse view of fetal head (occiput on the right). Cerebellum (**); 13 weeks of gestation.



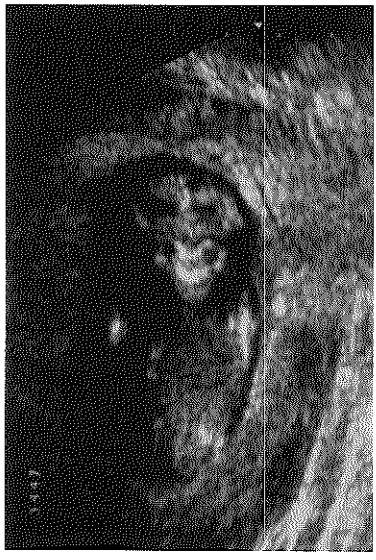
5. Transverse view of fetal head (occiput on the right). Lemon shaped skull, i.e. 'lemon sign', in a fetus with severe lumbar kyphosis (photograph 16); 14 weeks of gestation.



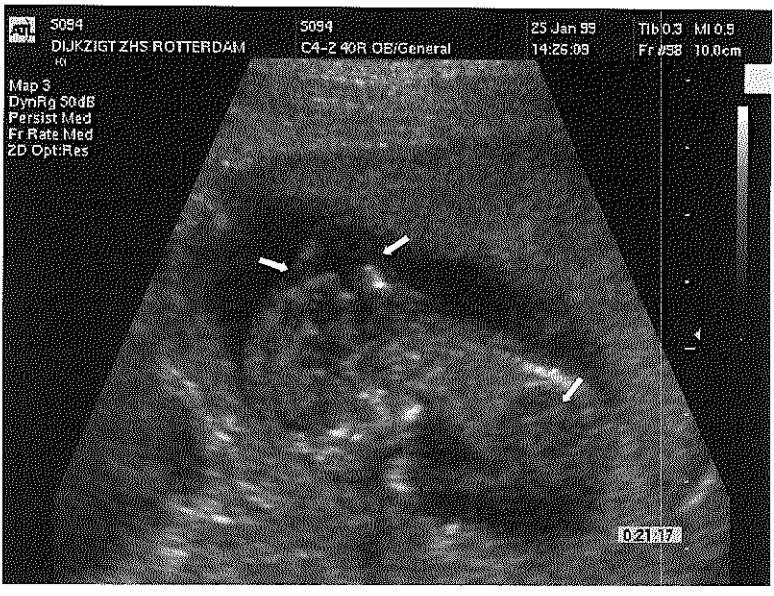
6. Transverse view of fetal head (occiput on the right). Banana shaped cerebellum, i.e. 'banana sign', (same fetus as in photograph 5 and 16); 14 weeks of gestation.



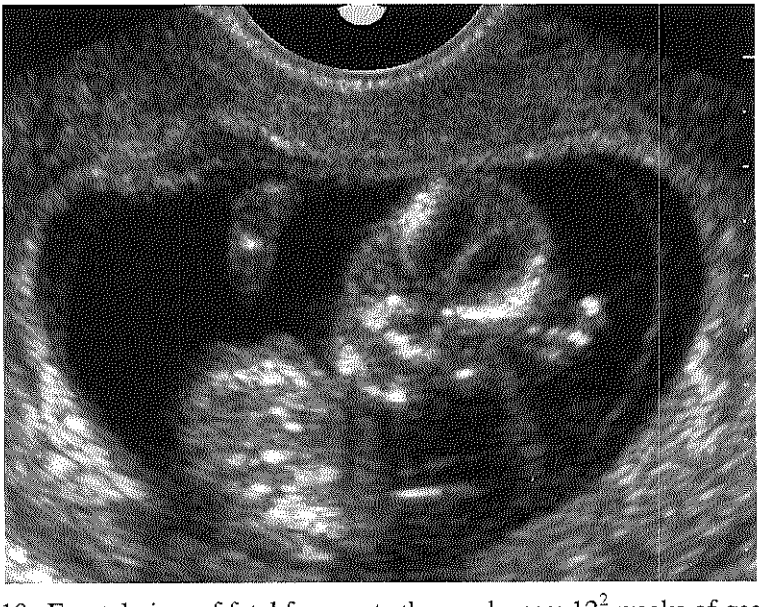
7. Alobair holoprosencephaly, note the monoventricle and absence of the midline; 14 weeks of gestation.



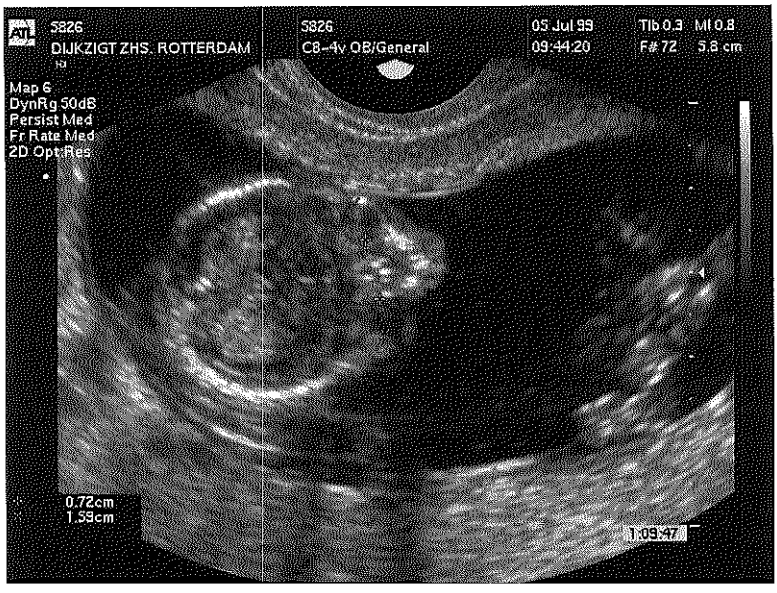
8. Frontal view of fetus with exencephaly; 12 weeks of gestation.



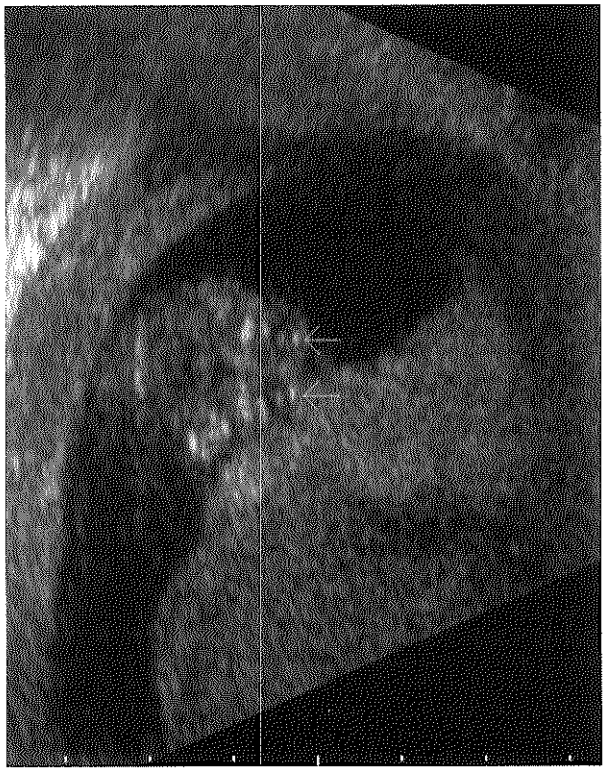
9. Longitudinal view of fetus with cystic cephalocele (arrows) associated with Meckel syndrome (a transverse view of the polycystic kidney (arrow) is shown in photograph 33); 13 weeks of gestation.



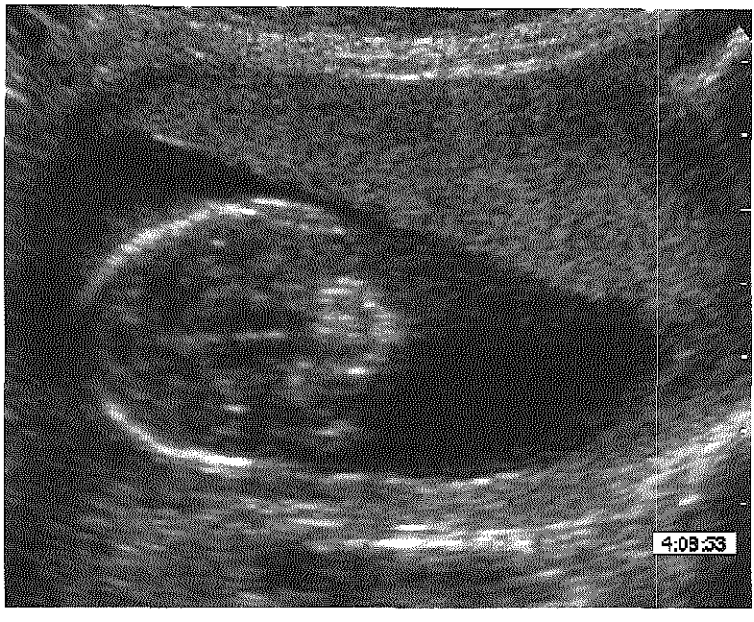
10. Frontal view of fetal face: note the eye lenses; 12^2 weeks of gestation.



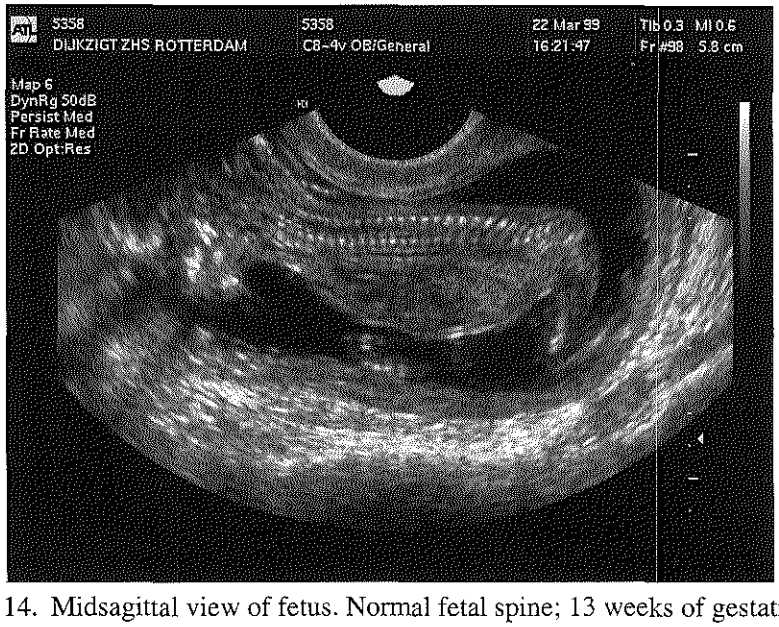
11. Transverse view of fetal head (occiput on the left). Measurement of orbitae; 13^3 weeks of gestation.



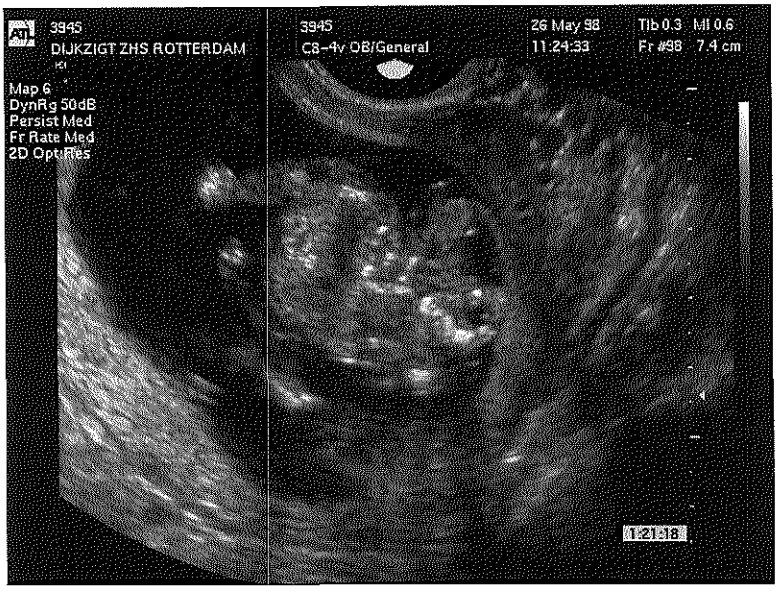
12. Transverse view of fetal head (occiput on the left). Hypotelorism in a fetus with holoprosencephaly associated with trisomy 13; 13 weeks of gestation.



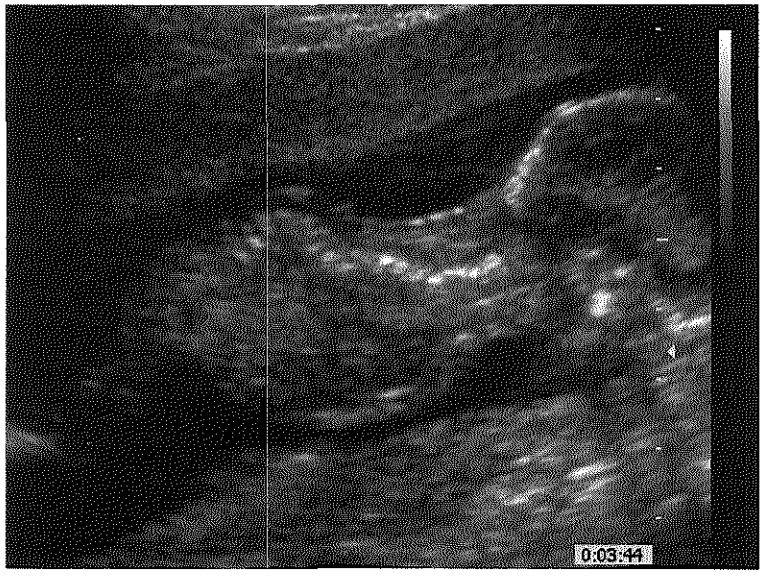
13. Hypertelorism in a fetus with severe facial anomalies associated with amniotic band syndrome (subchapter 2.1.1; Table 4A, patient 5); 14^2 weeks of gestation.



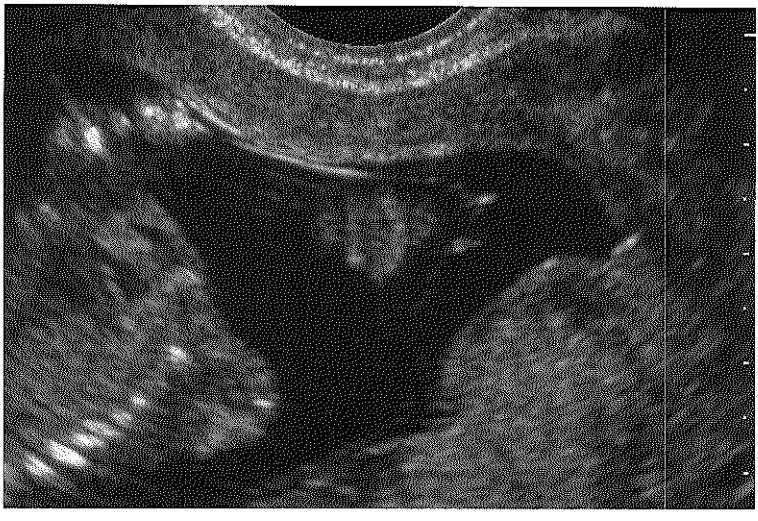
14. Midsagittal view of fetus. Normal fetal spine; 13 weeks of gestation.



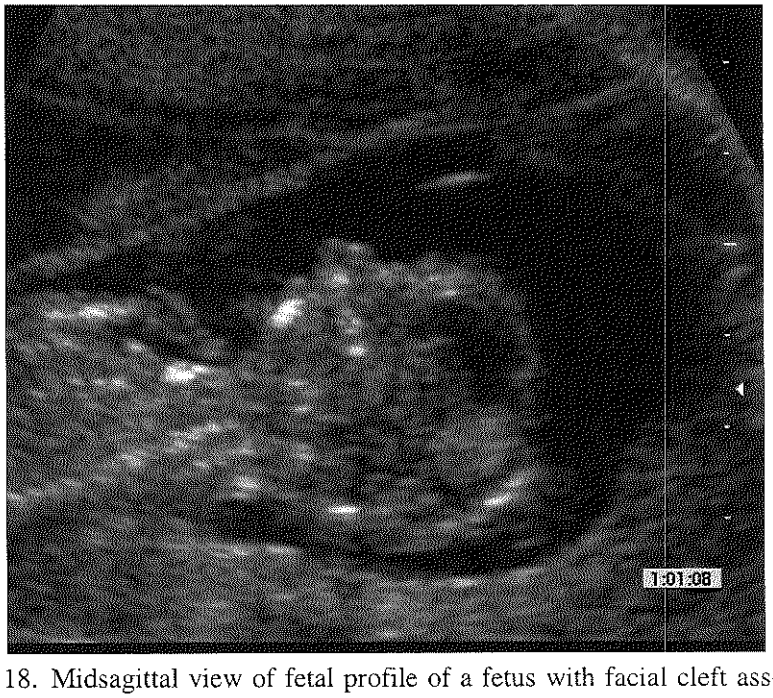
15. Midsagittal view of fetus. Fetus with iniencephaly; 12 weeks of gestation.



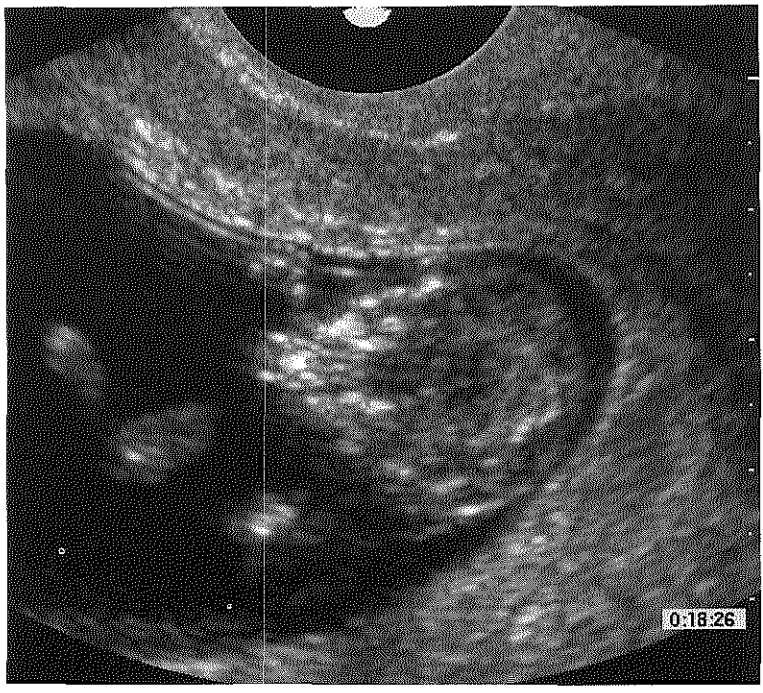
16. Fetus with severe lumbar kyphosis (see also lemon sign and banana sign in this fetus, photographs 5 and 6); 14 weeks of gestation.



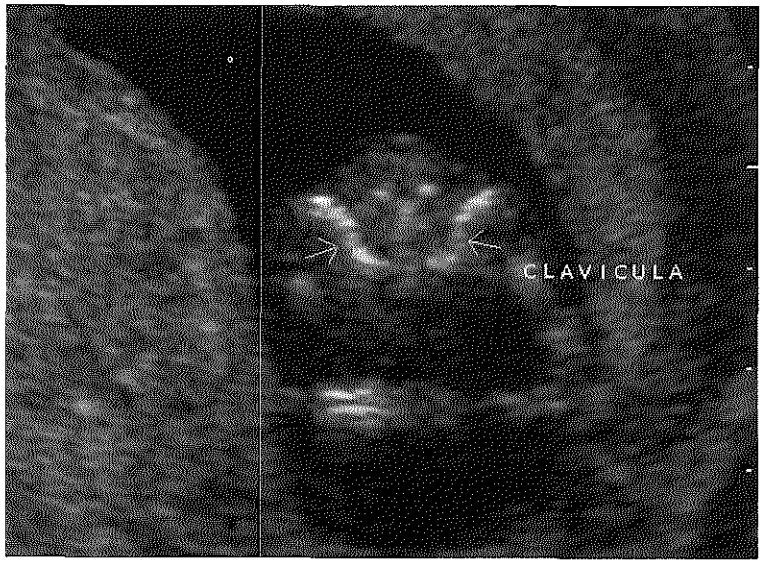
17. Frontal view of fetal nose, lips and chin; 14 weeks of gestation.



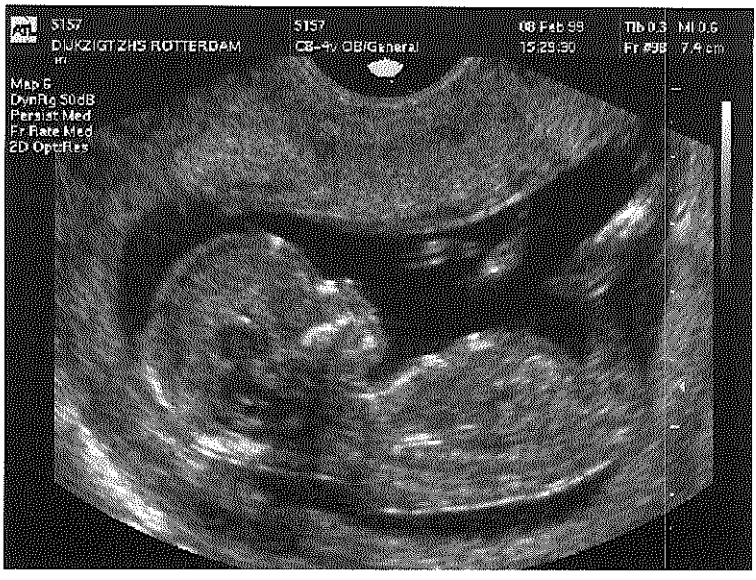
18. Midsagittal view of fetal profile of a fetus with facial cleft associated with an abnormal karyotype (unbalanced translocation); 14 weeks of gestation.



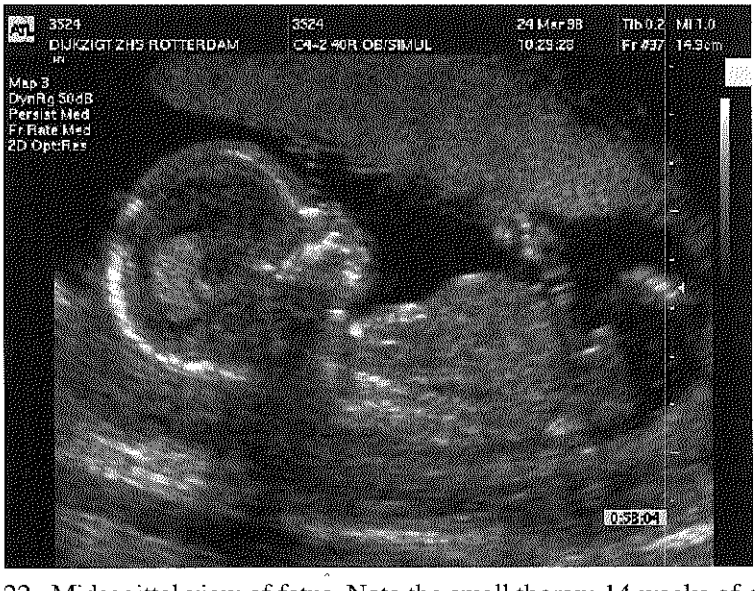
19. Transverse view of fetal head (occiput on the right) of a fetus with bilateral cheilognathopalatoschisis associated with trisomy 13; 12 weeks of gestation.



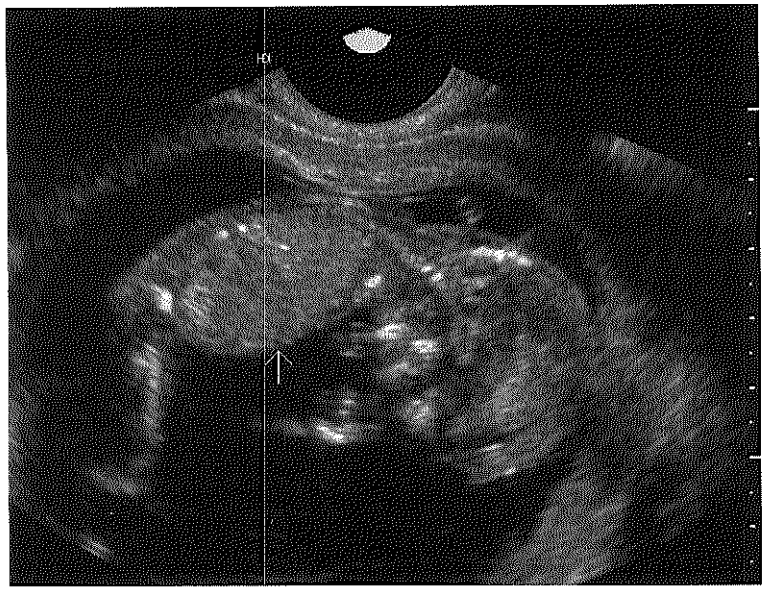
20. Transverse view of fetal upper thorax showing the clavicles; 13 weeks of gestation.



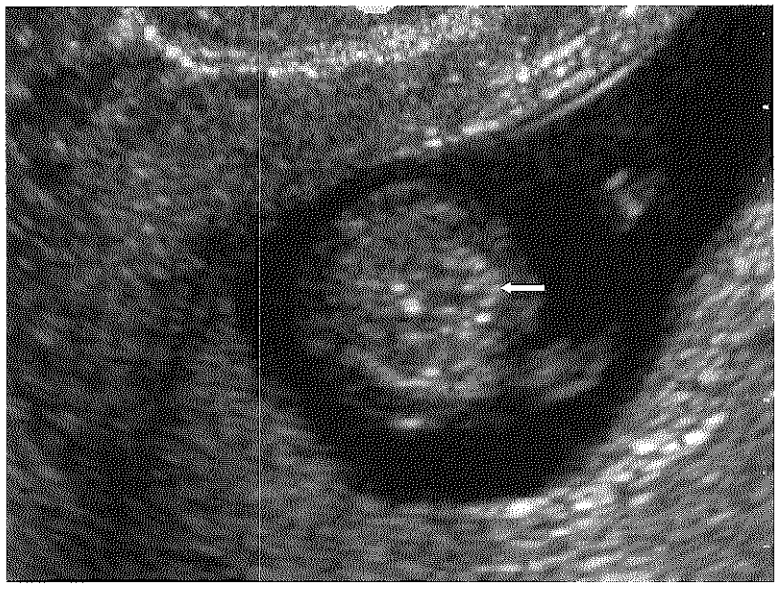
21. Midsagittal view of fetus. Note the thorax; 13^{0} weeks of gestation.



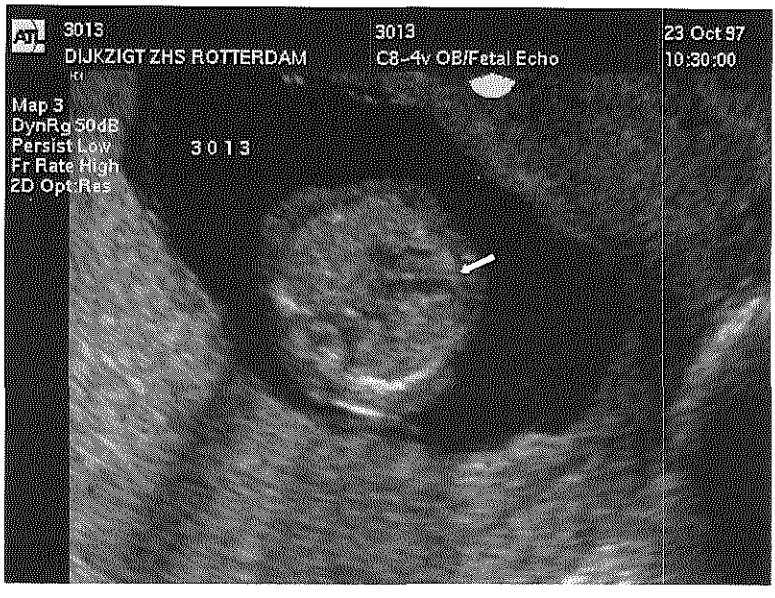
22. Midsagittal view of fetus. Note the small thorax; 14 weeks of gestation.



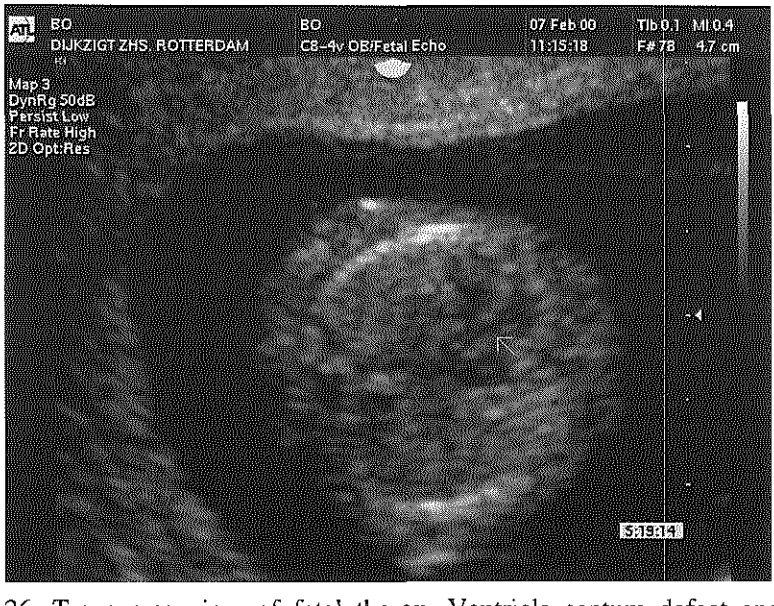
23. Midsagittal view of fetus. Diaphragm, note the lungs and the liver; 13 weeks of gestation.



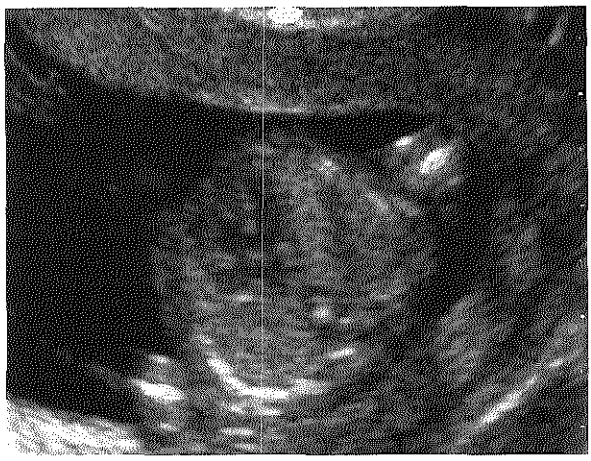
24. Transverse view of fetal thorax. Four chamber view (apex is marked); 12 weeks of gestation.



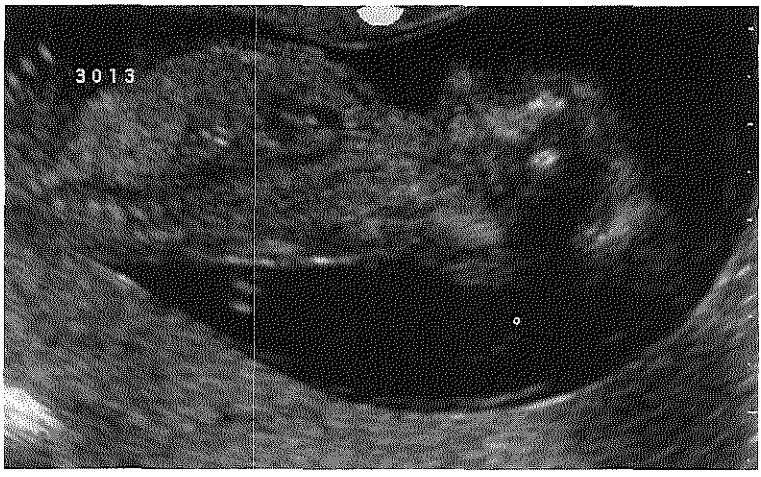
25. Transverse view of fetal thorax. Four chamber view (apex is marked); 13^4 weeks of gestation.



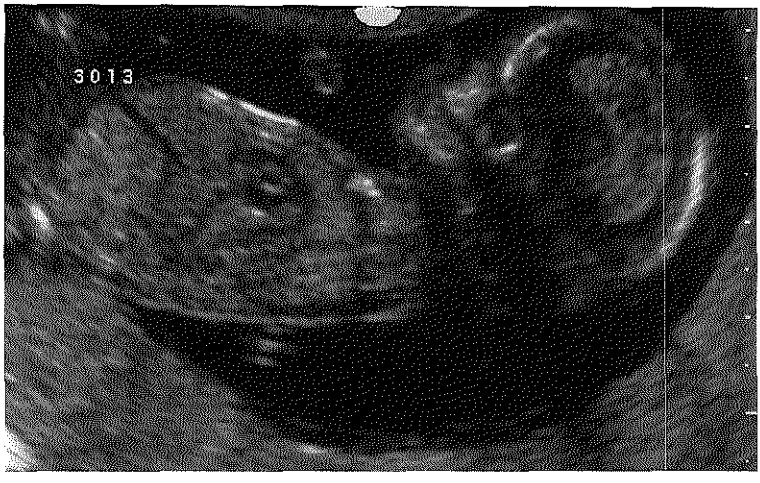
26. Transverse view of fetal thorax. Ventricle septum defect and overriding aorta (arrow); 12^2 weeks of gestation.



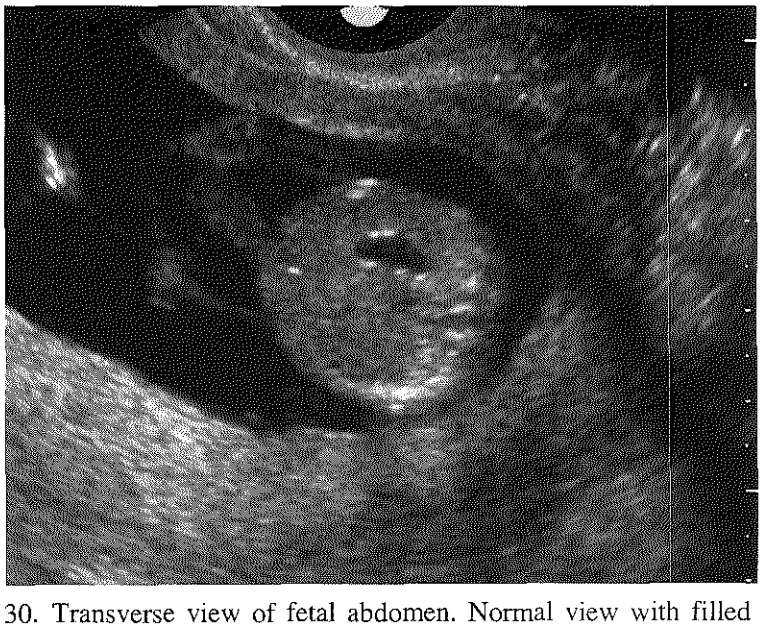
27. Transverse view of fetal thorax. Ventricle septum defect; 14 weeks of gestation.



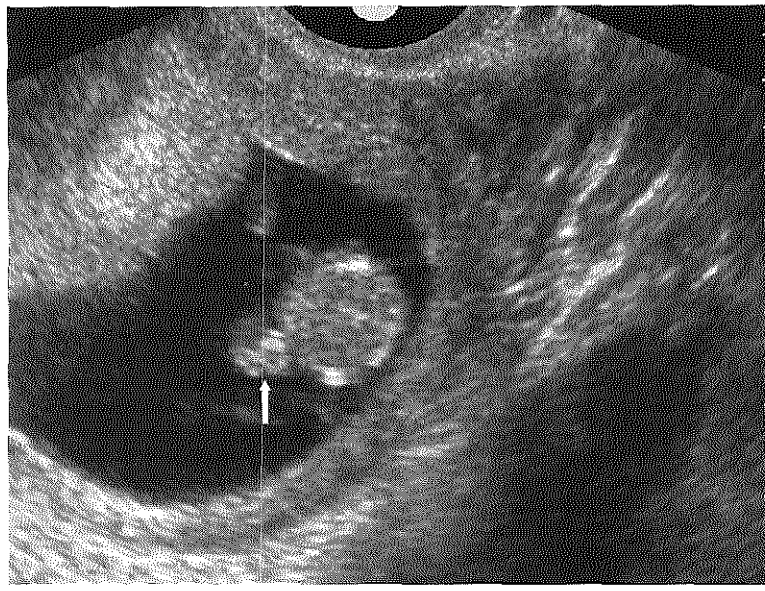
28. Sagittal view of fetus showing the aortic arch; 13^4 weeks of gestation.



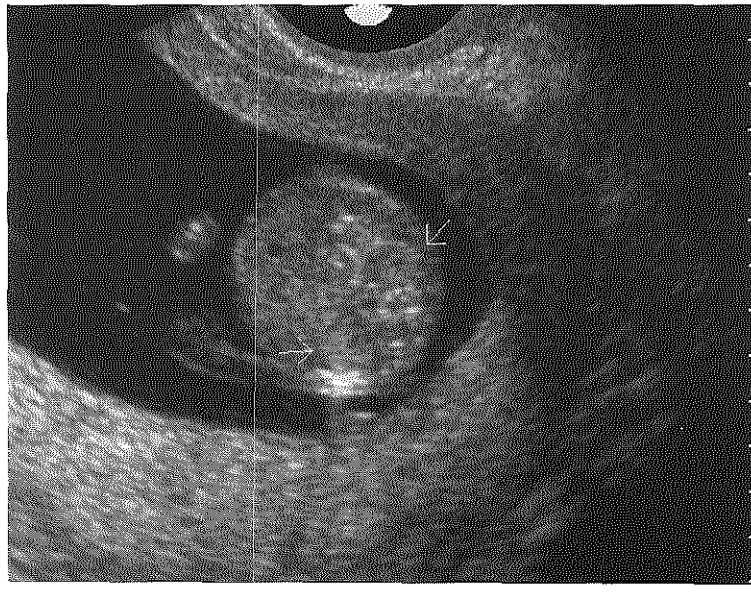
29. Sagittal view of fetus showing the short axis view of the heart; ductus arteriosus; 13^4 weeks of gestation.



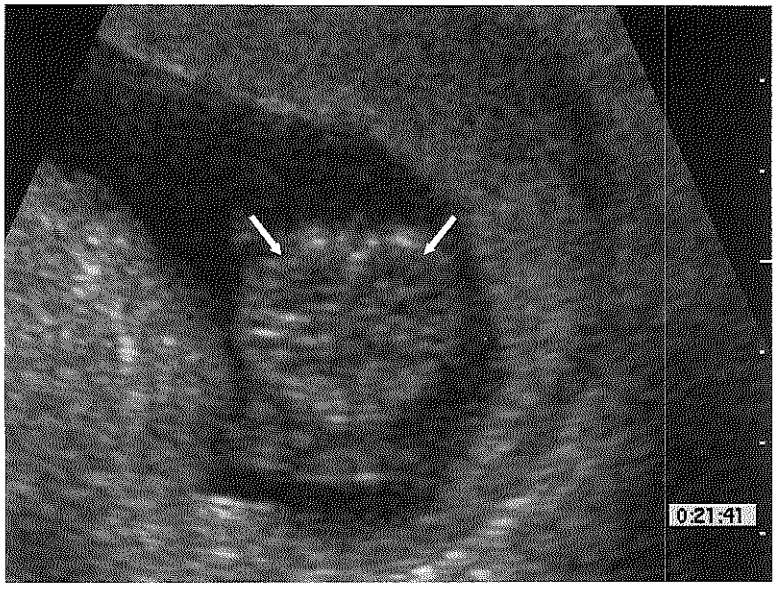
30. Transverse view of fetal abdomen. Normal view with filled stomach; 13^6 weeks of gestation.



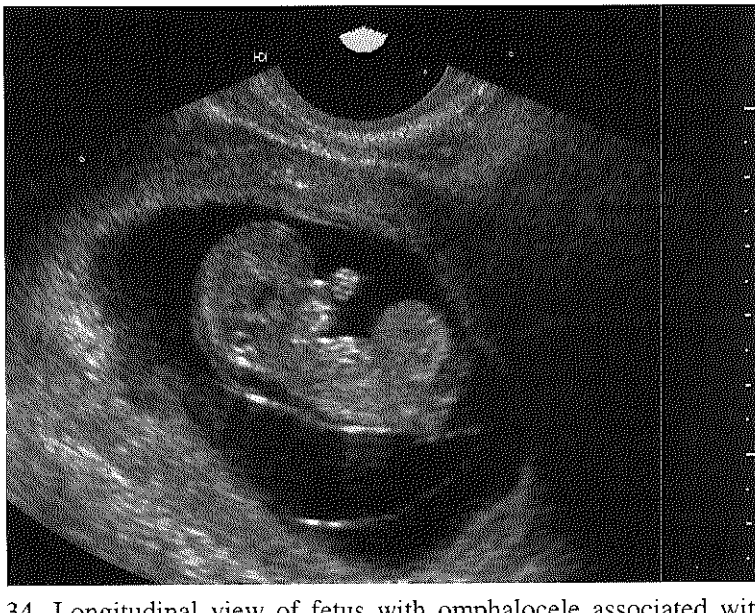
31. Transverse view of fetal abdomen. Physiological hernia (arrow); 10^5 weeks of gestation.



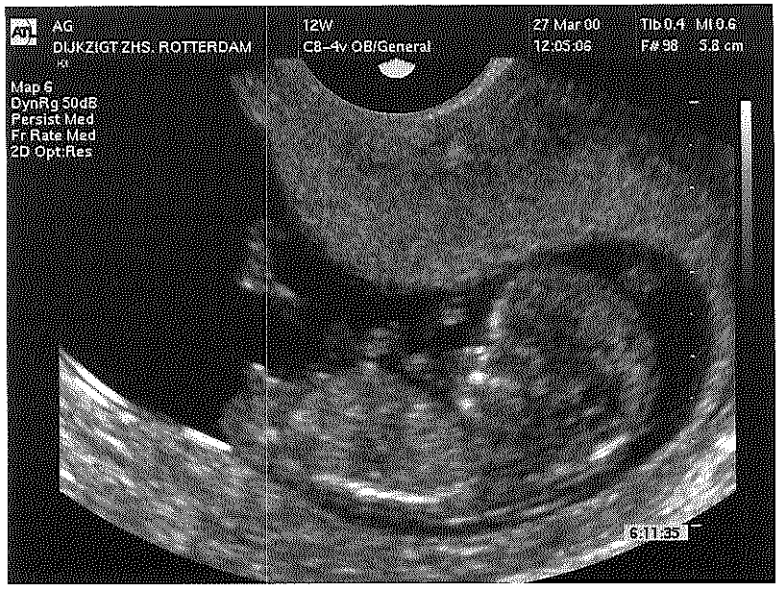
32. Transverse view of fetal abdomen. Normal kidneys (arrows); 13^6 weeks of gestation.



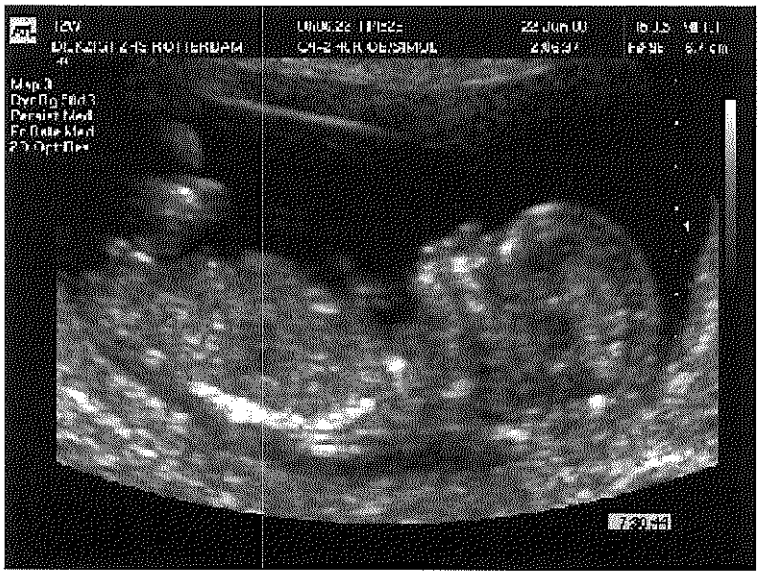
33. Transverse view of fetal abdomen. Polycystic kidneys (arrows) in a fetus with Meckel syndrome; 13 weeks of gestation.



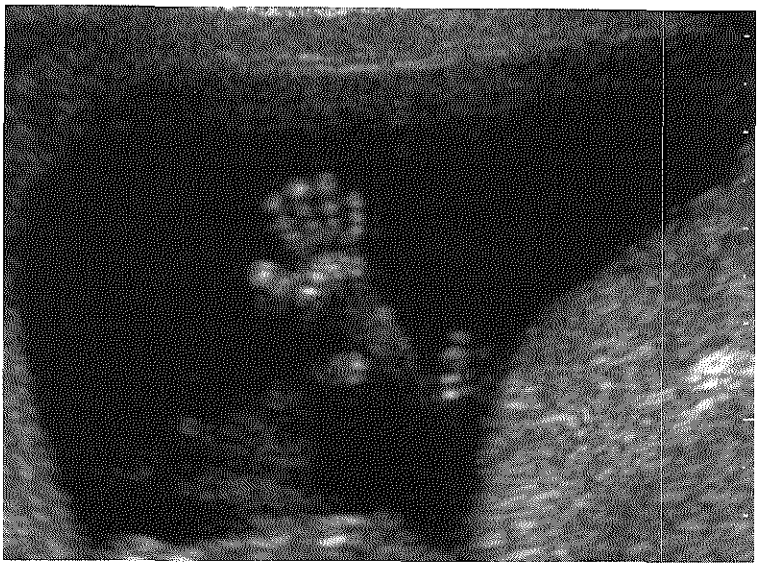
34. Longitudinal view of fetus with omphalocele associated with trisomy 18; 12^3 weeks of gestation.



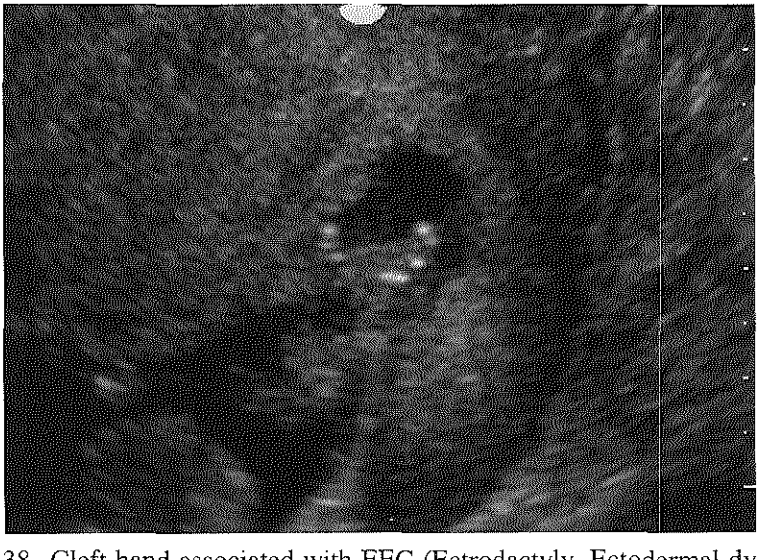
35. Midsagittal view of fetus. Male fetus; 12 weeks of gestation.



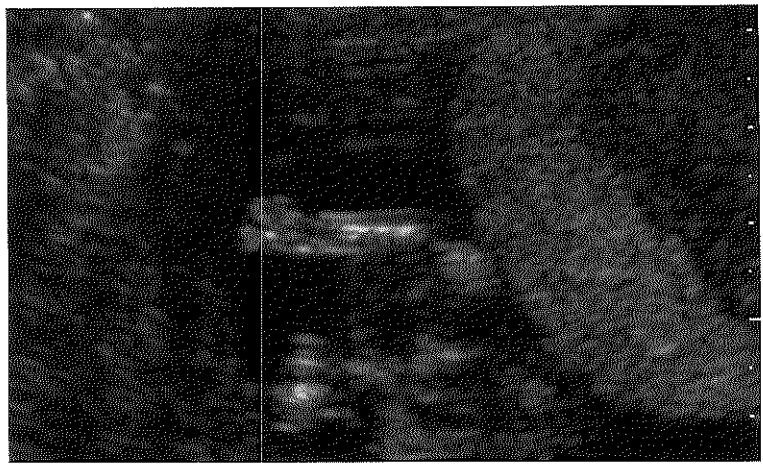
36. Midsagittal view of fetus. Female fetus; 12 weeks of gestation.



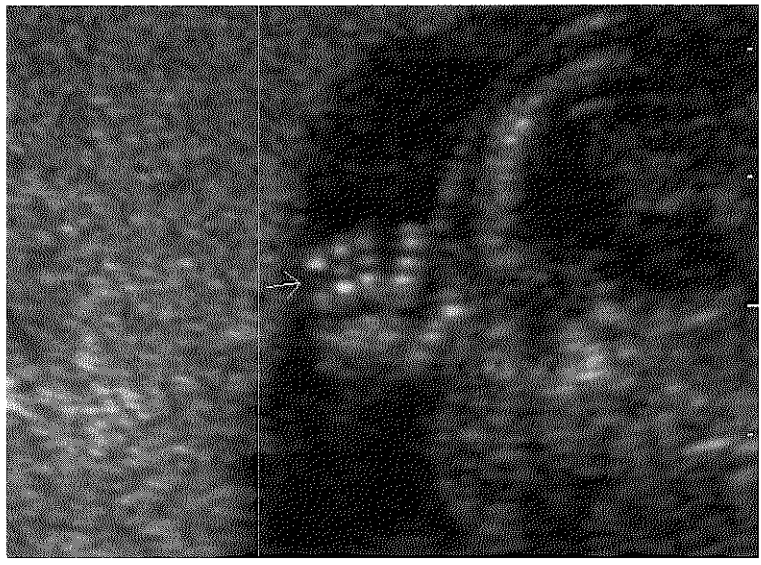
37. Normal fetal hand; 14 weeks of gestation.



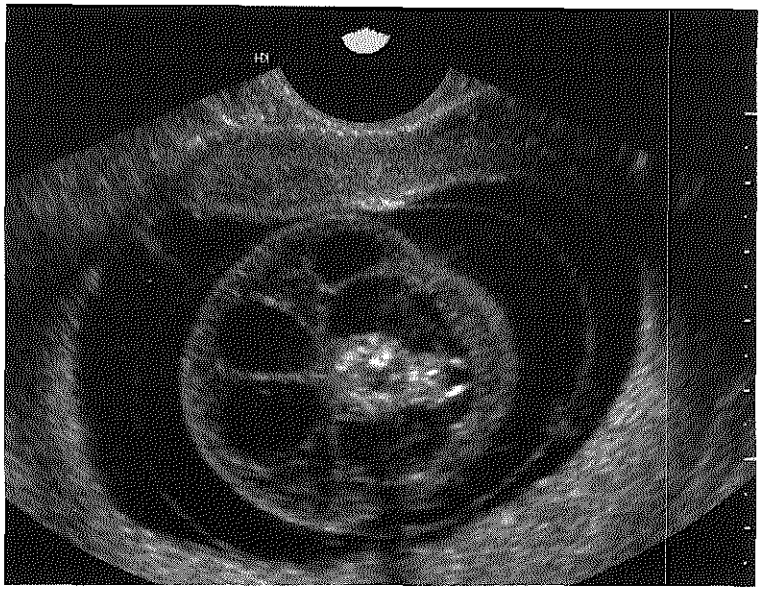
38. Cleft hand associated with EEC (Ectrodactyly, Ectodermal dysplasia, facial Clefting) syndrome (affected mother); 13 weeks of gestation.



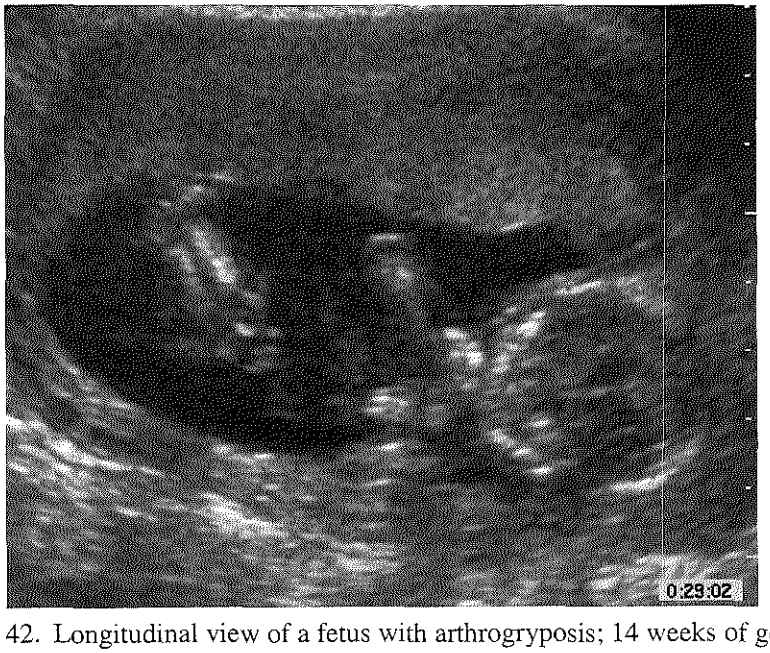
39. Clenched fist in a fetus with trisomy 18; 13 weeks of gestation.



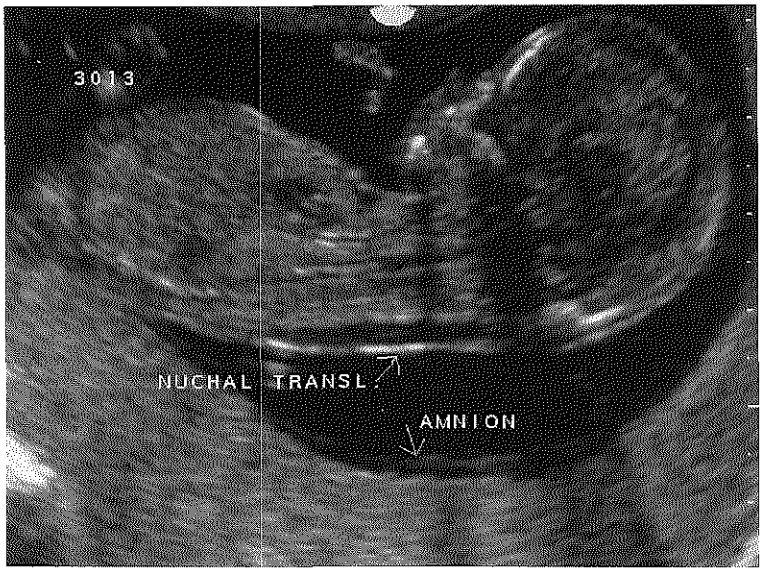
40. Hypoplastic mid-phalanx in a fetus with trisomy 21; 14 weeks of gestation.



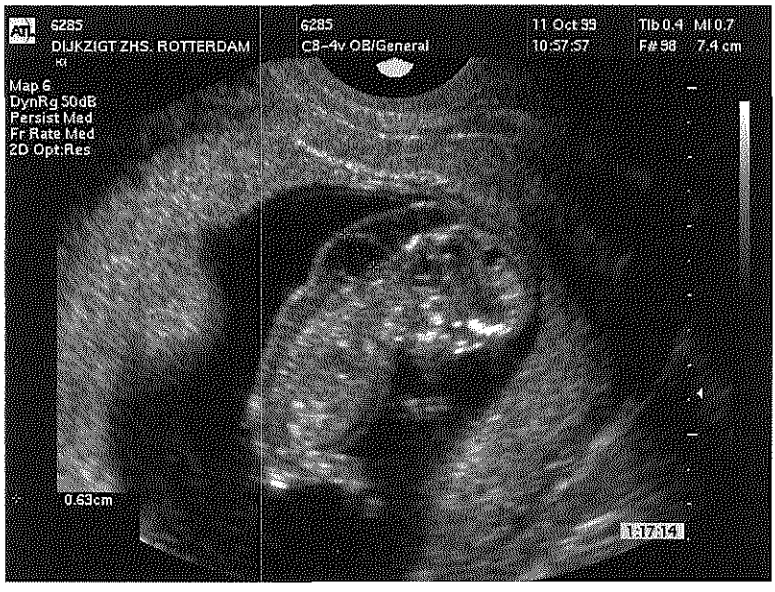
41. Cystic hygroma of the neck region; same fetus as photograph 40.



42. Longitudinal view of a fetus with arthrogryposis; 14 weeks of gestation.



43. Longitudinal view of a fetus; note the nuchal translucency and the amnion.



44. Longitudinal view of a fetus with enlarged nuchal translucency.

CHAPTER 2
IDENTIFICATION OF EARLY ABNORMAL FETAL SONOANATOMY; ITS VALIDITY AND IMPACT

2.1 Introductory remarks

Improvements in the resolution of ultrasound equipment as well as the introduction of transvaginal sonography have made it possible to describe normal anatomy of the living human embryo and fetus and to demonstrate a wide range of anomalies in the first and early second trimester of pregnancy. Especially, the sonographer should understand the dynamic processes of the development of fetal anomalies and be aware of the chronological order of development of the specific malformations. Some anomalies may develop as a transitory finding in early pregnancy and may not be present later in gestation. Other anomalies may present at varying gestational age. However, the majority of fetal anomalies can be diagnosed in the late first to early second trimester of pregnancy.

Although there is as yet no firm evidence that early termination of pregnancy for fetal malformations is psychologically more acceptable for a woman, to know that fetal development is normal as early as the late first to early second trimester of pregnancy may be very reassuring. This will be of particular advantage to those women who are at high risk of affected offspring.

In subchapter 2.1.1 a prospective study of 101 fetuses at high risk of congenital anomalies is presented to determine the validity and impact of early fetal anomaly scanning (11-14 weeks of gestation).

The detection of early fetal skeletal anomalies is introduced in subchapter 2.2. In subchapter 2.3 the topic is enlarged nuchal translucency.

2.1.1 Early fetal anomaly scanning in a population at risk of fetal anomalies

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Ultrasound Obstet Gynecol; accepted

Abstract

Objectives: To determine the validity and impact of early fetal anomaly scanning in a population at risk of fetal anomalies.

Design: A prospective study of 101 fetuses at risk of congenital anomalies at 11-14 weeks of gestation.

Results: A previously affected infant was the major reason for referral (93/101=92%). Nine (9/101=9%) fetuses demonstrated with structural anomalies at the 11-14 week scan. In five out of nine structurally affected fetuses, the nature of the anomalies was similar to that established in a previously affected pregnancy, four of which with a recurrence of an autosomal recessive syndrome.

In two fetuses with a normal 11-14 week scan, anomalies were detected at a 18-21 week scan (arthrogryposis) and at a 30 week scan (cardiomyopathy), respectively.

Conclusions: The majority of fetal anomalies can be diagnosed in the late first – early second trimester of pregnancy. This will be of particular advantage to those women who are at high risk for affected offspring. However, as fetal anomalies may present at varying gestational age, the standard 18-21 week scan can not be abandoned.

The validity of the early pregnancy scan depends on the natural history of anomalies (detectability at a certain gestational age) and the variable phenotypic expression of anomalies/syndromes.

Introduction

The ability to visualize the embryo and fetus in early pregnancy by transabdominal and/or transvaginal sonography and to detect fetal anomalies was investigated by many workers[1-12]. An increasing number of fetal structures

could be visualized with advancing gestational age. The optimal gestational age for a complete anatomical survey was suggested to be at 13 weeks of gestation[5-8,11]. This was also considered the optimal gestational age for nuchal translucency measurement[11]. An enlarged nuchal translucency is associated with a wide range of fetal chromosomal and structural anomalies and genetic syndromes[13-19].

When comparing the value of transvaginal versus transabdominal sonography in early pregnancy, the two scanning modes were found to be complementary because of the approach of the fetus from different angles[20,21].

Early pregnancy anomaly scans in high-risk patients were started in our center after considerable experience in scanning normal fetal anatomy in early pregnancy was obtained. The rationale of this study was to provide pregnant women at risk of fetal congenital abnormalities mostly because of a previously affected infant, with early information on fetal morphological development. This might either allow early reassurance with a positive impact on the quality of life of the woman in case of a normal result, or it might enable a decision on early termination of an affected pregnancy.

The following questions were addressed: (i) How successful is early fetal anomaly scanning in a population at risk of fetal congenital anomalies? (ii) What was the obstetric management adopted in affected cases? (iii) How often were sonographically established fetal anomalies confirmed by post mortem examination?

To this purpose, a total of 98 consecutive pregnancies in 93 women was studied at 11-14[4] weeks of gestation.

Methods

In a 3.5-year period, 93 couples with 98 consecutive pregnancies were referred to our center for Prenatal Diagnosis for a fetal anomaly scan in the late first or early second trimester of pregnancy. One couple presented with two consecutive pregnancies and one couple with three consecutive pregnancies. Mean maternal age was 30.4 years (range 20-39 years).

Before the anomaly scan took place, the parents were counseled, either at the Department of Clinical Genetics or at our Prenatal Diagnosis Unit, at their first visit. Table 1 shows that the referral reason was mostly a previously affected infant (93/101=92%). A considerable percentage 23/101=23% of the risk factors was determined by prevous genetic syndromes with external manifestations of the disease detectable by ultrasound and with a recurrence risk of 25-50% (Table 2).

A total of 101 fetuses, representing 95 singleton pregnancies and three twin pregnancies, were scanned (Toshiba, Tokyo, Japan, SSA 270A and Advanced Technical Laboratories (ATL), Bothell, Washington, HDI 3000) at a mean gestational age of 13 weeks (range 11-14[4] weeks). Gestational age was calculated from the first day of the last menstrual period and expressed in weeks

and days. The ultrasound scans were all performed transabdominally (Toshiba SSA 270A, carrier frequency 3.5 MHz; ATL HDI 3000, carrier frequency 2-4 MHz). An additional transvaginal scan was employed (Toshiba SSA 270A, carrier frequency 5 MHz; ATL HDI 3000, carrier frequency 4-8 MHz) when the fetus was at risk for a cardiac anomaly or to complete the anatomical survey (Table 3) when the scanning quality was not acceptable transabdominally.

A repeat ultrasound scan was routinely performed at 18-21 weeks of gestation and additionally at 30-32 weeks if the fetus was at risk for a potentially later developing (visible) anomaly, i.e. certain cardiac anomalies, arthrogryposis, obstructive uropathy, etc.

Fetal karyotyping was performed in most cases and outcome of each pregnancy was documented.

Table 1. Overview of the index patients. A previously affected infant was the major reason for referral.

Congenital anomalies of:	N
Previous child and patient/partner	5*
Previous child	93**
patient/partner	3
family member(s)	2
Total	103±

*two previously affected children in one family: n=2;
**two or more previously affected children in one family: n=9, in two families the two children displayed different congenital anomalies; ±two fetuses were at risk for two different anomalies

Table 2. The estimated genetic risk of the fetus for one or more congenital anomalies in the study group.

Genetic risk of the fetus	N
X-linked	2
Autosomal dominant (50%)	2
Autosomal recessive (25%)	19
10-25%	29
<10%	51**
Total	103**

**two fetuses were at risk for two different anomalies

Table 3. Ultrasound examination in early pregnancy. Biometrical and anatomical survey of the study group.

Ultrasound examination in early pregnancy
Biometry (CRL and/or BPD, HC, AC, FL)
Lateral ventricular width/ hemisphere width
Cerebellar diameter
Orbital distances
Nuchal translucency
Skull/ brain symmetry
Face: mandible, maxilla, orbitae, contour of upper lip, profile
Thorax: position of the heart, four chamber view, connection of the great arteries, diaphragm
Abdomen: position of the stomach, bowel, kidneys, bladder, anterior abdominal wall
Spine: longitudinal and transverse view
Extremities: position and length of arms and legs, position of hands and feet

CRL=crown rump length; BPD=biparietal diameter; HC=head circumference; AC=abdominal circumference;FL=femur length

Results

The results of the present study are summarized in Tables 4A and 4B.

Nine (9%) fetuses demonstrated structural anomalies at the first scan (Table 4A).

In five (56%) out of nine structurally affected fetuses, the nature of the anomalies was similar to that established in an earlier pregnancy (Table 4A: nos 1,2,3,4,7). Four fetuses were at risk of an autosomal recessive syndrome: short rib-(polydactyly) syndrome, SR(P)S type IV: Beemer-Langer[22], Blomstrand osteochondrodysplasia[23], Meckel-Gruber syndrome and Jeune syndrome[24], and in one instance (no.7) the recurrence risk was estimated to be less than 10%. There was parental consanguinity in two cases (nos 2,4).

A repeat ultrasound scan between 18-21 weeks was performed in every ongoing pregnancy (n=92). The second trimester scan revealed arthrogryposis in case no.10 (Table 4B). An ultrasound scan at 30 weeks of gestation in case no.11 revealed severe hydrops, cardiomegaly and thickened myocardium consistent with cardiomyopathy.

Table 4A. Fetuses with abnormal ultrasound findings at the 11-14 week scan

N3.	Referral reasons At risk for:	Risk (%)	Ultrasound findings	Karyotype	Outcome	Confirmation
1.	SR(P)S type IV: Beemer-Langer	25	12 wks: omphalocele and short limbs: recurrence of Beemer-Langer dysplasia[23]. Repeat scans at 14+16 wks	parents refrained	TOP 16 wks: female fetus, short limbs, small thorax, omphalocele, cleft lip and palate	+: X-ray, PM not permitted
2.*	Blomstrand osteochondrodysplasia	25	12 wks: nuchal translucency (6.3 mm), short limbs, long bone length at 5th centile with broad ends: recurrence of Blomstrand chondrodyspl.[24]	46,XX	TOP 12 wks: suction curettage	+: X-ray of suction curettage material
3.	Meckel-Gruber syndrome	25	13^4 wks: bilateral polycystic kidneys, occip.cystic cephalocele: recurrence of Meckel-G. syndrome	Asp.mat.: 46,XX	TOP 14 wks: suction curettage	-
4.*	Jeune syndrome	25	14 wks: small thorax and short ribs: recurrence of Jeune syndrome[25]	46,XX	At term; female infant 3755 g, died 30 min. after birth	+: X-ray, PM not permitted
5.*	Goldberg-Shprintzen syndrome	25	14^2 wks: amniotic bands, severe intracranial and facial anomalies, hand deformities: amniotic band syndrome	not performed (IUD)	IUD at 15 wks	+: PM
6.*	Arthrogryposis	25?	13^5 wks: situs inversus, DORV: isomerism	parents refrained	At term; male infant Alive at 2,5 years	+: postnatal echo(cardio)graphy: DORV and situs inversus: isomerism
7.	Diaphragmatic hernia and bilateral subpelvine stenosis	<10	13^2 wks: bilateral pyelectasis 5 mm; repeat scans: progressive hydronephrosis at 18^5 and 21^5 wks	46,XY	TOP 23^4 wks	+: PM: bilateral subpelvine stenosis
8.	Abnormal karyotype (previous infant trisomy 21)	<10	11 wks: nuchal translucency 5.5 mm; small left ventricle	47,XY,+18	TOP 11+ wks; IUD diagnosed; suction curettage	-: not possible because of maceration (IUD) and suction curettage
9.	Abnormal karyotype (advanced maternal age: 39 yrs)	<10	11^6 wks: bilateral pyelectasis and large urinary bladder: obstructive uropathy	46,XY	TOP 13.5 wks: male fetus, 43 g	+: PM: urethral valve

*parental consanguinity; SRPS=short rib-polydactyly syndrome; DORV=double outlet right ventricle; TOP=termination of pregnancy; IUD=intrauterine death; PM=post mortem examination

Table 4B. Fetuses with abnormal ultrasound findings at a repeat scan (nos. 10 and 11).

Nr	Referral reasons At risk for:	Risk (%)	Ultrasound findings	Karyotype	Outcome
10.*	Arthrogryposis	25?	13^3 wks: normal findings; 20^3 wks: hardly any movements of upper extremities visible; 21^5 wks: arthrogryposis	46,XX	TOP 22^4 wks fetus with arthrogryposis PM: no parental consent
11.*	HLHS	>10	13 + 20 wks: normal cardiac anatomy; 30 wks: cardiomegaly, thickened myocardium, diminished contractility, severe hydrops: cardiomyopathy?	30 wks: 46,XX	At term; female infant 3530g, died 3 weeks after birth PM: cardiomyopathy (mitochondrial). Diagnosis previous infant adjusted

*parental consanguinity; HLHS=hypoplastic left heart syndrome; PM=post mortem examination; TOP=termination of pregnancy

Discussion

In a population of 101 fetuses at risk of fetal anomalies, a total of 11 (11%) fetuses was diagnosed with one or more congenital anomalies. Nine out of 11 (82%) fetuses demonstrated with anomalies at the 11-14 week scan.

On the basis of their natural history, fetal anomalies can be classified into four groups[22]:

Class 1: early onset at gestational age. These anomalies occur very early in pregnancy and can be detected at a constant gestational age during the first trimester of pregnancy (i.e. anencephaly, holoprosencephaly, facial cleft, conjoined twins).

Class 2: transient conditions. The presence of transient conditions do not necessarily constitute a fetal anomaly but may indicate an underlying chromosomal defect or prelude an anomaly developing in another system (i.e. enlarged nuchal translucency, cystic hygromas, pericardial and pleural effusions, hydronephrosis).

Class 3: anomalies with variable onset. These anomalies may occur at a different gestational age in different patients (i.e. hydrocephaly, diaphragmatic hernia, talipes equinovarus, ovarian cysts, obstructive uropathy).

Class 4: late onset anomalies. These anomalies either affect those organs that develop late in pregnancy or they are the end of the natural history of anomalies affecting those organs that develop early in pregnancy (i.e. microcephaly, lissencephaly, corpus callosum agenesis, arachnoid cysts).

This classification indicates that early pregnancy scanning is feasible (class 1,2 and 3 anomalies) but a single ultrasound scan in pregnancy will not detect all fetal anomalies.

Based on the natural history of anomalies described above, in this study five (Table 4A: 1,2,3,4,6) anomalies could be classified as class 1 (early onset at gestational age), two (Table 4A: 3,7) as class 2 (transient conditions) and two (Table 4A: 4,9) as class 3 (variable onset). Case no.8 could be classified as class 2 or 3. Although the majority of the fetal anomalies was diagnosed in early pregnancy, a single (early) pregnancy scan will not detect all fetal anomalies. Therefore, the 18-21 week scan should not be abandoned[8,20,22,26,27].

Combining the 11-14 week scan and standard 18-21 week scan, congenital anomalies were detected in 10 out of 11 fetuses (91%). If case no.11 (Table 4B) is excluded from the study, as it is not likely that cardiomyopathy, which can be classified as a late onset (class 4) anomaly[22], will demonstrate in early pregnancy, the percentage of affected fetuses detected at the 11-14 week scan would be as high as 90% (9/10) and when combining the 11-14 week and 18-21 week scan as high as 100% (10/10).

Whitlow et al[26] reported a 59% detection rate of fetal anomalies at 11-14[6] weeks in an unselected population of 6634 pregnant women. When combining early and 18-20 week sonography the detection rate of fetal anomalies increased to 81%. Achiron et al[20] showed a 57% detection rate of fetal anomalies at 9-13 weeks in a population of 600 women, referred for a variety of indications and a 93% detection rate of fetal anomalies when combining the early and 18-20 week scan.

Termination took place in six (Table 4A, nos 1,2,3,7,8,9) out of nine (66%) pregnancies associated with one or more fetal anomalies diagnosed at 11-14 weeks of gestation. Confirmation of the anomalies detected by ultrasound was available in five of six cases (Table 4A, nos 1,2,7,8,9).

Overall confirmation of the prenatally diagnosed fetal anomalies by early pregnancy scanning was available in 8 of 9 cases (89%), either by X-ray examination (n=3), post mortem examination (n=3), fetal karyotyping (n=1) or postnatal echocardiography (n=1). Confirmation of fetal anomalies after suction curettage is of major concern[28]. In case no.2[24] (Table 4A) we proved that confirmation of anomalies is possible after suction curettage. Moreover, X-ray examination of the suction curettage material may be of use in confirming skeletal anomalies, as was demonstrated in case no.2.

It is suggested that detection of fetal anomalies as early as the late first trimester of pregnancy has advantages when compared with the 18-21 week scan on the basis of the nature (suction curettage) and location (out-patient) of the procedure and therefore favourable cost-benefit ratio[11,29]. But this probably does not reduce psychological morbidity in women and their partners[29].

Concerning patients at risk of fetal anomalies, the possibility and validity of the early pregnancy scan depends on the natural history of the anomalies the

patients are at risk for and on the knowledge of variable phenotypic expression of the anomalies and syndromes and thus indirectly depends on the availability of information, such as ultrasound assessment, post mortem evaluation and genetic counseling in case of a previously affected infant. This is highlighted in case no.11 (Table 4B), in which cardiomyopathy was diagnosed as late as 30 weeks of gestation. This was confirmed on post mortem examination and lead to a re-appraisal of the sib's heart. The result was that the original diagnosis of hypoplastic left heart had to be abandoned. Instead, a diagnosis of cardiomyopathy was made similar to the above case no.11. The consanguineous parents were re-counseled to be at 25% risk for cardiomyopathy in their offspring.

Finally, normal fetal development was demonstrated in 90% (90/101) of the fetuses and was confirmed postnatally. The re-assurance early in pregnancy may be of particular advantage to those women who are at high risk of affected offspring. Whereas our impression was that these women felt particularly re-assured, this was not the subject of the present study.

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2.2 Early skeletal pathology

Skeletal dysplasias are not easily detected in the first trimester of pregnancy[1].

The primary ossification centres are visualized by sonography as increased echogenicity of the bones. When measuring long bones by sonography, only the visible part, the ossification centre, is measured instead of the entire length of the long bone.

After establishing the relationship in a normal population between femur length, crown rump length and biparietal diameter (significant correlation), Gabrielli et al[2] evaluated the possibility of an early diagnosis of skeletal dysplasias in a high-risk population. In a total of 13 pregancies at risk of a skeletal dysplasia, they reported the diagnosis of two out of five skeletal dysplasias in the first trimester (10-11 weeks) of pregnancy. They concluded that biometric evaluation appears to be of value for the diagnosis of severe skeletal dysplasias but is of no value for the diagnosis of less severe cases.

In less severe skeletal dysplasias, the length of the ossification centre may not be measurably delayed in early pregnancy but may clearly be delayed later in gestation. Variable (intrafamilial) phenotypic expression of skeletal dysplasias, as in Jeune syndrome (subchapter 2.2.4) has to be taken into account and may interfere with early sonographic detection. The recurrence of less severe skeletal dysplasias may be established in early pregnancy by the presence of associated malformations (central nervous system anomalies, abdominal wall defects, etc.), as in Beemer-Langer dysplasia (subchapter 2.2.1). Furthermore, skeletal dysplasias may be associated with an enlarged nuchal translucency[3-5].

The rapidly increasing fundamental knowledge of basic cell-biological phenomena may assist in the ever closer co-operation between the ultrasonographer, paediatric pathologist, and basic-science laboratories. As in the family at risk of Blomstrand osteochondrodysplasia (subchapter 2.2.2), it may show the sequence of diagnosis by prenatal ultrasound of abnormal morphology and subsequent mutation detection in the type I parathyroid hormone/PTH related peptide receptor which makes detection by chorionic villus sampling in a following pregnancy possible as was reported by Karperien et al. (subchapter 2.2.3 as appendix to 2.2.2).

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2.2.1 Early transvaginal ultrasonographic diagnosis of Beemer-Langer dysplasia: report of two cases

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Ultrasound Obstet Gynecol 1998;11:298-302

Abstract

The early second trimester sonographic diagnosis of two infants with short rib (polydactyly) dysplasia type IV (Beemer-Langer dysplasia) is presented. In addition to short ribs, this syndrome is characterized by short limbs with or without polydactyly. There are often associated defects, particularly neural tube anomalies. The occurrence of consanguinity and of four affected sibs in this family support autosomal recessive inheritance.

Introduction

Beemer and colleagues[1] reported two cases of infants with short ribs and short limbs, but without polydactyly and suggested that their deformities represented a short rib syndrome which differed from the short rib polydactyly syndrome (SRPS) type II or Majewski syndrome. Yang and colleagues[2] described a case of a patient with the Beemer short rib syndrome but with polydactyly. They recommended the eponym "Beemer-Langer" dysplasia and the abbreviation SR(P) to indicate variable polydactyly. This was included as type IV in the short rib dysplasia group of the 1992 International Classification of Osteochondrodysplasias[3].

We report the prenatal sonographic diagnosis of Beemer-Langer short rib dysplasia in two fetuses from a consanguineous couple, whose first affected two children born 15 years previously were included in the study of Beemer and colleagues[1] on a new short rib dysplasia distinct from SR(P) type II.

Clinical report

The two affected fetuses represent the fifth and sixth pregnancy of a consanguineous couple with the following obstetric history. The first infant was born at term in early 1980 and diagnosed as having "Majewski-like" dysplasia[4]. The second pregnancy was evaluated in our centre in the same year. Ultrasonography revealed the same skeletal anomaly at 18 weeks of gestation. The pregnancy was terminated and the diagnosis was confirmed by post mortem radiography[4]. In 1981 and 1985 a healthy male and female infant were born. During the fifth pregnancy in 1993, a fetal anomaly scan was carried-out at our centre at 17 weeks of gestation revealing a small thorax, short ribs, short limb bones with bowing of the femura and normal echogenicity. The tibia was slightly longer than the fibula (Table 1). There was no polydactyly. Biometric data, with the exception of the thoracic circumference, were related to the normal reference charts of Chitty et al[5] and, apart from the head measurements, were found to be below the 5th centile (Table 1). The thoracic circumference was below the fifth centile according to our own unpublished data of thoracic circumference measurements in 144 normal fetuses (Table 1, Figure 1). Ventriculomegaly was also present. The parents opted for termination of pregnancy resulting in the delivery of a female infant weighing 92 g. Post mortem X-ray and the autopsy confirmed the diagnosis Beemer-Langer dysplasia which included a median cleft upper lip, cleft palate, micrognathia and low set ears.

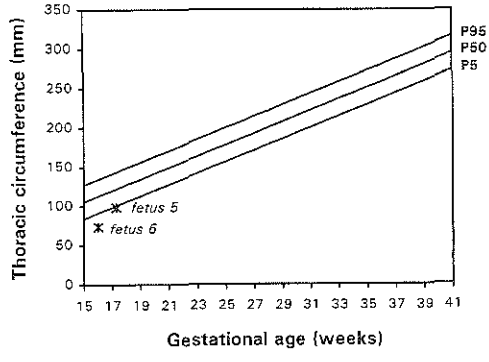

Figure 1. Reference chart for thoracic circumference, measured in 144 normal fetuses, giving 5th, 50th and 95th centiles. Measurements in fetus 5 and 6 are marked.

In 1995, the patient was again referred to our prenatal center for monitoring of her sixth pregnancy. An initial examination was performed using transvaginal sonography at 12 weeks of gestation. Already at this early stage of pregnancy limb size was reduced according to the reference charts of Chitty and colleagues[5]

(Table 1). There was normal echogenicity and no bowing of the long bones. An omphalocele was also seen. At this stage, a physiological midgut herniation could be ruled out. A repeat transabdominal scan at 14 weeks of gestation confirmed the presence of short limbs (Table 1) and an omphalocele (Figure 2). A recurrence of Beemer-Langer dysplasia was suspected. At 16 weeks of gestation, limb length was consistent with that of 14 weeks' gestation but there was no bowing and the bone was of normal echogenicity. The tibia appeared to be slightly longer than the fibula. There was no polydactyly. The cerebellum appeared 'banana' shaped (Figure 3) and a cyst was observed in the posterior cerebral fossa (Figure 4). A Dandy Walker variant was considered. There was general oedema measuring 5.5 mm in the neck region. The fetal face could not be seen. The thorax was small (Table 1, Figure 1) and short with short ribs (Figure 5). No other abnormalities could be demonstrated. The couple opted for pregnancy termination which was carried out by prostaglandin induction and a female fetus weighing 130 g was delivered (Figure 6). The fetus had a small thorax, short limbs, a ruptured omphalocele and a cleft lip and palate (Figure 6). An autopsy was refused but X-rays (Figure 7) showed a very small thorax, short ribs and short long bones with smooth metaphyses. The tibiae were relatively normally shaped and they were longer than the fibulae, which is a typical finding in Beemer-Langer dysplasia.

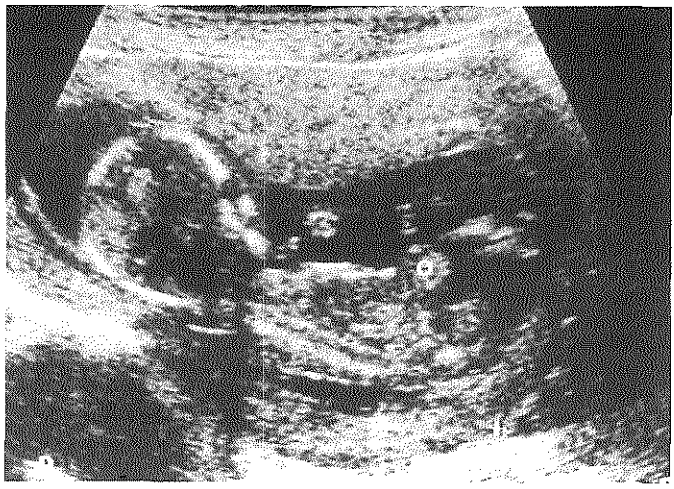

Figure 2. Fetus 6 at 14 weeks of gestation demonstrating an omphalocele (o).

Table 1. Biometric data from two fetuses with Beemer-Langer dysplasia in the 5th and 6th pregnancy of a couple whose first two infants were affected by the same syndrome

	Fetus 5 (1993)	Fetus 6 (1995)		
Length (mm)	17 wks 3 days	12 wks	14 wks	16 wks
BPD	39.3	20	31	38
HC	147	83	113	138
AC	93*	59*	76*	-
TC	98.5**	-	-	73.9**
Tibia	11.2*	4.6*	8.1*	11.0*
Fibula	10.4*	-	-	8.8*
Femur	15.5*	5.4*	10.4*	14.8*
Radius/ulna	12.5*	-	7.5*	10.4*
Humerus	14.8*	6.7*	12.4*	14.0*

*= below the 5th centile according to the normal reference charts of Chitty et al[5],
**= below the 5th centile according to our own unpublished data (Figure 1),
BPD: biparietal diameter, HC: head circumference, AC: upper abdominal circumference, TC: thoracic circumference

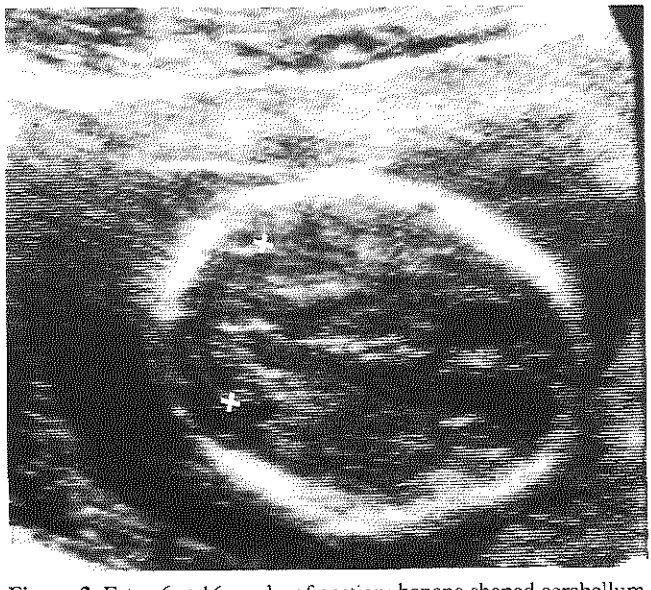

Figure 3. Fetus 6 at 16 weeks of gestion; banana shaped cerebellum (between +).

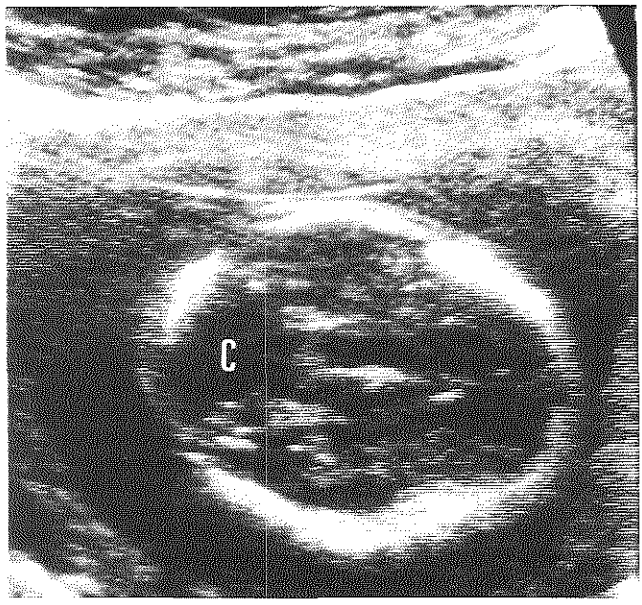

Figure 4. Fetus 6 at 16 weeks of gestation; posterior fossa cyst (C).

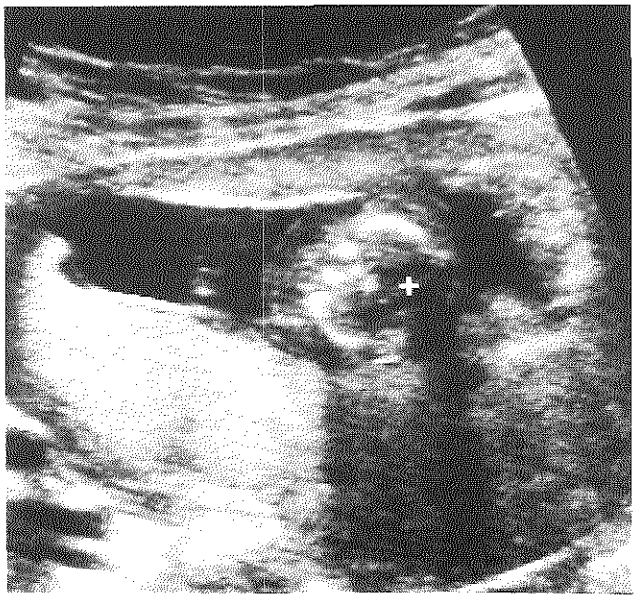

Figure 5. Fetus 6 at 16 weeks of gestation; small thorax with short ribs (+ = heart).

Discussion

Since the initial clinical debate on short-rib syndromes[6], a number of cases have been reported[1,7] and four types, based on skeletal radiography have been included in the international classification[3]: type I (Saldino-Noonan); type II (Majewski); type III (Verma Naumoff) and type IV (Beemer-Langer). Paramount to the diagnosis of short-rib dysplasia is a distinct short rib thoracic cage with extreme lung hypoplasia and marked shortening of the limbs. Polydactyly was once considered one of the most important characteristics to separate the Beemer-Langer from the Majewski type. Yang and colleagues[2] ,however, provided a detailed radiographic description of a case classified as the Beemer-Langer type but which presented with polydactyly. A similar case was reported by Lin and colleagues[8] characterized by microcephaly, flat face, hypertelorism, median cleft lip, short ribs and limbs in combination with polydactyly. Short-rib dysplasia has also been associated with central nervous system anomalies such as holoprosencephaly, Dandy Walker malformation[2] or hydrocephalus[9]. Table 2 represents possible sonographic features of Beemer-Langer dysplasia.

A *de novo* 17q paracentric inversion mosaicism has been detected in a fetus with Beemer-Langer dysplasia[10]. A possible location of the gene or cluster of linked genes responsible for this dysplasia is suggested to be 17q21 and 17q23. Brenner and colleagues[11] found that the response to transforming growth factor ß1 was markedly elevated in cultured articular chondrocytes in their case. Just as the other short-rib dysplasias, the Beemer-langer dysplasia is inherited as an autosomal recessive trait.

Ultrasound plays a pivotal role in the early diagnosis of this dysplasia in affected families. After a diagnosis was made twice in the same patient in 1980 and identified as a Majewski-like short-rib syndrome, the next case was diagnosed in 1993 by transabdominal ultrasound at 17 weeks of gestation and again in 1995 by transvaginal ultrasound at 12 weeks of gestation and confirmed at 14 weeks by transabdominal ultrasound. After the publication of Beemer and colleagues[1], these last two cases were diagnosed as Beemer-Langer dysplasia. Both fetuses had short ribs and limbs and central nervous system anomalies (hydrocephaly, posterior fossa cyst, banana shaped cerebellum) as well as a cleft palate and micrognathia. The last fetus also displayed an omphalocele which did not exist in the previous fetus, but was present in the first infant (of the six pregnancies). Transposition of the great arteries was also observed in this first infant. It is important to stress the high incidence (> 60%) of brain abnormalities in Beemer-Langer short-rib dysplasia[12]. This high incidence together with a greater clinical severity and a wider spectrum of brain defects are important in distinguishing between Beemer-Langer and Majewski short-rib dysplasia. Since all neurological anomalies involve midline defects, it was proposed by Lurie[12] that a single primary insult, affecting the midline of the early neural tube, may be responsible for all the brain defects seen in this dysplasia. As has been strongly suggested for all skeletal dysplasias, detailed post mortem radiography and autopsy of an affected fetus are essential to

confirm, in this case, the diagnosis of Beemer-Langer short-rib dysplasia. The complete autopsy should include histological examination of at least the growth plate (physis) of one or two of the long bones, the costochondral junction of the rib and samples of the vertebral bodies[13]. The central nervous system should be examined in detail, and spleen and cartilage frozen for DNA-studies[14]. Karyotyping should always be carried out, especially when multiple anomalies are observed. A skin biopsy for fibroblast culture and storage for future biochemical and molecular genetic investigations is advocated.

It may be concluded that, in case of a history of Beemer-Langer dysplasia, a fetal anomaly scan should be carried out as early as the late first or early second trimester of pregnancy. The occurrence of consanguinity and of four affected sibs in this family support autosomal recessive inheritance.

Table 2. Possible sonographic features of Beemer-Langer dysplasia.

Skeletal
Short limbs
Tibia longer than fibula
Bowed ulna and radius
Narrow thorax
Short ribs
Small scapula
Small ilia
Polydactyly
General
Hydrops
Abnormal ears
Midline cleft lip
Micrognathia
Other[1,2,8,10,12]
Abnormalities of:
gastrointestinal tract (omphalocele)
central nervous system (anencephaly, encephalocele, holoprosencephaly, Dandy-Walker malformation, hydrocephalus, agenesis of the corpus callosum)
cardiac system (transposition of the great vessels, ventricular septal defect, coarctation of the aorta, atresia ascending aorta)
genitourinary tract (ambiguous genitalia, abnormal kidneys)

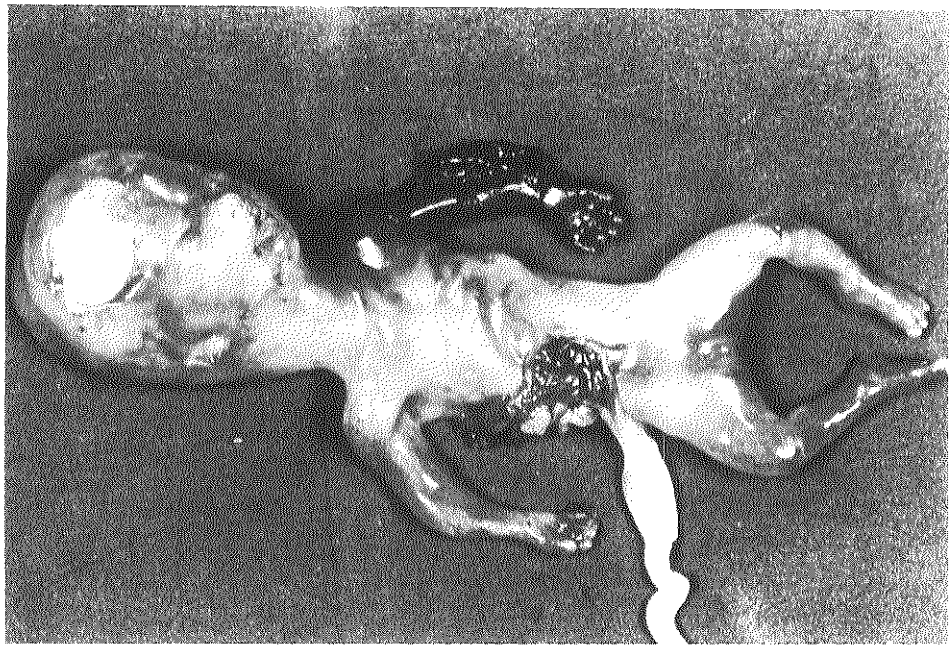

Figure 6. Beemer-Langer short-rib dysplasia type IV (fetus 6) characterized by a narrow thorax and short limbs. Polydactyly is lacking. A ruptured omphalocele and a blood clot, attached to the left arm, are visible.

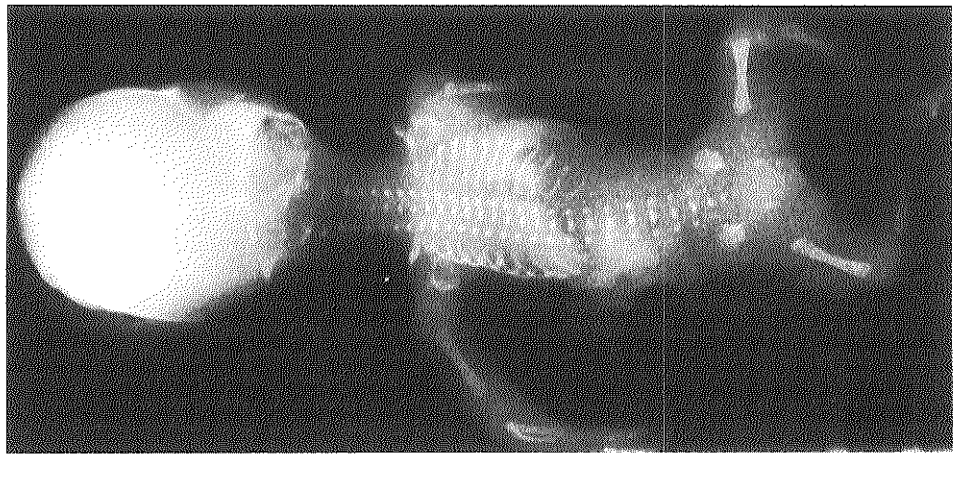

Figure 7. Post mortem radiograph of fetus 6. There are short ribs and short appendicular bones. Note the aspect of the tibiae.

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2.2.2 First trimester diagnosis of Blomstrand lethal osteochondrodysplasia

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Abstract

Blomstrand chondrodysplasia is a rare lethal skeletal dysplasia with presumed autosomal recessive inheritance. A family with two affected fetuses was studied. One fetus demonstrated a severe skeletal dysplasia at routine transabdominal ultrasound examination at 18.5 weeks of gestation. The pregnancy was terminated and the diagnosis Blomstrand chondrodysplasia was made at autopsy. A second affected fetus was identified by first trimester transvaginal ultrasound at 12 weeks of gestation. In this case the diagnosis was confirmed by post-termination radiography and histopathology.

From these observations, Blomstrand chondrodysplasia seems a lethal rhizo/mesomelic short-limb, early onset dysplasia with autosomal recessive inheritance. Easy detectability by transvaginal ultrasound is demonstrated, but general applicability awaits further studies on the intra- and interfamilial variability of this disorder.

Introduction

Blomstrand chondrodysplasia is a rare lethal skeletal dysplasia first reported in 1985[1]. The most characteristic finding is advanced skeletal maturation. Autosomal recessive inheritance was suggested by parental consanguinity. The latter was subsequently confirmed by other reports[2-4] of cases born to consanguineous parents. An increase in bone density[2] was identified as a characteristic of this skeletal dysplasia.

We present a case of Blomstrand chondrodysplasia diagnosed as early as the 12th week of gestation in consanguineous parents who were at risk for this lethal skeletal dysplasia.

Clinical report

A healthy Caucasian 31-year-old primigravid woman was referred for a detailed anomaly scan because of suspected fetal skeletal abnormalities at 18.5 weeks of gestation. The family history was unremarkable. The parents were consanguineous (second cousins in a multilaterally related pedigree).

Ultrasound examination demonstrated nuchal edema, macroglossia and a protuberant abdomen. There was severe shortness of all long bones (Figure 1A), with bone length measurements well below the 5th centile for gestational age according to the reference charts by Chitty et al[5], (Table I). Amniocentesis showed a normal female karyotype and normal alpha-fetoprotein level. Because of suspected lethal fetal skeletal dysplasia the parents opted for termination of the pregnancy at 19.5 weeks of gestation. The 250 g female fetus (Figure 2) showed macroglossia, exophthalmus and severe rhizo-meso-acromelic short-ness of the limbs. Radiographs showed extreme shortness of the long bones with broad metaphyses and narrow diaphyses and short ribs (Figure 3A and 3B). The skull and the laryngeal cartilage were mineralised. There was under-development of the viscerocranium. At autopsy no abnormalities of the viscera were found apart from hemosiderosis of the liver. Histologic study of the long bones showed reduction of the epiphyseal cartilage, the cortical bone of the widened metaphysis and narrowed diaphysis was thickened (Figure 4A). The enchondral growth plate showed a marked reduction in the zone of proliferating cartilage, but columns of hypertrophic chondrocytes were formed. The primary spongiosum was lattice-like (Figure 4B), normal amounts of osteoblasts and osteoclasts were present, the bone marrow cells appeared to be normal.

Based on the radiologic findings and supported by the osteochondral histology the diagnosis of Blomstrand lethal osteochondrodysplasia was made. The parents were informed that the recurrence risk was 25% as the inheritance was most probably autosomal recessive and that the early onset of severe limb shortness might enable early detection of an affected pregnancy by ultrasound in the 12-13th week of gestation.

In the second pregnancy a transvaginal ultrasound scan at 12 weeks was performed. There was nuchal edema measuring 6.3 mm in diameter. All bone length measurements were below the 5th centile for gestational age according to Chitty et al[5] (Table I). The long bones displayed wide proximal and distal ends (Figure 1B). The hands did not reach each other in the median plane demonstrating rhizo-meso-acromelic shortness. Chorion villus sampling showed a normal female karyotype. The diagnosis 'Blomstrand chondro-dysplasia' was made. The parents opted for termination. In the aspirotomy material, most of the organs and tissues

were available for study, including the skeletal parts. Soft-tissue radiography of the bony parts demonstrated the same severely short long bones with broad metaphyses and short ribs as were seen in the previous fetus (Figure 5).

Table 1. Biometry (mm) of the first, second and third fetus.

	FETUS 1 18.5 wks (affected)	FETUS 2 12 wks (affected)	FETUS 3 12.3 wks (unaffected)
Biparietal diameter		20.6	21
Head circumference	156		77.3
Abdominal circumf.	134		67.5
Thorax circumf.	104		
Humerus	11.0*	4.2*	8.8
Ulna	8.7*	3.3*	7.1
Radius	5.0*	3.3*	7.1
Femur	9.5*	4.2*	7.7
Tibia	9.0*	3.3*	6.9
Fibula	7.9*	3.3*	6.9
Foot	21.3*	9.5*	10.8

Below the 5th centile for gestational age according to the reference charts by Chitty et.al[5]

Histology of the osteochondral tissue displayed the same relative thickness of periostal bone of metaphyses and diaphyses and the same appearance of the enchondral growth plate as described in the sib above. Apart from a duplication of the adrenal gland, and hemosiderosis of the liver, no abnormalities of the visceral organs were found.

In the third pregnancy normal long bone lengths according to Chitty et al[5] (Table I) and normal bone aspect (Figure 1C) were established at 12, 13 and 16 weeks of gestation. Nuchal oedema (7.5 mm) was observed at 12 weeks of gestation, which had disappeared by 16 weeks of gestation. Chorion villus sampling showed a normal male karyotype. A fetal anomaly scan at 16 and 23 weeks of gestation did not demonstrate any structural abnormalities. A healthy male infant was born at term.

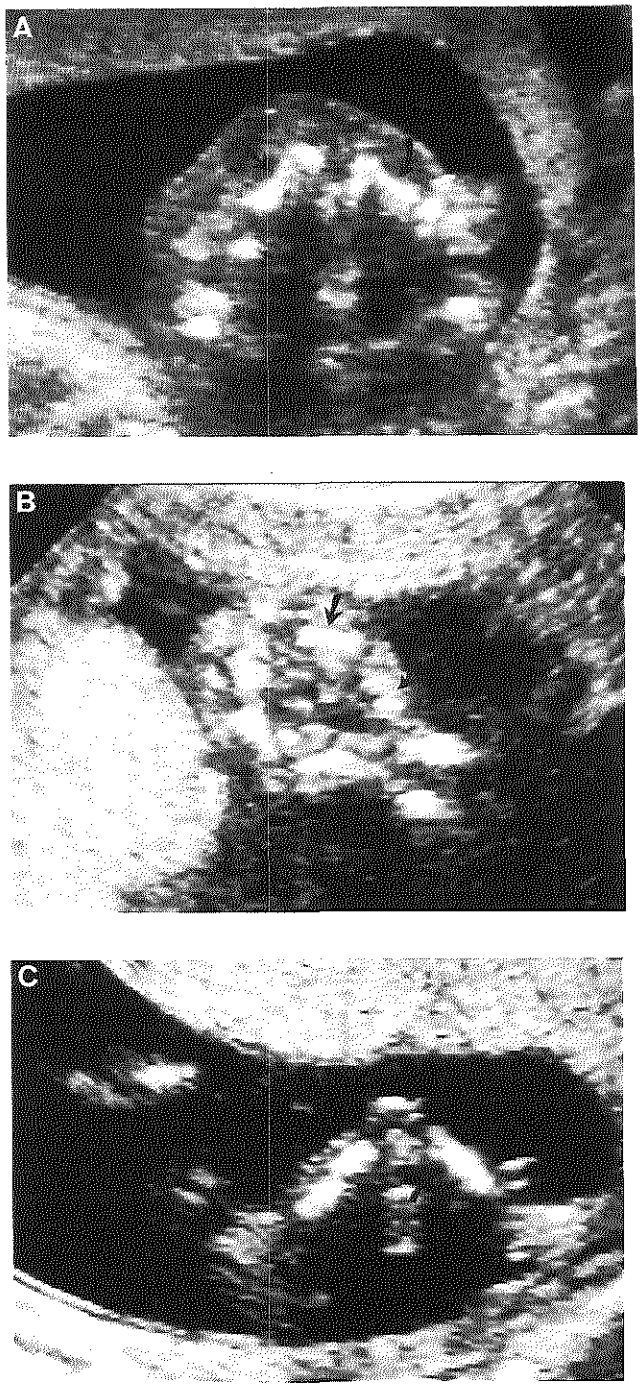

Figure 1. Femur (straight arrow) and tibia (curved arrow) of the first (A), second (B) and third (C) fetus. Femur and tibia of the first and second fetus are short and display wide proximal and distal ends. Length and aspect of femur and tibia of the third fetus are normal.

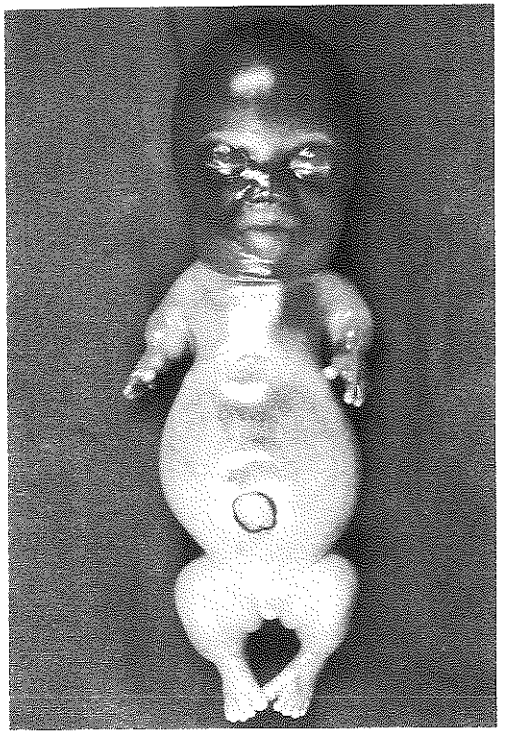

Figure 2. First fetus (index case) with protruding tongue, exophthalmus and severe micromelia. The abdomen is protuberant because of the small thorax.

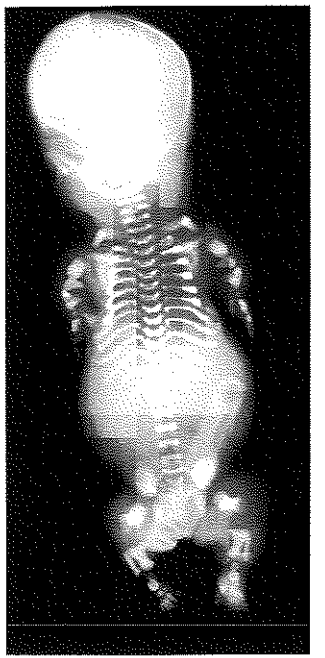

Figure 3A. Antero-posterior view of index case. The skeleton shows accelerated bone growth with increased bone density. Mid-face and skull base are hypoplastic, the ribs are short as are the tubular bones showing wide metaphyses.

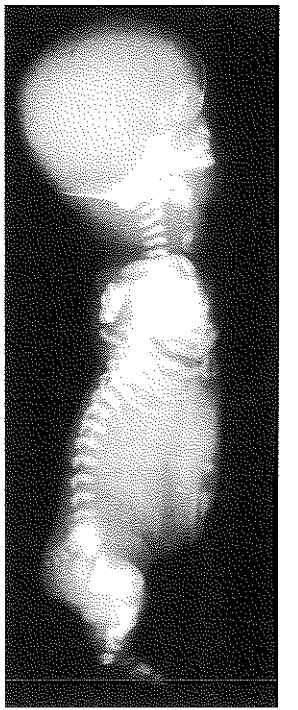

Figure 3B. Lateral view of index case showing a coccyx and mineralization of the pharyngeal cartilage.

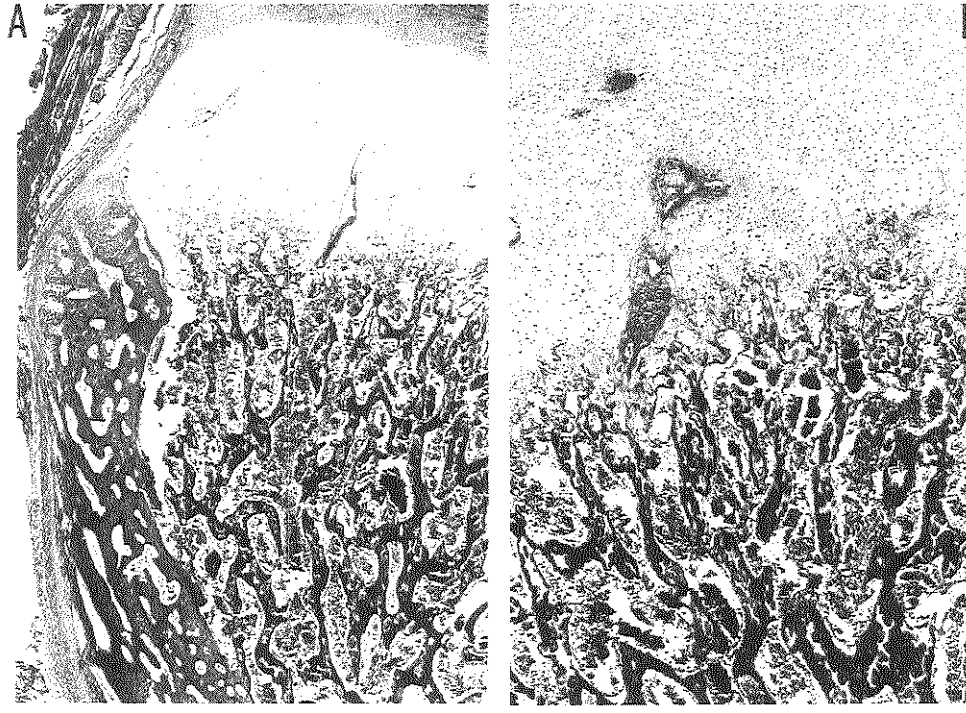

Figure 4.
A. Humeral head (index case) shows overgrowth of the enchondral growth plate by the thickened cortical bone at the left (Ladewig x 20.6).
B. Growth plate (index case) with marked reduction of the resting and proliferating cartilage. The border between the hypertrofic cartilage and the primary spongiosum is irregular (Ladewig x 41.3).

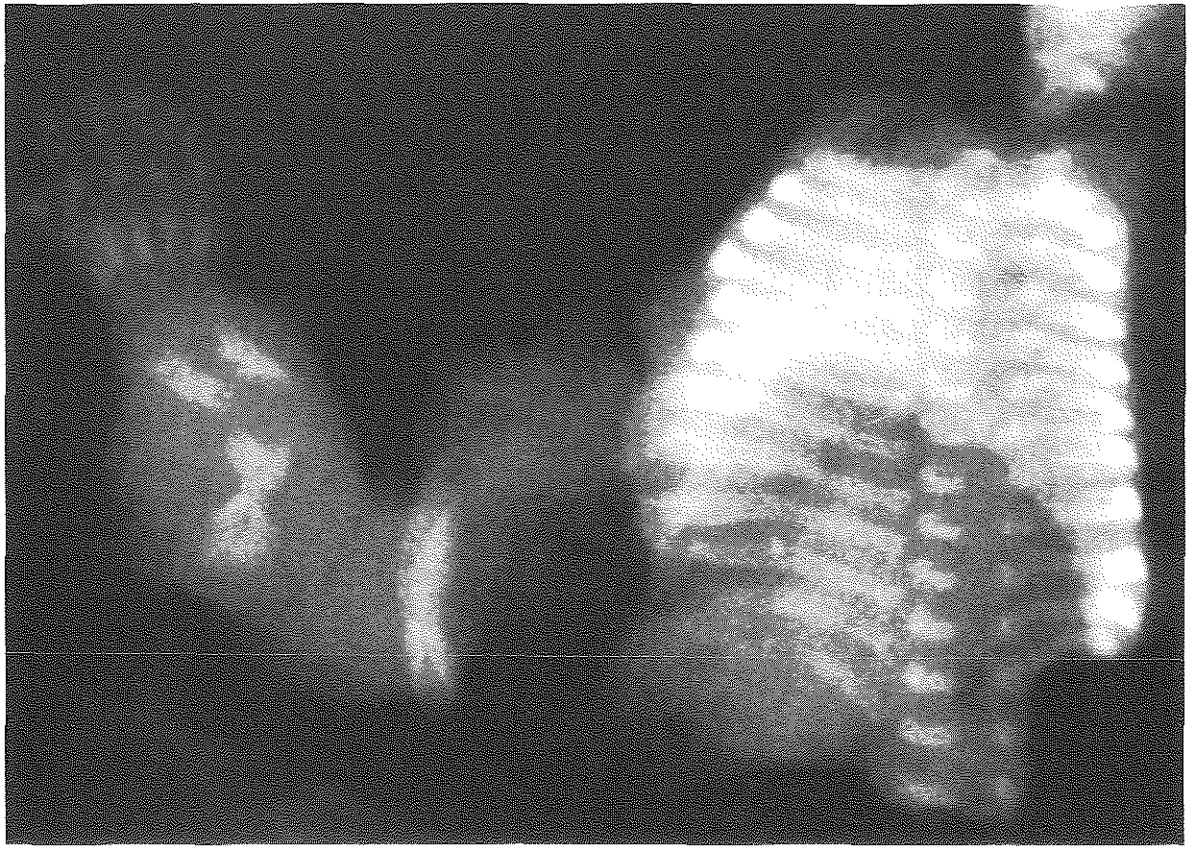

Figure 5. Detail of the soft-tissue radiograph of the second fetus showing the thorax and the left arm. The wide proximal and distal ends of the humerus are clearly visible. The ribs are short and broad.

Discussion

This report confirms the apparently invariably lethal nature of Blomstrand chondrodysplasia, as well as the autosomal recessive inheritance pattern of the syndrome and the expression at a very early stage of pregnancy enabling prenatal detection by ultrasound at 12-13 weeks of gestation. Until now four cases of Blomstrand osteochondrodysplasia have been reported[1-4]. In two cases published recently by Young et al[3] and Leroy et al[4], no postmortem radiography and autopsy were undertaken.

Non-skeletal anomalies include single observations of preductal aortic coarctation[1], malrotation of the bowel[3] and hemosiderosis of the liver[4]. Interestingly, hemosiderosis was found in both cases reported in this paper. Macroglossia may be seen as a deformation and is in fact protrusion of a normal tongue because of the small oral cavity[1,2,4].

In all described cases of Blomstrand lethal osteochondrodysplasia, including ours, there was parental consanguinity supporting an autosomal recessive inheritance pattern.

Due to the severe shortness of the long bones and increased bone density, ultrasound diagnosis in the first trimester was possible. Postmortem radiography and histology of osteochondral tissue is needed to confirm the diagnosis[6] because definite typing is not only important for genetic counseling, but also for biochemical or molecular genetic classification which may be expected in the near future. It can be concluded that Blomstrand chondrodysplasia can be diagnosed by transvaginal ultrasound as early as 12 weeks of gestation. However, general applicability awaits further studies on the intra- and interfamilial variability of this disorder.

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2.2.3 Appendix to subchapter 2.2.2

A frame-shift mutation in the type I Parathyroid Hormone/Parathyroid Hormone-related Peptide Receptor causing Blomstrand lethal osteochondro-dysplasia

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Abstract

Blomstrand osteochondrodysplasia (BOCD) is a rare lethal skeletal dysplasia characterized by accelerated endochondral and intramembranous ossification. Comparison of the characteristics of BOCD with type I Parathyroid Hormone (PTH)/PTH-related Peptide (PTHrP)-receptor ablated mice reveals striking similarities that are most prominent in the growth plate. In both cases, the growth plate is reduced in size due to a strongly diminished zone of resting cartilage and the near absence of columnar arrangment of proliferating chondrocytes. This overall similarity suggested that an inactivating mutation of the PTH/PTHrP-receptor might be the underlying genetic defect causing BOCD. Indeed, inactivating mutations of the PTH/PTHrP-receptor have been recently identified in two cases of BOCD.

We describe here a novel inactivating mutation in the PTH/PTHrP-receptor. Sequence analysis of all coding exons of the type I PTH/PTHrP-receptor gene and complementary DNA of a case with BOCD identified a homozygous point mutation in exon EL2 in which one nucleotide (G at position 1122) was absent. The mutation was inherited from both parents, supporting the autosomal recessive nature of the disease. The missense mutation resulted in a shift in the

open reading frame, leading to a truncated protein that completely diverged from the wild-type sequence after amino acid 364. The mutant receptor, therefore, lacked the transmembrane domains 5, 6 and 7; the connecting intra- and extracellular loops; and the cytoplasmic tail. Functional analysis of the mutant receptor in COS-7 cells and of dermal fibroblasts obtained from the case proved that the mutation was indeed inactivating. Neither the transiently transfected COS-7 cells nor the dermal fibroblasts responded to a challenge with PTH or PTH(rP) with a rise in intracellular cAMP levels, in sharp contrast to control cells. Our results provide further evidence that BOCD is caused by inactivating mutations of the type I PTH/PTHrP-receptor and underscore the importance of this receptor in mammalian skeletal development

Introduction

The common receptor for PTH and PTH-related peptide (PTHrP), the type I PTH/PTHrP-receptor, plays an essential role in regulating calcium homeostasis in adult vertebrates[1]. The receptor belongs to a subclass of G-protein coupled receptors, that share typical structural features. These include an extracellular N-terminus, a midregion coding for seven transmembrane domains, and an intracellular C-terminus[2]. Besides its role in calcium homeostasis, recent experiments in genetically manipulated mice have indicated a pivotal role for this receptor in embryonic development. In combination with its auto- or paracrine acting ligand PTHrP, the receptor is involved in the formation of the extra-embryonic endoderm of the parietal and visceral yolk sac[3], in skin and mammary duct development[4,5] and, most prominently, in the formation of the skeleton[3]. Mice lacking both copies of the PTH/PTHrP-receptor gene die during midgestation but some genetic backgrounds allow survival until birth, displaying severe skeletal malformations. Prominent features of these knockout mice are a domed skull, a protruding tongue, short extremities due to short long bones, and an advanced state of maturation of all skeletal components. Histology of the strongly reduced growth plate of long bones shows a decrease in resting cartilage and the near absence of columnization of proliferating chondrocytes. This is the result of accelerated chondrocyte differentiation and premature ossification[3]. The skeletal aberrations are similar to but more severe than the malformations found in mice ablated for the PTHrP-gene[6]. It has, therefore, been suggested that during embryonic bone formation PTHrP, but not PTH, acts as the main ligand activating the PTH/PTHrP-receptor[3,6].

These and other observations have led to the identification of a locally acting negative feedback loop that regulates the rate of chondrocyte differentiation in the embryonal growth plate[7]. Besides the PTH/PTHrP-receptor, this loop involves the morphogene Indian hedgehog (Ihh), its receptor complex Patched and Smoothened, and PTHrP. Chondrocytes making the transition from the proliferative to the hypertrophic zone express Ihh. Via an as yet unknown

mechanism, Ihh increases the expression of PTHrP in the periarticular perichondrium. PTHrP in turn binds to PTH/PTHrP-receptor-expressing proliferating chondrocytes and inhibits their further differentiation. This results in fewer Ihh-producing cells, which closes the feedback loop. In this model, the level of PTHrP expression critically determines the rate of chondrocyte differentiation. This is underscored by observations in transgenic mice either lacking or overexpressing PTHrP, in which chondrocyte differentiation is respectively accelerated or delayed[6,8].

The pivotal role of the PTH/PTHrP-receptor in endochondral bone formation makes this receptor a potential candidate gene involved in the pathogenesis of human skeletal disorders. Indeed, constitutively activating mutations have been detected in the PTH/PTHrP-receptor as the most likely cause of Jansen's metaphyseal chondrodysplasia[9,10]. More recently, inactivating mutations were detected in the human PTH/PTHrP-receptor gene as the most likely cause of Blomstrand lethal osteochondrodysplasia (BOCD)[11,12]. This rare dysplasia is characterized by advanced skeletal maturation and premature ossification of the skeleton[13,14,15]. The phenotype of BOCD closely resembles the malformations in the skeleton observed in PTH/PTHrP-receptor knockout mice. Here we describe and characterize a novel homozygous inactivating mutation in the type I PTH/PTHrP-receptor in a third case of BOCD.

Materials and methods

Description of the case

A 19-week-old fetus was obtained from a terminated pregnancy of a healthy Caucasian 31-year-old primigravida who was referred for a second opinion ultrasound at 18.5 weeks' gestation because of suspected fetal skeletal abnormalities. The parents were consanguineous (second cousins in a multilaterally related pedigree). Postmortem radiography and osteochondral histopathology classified the skeletal dysplasia as BOCD. A detailed description of the case is provided previously[16]. At termination, a skin biopsy was taken and used for establishing a cell culture of dermal fibroblast according to standard protocols. For comparison of the osteochondral histology, a humeral head of a normal 19 weeks fetus was used. Parental consent was obtained for this study.

Cell culture, ribonucleic acid (RNA) extraction, transient transfection assays, and cAMP production

Dermal fibroblasts were cultured in αMEM supplemented with 10% Fetal Calf Serum (FCS) and antibiotics (all from Life Technologies, Inc., Rockville, MD). For the isolation of total RNA, cells were seeded at a density of 15 000 cells/cm^2 in a 56 cm^2 tissue culture disk. After confluence, total RNA was extracted according to the method of Chomzynski and Sacchi[17]. COS-7 cells were

cultured in bicarbonate-buffered DMEM supplemented with 7.5% FCS and antibiotics. For transient transfection assays, cells were seeded in a 75-cm^2 disk. At 80% confluence, cells were transfected with 6 μg of the pcDNA3 expression vector (Invitrogen, San Diego, CA) containing either the wild type or mutant human PTH/PTHrP-receptor complementary DNA or no insert (mock) using Fugene (Roche Molecular Biochemicals, Indianapolis, IN) overnight. The next day, cells were trypsinized and seeded at a density of 15 000 cells/cm^2 in a 24-well tissue culture plate. After two days, cells were used for determination of intracellulair cAMP. For this, cells were washed twice with prechilled phosphate-buffered saline (PBS) and covered with 500μL of stimulation medium (DMEM containing 20 mM HEPES (pH 7.5), 0.1% fat-free BSA (Sigma Chemical Co., St. Louis, MO), 0.5 μg/μL aprotinin, and 2 mM of the phosphodiesterase inhibitor isobutylmethylxanthine (Sigma Chemical Co.)) in the absence or presence of human (h) PTHrP(1-34), bovine (b) PTH(1-34) (both from Bachem, Basel, Switzerland) or forskolin (Sigma Chemical Co., dissolved as a 10^{-2} mol/L in ethanol). After incubation for 15 minutes at 37°C, the stimulation medium was removed and the reaction was stopped by quickly freezing the cells on dry ice. Intracellular cAMP was released from the cells by the addition of 500 μL 50 mmol/L HCl and 20 μL was used for determination of cAMP content using a commercially available RIA (Innogenetics, Nijmegen, the Netherlands) according to the protocol of the manufacturer. Samples were measured in triplicates.

Semiquantitative RT-competitive PCR

Denatured DNase treated total RNA (1 μg, 5 minutes at 70°C and quickly chilled on ice) was reverse transcribed into cDNA in a 20-μl reaction volume containing first strand buffer (75 mmol/L KCl, 3 mmol/L $MgCl_2$, 50 mmol/L Tris-HCl pH8,3), 10 mmol/L dithiothreitol, 0.5 mmol/L deoxy-NTPs, 200 ng of random hexanucleotide primers, 1 U RNAsin/μL and 2.5 U Moloney murine leukemia virus reverse transcriptase (MMLV) /μL (Life Technologies, Inc.). Reverse transcription was performed at 37°C for 60 minutes after which fresh MMLV and RNAsin was added. Enzymes were inactivated by incubation at 70°C for 5 minutes and samples were diluted to a theoretical concentration of 10 ng/μL (assuming 100% efficiency of reverse transcription), aliquoted and stored at -20°C for later use.

To correct for variation in RNA content and cDNA synthesis between the different samples, cDNAs were equalized on the basis of their content of the β_2-microglobulin house keeping gene by competition PCR. This method has been described in detail elsewhere[18]. In short, 5 ng cDNA was coamplified in the presence of 4-fold serial dilutions of internal standard plasmid pQA1[19]. Competition PCR was performed in 25 μL reaction volume containing reaction buffer (75 mmol/L TrisHCl pH 9.0, 20 mmol/L $(NH_4)_2SO_4$, 0.01% (wt/vol) Tween 20), 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 0.25 μmol/L sense and

antisense primer and 0.125 U Goldstar TAQ DNA polymerase (Eurogentec, Seraing, Belgium). PCR was performed on a Gene Amp 9700 Thermocycler (Perkin Elmer, Norwolk, CT). Samples were analyzed by ethidium bromide staining of agarose gels. The intensity of the PCR products was determined by densitometry and the ratio cDNA/internal standard was plotted against the number of copies of the internal standard added in the PCR reaction. As the amplicons for β_2-microglobulin cDNA and internal standard amplified with similar efficiency, the point at which the cDNA/internal standard ratio equals 1 indicated the exact number of copies of β_2-microglobulin in the cDNA preparations. The corrected cDNA preparations were used for a semiquantitative PCR-reaction (conditions described above) to detect various parts of hPTH/PTHrP-receptor cDNA. The primer combinations are listed in Table 1 and were ordered from Eurogentec (Seraing, Belgium).

Table 1. Oligonucleotides used for PCR analysis.

Name:	S/ AS[2]	Sequence	Size	Tm[3]
β2-microglobulin	S	5' CCA GCA GAG AAT GGA AAG TC	268bp	56°
	AS	5' GAT GCT GCT TAC ATG TCT CG		
PTHR-S4 (551[1])	S	5' ACC AAT GAG ACT CGT GAA CG	420bp	56°
PTHR-AS4 (971[1])	AS	5' AGA AGG CCA TGA AGA TGA GG		
PTHR-S6 (903[1])	S	5' TGG CCA CCA ACT ACT ACT GG	398bp	56°
PTHR-AS5 (1301[1])	AS	5' TGA AGA CAA TGT AGT GGA CG		
PTHR-AS3 (1437[1])	AS	5' ATC TCA GCT TGT ACC TCG CC	534bp	56°
exon S	S	5' AGC TCT GCA CCC CCT ACC	200bp	60°
	AS	5' GCA GCC TGT CCC GGA GTG TTG G		
exon M2	S	5' CAC GGT CAT GTC GCG CGC	256bp	60°
	AS	5' GGC GGC ACC GGG AGC GGG CG		

[1] Number corresponds to the 5' nucleotide in the PTH/PTHrP-receptor mRNA (33).

[2] Orientation of the oligonucleotide: Sense (S) or Antisense (AS).

[3] Annealing temperature used in the PCR-reaction.

Sequence analysis of genomic DNA and site directed mutagenesis

Genomic DNA of fibroblasts was isolated by sequential proteinase K treatment and high salt precipitation. Genomic DNA was isolated from whole blood using the DNA isolation kit of Roche Molecular Biochemicals. Primer sets for the amplification of exons of the hPTH/PTHrP-receptor gene were as described[20] except that T7 promoter sequences were incorporated in the sense and Sp6 promoter sequences were incorporated in the antisense primers to facilitate sequencing. Oligonucleotides were ordered from Eurogentec. Different primer sets were used for the amplification and sequencing of exons S and M2 (Table 1). Automated sequencing was performed on an ABI thermal sequencer (PE Applied Biosystems, Foster City, CA). Some sequencing was performed by Eurogentec.

PCR-based site directed mutagenesis was used to introduce the missense mutation in the wt human PTH/PTHrP-receptor cDNA. The mutated receptor was controlled by partial sequencing and restriction enzyme digestion. The wild type and mutant receptor cDNAs were cloned in the eukaryotic pcDNA3 (Invitrogen, San Diego, CA) expression vector.

Results

Mutation analysis of the PTH/PTHrP-receptor gene identifies a novel homozygous missense mutation in a case of BOCD

BOCD is a lethal short limbed skeletal dysplasia. Our case had the typical features of BOCD, namely generalized sclerosis and advanced skeletal maturation, a hypoplastic viscero-cranium, a protruding tongue, calcified laryngeal cartilage and hyoid bones, extreme short ribs and extremities. As shown in Figure 1A and B, the cartilage part of the humeral head is extremely reduced compared to an age-matched control. Histological analysis of the humeral growth plate demonstrated a dramatically reduced number of chondrocytes in the resting zone, the near absence of columnization of proliferating chondrocytes and a diminished zone of hypertrophic chondrocytes (Figure 1C and D). Furthermore, there is an irregular boundary between the growth plate and the metaphysis and a thickening of subcortical bone. These features are similar to those found in PTH/PTHrP-receptor gene knock out mice.

Sequence analysis of all coding exons and flanking intron-exon boundaries of the PTH/PTHrP-receptor gene of DNA isolated from our case identified a homozygous point mutation in exon EL2 of the PTH/PTHrP-receptor gene. In this exon, one nucleotide (G, corresponding to nucleotide (nt)1122 of the cDNA sequence) was missing (Figure 2A). This mutation caused a shift in the open reading frame resulting in a truncated protein which in sequence completely diverged from the wt receptor C-terminal of amino acid 364 (Figure 2B).

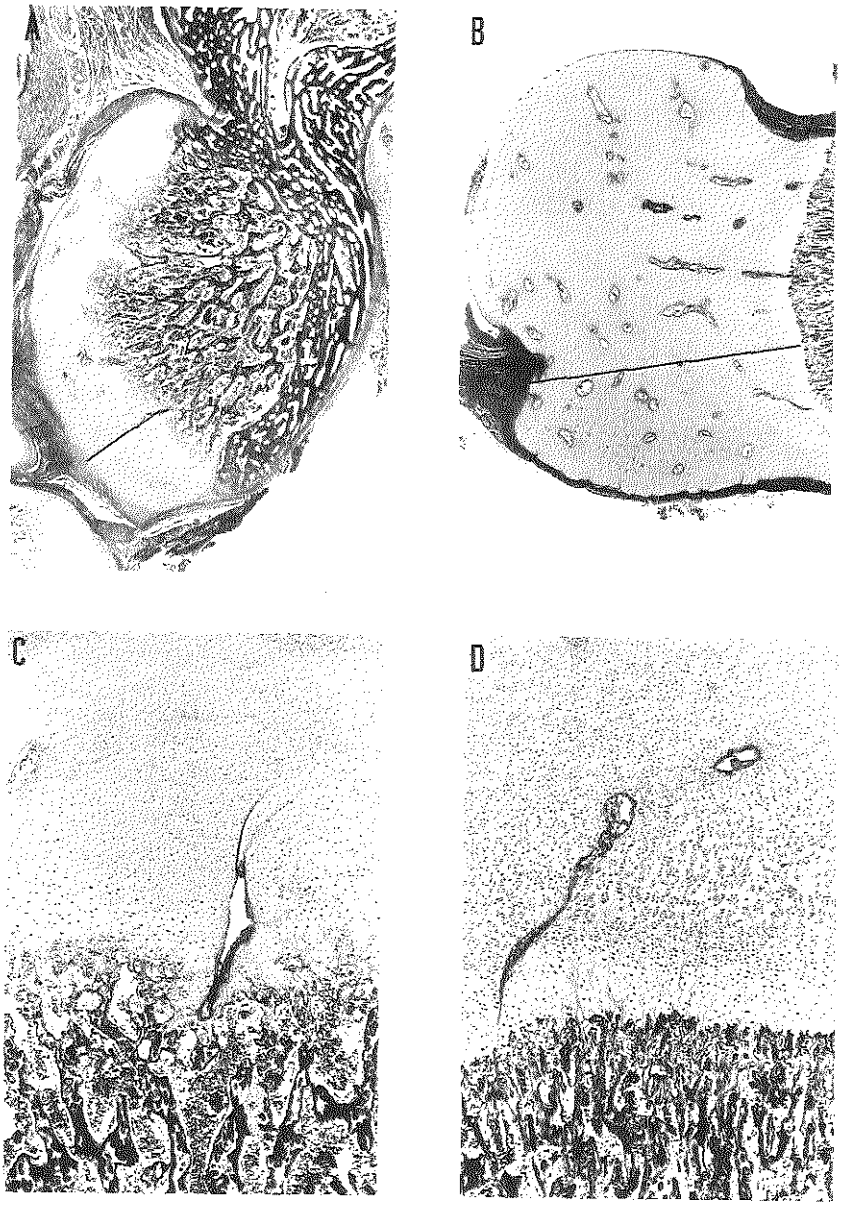

Figure 1. Comparison of humeral heads of BOCD and an age-matched control.
A) Ladewig stained mid-sagital section of a humeral head from a 19 week old fetus with BOCD and **B)** from an age-matched normal control. Note the severely reduced size of the growth plate, the increased cortical bone mass and the irregular boundary between the growth plate and the primary spongiosa. Magnification (A and B) x11.25. **C)** Detail of the growth plate of the humerus of BOCD and **D)** an age-matched control. Note the reduced zone of resting chondrocytes (r), the near absence of proliferating chondrocytes (p) and the decreased zone of hypertrophic chondrocytes (h). Magnification (C and D) x40.

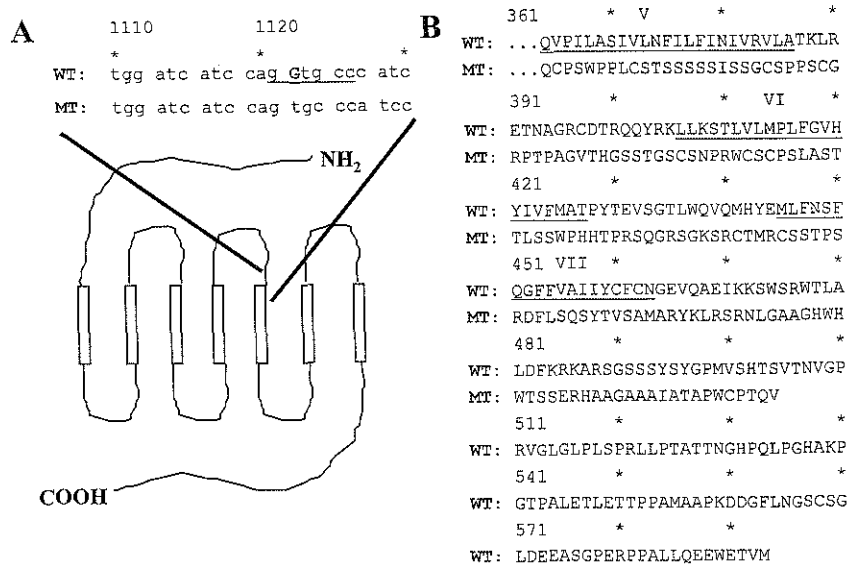

Figure 2. The PTH/PTHrP-receptor of BOCD contains a missense mutation.
A) Schematic representation of the structure of the PTH/PTHrP-receptor showing the extra-cellular N-terminus, the 7 transmembrane domains (boxed) and the intracellular C-terminus. The point mutation was found at the border of the 2nd extra-cellular loop and the 5th transmembrane domain and consisted of the loss of 1 nucleotide (indicated in bold) at nucleotide position 1122[33]. The mutation resulted in the loss of a BanI restriction site (underlined).
B) Comparison of the amino acid sequence of the wild type (WT) and mutant (MT) receptor. The frame shift yielded a truncated protein that completely diverged from the wt sequence after amino acid 364. The hydrophobic transmembrane domains 5, 6 and 7 in the wt sequence are underlined and numbered V, VI and VII, respectively.

The frame-shift mutation resulted in the loss of a BanI restriction site in the mutant receptor (Figure 2A). The absence of this restriction site in genomic DNA was confirmed by enzymatic digestion of a PCR amplicon of 151 basepairs encoding exon EL2. As expected, DNA of an unrelated control was completely digested by incubation with BanI resulting in restriction fragments of 64 and 87 basepairs (Figure 3A). The amplicon of the affected proband was resistant to enzymatic digestion, while both the father and mother were heterozygous having one wild type digested allele and one BanI resistant mutant allele (Figure 3A). In addition, an unaffected proband was homozygous for the wt allele. These results were in agreement with the consanguinity of the parents and the autosomal recessive mode of inheritance. We then verified whether the BanI restriction site was also absent in PTH/PTHrP-receptor cDNA. For this, a reverse transcription PCR reaction was performed on RNA isolated from dermal fibroblasts from the case resulting in an amplicon of 401 basepairs. As shown in

Figure. 3B, the amplicon of BOCD was resistant to BanI while digestion of an amplicon obtained from a normal control resulted in the appearance of the expected restriction fragments of 218 and 181 basepairs.

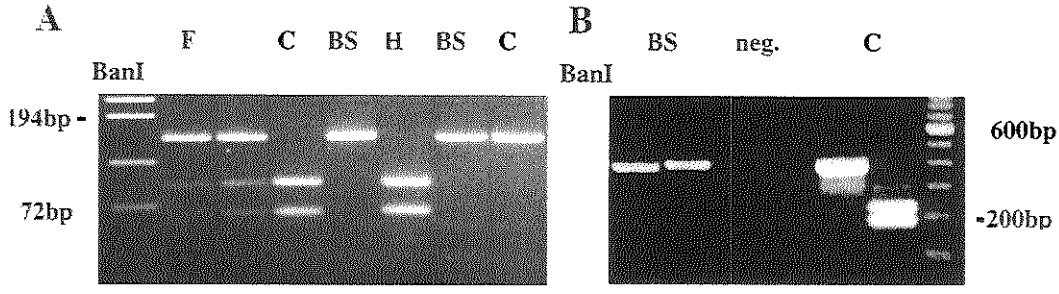

Figure 3. The mutation is recessively inherited. **A)** Exon EL-2 of the PTH/PTHrP-receptor gene was amplified by PCR using DNA derived from the father (F), the mother (M), an unrelated control (C), the affected proband (BS), and an unaffected proband (H), as template. The amplicons were incubated in the presence (+) or presence (-) of the restriction enzyme BanI. The reactions were analyzed by ethidium bromide stained agarose gel electrophoresis. BanI digestion of the wt amplicon of 151 bp results in restriction framents of 64 and 87 bp.
B) Total RNA from dermal fibroblasts from BOCD (BS) and a normal control (C) was reversed transcribed into cDNA. Part of the PTH/PTHrP-receptor cDNA (between nucleotide 903 and 1301) was amplified by PCR and incubated without (-) or with (+) BanI. The PCR product of BOCD was resistant to the restriction enzyme, while digestion of the control resulted in the appearance of the two expected restriction fragments of 218 and 181 bp, respectively.

The missense mutation inactivates the PTH/PTHrP-receptor

In the wild type receptor, amino acids C-terminal of residue 364 encode the transmembrane domains 5, 6 and 7, the intervening extra- and intracellulair loops and the C-terminal cytoplasmic tail. In contrast to the wt receptor, the mutant receptor did not contain hydrophobic domains capable of spanning the cell membrane beyond amino acid 364 (Figure 4), indicating that the mutation created a truncated receptor containing only 4 instead of 7 transmembrane domains. The lacking structural domains play a crucial role in the proper incorporation of a G-protein coupled receptor in the membrane, in ligand binding and in signal transduction and the mutation was, therefore, expected to inactivate the receptor. To test this, we performed functional analysis of dermal fibroblasts derived from the case with BOCD, since these cells are well known to express functional type I PTH/PTHrP-receptors[21]. We first tested whether the mutation affected PTH/PTHrP-receptor mRNA expression. For this, total RNA was isolated from BOCD and control fibroblasts and reversed transcribed into

cDNA. To correct for differences in amounts of cDNA a semiquantitative competition PCR reaction was performed. A fixed amount of cDNA was mixed with a series of 4-fold dilutions of an internal standard which co-amplified with β_2-microglobulin cDNA in a PCR reaction. As shown in Figure 5A, the intensity of the PCR products of cDNA and internal standard were identical at the same dilution of the internal standard (lane 3 for BOCD and lane 8 for control fibroblasts). This indicated that both samples contained equal amounts of cDNA. Subsequently, semi-quantitative PCR-reactions were performed with primer sets amplifying various parts of the hPTH/PTHrP-receptor cDNA. Both BOCD and control fibroblasts expressed comparable levels of PTH/PTHrP-receptor mRNA suggesting that the mutation did not have major effects on gene expression (Figure 5B).

We then tested whether dermal fibroblasts responded to a challenge with high doses of hPTHrP(1-34) with an increase in intracellular cAMP levels. As shown in Figure 5C, dermal fibroblasts from the case did not accumulate intracellular cAMP levels in response to hPTHrP(1-34) in sharp contrast to control fibroblasts in which an approximately 8 fold increase was observed. The absence of a rise in intracellular cAMP levels was not caused by dysfunction of the cAMP signalling cascade as forskolin, an activator of adenylate cyclase, efficiently increased cAMP levels. This increase was higher in BOCD fibroblasts, and may reflect an increased sensitivity of adenylate cyclase in cells lacking a functional PTH/PTHrP-receptor.

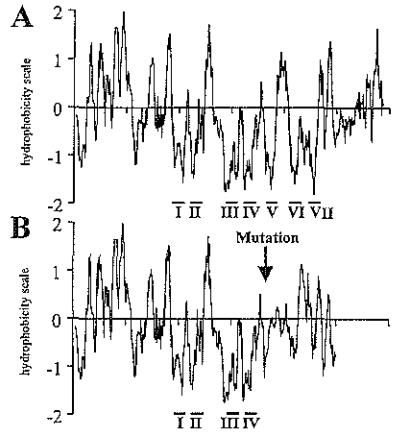

Figure 4. The mutation results in a truncated receptor lacking the last 3 transmembrane domains. Hydrophobicity plots of the amino acid sequence of the wt **(A)** and of the mutant hPTH/PTHrP-receptor **(B)** were generated using the algoritm of Hopp and Woods with a 7-residue window (http://www.expasy.ch). Scores below 0 are indicative for hydrophobic stretches of amino acid residues. The transmembrane domains are underlined and are labelled I through VII. The position of the point mutation in the mutant sequence is marked with an arrow.

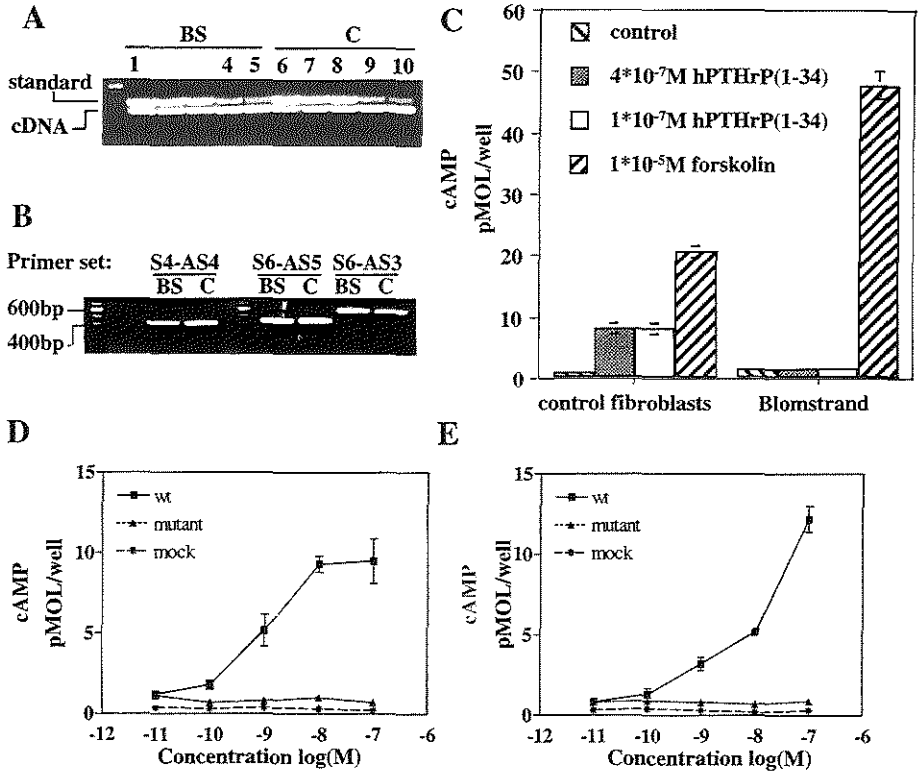

Figure 5. The frame shift mutation inactivates the PTH/PTHrP-receptor.

A) Total RNA isolated from Blomstrand dermal fibroblasts and normal control was reversed transcribed into cDNA. The amount of cDNA of both samples was standardized by semiquantitative competition PCR. Five samples of fixed amounts of cDNA were mixed with a series of 4-fold dilutions of an internal standard construct (lanes 1 - 5 for BOCD and lanes 6 - 10 for control) and subjected to a standard PCR reaction for β2-microglobulin. The relative intensity of the amplicons for the cDNA and the internal standard equalled 1 at the same dilution of the interal standard (lane 3 for BOCD, lane 8 for control) indicating that both samples contained equal amounts of cDNA.

B) Semiquantitative PCR for the PTH/PTHrP-receptor using standardized cDNA of BOCD dermal fibroblasts and control as template and various primer sets amplifying different parts of the receptor.BOCD and control dermal fibroblasts expressed similar levels of PTH/PTHrP-receptor mRNA.

C) Dermal fibroblasts of BOCD and a control were stimulated with high doses of hPTHrP(1-34) and intracellular cAMP accumulation was measured as described in material and methods. Dermal fibroblasts of BOCD did not accumulate intracellular cAMP after a challenge with PTHrP while stimulation with forskolin ($1x10^{-5}$mol/L) efficiently increased intracellular cAMP levels. Values are expressed as the mean of two independent triplicate experiments +/- SEM.

D) COS-7 cells were transiently transfected with a wild type (wt) or mutant hPTH/PTHrP-receptor expression vector or an empty expression vector (mock). Subsequently, cells were challenged with an increasing dose of hPTHrP(1-34) and intracellular cAMP levels were determined. Values are expressed as the mean of two independent quadruplicate experiments +/- SEM.

E) Similar as D, but cells were now challenged with bPTH(1-34).

We finally introduced the frame shift mutation in a wt PTH/PTHrP-receptor expression vector and tested its effect on receptor functioning in transiently transfected COS-7 cells. Challenging COS-7 cells, transiently transfected with the wt PTH/PTHrP-receptor expression vector with either hPTHrP(1-34) or bPTH(1-34), induced a dose-depedent increase in intracellulair cAMP levels (Figure 5D and E, respectively). In sharp contrast, COS-7 cells transfected with the mutant receptor or an empty expression vector did not respond to challenges with hPTHrP(1-34) or bPTH(1-34).

These results indicated that the mutation indeed inactivated the PTH/PTHrP-receptor.

Discussion

Studies using transgenic mice with targeted disruption of both copies of the type I PTH/PTHrP-receptor gene have revealed a crucial role for this receptor in skeletal development[3]. This made the hPTH/PTHrP-receptor a potential candidate gene involved in the development of human skeletal dysplasias. This was supported by the detection of two different point mutations in the PTH/PTHrP-receptor gene of patients with Jansen's metaphyseal chondrodysplasia. One mutation was located in exon M2 causing the replacement of a Histidine at position 233 by an Arginine while the second mutation was located in exon M6 causing the replacement of a Threonine by a Proline at position 410[9,10]. Both mutations resulted in ligand independent constitutively active PTH/PTHrP-receptors, and were the most likely cause of the severe aberation in bone formation in this rare form of skeletal dysplasia. This was recently corroborated by observations made in transgenic mice in which the wt PTH/PTHrP-receptor was replaced by a constitutively active hPTH/PTHrP-receptor. In these mice, similar malformations of the skeleton were observed as in Jansen type chondrodysplasia[22].

More recently, inactivating mutations were detected in the PTH/PTHrP-receptor gene of two cases with Blomstrand osteochondrodysplasia[11,12]. This severe lethal skeletal dysplasia has many features in common with the skeletal aberrations found in transgenic mice lacking both copies of the type I PTH/PTHrP-receptor gene. This is most prominent in the physis and the metaphysis of the long bones. The phenotypic similarity suggested that BOCD was caused by an inactivating mutation of the PTH/PTHrP-receptor. Analysis of the PTH/PTHrP-receptor genes in these two cases identified a heterozygous nucleotide substitution in exon M5 coding for the 5th transmembrane domain in one case, while in the second case a homozygous point mutation was detected in exon E3 coding for part of the extra-cellular N-terminus of the receptor. In contrast to the second case, the first case was from non-consanguineous parents. The point mutation in exon M5 created a novel splice acceptor site leading to a defect in mRNA splicing, resulting in a protein that lacks amino acids 373 to

383 compared to the wt receptor. Functional studies in transiently transfected COS-7 cells demonstrated that this mutation inactivated the receptor. The mutation was inherited from the mother while the paternal allele did not contain mutations in the coding exons of the PTH/PTHrP-receptor gene. Analysis of chondrocytes from this case demonstrated, however, that the paternal allele was not expressed. Which genetic defect underlies the absence of expression of the paternal allele is presently unknown, but might be caused by a mutation in a region that is involved in regulation of PTH/PTHrP-receptor gene expression[11].

The homozygous point mutation in the second case resulted in the replacement of a Proline at position 132 for a Leucine. Functional analysis in transiently transfected COS-7 cells demonstrated that this receptor was equally well expressed as wt receptors but binding of either PTH(1-34) or PTHrP(1-34) was less than 10% of the wild type control. Furthermore PTH-induced cAMP accumulation was severely reduced and inositol phosphate accumulation was not detectable. It seems likely that the Proline at position 132 plays a crucial role in ligand binding[12].

In this study we describe a novel inactivating mutation in the PTH/PTHrP-receptor gene in a third unrelated case of BOCD. The identified mutation was located in exon EL2 coding for the second extracellular loop and consisted of a loss of 1nt at position 1122 of the cDNA sequence. Consequently, a frame-shift was induced in the open reading frame of the PTH/PTHrP-receptor mRNA. The resulting mutant protein completely diverged from the wild type sequence C-terminal of amino acid 364. As shown by analysis of hydrophobicity plots, the mutation created a truncated receptor containing only 4 instead of 7 transmembrane domains. Structure function analysis of the PTH/PTHrP-receptor has indicated a pivotal role in receptor functioning for the domains that are lacking in the mutant receptor. For example, it has been shown that residues in the third extracellular loop are essential for ligand binding[23,24] and that the lacking intracellular domains are involved in signal transduction[25,26]. Furthermore, it seems highly unlikely that a G-protein coupled receptor lacking 3 of the 7 transmembrane domains can be normally incorporated in the cell membrane, due to the complete disruption of its secondary and tertiary structure. The mutation is, therefore, expected to be inactivating. Functional analysis of the mutant receptor proved indeed that the mutation inactivated the receptor. This was shown by functional analysis of dermal fibroblasts from the case itself. Dermal fibroblasts are well known to express functional PTH/PTHrP-receptors[21]. Analysis of PTH/PTHrP-receptor mRNA expression in dermal fibroblasts from BOCD and from a normal control demonstrated that both cells expressed comparable levels of PTH/PTHrP-receptor mRNA, suggesting that the mutation did not have major effects on PTH/PTHrP-receptor mRNA expression. In marked contrast to the control fibroblasts, BOCD dermal fibroblasts did not respond to a challenge with high doses of hPTHrP(1-34). These observations were furthermore corroborated by analysis of the mutant

receptor in COS-7 cells. Unlike the wt receptor, COS-7 cells transiently transfected with the mutant receptor did not respond to both PTH and PTHrP, furthermore providing evidence for the inactivating nature of the mutation. Analysis of parental DNA demonstrated that the mutation was inherited from both parents in agreement with the consanguinity and the autosomal recessive mode of inheritance of the disease.

The existence of both activating and inactivating mutations has been described for a number of G-protein coupled receptors and has been implicated as the underlying cause of a variety of human diseases (for recent reviews see[27,28]. The mutations involve single amino acid substitutions, frame-shift mutations, or the introduction of premature stop codons. For example, loss of function mutations in the calcium sensing receptor cause familial hypocalciuric hypercalcemia and neonatal severe primary hyperparathyroidism[29], while constitutively activating mutations cause familial hypoparathyroidism[30]. In addition, loss of function mutations in the LH-receptor cause male-pseudohermaphroditism[31] while gain of function mutations cause familial precocious puberty[32]. The identification of loss and gain of function mutations places the type I PTH/PTHrP-receptor on the expanding list of G-protein coupled receptors involved in the pathogenesis of various human diseases.
In conclusion, our results in combination with the previously identified inactivating mutations in the PTH/PTHrP-receptor clearly demonstrate that BOCD is the human mirror image of PTH/PTHrP-receptor ablated mice and underscore the importance of this receptor in human skeletal development.

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2.2.4 Early prenatal sonographic diagnosis and follow-up of Jeune Syndrome

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Abstract

Jeune syndrome or asphyxiating thoracic dysplasia is an autosomal recessive osteochondrodysplasia. It is one of the six short-rib (polydactyly) syndromes (SR(P)S). The disease has a wide spectrum of manifestations, ranging from a latent to a mild or lethal condition.

We describe the prenatal sonographic diagnosis of Jeune syndrome at 14 weeks of gestation in a fetus at risk for this condition and the development of this syndrome throughout the pregnancy.

Introduction

Jeune syndrome or asphyxiating thoracic dysplasia (ATD) is an autosomal recessive osteochondrodysplasia. In 1992 the International Working Group on Constitutional Diseases of Bone classified Jeune syndrome as one of six short rib (SR) dysplasia syndromes (S) with or without polydactyly (P), SR(P)S: Type I: Saldino-Noonan, Type II: Majewski, Type III: Verma-Naumoff, Type IV: Beemer-Langer, Jeune syndrome and Ellis-van Creveld syndrome[1]. Yang et al[2] differentiated between two types of Jeune syndrome, type 1 ATD and type 2 ATD, based on radiologic and histopathologic characteristics.

Pathognomonic radiographic features of Jeune syndrome are short horizontal ribs, square iliac wings and horizontal acetabular roofs with medial spur-shaped projections on each side[3]. The long bones are either normal or mildly shortened. In about two thirds of the cases the proximal femoral ossification center is present at birth. The disease has a wide spectrum of manifestations ranging from a latent form to a mild or lethal condition[2,4,5]. In the severe form respiratory failure leads to death in early infancy. The mild form of Jeune syndrome is characterized by a progressive renal failure which is the main prognostic factor[4].

However, clinically significant hepatic dysfunction, leading to hepatic fibrosis and later to biliary cirrhosis, has been reported[6,7]. Respiratory distress in milder forms might be relieved by surgical procedures to expand the thoracic cavity[8].

Overlap of Jeune syndrome with SRPS type III: Verma-Naumoff, Ellis-van Creveld syndrome and oro-facio-digital syndromes has been described[5,9].

Detection of Jeune syndrome in the second and third trimester has been reported[3,10-13]. We report on the prenatal sonographic diagnosis of Jeune syndrome as early as 14 weeks of gestation in a fetus at risk for this condition.

Case report

The first child of a consanguineous Turkish couple died at the age of six weeks of respiratory insufficiency caused by Jeune syndrome, based on clinical and radiological features. This female infant had a small thorax and short extremities. Radiographs demonstrated a small transverse and antero-posterior diameter of the thorax with extremely short horizontal ribs and handlebar clavi-

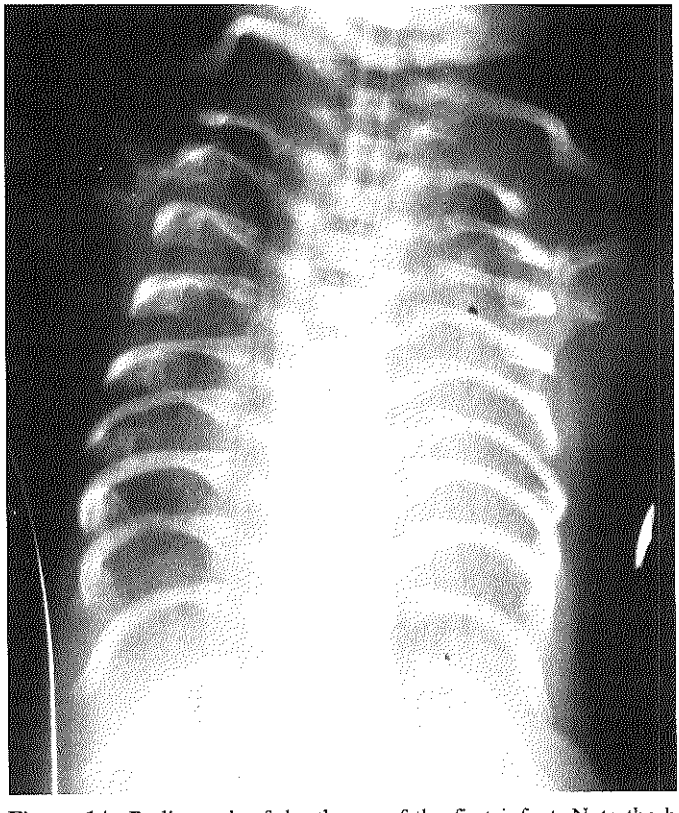

Figure 1A. Radiograph of the thorax of the first infant. Note the handlebar clavicles, short horizontal ribs.

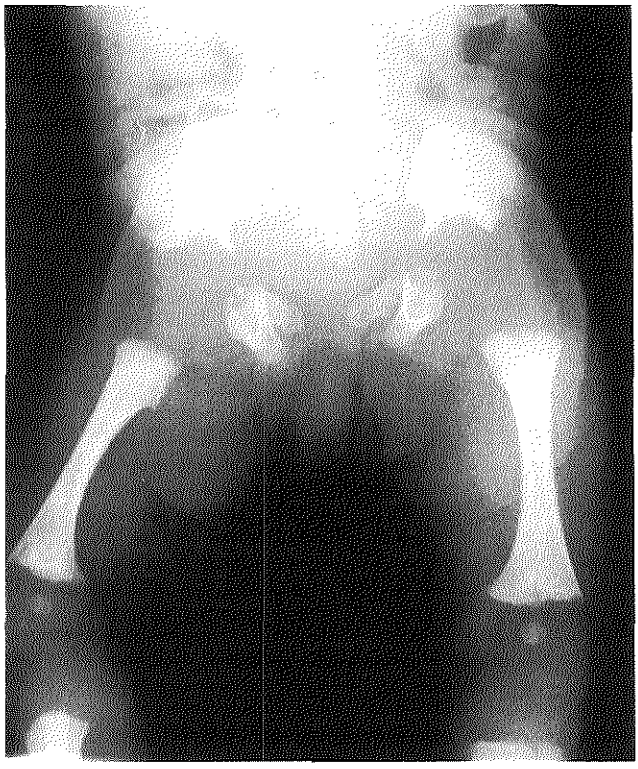

Figure 1B. Radiograph of the pelvis of the first infant. Note the trident shaped pelvis with medial spurs of both acetabular roofs and the small, square-shaped iliac bones.

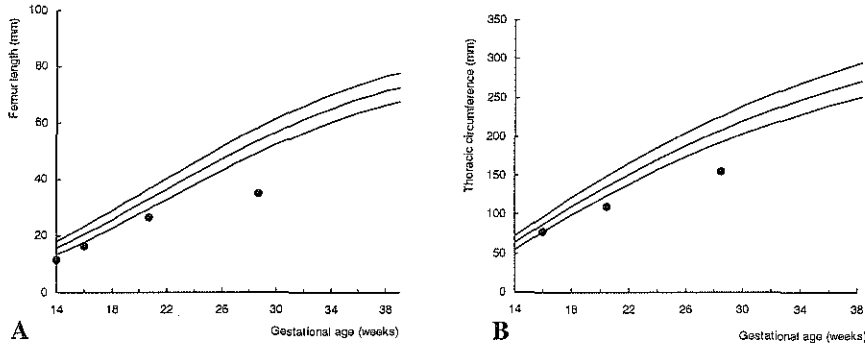

Figure 2. A,B: Biometric data of the fetus affected with Jeune syndrome (A=femur length, B= thoracic circumference).

cles (Figure 1A). Radiographs of the pelvis and the long bones displayed a trident shaped pelvis with medial spurs of both acetabular roofs, square-shaped iliac bones (Figure 1B) and shortened long bones with smooth metaphyses. Post mortem investigation was declinded.

During the couple's second pregnancy ultrasound examination at 19 weeks of gestation demonstrated a normal developing male fetus and a healthy infant was born at term.

In their third pregnancy, the first ultrasound examination at 14 weeks of gestation (HDI 3000, Advanced Technology Laboratories, Bothell, Washington) showed normal biometric data (biparietal diameter (BPD), humerus and ulna length) according to the normal reference charts of Snijders and Nicolaides[14]. The femur length (FL), however, was below the 5th centile for gestational age[14] (Figure 2A) and the thorax was shallow with short ribs (Figure 3). There was a small nuchal translucency of 1.6 mm. Recurrence of Jeune syndrome was suspected because of the shallow thorax and short ribs.

The parents decided to continue the pregnancy and consented to serial sonographic follow-up. Amniocentesis at 16 weeks of gestation was performed to exclude a chromosomal anomaly. A normal female karyotype and normal alphafetoprotein levels were established.

A repeat ultrasound examination at 16 weeks demonstrated a thoracic circumference on the 5th centile for gestational age according to our own unpublished data (Figure 2B). The long bones, especially femura and tibiae demonstrated broad ends and slight bowing of the tibiae (Figure 4).

At 20 weeks BPD, head and upper abdominal circumference were appropriate for gestational age. The thorax was very narrow with an everted sternum (Figure 5). The thoracic circumference was below the 5th centile (Figure 2B). The left atrium was situated against the spine (Figure 5). There was rhizomelic shortening of the upper and lower extremities and there were broad distal ends of the femora, tibiae (Figure 4) and humeri.

At 28 weeks of gestation BPD, head and abdominal circumference were appropriate for dates (50th centile) with severe rhizomelic shortening of both upper and lower extremities, with a severely shortened femur (Figure 2A). The tibiae and femora were slightly bowed (Figure 4) and the thorax had become increasingly narrow (Figure 2B). The left atrium of the heart could not be visualized as it was compressed by the spine. The right kidney appeared to be small. The left kidney could not be visualized because of the fetal position but had been demonstrated on previous ultrasound scans and was considered to be normal.

At term a female infant with a birth weight of 3755 g was delivered. The Apgar scores after 1 and 5 minutes were 1 and 4, respectively. Despite assisted ventilation the infant died of respiratory insufficiency 30 min after birth.

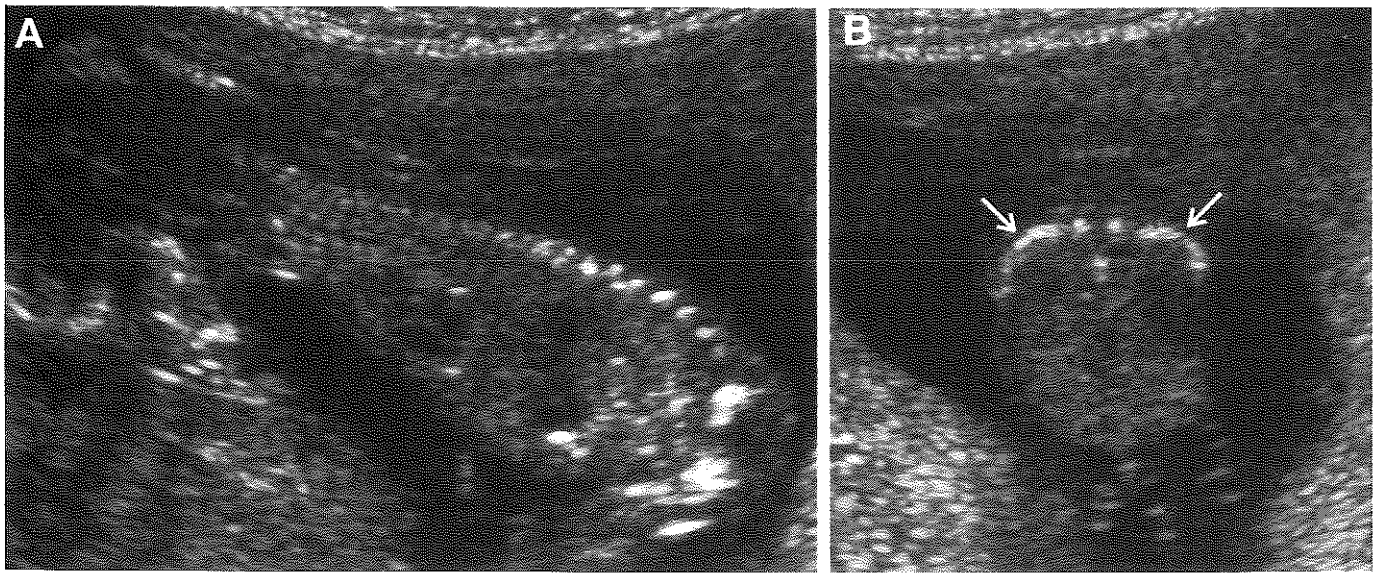

Figure 3. The shallow thorax (A:longitudinal section) and short ribs (B:transverse section; arrows) at 14 weeks of gestation. Note the short ribs (arrows) in the transverse section.

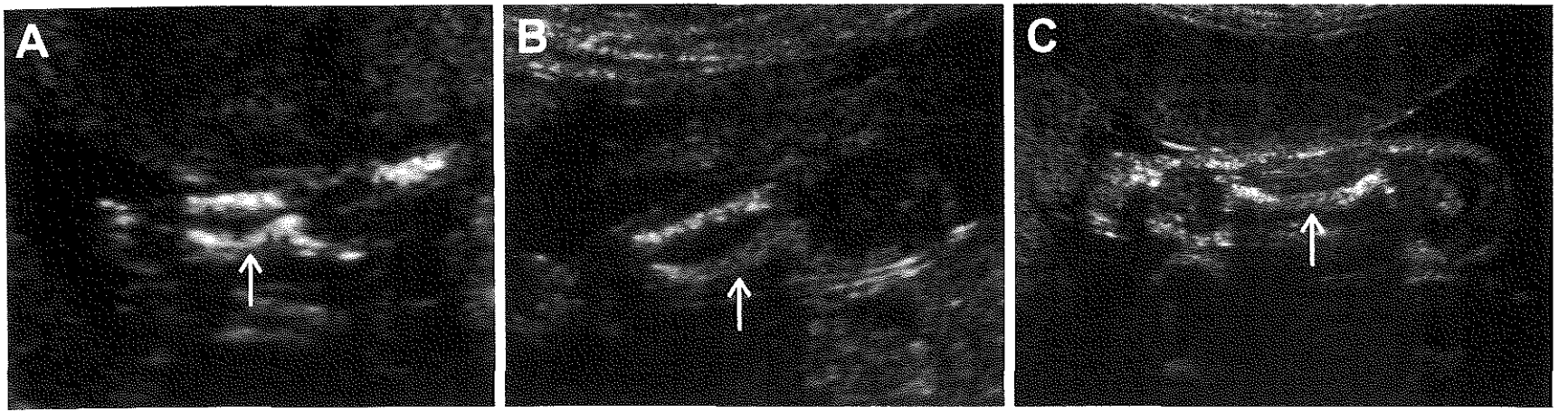

Figure 4. Ultrasound images of the second affected fetus showing bowing and broad ends.of the tibia (arrows) at 16 (A), 20 (B) and 28 (C) weeks of gestation.

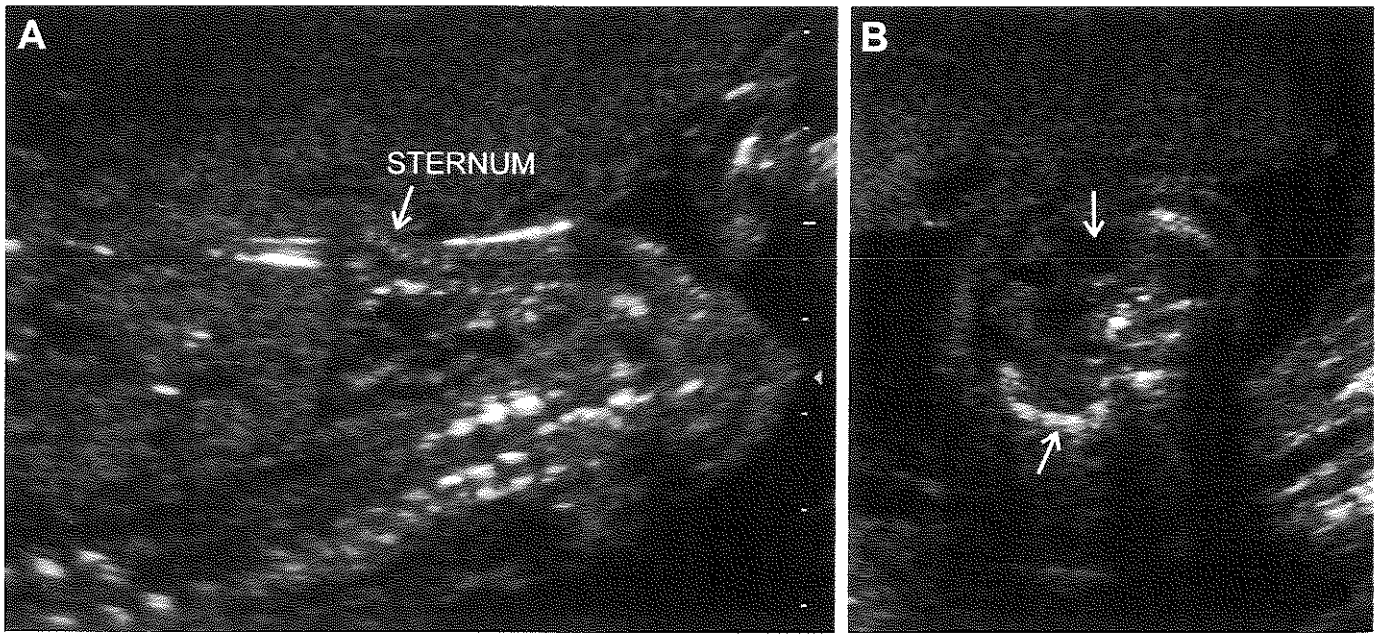

Figure 5. A. Longitudinal section through the thorax at 20 weeks of gestation. Note the everted sternum. B. Transverse section through the hypoplastic thorax at 20 weeks of gestation. Upper arrow: right atrium; lower arrow: short rib.

Post mortem investigation was not permitted. Radiographs of the skeleton (Figure 6) were compatible with the diagnosis of Jeune syndrome. The thorax was very small in both transverse and antero-posterior projections with short horizontal ribs and handlebar clavicles. The square-shaped iliac bones showed horizontal acetabular roofs with medial spur-shaped downward projections on both sides. The proximal femoral ossification centers were present. All long bones were shortened. There was slight bowing of the femora.

Discussion

Several authors[3,12,13] have concluded that the diagnosis of Jeune syndrome can not be made before 17-18 weeks of gestational age. However, due to the improved resolution of ultrasound equipment early prenatal sonographic diagnosis is now possible when the condition is severe.

Apparently, in this family, the second affected fetus demonstrated a development of Jeune syndrome quite similar to the index case, enabling prenatal detection a early as 14 weeks of gestation. Repeat ultrasound scans demonstrated severe rhizomelic shortening of the extremities in the late second trimester and a hypoplastic thorax causing increasing compression of the heart with advancing gestational age. Between 14 and 28 weeks of gestation, the BPD, head circumference and abdominal circumference were appropriate for gestational age. At 28 weeks of gestation the right kidney appeared to be small which may have represented renal involvement[4] described for Jeune syndrome (the left kidney could not be visualized at this stage). Post mortem examination was not permitted, therefore no information was available on the size, shape and microscopic evaluation of the kidneys.

Radiographs of the skeleton of both infants affected with Jeune syndrome described here were strikingly similar. However, the second affected infant seemed to have had a more severely hypoplastic thorax as she died 30 minutes after birth despite assisted ventilation. The presence of the proximal femoral ossification centers at birth, seen in two thirds of the cases, was only demonstrated in the second affected infant.

Yang et al[2] differentiated between two types of Jeune syndrome, type 1 ATD and type 2 ATD, based on radiologic and histopathologic characteristics. Type 1 ATD is characterized by radiologically irregular metaphyseal ends and a histopathologically irregular cartilage bone junction, whilst type 2 ATD is characterized by radiologically smooth metaphyseal ends and histopathologically diffusely retarded and disorganized physes with smooth cartilage-bone junctions, the type 2 ATD seemed to represent the most commonly described form of the Jeune syndrome and although bone and cartilage histology were not available, the radiographic features of both affected infants in our report are compatible with the type 2 ATD[2].

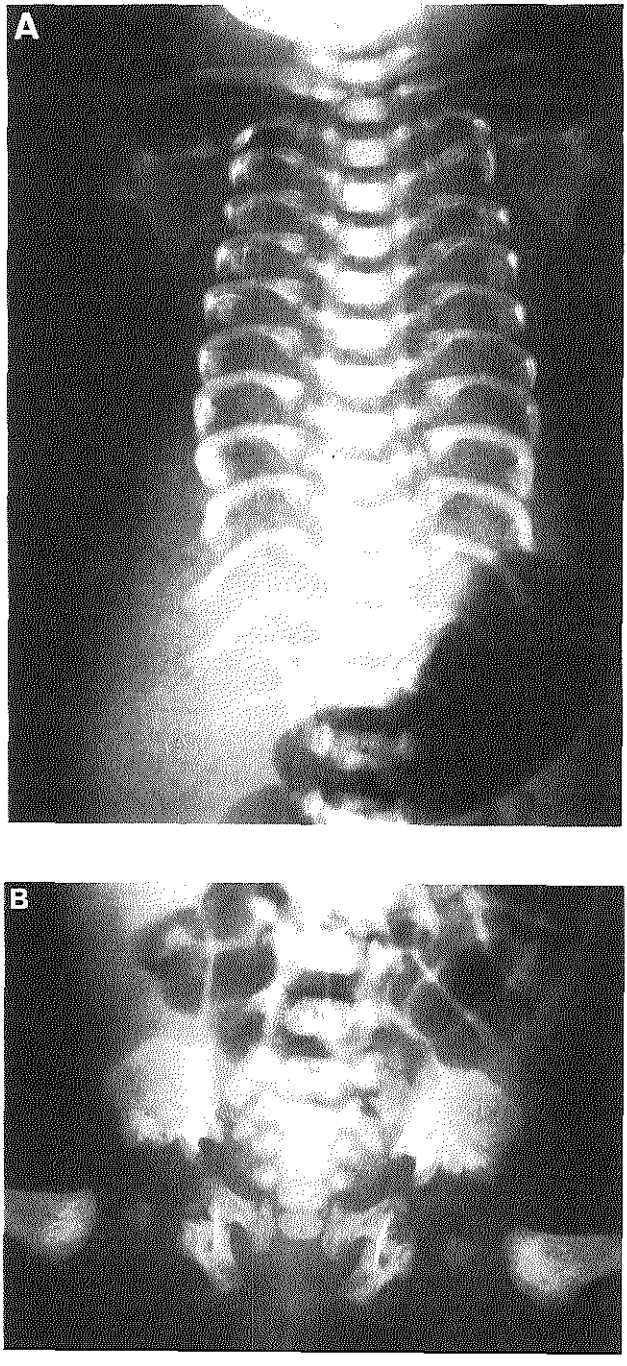

Figure 6. Radiographs of the thorax (A) and pelvis (B) of the second affected infant. Note (A) the handlebar clavicles, short horizontal ribs, (B) the square-shaped iliac bones, the medial spurs of both acetabular roofs and the presence of the proximal femoral ossification centers.

The radiological differential diagnosis of type 2 ATD is Ellis-van Creveld syndrome. However, frequent clinical features of Ellis-van Creveld syndrome are bilateral, postaxial polydactyly, congenital heart defect and nail and teeth abnormalities[2,3]. Recently, the gene that is mutated in individuals with Ellis-van Creveld syndrome was identified and mapped to chromosome 4p16[15]. As there are features of Ellis-van Creveld syndrome and Jeune syndrome that overlap, they may be allelic conditions.

The radiological and pathological differential diagnosis of type 1 ATD[2] is the short-rib polydactyly syndrome type III (Verma-Naumoff syndrome). In the latter condition the long bones are shorter and the ribs are extremely short with a very narrow cylindrically shaped thorax. Moreover, polydactyly is more common in the Verma-Naumoff short-rib polydactyly syndrome[2]. Recently, Ho et al[5] presented a non-consanguineous family in which two siblings displayed a mild form of Jeune syndrome and one infant, of related parents, demonstrated SRPS type III (Verma-Naumoff syndrome). This family provides evidence that these conditions may represent variants of the same disorder. The intrafamilial variability may reflect the effects of modifying loci on gene expression.

In Table 1 the present case is compared with structural anomalies in Jeune syndrome, Ellis-van Creveld syndrome and SRPS type III (Verma-Naumoff syndrome).

Majewski et al[9] reported on the overlap of oro-facio-digital (OFD) syndromes and SR(P)S (including Jeune syndrome) which may relate to deletions of different size within contiguous genes or compound heterozygosity for two or more mutations.

Recently, it was postulated that in a fetus with Jeune syndrome, diagnosed at 22 weeks of gestation, a nuchal translucency that was established at 14 weeks was caused by the constrictive pressure on the heart by the hypoplastic thorax[10]. However, in our case the nuchal translucency measured only 1.6 mm at 14 weeks. A severely hypoplastic thorax had already been established as early as 14 weeks of gestation. Whereas, the nuchal translucency could have been more pronounced earlier in pregnancy the constrictive pressure on the heart caused by the hypoplastic thorax would not have decreased with advancing gestational age. This emphasizes the unexplained origin of the nuchal translucency in the case reported by Ben Ami et al[10]. An enlarged nuchal translucency may be an additional finding in fetuses affected with Jeune syndrome.

It can be concluded that early prenatal sonographic diagnosis of the severe form of Jeune syndrome can be made in the late first and early second trimester of pregnancy by examining the size and shape of the thorax and ribs. Milder forms, however, may not be identified prenatally.

Table 1. Structural anomalies in Jeune syndrome, Ellis-van Creveld syndrome, SRPS type III: Verma-Naumoff and the present case.

	this case	Jeune syndrome	Ellis-van Creveld syndrome	SRPS type III: Verma-Naumoff
long bones	shortened	moderately shortened	shortened (rhizomelia)	severely shortened
ribs	short	short, horizontal		extremely short, horizontal
thorax	narrow	mild to severely narrow		narrow cylindrically shaped
hands/ feet		polydactyly of hands and feet (inconstant feature)	all cases: postaxial polydactyly of the hands (in minority of cases polydactyly of feet) all cases: dysplasia of fingernails	postaxial polysyndactyly
face			partial harelip (associated with natal teeth)	flat face
visceral anomalies		renal and liver failure starting in infancy	>50%: heart defect (atrial septum defect; primum type); epispadia; Dandy Walker malformation	urogenital anomalies (ambiguous genitalia) hydrops fetalis
X-ray features	as in Jeune syndrome	- short horizontal ribs - trident shaped pelvis - square iliac wings - horizontal acetabular roofs with medial spurs - proximal femoral ossification centre present at birth (two thirds of the cases)	- wrist: fusion of hamate and capitate bones (characteristic) - trident shaped pelvis - erosion of lateral aspects of the proximal tibial metaphysis	- short horizontal ribs - horizontal trident lower iliac margins - flat acetabulae - severely shortened long bones - widened metaphyses - longitudinal metaphyseal spurs - vertebral abnormalities

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2.3 Enlarged nuchal translucency

The aetiology of the (enlarged) nuchal translucency has not been elucidated sofar. The enlarged nuchal translucency may be based on several different aetiologies as it is associated with a wide range of chromosomal and structural anomalies and genetic syndromes[1-3]. Pandya et al[2] established that screening for fetal trisomies by maternal age and nuchal translucency measurement can be carried out effectively during the first trimester of pregnancy. In their study, the nuchal translucency thickness was above the 95th centile in 77% of fetuses with trisomy 21 and in 78% of those with other chromosomal defects. They proposed a new method of screening which involved the assessment of an individual risk based on the combination of the nuchal translucency measurement, crown-rump length and maternal age. Recently, several studies on early pregnancy screening achieved even a higher detection rate of trisomy 21 by combining nuchal translucency measurement with measurement of maternal serum PAPP (pregnancy associated plasma protein) -A and free-ß-hCG[4-8].

In chromosomally normal fetuses with an enlarged nuchal translucency there is a substantially increased prevalence of major cardiac defects, diaphragmatic hernia, omphalocele, body stalk anomaly and fetal akinesia sequence[1,3]. Furthermore, an enlarged nuchal translucency has been detected in combination with a wide range of skeletal dysplasias and genetic syndromes[1,9,10].

In subchapter 2.3.1 a fetus with an enlarged nuchal translucency at 13 weeks of gestation, which developed non-immune hydrops caused by muco-polysaccharidosis type VII is presented. This paper supports the heterogeneous origin of the enlarged nuchal translucency in the presence of a normal fetal karyotype and emphasizes the difficulty of counseling parents in these cases.

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2.3.1 In-utero diagnosis of mucopolysaccharidosis type VII in a fetus with an enlarged nuchal translucency

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Abstract

Mucopolysaccharidosis type VII was diagnosed prenatally during the first pregnancy of a Turkish consanguineous couple, following diagnostic work-up of an enlarged nuchal translucency detected by ultrasound at 13 weeks of gestation.

Mucopolysaccharidosis type VII (MPS VII) or Sly syndrome is a rare autosomal recessive lysosomal storage disease, caused by the deficiency of the enzyme ß-glucuronidase. The most severe form of MPS VII manifests itself by non-immune fetal hydrops. Tests for the diagnosis of metabolic disorders, especially lysosomal diseases, are essential when the major causes of hydrops fetalis have been excluded.

The presence of a ß-glucosidase deficiency, Gaucher's disease, in the infant of the patient's sister emphasizes the importance of a complete family history in consanguineous couples and the risk for several recessive diseases in some families.

Introduction

Mucopolysaccharidosis type VII (MPS VII) or Sly syndrome is a rare autosomal recessive storage disease caused by the deficiency of the enzyme ß-glucuronidase. The first case was reported in 1973[1]. Various phenotypes of this metabolic disorder have been described[2,3]. The most severe form of MPS VII, non-immune fetal hydrops, has been reported in several fetuses[4-10]. Clinical signs at birth or within the first years of life are coarsened facies, hepatosplenomegaly, corneal clouding, frequent respiratory infections, umbilical or additional inguinal herniae, leukocyte inclusions, short stature and developmental retardation. Another mild phenotype becomes manifest in the second decade and is presented by mild bony changes (X-ray), mild facial coarsening, normal growth and normal mental development.

Diagnosis of MPS VII is established by demonstrating ß-glucuronidase deficiency in tissues, fibroblasts, leukocytes or serum. This enables reliable prenatal diagnosis by chorionic villus sampling[10] or amniocentesis. The ß-glucuronidase gene has been mapped to 7q21.2-q22[11]. Analysis of the ß-glucuronidase gene of MPS VII patients has revealed a broad mutational heterogeneity[3,12].

A case of MPS VII diagnosed during the first pregnancy of a consanguineous couple is presented.

Case report

A 21-year old primigravida was referred to our Division of Prenatal Diagnosis because of a nuchal translucency at 13 weeks of gestation, based on the first day of the last menstrual period. She was admitted at the same time due of hyperemesis. The family history initially revealed no abnormalities. The parents were consanguineous (first cousins) and of Turkish ancestry. Ultrasound examination (HDI 3000, Advanced Technical Laboratories (ATL), Bothell, Washington) revealed a nuchal translucency of 5.7 mm and generalized skin edema. Chorionic villus sampling demonstrated a normal female karyotype.

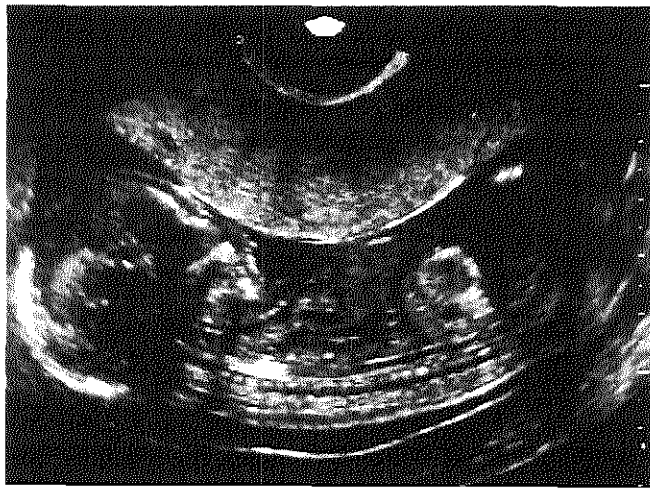

Figure 1. Fetus 1 at 15 weeks of gestation. Note the pronounced nuchal translucency, generalized skin edema and echodense bowel.

Ultrasound examination was repeated at 15 weeks of gestation (Figure 1). In addition to generalized edema, a hydrothorax and echodense bowels were present. There were no other (cardiovascular) abnormalities which could explain the fetal hydrops. Maternal serum titers indicated no recent infection (cytomegalovirus, rubellavirus, parvovirus B19, toxoplasmosis). The maternal blood group was A rhesus positive. A maternal hemoglobinopathy was already excluded before the pregnancy, thus ruling out the most common causes of fetal hydrops. Based on the presence of fetal hydrops and parental consanguinity an autosomal recessive metabolic disorder was suspected. Amniocentesis was carried out at 17 weeks of gestation to screen for a series of metabolic disorders (Table 1; fetus 1). This enabled the detection of mucopolysaccharidosis type VII by demonstrating a ß-glucuronidase deficiency. Following counseling, the parents opted for termination of pregnancy, which was carried out at 20 weeks of gestation. Post mortem investigation was not permitted.

Additional information on the family history was revealed at the time of enzyme analysis of fetus 1. It appeared that the patient's sister had a previous stillbirth (Figure 2; fetus 2) at 22 weeks of gestation of a severely hydropic fetus. Gaucher's disease was suspected by the finding of large, atypical histiocytes in many organs at post mortem investigation. A ß-glucosidase deficiency (Table 1; fetus 2) established the diagnosis of Gaucher's disease and subsequent mutation analysis showed homozygosity for a null mutation in the glucocerebrosidase gene[13].

The second pregnancy of our patient (fetus 3) revealed normal activity of ß-glucuronidase and ß-glucosidase in chorionic villi. A healthy infant was born at term.

Table 1. Enzyme activities in amniocytes (fetus 1) and fibroblasts (fetus 2).

Enzyme	Metabolic disorder	Fetus 1	Controls amniocytes	Fetus 2	Controls fibroblasts
ß-glucuronidase	MPS VII/ Sly syndrome	0.5	139;207		
ß-glucosidase	Gaucher disease	116	159;174	0.5	130-400
ß-galactosidase	GM1-Gangliosidosis	943	652;1580	332	450-1250
α-fucosidase	Fucosidosis	287	244;364		
α-mannosidase	α-Mannosidosis	110	108;165		
ß-mannosidase	ß-Mannosidosis	76	81;103		
ß-hexosaminidase	Sandhoff disease	6630	3800;5280		

Discussion

Non-immune fetal hydrops occurred in each of the previously reported perinatal cases of MPS VII, but different pathways led to the diagnosis[4-10].

The first in-utero diagnosis of MPS VII was reported in 1992[6]. Two previous hydropic infants of a consanguineous couple were stillborn at 35 and 28 weeks of gestation, respectively. ß-Glucuronidase deficiency was diagnosed in both infants. The origin of fetal hydrops in a following pregnacy, which was diagnosed at 18 weeks of gestation, remained unclear. A normal fetal karyotype was present; intra-uterine infections and Rhesus iso-immunisation could be excluded. Repeated albumin transfusions were administered to the fetus until ß-glucuronidase deficiency was diagnosed in cultured amniotic cells and lymphoblasts. Fetal death occurred in the third trimester of pregnancy.

A similar case of parental consanguinity and two previous stillborn infants with ascites was described by Nelson et al[7]. Post mortem examination was not permitted. In a subsequent pregnancy fetal hydrops was demonstrated at 22 weeks and fetal death was diagnosed six weeks later. In this case, placental histology provided an important clue to the diagnosis. The presence of pronounced foamy cytoplasmic changes in the villous Hofbauer cells of the placenta raised the possibility of a lysosomal storage disorder. The parents were shown to have ß-glucuronidase activity in the heterozygous range in leucocyte and fibroblasts, which suggested that the non-immune hydrops was caused by MPS VII.

In another case MPS VII was suspected on the basis of histopathological examination[8]. Finely vacuolated interstitial foamy cells were present in many organs, especially in the spleen, lung, myocardium, bowel mucosa and bone marrow. The placenta showed vacuolation of villous Hofbauer cells. The diagnosis MPS VII was confirmed by quantitation of enzyme activity in cultured fibroblasts.

In a further case of parental consanguinity and non-immune hydrops fetal death occurred at 26 weeks of gestation[5]. Again the fetal karyotype was normal, intra-uterine infections were excluded but post mortem examination was not permitted. A skin biopsy of the infant was taken and ß-glucuronidase deficiency was diagnosed in the cultured fibroblasts.

Three cases of MPS VII were reported from different, non-consanguineous families[4,9,10]. In the first case lysosomal overloading in the fetal kidneys, liver and spleen was seen by light and electron microscopy. Deficiency of ß-glucuronidase was diagnosed in cultured amniotic cells and fetal plasma.
In the second case a Caesarean section was carried out at 32 weeks of gestation because of fetal hydrops that was first diagnosed at 22 weeks. The major causes of fetal hydrops had been excluded. Cultured skin fibroblasts revealed a deficiency of ß-glucuronidase activity. The infant died at seven months of age. Intra-uterine growth acceleration (birth length, femur length and thoracic spine

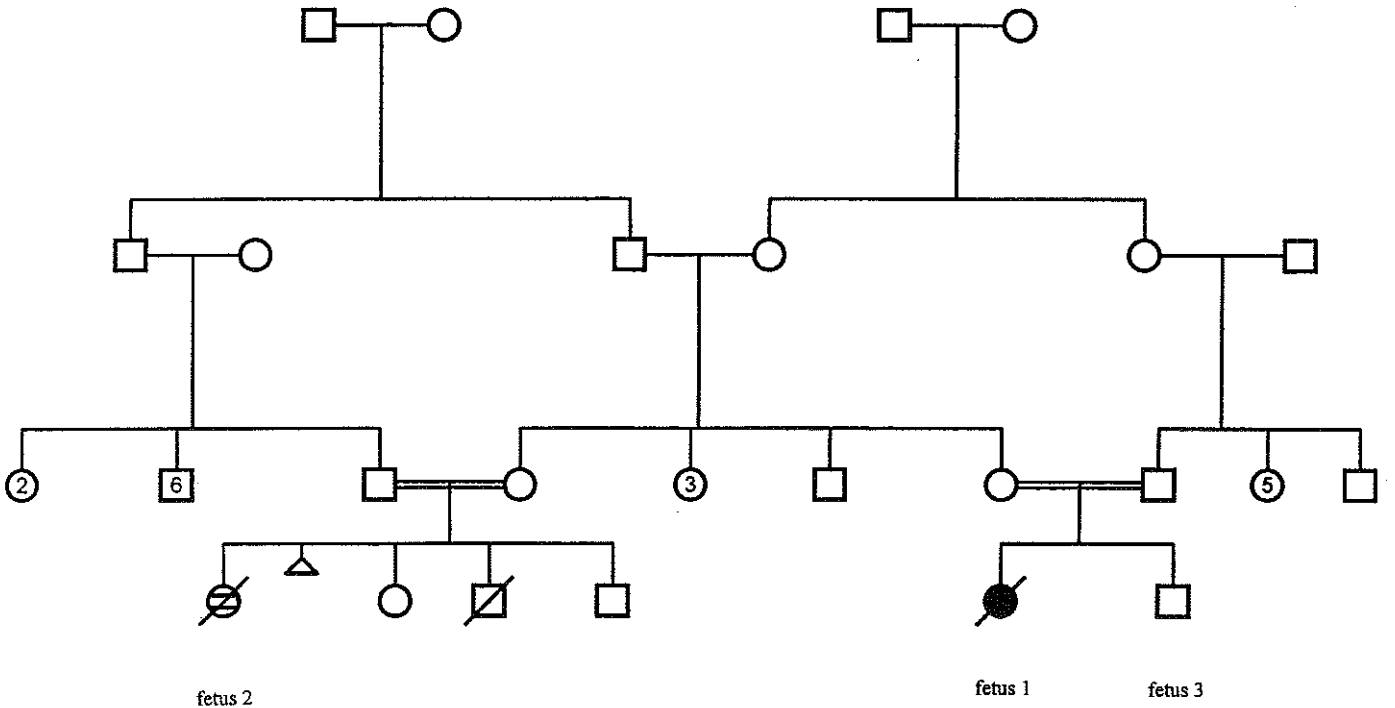

Figure 2. Pedigree of family with pregnancies affected with mucopolysaccharidosis type VII (fetus 1) and β-glucosidase deficiency, Gaucher's disease (fetus 2).

length) and bone maturation were reported in this infant with MPS VII associated with fetal hydrops[9].

In the third case enzyme analysis of fibroblasts cultured from the skin of a second hydropic, stillborn infant revealed a deficiency of ß-glucuronidase[10]. In the next pregnancy a transcervical chorion villus sampling was carried out at 11 weeks of gestation and ß-glucuronidase activity in the villi appeared deficient. Aspirotomy was performed and histo-pathological examination demonstrated edematous fetal parts.

In our case, the patient obviously did not acknowledge the importance of her sister´s infant with Gaucher´s disease since this was not mentioned at the first visit for ultrasound examination. When this diagnosis became clear we expected to find a ß-glucosidase deficiency in fetus 1 rather than a ß-glucuronidase deficiency.

This case report emphasizes the importance of the family history and the importance of paying attention to family members being at risk when counseling a (consanguineous) couple. The patient and her sister are both potentially at risk for two autosomal recessive diseases in their offspring.

Analysis of the ß-glucuronidase gene of MPS VII patients has revealed extensive genetic heterogeneity, even in a group of patients presenting with fetal hydrops[3,12].

MPS VII is a rare disorder. Between 1985 and 1996, six cases of MPS VII, all associated with fetal hydrops have been diagnosed in the Netherlands[14]. This suggests a prevalence of 1:417 000 live births, which is 5% prevalence of the whole group of mucopolysaccharidoses (1:22 000[14,15]) and 1.7% of the combined prevalence for all lysosomal storage disorders (1:7100[14] and 1:7700[15]) which have recently been established in the Netherlands[14] and Australia[15] respectively. It seems likely that the prevalence of fetal hydrops due to lysosomal enzyme defects has been underestimated as enzyme investigations have not routinely been performed in such cases. Diagnosing a metabolic disorder is important because of the high risk of recurrence and the possibility of early diagnosis, for example by chorionic villus sampling[16]. Tests for the diagnosis of metabolic disorders should be performed when major causes of hydrops fetalis have been excluded.

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PART TWO

LATE FETAL ANOMALY SCANNING:
second and third trimester of pregnancy

CHAPTER 3
LATE FETAL ANOMALY SCANNING

3.1 Introductory remarks

As discussed in subchapter 1.2 a single ultrasound scan in pregnancy will not detect all fetal anomalies. Some anomalies may present at varying gestational age, such as hydrocephaly. Other anomalies may develop as late as the third trimester of pregnancy, such as microcephaly, porencephaly, intracranial arachnoid cysts, etc.

Combining the 11-14 week scan and the standard 18-21 week scan, between 80-90% of the fetal anomalies will be detected[1-3]. The remainder of the fetal anomalies may be recognized in the third trimester of pregnancy or not at all. For example, normal biometry on a second trimester scan does not exclude the development of microcephaly in a fetus or infant. This is not surprising as microcephaly may even develop as late as the neonatal period or in childhood[4]. The same applies to the diagnosis of hydrocephaly. Both hydrocephaly and microcephaly are conditions with a high percentage of poor outcome, such as mental disability. The possibility of a prenatal diagnosis will also be determined by the presence of associated anomalies.

Both microcephaly and hydrocephaly may be associated with an abnormal karyotype, or develop *in utero* as a result of infection, haemorrhage, drugs, intoxications or represent a heterogeneous group of monogenic and multifactorial genetic conditions.

Hydrocephaly is associated with other intracranial malformations in 37% and extracranial malformations are present in 63% of cases[5]. In isolated hydrocephaly or ventriculomegaly, the prognosis depends on the intracranial pressure dynamics. Intraventricular pressure first affects the development of the white matter of the brain. The gray matter is said to be protected from permanent damage for a longer period. If ventriculomegaly is severe, prenatal differential diagnosis between cortical mantle thinning with normal developmental capacity and the existence of permanent cortical atrophy or dysplasia is hardly possible[6]. *Pediatric data,* however, suggest a correlation between cortical mantle thickness and long-term intellectual capacity. To minimize the deleterious effects of intracranial pressure on the preterm brain, it is suggested that hydrocephaly should be 'treated' in the early period of neuronal maturation, that is in the last trimester of pregnancy by induction of delivery[5].

In our retrospective study of 42 fetuses with sonographically diagnosed fetal ventriculomegaly (subchapter 3.1.1), we concluded that the prediction of

postnatal outcome of prenatally diagnosed ventriculomegaly is disappointing. Our assumption was that fetal outcome was directly determined by the nature and severity of the ventriculomegaly as major other (extracranial) anomalies were not diagnosed at the time of prenatal diagnosis. However, postnatally, the subset of patients proved to be heterogeneous. Minor and major anomalies occurred in nearly half of the infants, with a majority in the subset of retarded psychomotor development. Postnatal examination revealed syndromal anomalies in at least five infants, four of which were in the subset of retarded psychomotor development. These observations emphasize the importance of combining dysmorphological evaluation, post mortem examination, cytogenetic/DNA studies and genetic counseling for future pregnancies.

As routine scanning at 11-14 weeks as well as routine scanning at 18-21 weeks is still subject to debate in The Netherlands, a considerable percentage of pregnancies is referred to tertiary centres in the late second or third trimester of pregnancy with divergent fetal anomalies. This is clearly demonstrated in the subchapters 3.1.1, 3.1.2, 3.1.3.

Although isolated microcephaly is not likely to be diagnosed in the first or early second trimester of pregnancy, in the retrospective study presented in subchapter 3.1.2, in 25 out of 30 infants microcephaly proved to be part of a complex problem emphasizing the need for a meticulous search for structural anomalies and fetal karyotyping and again stressing the importance of post mortem examination, follow-up and genetic counseling.

A combination of certain malformations may lead to the prenatal diagnosis of a rare syndrome as is reported in subchapter 3.1.3. We describe the prenatal sonographic diagnosis together with the post mortem results of a fetus with an acrofacial dysostosis syndrome at 25^5 weeks of gestation. As the limb and facial anomalies associated with this syndrome are obvious at an early developmental stage, parents should be offered a first trimester ultrasound scan in a tertiary centre in successive pregnancies.

In subchapters 3.1.4 and 3.1.5 the prenatal assessment of brachydactyly types A1 and A3 are described. The careful observation of hand/foot malformations is not only useful to inform parents about a possible recurrence of their often dominantly transmitted disorder, but may especially be helpful in a multitude of multiple anomaly syndromes associated with such anomalies. With the improving resolution of ultrasound equipment these anomalies might even become detectable as early as the late first to early second trimester of pregnancy (Appendix photograph 40).

When counseling is requested, collecting as much significant information about the index patient is essential. In every individual case, extensive paediatric

dysmorphological examination or post mortem examination in case of death of the index patient is important in order to confirm the prenatally established anomalies and/or to determine the association with other malformations to allow a diagnosis. As post mortem examination is not always consented or performed or informative (in case of maceration), ultrasound findings may be invaluable in genetic counseling. The value of a detailed sonographic dysmorphology will remain even when a slowly increasing number of syndromes becomes detectable at DNA-level. An early suspicion of a diagnosis in the family will be essential to start a diagnostic work up, which may often lead to years of waiting before a family specific mutation can be identified, frequently by international collaboration, if this is accessible to the family. In the 'waiting' period, many parents will opt for a pregnancy using available technology and expertise. This integration of sonographic and dysmorphological expertise is as important as the increasing resolution of ultrasound equipment in this respect.

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3.1.1 Prenatally diagnosed fetal ventriculomegaly; prognosis and outcome

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Abstract

The purpose of the present study was to determine the postnatal outcome and prognostic factors of prenatally diagnosed ventriculomegaly and to establish the relationship between prenatal sonographic measurements and postnatal psychomotor development.

A total of 42 singleton pregnancies with sonographically determined fetal ventriculomegaly at 20-38 weeks' gestation were reviewed, together with follow-up data on postnatal outcome at a mean of 29 months after delivery. Sonographic measurements included head circumference, cerebral lateral ventricular diameter at the anterior and posterior horn level and hemisphere diameter. Classification of psycho-motor development consisted of assessment of motoric behavior, speech, communication and social skills ('Van Wiechen' classification).

Perinatal mortality rate was 38%, of which half was directly associated with cephalocentesis. Only the ventricle/hemisphere ratio for the anterior and posterior horn of the lateral cerebral ventricles was significantly higher among perinatal deaths than amongst the survivors. Within the subset of survivors (n=26), psycho-motor development was normal in 46%. Postnatal examination revealed syndromal anomalies in five infants, of which four were associated with psycho-motor retardation.

Prenatally diagnosed ventriculomegaly has a poor postnatal outcome with more than 50% of the live-born infants demonstrating abnormal psycho-motor development. The predictive value of fetal biometric measurements is poor. The presence of syndromal anomalies emphasises the need for genetic counseling in future pregnancies.

Introduction

Ventriculomegaly represents an abnormal increase in the volume of cerebral ventricles. It is almost always due to obstruction of the flow of cerebrospinal fluid and the resulting increase in intracranial pressure. Ventriculomegaly may be associated with dysmorphogenesis, an abnormal karyotype or defects acquired *in utero* (i.a. infection, intraventricular hemorrhage). The prognosis of ventriculomegaly depends on the primary aetiology[1].

Several papers have been published concerning the prognosis of prenatally diagnosed ventriculomegaly/hydrocephaly[2-9]. These studies differ in number of patients studied, inclusion criteria, outcome and prognosis. The differences in outcome and prognosis may be the result of the heterogeneity of the studied groups. The most homogeneous group is probably that studied by Renier et al.[4] They published the clinical records of 108 infants presenting with hydrocephaly, Dandy Walker Malformation or brain cysts, at birth. Out of 52 children who had reached school age, only 29% achieved a normal academic level. The psychological status was normal or borderline in 46% of the infants. The same authors found that head enlargement at birth, ventricular size and the age at the time of surgery (shunt placement) are not related to later functional development.

We present 42 cases of fetal ventriculomegaly that were diagnosed by ultrasound whilst there was no evidence of major extracranial fetal structural abnormalities. Fetuses with an abnormal karyotype were excluded. Thirty-five (35/42 = 83%) women were referred to our centre after an ultrasound scan was performed at 24 weeks of gestation or later. Of these women, 12 (12/35 = 34%) had undergone a first trimester ultrasound scan. In the remaining seven (7/42 = 17%) women an ultrasound scan was first performed between 19-24 weeks of gestation. Only in one case this did this lead to an early referral to our centre at 20 weeks of gestation.

The purpose of this retrospective study was to determine the prognosis of prenatally diagnosed ventriculomegaly and to establish the relationship between prenatal sonographic measurements and postnatal psycho-motor development.

Materials and methods

Between 1st January 1989 and 1st January 1994, 86 fetuses with ventriculmegaly without major extracranial abnormalities or neural-tube defects were diagnosed by prenatal ultrasound. All patients were referred to the Division of Prenatal Diagnosis for a detailed anomaly scan following suspected fetal ventriculomegaly in regional community hospitals. There were 69 singleton pregnancies, 15 twin pregnancies with one abnormal fetus and one twin pregnancy with two fetuses with ventriculomegaly. Of the 69 singleton pregnancies there were five fetuses with an abnormal karyotype. All other fetuses were chromosomally normal. Eighteen pregnancies were terminated, of which 13 before and five beyond 24

weeks of gestation because of ventriculomegaly (n=15) or Dandy Walker malformation (n=3). Since the purpose of this study was to evaluate the prognosis of prenatally diagnosed ventriculomegaly in singleton pregnancies, twin pregnancies, pregnancy terminations and karyotypically abnormal fetuses were excluded from further analysis. Thus, 46 singleton pregnancies were reviewed retrospectively for diagnosis and outcome. Pregnancy duration varied between 20 and 38 weeks of gestation (mean 31 weeks). From each fetus, measurements of head circumference, cerebral ventricular diameter at the level of the anterior and posterior horn, and maximum hemisphere diameter were collected from a transverse cross section through the fetal head at the level of the cavum septum pellucidum. The ventricle/hemisphere ratios at the level of the anterior (Va/H) and posterior (Vp/H) horns were calculated. All data were related to normal reference charts according to Snijders and Nicolaides[10].

Mild ventriculomegaly was defined as a dilatation of the posterior horns of the lateral ventricles between 10-15 mm and ventriculomegaly as a dilatation of the anterior and posterior horns of the lateral ventricle of 16 mm or more. Dandy Walker malformation was diagnosed in case of a cerebellar vermis defect and a posterior fossa cyst.

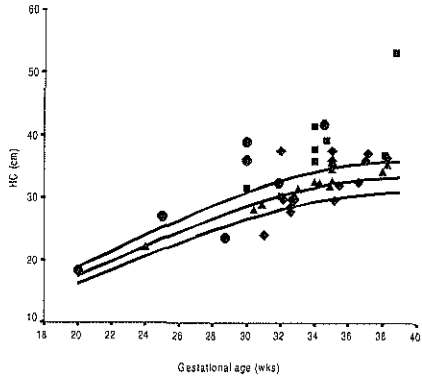
Screening for TORCH was carried out in each instance. Mode of delivery and the need for cephalocentesis during delivery in the presence of cephalo-pelvic disproportion were noted. In the case of perinatal mortality, postmortem reports were reviewed for confirmation of the diagnosis made by ultrasound.

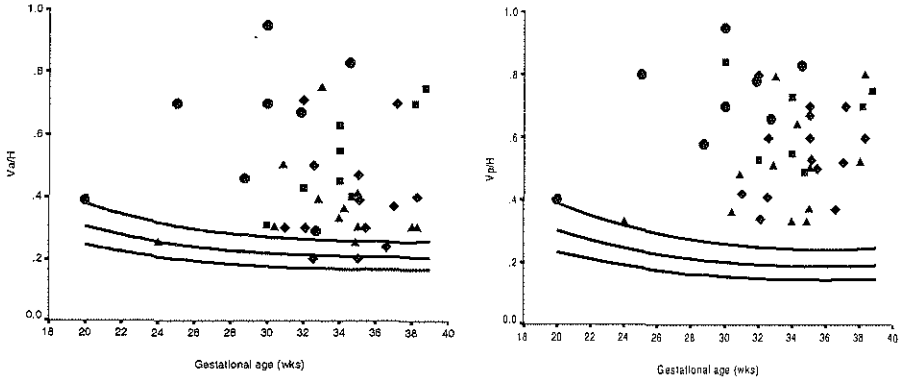
The parents of the surviving infants were asked to give permission to obtain information from the attending pediatrician regarding diagnosis, treatment and psycho-motor development of their infant at the age of six months or older. Four infants were lost to follow-up since permission from the parents could not be obtained, resulting in 42 cases for further analysis.

Within several hours to days after delivery an ultrasound scan of the cerebrum was performed in case of postnatal survival. Depending on the results of the ultrasound scan and the infant's well being a CT-scan and/or MRI was performed.

Classification of the psycho-motor development was carried out according to a standardized and validated model called 'Van Wiechen' classification[11]. This classification system is applied in all Child Health Care Centres and Pediatric Departments in the Netherlands at the age of four weeks to four and a half years. The classification system consists of age dependent assessment of motor behavior, speech, communication and social skills. Retardation was considered to be present when development in one or more of the above domains was retarded. Absent or only minimal psycho-motor development was considered a state of severe retardation.

Figure 1. Head circumference (HC) in 42 fetuses with prenatally diagnosed ventriculomegaly; upper and lower limits represent 95th and fifth centiles. (normal psychomotor development (▲), retarded psychomotor development (◆), perinatal deaths cephalocentesis (●), perinatal deaths (■).)





Figures 2 and 3. Ventricle/hemisphere (V/H) ratio for the anterior horn (Va/H), left; for the posterior horn (Vp/H), right, in 41 fetuses with prenatally diagnosed ventriculomegaly; upper and lower limits represent 95th and fifth centiles. (normal psychomotor development (▲), retarded psychomotor development (◆), perinatal deaths cephalocentesis (●), perinatal deaths (■).)

Results

There were 35 fetuses with ventriculomegaly and five fetuses with mild ventriculomegaly. Moreover there were two fetuses with ventriculomegaly and a Dandy Walker Malformation. Perinatal death occurred in 16 fetuses. Of the 26 survivors, assessment of psychomotor development at six months or later showed normal development in 12 infants and retarded development in 14 infants.

Perinatal deaths (n=16; Table 1)

Marked ventriculomegaly was diagnosed prenatally in 15 fetuses, and ventriculomegaly with Dandy Walker malformation in one fetus. Mean gestational age at diagnosis was 30 weeks (range: 20 - 38 wks). Intra-uterine death occurred in one fetus (no. 1) at 28 weeks as a result of placental insufficiency. Intrapartum

death took place in seven fetuses (nos 2-8), six of these resulting from cephalocentesis which was conducted because of cephalo-pelvic disproportion. Early neonatal death was established in eight infants (nos 9-16), two of these resulting from cephalocentesis during labour. Postmortem examination was available in nine out of 16 fetuses. Parents refused postmortem examination or no examination was requested in four fetuses. No information could be gathered in the remaining three fetuses. Postpartum examination revealed additional anomalies in five infants: a horse shoe kidney, an intracranial teratoma with anencephalic embryos[12], spina bifida, alobar holoprosencephaly and VATER-hydrocephaly syndrome.

Survivals with normal psychomotor development (n=12; Table 2)

Mild ventriculomegaly existed in three fetuses and ventriculomegaly in nine fetuses. Mean gestational age at diagnosis was 31 weeks (range: 24 - 38 wks). In three infants with mild ventriculomegaly and one infant with ventriculomegaly, postnatal examination revealed no abnormalities (nos 17,21,23,27). Aquaduct stenosis was diagnosed in two infants. Complete corpus callosum agenesis was established postnatally in three infants (nos 18 and 28 by CT-scan; no. 19 by ultrasound of the cerebrum). The unilateral dysplastic kidney in patient 24 was confirmed postnatally. A ventriculo-peritoneal shunt (VP-shunt) was placed in three out of 12 infants (25%) at two weeks to two months after delivery. Information on normal psychomotor development at the age of two years or more was available in eight infants and at the age of four years or more in three infants. There were no syndromal anomalies in this subset of patients.

Survivals with retarded psychomotor development (n=14; Table 3)

In this subset, mild ventriculomegaly was established prenatally in two fetuses and ventriculomegaly in 11 fetuses. There was one fetus with ventriculomegaly and Dandy Walker malformation. Mean gestational age at diagnosis was 32 weeks (range 25-38 weeks). No intrapartum cephalocentesis was performed. A VP-shunt was placed in seven out of 14 infants (50%) at four days to four months after delivery.

Minor and major central nervous system and other anomalies were diagnosed postnatally in 11 out of 14 patients (80%). In four instances syndromal pathology was established (nos 31,34,38,40). Corpus callosum pathology was established postnatally in five patients (nos 29,30,34,35,41). In patient 30 lobar holoprosencephaly and in patient 35 semilobar holoprosencephaly was diagnosed postnatally. In patient 37 ventriculomegaly happened to be the result of massive bleeding due to immuno-thrombocytopenia.

Information on psycho-motor development at the age of two years or more was available in seven infants and at the age of four years or more in three infants. In patient 42 there only was a slightly retarded motor development at the age of 1.5 years.

Table 1. Prenatal ultrasound findings and menstrual age (wks) at first visit and post mortem results of 16 perinatal deaths

Ultrasound findings/ menstrual age (wks)	Cephalo-centesis (cc)	Delivery (wks) M/F	Postmortem results
1. Ventriculomegaly 28		31 F	Hydrocephaly, horse-shoe kidney, placental infarctions
2. Ventriculomegaly 28	300	35 F	n.c.
3. Ventriculomegaly 30		37 F	Communicating hydrocephaly
4. Ventriculomegaly 32	750	35 F	*Teratoma* with anencephalic embryo's
5. Dandy Walker Malformation 32	yes	37 M	n.p.
6. Ventriculomegaly 34	yes	37 M	Hydrocephaly
7. Ventriculomegaly 38	150	40 M	*Spina bifida*, hydrocephaly
8. Ventriculomegaly 38	1500	38 M	n.p.
9. Ventriculomegaly 20		21 F	n.i.
10. Ventriculomegaly 24		31 F	*Alobar holoprosencephaly*, hypertelorism, retrognathia, low set ears, oesophageal atresia
11. Ventriculomegaly 25		25 F	Aquaduct stenosis
12. Ventriculomegaly 27		36 M	n.c.
13. Ventriculomegaly 27	500	36 F	n.i.
14. Ventriculomegaly 30	200	33 F	Aquaduct stenosis
15. Ventriculomegaly 31		35 M	Aquaduct stenosis, horse shoe kidney, low set ears, anal atresia, etc. *VATER-hydrocephaly Syndrome*
16. Ventriculomegaly 32		34 M	n.i.

F= female, M= male; n.p. = not performed, n.c. = no parental consent, n.i. = no information.
Case 1 = IUD, case 2-8 = intrapartum death, case 9-16 = neonatal death.

Table 2. Survivals with normal psychomotor development (n=12)

Ultrasound findings/ menstrual age (wks)	Delivery (wks) M/F	Postnatal diagnosis by pediatrician/ geneticist	VP shunt	Psychomotor development
17. Mild ventriculomegaly 24	39 M	Normal		1 year: normal
18. Ventriculomegaly 26	39 M	Mild hydrocephaly, corpus callosum agenesis		2.5 years: normal
19. Ventriculomegaly and corpus callosum agenesis 28	40 F	Hydrocephaly, corpus callosum agenesis		2.5 years: normal
20. Ventriculomegaly 29	40 M	Aquaduct stenosis	4 wks	2.5 years: normal
21. Mild ventriculomegaly 30	42 M	Normal		4 years: normal
22. Ventriculomegaly 30	37 F	Aquaduct stenosis	2 wks	3 years: normal
23. Mild ventriculomegaly 33	40 M	Normal		10 months: normal
24. Ventriculomegaly and unilat.dyspl kidney 33	38 F	Hydrocephaly, unilat.dysplastic kidney	2 months	1 year: normal
25. Ventriculomegaly 33	39 F	Ventriculomegaly		7 months: normal
26. Ventriculomegaly 34	37 M	Hydrocephaly, choroid plexus bleeding		5 years: normal
27. Ventriculomegaly 35	38 F	Normal		2 years: normal
28. Ventriculomegaly 38	39 M	Ventriculomegaly, corpus callosum agenesis		4 years: normal

Table 3. Survivals with retarded psychomotor development (n=14)

Ultrasound findings/ menstrual age (wks)	Delivery (wks) M/F	Postnatal diagnosis by pediatrician/ geneticist	VP shunt	Psychomotor development
29.Mild ventriculomegaly 25	40 F	Postnatal diagnosis: ventriculomegaly. Diagnosis at 3 years: hypoplastic corpus callosum, mild Arnold Chiari malformation, diffuse encephalopathy		3 years: retarded, vocabulary < 10 words
30.Ventriculomegaly asymmetrical 27	36 M	**Lobar holoprosencephaly**, corpus callosum agenesis	2 wks	1 year: retarded, epilepsy
31.Ventriculomegaly, eye deformity 28	41 M	Hydrocephaly, microphtalmia, ear deformity, hypoplastic genitals - **Walker Warburg Syndrome**		severely retarded; died at 1.5 years
32.Ventriculomegaly 29	38 F	Extreme hydrocephaly, thin cortical mantle, retrognathia	1 month	22 months: retarded
33.Ventriculomegaly 31	37 F	Ventriculomegaly, **microcephaly**		1 year: severely retarded, spastic
34.Ventriculomegaly 32	38 M	Corpus callosum agenesis, ventriculomegaly, micrognathia, finger and toe abnormalities – **Rubinstein Taybi Syndrome**		5 years: retarded; walks, imitates sounds, does not form words
35.Mild ventriculomegaly 32	37 F	Dilated posterior horns, partial corpus callosum agenesis – **Semilobar holoprosencephaly**		1.5 years: severely retarded (developmental age: 4 months)
36.Ventriculomegaly asymmetrical 33	36 M	Asymmetrical hydrocephaly	3 wks	1 year: retarded, right-sided hemianopsia and hemiplegia, left-sided hemi-hypsarrhythmia
37.Ventriculomegaly, unilat. plexus chor. abn. 34	36 F	Hydrocephaly, **Immunothrombocytopenia** causing severe intracranial bleeding	4 months	3 years: severely retarded, spastic tetraplegia, epilepsy

Table 3. *continued*

Ultrasound findings/ menstrual age (wks)	Delivery (wks) M/F	Postnatal diagnosis by pediatrician/geneticist	VP shunt	Psychomotor development
38.Ventriculomegaly 35	40 M	Hydrocephaly, exophtalmus **Walker Warburg syndrome**		4.5 years: severely retarded, spastic tetraplegia, epilepsy
39.Ventriculomegaly 35	40 M	Communicating hydrocephaly		3 years: severely retarded; no psychomotor development
40.Ventriculomegaly 36	40 F	Hydrocephaly, intracranial cysts, webbing of the hands, gnatho- palato schizis, lobated tongue - **Oro-facio-digital Syndrome, type 1**	2 months	2 years: retarded; does not sit steadily; speech: several words
41.Dandy Walker Malformation 37	38 F	Dandy Walker Malformation, corpus callosum agenesis, low set ears	2.5 months	4 years: severely retarded; 'sign-language'
42.Ventriculomegaly 38	39 F	Hydrocephaly	4 days	1.5 years: slightly retarded motor development

In patient 38 it was impossible to measure the anterior horn of the lateral ventricle and in patient 2 it was impossible to measure the posterior horn.

Fetal head circumference (Figure 1), ventriculo/hemisphere ratio for the anterior horn (Va/H) (Figure 2) and posterior horn (Vp/H) (Figure 3) were compared between each of the three subsets to determine the predictive value for fetal outcome. Pregnancy duration was not significantly different between the three subsets.

A statistically significant difference (Student *t*-test) was established between survivors and perinatal deaths for the Va/H (Figure 2; $P< 0.005$) and Vp/H (Figure 3; $P< 0.002$). Statistical significance was maintained ($P< 0.01$; $P< 0.006$) when excluding the eight cases of cephalocentesis.

Discussion

Prediction of postnatal outcome of prenatally diagnosed fetal ventriculomegaly is thus far disappointing. Its diagnosis represents a formidable challenge to the obstetrician since the ventriculomegaly is often detected late in pregnancy precluding pregnancy termination. Moreover, ventriculomegaly may result in cephalo-pelvic disproportion with the obstetrician facing a dilemma as to whether to perform a Caesarean section or cephalocentesis with the objective of a vaginal delivery. The patient cohort in the present study comprises of fetuses with ventriculomegaly without evidence of other major non-central nervous system anomalies or neural tube defects at the time of prenatal diagnosis. Therefore, our assumption was that fetal outcome is directly determined by the nature and severity of the ventriculomegaly. Of the 42 fetuses with ventriculomegaly, the perinatal death rate was 38%. A considerable percentage (8/16 = 50%) of these deaths was caused by cephalocentesis in the presence of cephalo-pelvic disproportion. Whereas normally a Caesarian section is contemplated under these circumstances, this was not persued in the presence of severe ventriculomegaly. Within the subgroup of survivors (26/42 = 62%), normal psychomotor development was observed in 46% (12/26). This percentage is similar to that reported by Renier et al.[4]

The predictive value of the different biometric variables was disappointing. Only the anterior and posterior horn of the lateral ventricles were significantly larger amongst the perinatal deaths than amongst survivors. Also, when the eight cases of cephalocentesis are excluded from the study, the difference is still significant. Nowadays, lateral ventricular atrium measurements are also included in the differentiation between a normal and dilated ventricular system[13,14]. This measurement was introduced in our department in 1992, which would exclude nearly half of the data in the present study.

In the subset of normally developing infants prenatally established ventricular dilatation was not confirmed postnatally in four infants. Three of these four infants represented with prenatally diagnosed mild ventriculomegaly.

Within hours to several days after delivery, a sonographic scan of the brain was carried out. Depending on the degree of ventriculomegaly, MRI and/or CT scans were performed. In case of normal findings or mild ventriculomegaly, follow-up consisted of repeat sonographic brain scans and discharge at the age of 12-18 months.

Postnatal examination revealed that minor and major anomalies other than mild ventriculomegaly occurred in nearly half (20/42) of the infants, with a majority (11/20) in the subset of retarded psychomotor development. Spina bifida, which is potentially detectable by ultrasound was not detected in patient number 7, probably due to breech position at 38 weeks of gestation. Although finger and toe abnormalities and micrognathia in patient number 34 may be detectable by ultrasound, other postnatally diagnosed anomalies in the syndromal cases such as ear deformity, hypoplastic genitals, webbing of the hands, gnatho-palato schisis

and lobated tongue are less subject to detection by ultrasound. Ventriculomegaly and (semi)lobar holoprosencephaly are often difficult to distinguish, as was the case in three fetuses in the present study. Amongst the survivors there were eight infants with corpus callosum pathology (31%) with a nearly equal distribution between normally developing (n=3) and retarded infants (n=5). The prognosis of psychomotor development in case of corpus callosum agenesis is not related to the degree of absence of this structure but rather depends on the accompanying cerebral developmental anomalies. Some syndromes have partial rather than complete agenesis; there are also individuals with complete agenesis who are phenotypically normal[15].

Genetically, ventriculomegaly represents a heterogeneous group of anomalies. This applies to ventriculomegaly due to X-linked aquaduct stenosis as well as ventriculomegaly associated with central nervous system and non-central nervous system anomalies.

Postnatal examination revealed syndromal anomalies in five infants, four of which in the subset of retarded psychomotor development. This observation emphasises: (i) the possiblity of syndromal appearance of prenatally diagnosed ventriculomegaly; (ii) the limitation of diagnostic ultrasound in detecting other minor anomalies; (iii) the need for genetic counseling of the parents, since syndromal anomalies may follow a relevant inheritance pattern. In the present study, the recurrence risk varied from sporadic as presented by the VATER-hydrocephaly syndrome and Rubinstein-Taybi syndrome to X-linked dominant (Oro-facio-digital syndrome, type 1) or autosomal recessive (Walker-Warburg syndrome). Not all parents were referred for genetic counseling. Therefore, it is possible that there are more syndromal cases in this group.

Some caution is needed to extrapolate the current findings to other obstetric settings. The present study is of a retrospective nature and only women referred for further diagnostic evaluation by ultrasound were analysed, thereby creating a potential selection bias towards fetuses with poor prognosis in terms of both mortality and morbidity. If all prenatally detected intra- and extracranial abnormalities are considered (nos 5, 19, 24, 30, 31, 36, 37 and 41), then only two of eight infants had an apparently normal outcome. Of the six fetuses (nos 5, 19, 30, 36, 37 and 41) with prenatally detected intracranial abnormalities or asymmetrical ventriculomegaly, five had a poor outcome. The prognosis of infants with ventriculomegaly that is only detected at birth is probably different from that of fetuses with prenatally detected ventriculomegaly.

In our series prenatally diagnosed ventriculomegaly had a poor postnatal outcome. Of the live-born infants, over 50% demonstrated abnormal psychomotor development. The predictive value of fetal biometric measurements is disappointing. Only the ventricle/hemisphere ratio for anterior and posterior horn of the lateral ventricle was significantly higher amongst perinatal deaths than amongst survivors. Prenatally diagnosed ventriculomegaly has a relatively better prognosis in the absence of other intracranial malformations. The presence of

syndromal anomalies in prenatally diagnosed ventriculomegaly necessitates genetic counseling for future pregnancies.

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3.1.2 Congenital microcephaly detected by prenatal ultrasound: genetic aspects and clinical significance

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Abstract

Objective The aim of this study was to analyse fetuses with prenatally diagnosed microcephaly including the nature of associated anomalies and the genetic-diagnostic implications.

Design Retrospective study design.

Methods A total of 30 fetuses with reliable dates and with prenatally diagnosed microcephaly as a common feature were analysed.

Results Microcephaly was diagnosed at a mean gestational age of 28 weeks. More than half of the fetuses were also small for gestational age. Five subsets of microcephaly emerged from this study: (1) isolated microcephaly (16.7%), (2) microcephaly due to holoprosencephaly (16.7%), (3) microcephaly associated with chromosomal disorders (23.3%), (4) microcephaly as part of a genetic syndrome (20.0%) and (5) microcephaly as part of multiple anomalies (23.3%).

Conclusions In 25 out of 30 infants microcephaly proved to be part of a complex problem, emphasizing the need of a meticulous search for structural anomalies and fetal karyotyping when biometric data are not according to gestational age. The etiologic heterogeneity and variability of microcephaly in genetic syndromes are among the more difficult issues in prenatal ultrasound in pregnancies either with an incidental finding of this anomaly, or in cases with a recurrence risk. The complex situations described in this study demonstrate the importance of follow up, post-mortem investigation and careful genetic counseling.

Introduction

Microcephaly is characterized by a small size of the brain as evidenced by an abnormally small head circumference and has an estimated incidence of 1:6200-

1:8500 neonates[1-4]. The true incidence may be higher as the disorder often escapes detection in intra-uterine death and stillborn infants.

The causes of microcephaly are heterogeneous and range from teratogenic, infectious, to multifactorial and chromosomal.

Microcephaly has been defined as a head circumference which is more than 2SD below the mean[5] and by others as a head circumference of more than 3SD below the mean[6].

The aetiologic heterogeneity and variability of microcephaly in genetic syndromes are among the more difficult tasks in prenatal ultrasound imaging in pregnancies either with an incidental finding of this anomaly, or in cases with a risk of recurrence. This study presents a retrospective analysis of 30 cases of microcephaly including the genetic-diagnostic implications.

Methods

Between 1 january 1990 and 1 january 1997, a total of 29 consecutive couples with prenatally established fetal microcephaly was seen in our centre. There were 30 affected fetuses, since in one couple there were two successively affected pregnancies. Referral indications for ultrasound investigation are presented in Table 1. Mean gestational age at the time of referral was 27 weeks (range 16-36 weeks). Pregnancy duration was established from a certain last menstrual period and/or fetal crown-rump length measurement (n=24) during the first trimester of pregnancy. Mean maternal age was 28 years (range 19-37 years). Details on obstetric and familial history were noted.

Anomaly scans were performed on a Toshiba SSA 270 (carrier frequency 3.75 mHz). All data were related to normal reference charts according to Snijders and Nicolaides[7]. Microcephaly was defined as a fetal head circumference which was more than 3SD below the mean[6]. Selection did not take place on the basis of a postnatal diagnosis of microcephaly. A small for gestational age fetus was diagnosed when the fetal upper abdominal circumference was situated below the fifth centile of the reference curve[8]. Head circumference/abdominal circumference (HC/AC) ratio and head circumference/femur length (HC/FL) ratio were calculated.

Karyotyping was performed in 27 out of 30 (90%) fetuses by means of amniocentesis (n=17), transabdominal chorionic villus sampling (n=4) or cordocentesis (n=6). In one infant the karyotype was determined by postnatal skin biopsy.

Decisions regarding obstetric management were based on structural ultrasound findings and fetal karyotype. The outcome of each pregnancy was documented. Information from the attending paediatrician, pathologist and/or geneticist regarding postnatal diagnosis was obtained in most cases.

Postnatally, the 30 infants were divided into five groups: isolated microcephaly (group 1), microcephaly due to holoprosencephaly (group 2), microcephaly

associated with chromosomal disorders (group 3), microcephaly as part of a genetic syndrome (group 4) and microcephaly as part of multiple anomalies (unknown malformation syndrome; group 5).

Postnatally, genetic counseling was requested by 18 out of 29 (62%) couples.

Table 1. Prenatal detection of microcephaly: referral indications for ultrasound investigation.

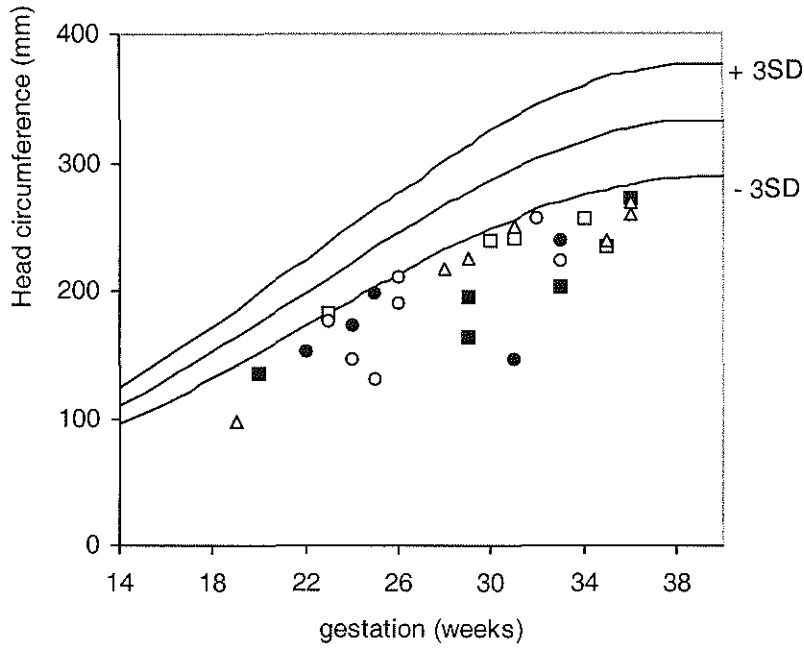
REASONS FOR REFERRAL	N
Reduced head size or suspected IUGR	16
Previous microcephaly	1
Intracranial abnormalities	5
Extracranial abnormalities	3
Hydrops	2
Polyhydramnios	1
Previous multiple abnormalities	1
Echodense bowel	1
Total	30

Results

Measurements of head circumference and upper abdominal circumference are shown in Figure 1A and B respectively. Calculated values of HC/AC and HC/FL are presented in Figure 1C and D respectively. The fetal abnormalities diagnosed by ultrasound and the postnatal findings are presented in Tables 2-6.

Group 1: Isolated microcephaly

Prenatally, four out of five fetuses displayed minor ultrasound abnormalities. Fetus no.4 developed microcephaly after its twin-brother died in utero at around 23 weeks of gestation. This is probably an example of a twin to twin transfusion syndrome with ventriculomegaly and microcephaly being the result of severe hemodynamic changes after the death of the cotwin[9]. Karyotyping was not carried out in this pregnancy. The remaining fetuses all showed a normal karyotype. One pregnancy (no.5) was terminated at 24 weeks of gestation. The parents of this fetus had had a previous child with microcephaly, who died at the age of four months. The consanguineous couple (no.2) did not have previous infants (Table 2).



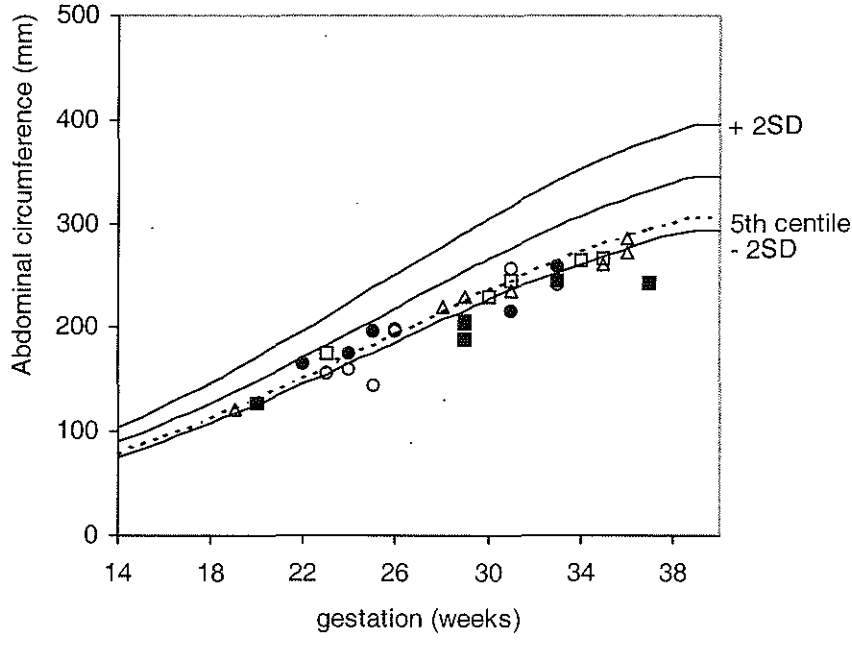
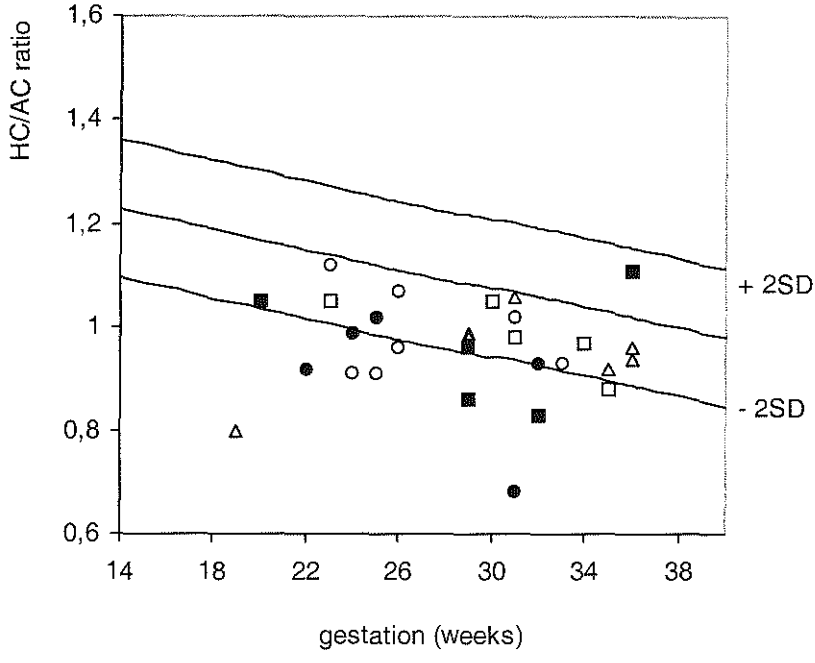


Figure 1A. (above) Head circumference (n=29); **1B** (below). Upper abdominal circumference (n=29). □, Group 1; ●, group 2; ○, group 3; ■, group 4; ▲, group 5.



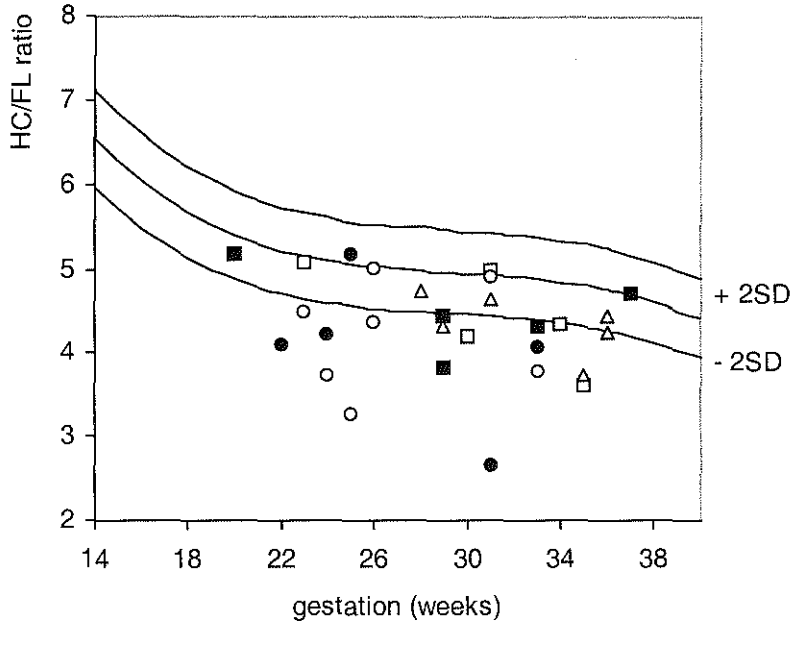


Figure 1C. (above) HC/AC ratio (n=29); **1D**. (below) HC/FL ratio (n=28).
□, Group 1; ●, group 2; ○, group 3; ■, group 4; ▲, group 5.

Table 2. Group 1: Isolated microcephaly.

No	First scan (wks)	Associated ultrasound anomalies	Outcome (wks), sex, weight (g)	Postnatal diagnosis	PM	Remarks
1	35	Heart:dysplastic atrio-ventricular valves, abnormal hand/foot position	Alive, 43 F, 2810	Microcephaly		
2	30	-	Alive, 41 F, 2620	Microcephaly		Consanguinity of parents
3	31	Dilated posterior horns, abnormal foot position	Alive, 38 M, 2105	Microcephaly		
4	23*	Ventriculomegaly, facial edema, cardiomegaly	Alive, 38 M, 2300	Microcephaly**		
5	16*	Two vessel cord	TOP, 24 M, ?	Microcephaly	+	Previous child microcephaly

F=female, M=male; PM= Post-Mortem investigation (+ = performed); *=two or more ultrasound investigations in tertiary centre; **= unknown karyotype; TOP=termination of pregnancy

Table 3. Group 2: Microcephaly due to holoprosencephaly.

No	First scan (wks)	Associated ultrasound anomalies	Outcome (wks), sex, weight (g)	Postnatal diagnosis	PM	Remarks
6	20*	Holoprosencephaly abnormal facial features, abnormal right ear	NND, 29 M, 1000	Holoprosencephaly, cyclops, absent nose, small mouth, abnormal ears**	-	IVF pregnancy; Consan-guinity of parents
7	31	Holoprosencephaly hypotelorism, exophthalmus,cleft lip/palate	TOP, 31 M, 840	Holoprosencephaly, severe hypotelorism, absent nose,cleft palate, abnormal ears	-	
8	33	Holoprosencephaly nuchal edema, hypotelorism, flat nose	NND, 36.5 F, 2300	Holoprosencephaly, median cleft lip/palate	-	
9	22	Holoprosencephaly hypotelorism, absent nose, cleft lip, horse shoe kidney	TOP, 22.5 F, 432	Holoprosencephaly and facial anomalies as described	-	
10	25	Holoprosencephaly cleft lip, hypoplastic nose, stomach not visible	NND, 37 M, 1725	Holoprosencephaly, hypotelorism, flat nose, abnormal ears, bilateral cleft lip/palate	-	Consangui-nity of parents

F=female; M= male; PM= Post-Mortem investigation (-=not performed); *=two or more ultrasound investigations at tertiary centre;**=karyotyping by skin biopsy; NND=neonatal death; TOP=termination of pregnancy

Group 2: Microcephaly due to holoprosencephaly

Microcephaly due to holoprosencephaly was present in five out of 30 cases (16.7%). A normal karyotype was present in all fetuses. Two pregnancies were terminated, three infants died after birth. Both consanguineous couples (nos 6 and 10) had healthy previous infants (Table 3).

Group 3: Microcephaly associated with chromosomal disorders

Chromosomal disorders were diagnosed in seven out of 30 cases (23.3%). Four pregnancies were terminated and two infants died after birth. In case no.12, prenatal karyotyping initially revealed a normal karyotype. However, postnatal rekaryotyping demonstrated a deletion 7q34. In one twin pregnancy established by intracytoplasmic sperm injection (ICSI), a translocation trisomy 13 was found in the fetus with microcephaly (no.17) while a balanced Robertsonian translocation was present in the other normally developing fetus. The father was subsequentially found to be carrier of a balanced Robertsonian translocation 45,XY,der(13;14)(q10;q10)[10]. The fetus with microcephaly died in utero at 34 weeks of gestation (Table 4).

Group 4: Microcephaly as part of a genetic syndrome

A genetic syndrome was diagnosed in six out of 30 infants (20.0%). Normal karyotypes were presented in all cases.

In one fetus (no.23), being a sib of an infant (no.22) with Pena Shokeir syndrome type 1, a recurrence of this syndrome was diagnosed by ultrasound. This pregnancy was terminated. Prior to ultrasound investigation, prenatal cytogenetic and DNA investigations were carried out in both cases, turning out normal, because of maternal carriership of FMR-1 (Fragile X) mutation.

Four infants died in the neonatal period. One infant with a postnatally diagnosed Cornelia de Lange syndrome (no.18) is alive (Table 5).

Group 5: Microcephaly as part of multiple anomalies

In seven cases microcephaly combined with other anomalies did not match with known syndromes. Karyotyping was not carried out in patient no.29. All other fetuses showed normal karyotypes. One pregnancy was terminated, one infant died in the neonatal period. Three infants are alive (Table 6).

Overall, in this study (group 1-5) microcephaly was diagnosed at a mean gestational age of 28 weeks (range 18-36 weeks). The fetal upper abdominal circumference was below 2SD in 19 out of 29 fetuses (Figure 1B; 66%), suggesting that more than half of the fetuses were also small for gestational age. Doppler flow measurements of the umbilical artery were performed in nine fetuses and were found to be normal, ruling out placental insufficiency.

The birth weights of 25 out of 30 infants are shown in Tables 2-6. In 16 out of 25 (64%) infants, birth weight was below -2SD and in 40% (10 infants) even below -3SD of the mean, proving growth retardation.

The overall mortality rate was 70% (21 out of 30 infants), of which 43% was represented by pregnancy terminations. Nine infants (30%) are alive; all are mentally retarded.

Post mortem investigation was performed in eight out of 21 (38%) fetuses/infants.

Table 4. Group 3: Microcephaly associated with chromosomal disorders.

No	First scan (wks)	Associated ultrasound anomalies and karyotype	Outcome (wks), sex, weight (g)	PM	Remarks/ Postnatal diagnosis
11	24	Oligohydramnios, intracranial anatomy not seen, no bladder filling, kidneys not visible 46,XY,der(7)t(7;12)(q34;p13) paternal	TOP 26 M ?	+	PM: holoprosencephaly, cyclopia, proboscis, cardiac abnormality
12	26*	Right sided hydronefrosis; 31 weeks: microcephaly 46,XY,del(7)(q34)	NND 7 M 2250	-	Micrognathia, broad nasal bridge, short neck, bell shaped thorax, hydronefrosis, megaureter
13	25	Hypotelorism, exophthalmus, thickened myocardium, short ulna/radius 47,XY,+8[4]/46,XY[61] in fetal blood	TOP 26.5 M 375	-	Ventriculomegaly, large ears, exophthalmus, small cleft lip, choanal stenosis/atresia, abnormal lower arms and hands
14	33	Ventriculomegaly, two vessel cord 46,XX,+13,der(13;14)(p11;q11), de novo	NND 36 F 1780	+	PM: holoprosencephaly, ventricular septum defect, polydactyly, cleft lip/palate, abnormal ears
15	26	Holoprosencephaly, hypotelorism, left/right heart asymmetry 47,XY,+13	TOP 29 M 1075	-	Abnormal skull, hypotelorism, one nostril, polydactyly (fingers)
16	23	Holoprosencephaly, cleft lip, echodense kidneys, ambiguous genitalia, left/right heart asymmetry 47,XY,+13	TOP 25 M 490	?	No information
17	26	Abnormally shaped cavum septum pellucidum, bilateral cleft lip, ambiguous genitalia, dysplastic heart valves, abnormal kidneys 46,XY,+13,der(13;14)(q10;q10) paternal	IUD (34) 37 M ?	-	ICSI pregnancy, consanguinity of parents no post mortem examination (maceration)

F=female, M=male, PM=Post-Mortem investigation (+=performed; -=not performed); *= two or more ultrasound investigations in our centre; TOP=termination of pregnancy; NND=neonatal death; IUD=intrauterine death

Table 5. Group 4: Microcephaly as part of a genetic syndrome.

No	First scan (wks)	Associated ultrasound anomalies	Outcome (wks), sex, weight (g)	Postnatal diagnosis	PM	Remarks
18	24*	Oligohydramnios two vessel cord 37 wks: microcephaly	Alive 38, F,1700	Cornelia de Lange syndrome		
19	29	Craniosynostosis, hypotelorism, micro/retrognathia, abnormal hand/foot position, no movements	NND 32,M, 1050	Fetal akinesia syndrome	-	
20	29	Craniosynostosis, dilated posterior horns, cerebellar abnormality	NND 38, F, 1430	Seckel-like syndrome	+	Consanguinity of parents
21	27*	Hypoplastic cerebellum, micrognathia, pes equinovarus, echodense lenses, small stomach	NND 42,F, 1400	Neu-Laxova syndrome	+	Consanguinity of parents
22	29	Hypertelorism, micrognathia, arms: flexion contractures, legs: extension contractures, no movements	NND 32,M, 890	Pena Shokeir type I	+	Consanguinity of parents sib of patient 23; previous child Fragile X syndrome
23	18*	Corpus callosum agenesis, hypoplastic cerebellum, echodense lenses, micrognathia, generalised skin edema, no movements	TOP 23, M,?	Pena Shokeir type I	+	Sib of patient 22

F=female, M=male; PM= Post-Mortem investigation (+=performed; -=not performed); *= two or more ultrasound investigations in tertiary centre; NND= neonatal death; TOP= termination of pregnancy

Table 6. Group 5: Microcephaly as part of multiple anomalies (unknown malformation syndrome).

No	First scan (wks)	Associated ultrasound anomalies	Outcome(wks), sex, weight (g)	Postnatal diagnosis	PM	Remarks
24	35	Oligohydramnios, abnormal right hand position, ventricular septum defect	Alive 39,F, 1830	Bilateral microcornea, ventricular septum defect, pulmonary atresia, persistent ductus arteriosus		
25	29	Small cavum septum pellucidum, left/right heart asymmetry, dysplastic valves, left pes equinovarus	Alive 29, M, 2690	Pes equinovarus left, cardiac abnormality		IVF pregnancy
26	19	Hydrops, hygroma colli, stomach not filled, contractures, no movements	TOP 20, ?	Arthrogryposis associated with microcephalia vera	+	
27	28	Caudal vermis defect, large cisterna magna, micrognathia, double outlet right ventricle, stomach not filled, abnormal hand position	IUD 29, M, 1100	Micrognathia, hypertelorism, low ear implant, webbed neck, rocker bottom feet		
28	24*	Ventriculomegaly, micrognathia, nuchal edema, left diaphragmatic hernia, pectus excavatum, double outlet right ventricle, overlapping fingers	NND 33, M, 1580	Low set ears, hypo/epispadia	-	
29	36	SGA; left/right heart asymmetry	Alive 38, F, 1935	Porencephalic cyst**		
30	20.5*	Echodense bowel 34 wks: abnormal intracranial anatomy?	Alive 38, M, 2390	Ventriculomegaly, mega cisterna magna, hypoplastic cerebellum, hypertelorism, microsferofaki, low set right ear, small thorax, wide spaced nipples, small penis		Consan-guinity of parents

F=female, M=male; PM= Post-Mortem investigation (+=performed; -=not performed); *= two or more ultrasound investigations in tertiary centre; **= unknown karyotype; TOP= termination of pregnancy; NND= neonatal death

Discussion

In the present study, a heterogeneous group of 30 affected infants is presented with prenatally diagnosed microcephaly as a common feature. Prenatal diagnosis consisted of a fetal head circumference at or below -3SD of the mean. Head circumference measurements even slightly less than -3SD turned out postnatally to be microcephalic. This may be partly due to selection, since all small for gestational age fetuses with abnormal Doppler findings were excluded from this study. Sloping of the forehead was seen in most cases of microcephaly. Since data were collected over a 7-year time period, no information was available on a possible deviation in normal blood flow to the fetal brain, as recently described by Pilu et al[11]. In five cases isolated microcephaly was established postnatally; minor associated anomalies, suspected prenatally in three cases, were not confirmed after birth. In the remaining 25 out of 30 (83%) infants, microcephaly proved to be part of a complex problem, emphasizing the need of a meticulous search for structural anomalies and fetal karyotyping when biometric data are not according to gestational age.

In the presence of microcephaly one would expect the HC/AC ratio to be reduced. However, 19 out of 29 (66%) HC/AC ratio values (Figure 1C) and 10 out of 28 (36%) HC/FL ratio values (Figure 1D) were situated within the normal range (above -2SD). This is due to the reduced upper abdominal circumference (below the fifth centile) in 19 out of 29 (66%) cases reflecting the presence of an SGA fetus. This high percentage of SGA fetuses may be determined by the referral pattern in this study. Approximately half of the cases was sent to our center for reasons of abnormal biometry, suggesting abnormal fetal growth.

Microcephalic infants have brains which are not only small, but may also be dysmorphic as expressed by the presence of holoprosencephaly (n=6) and cerebellar abnormalities (n=6), the latter ranging between hypoplastic cerebellum (n=4) and caudal vermis defect (n=2). Facial anomalies (hypotelorism, exophthalmus, abnormal/absent nose and cleft lip/palate) were nearly always associated with holoprosencephaly.

An abnormal fetal karyotype was established in seven out of 30 pregnancies (23.3%), of which five represented a structural chromosomal abnormality. Structural anomalies included central nervous system, facial, cardiac and genito-renal anomalies.

Genetic counseling was requested by 18 out of 29 (62%) couples (infants 22 and 23 are sibs). The mother of pregnancies 22 and 23 was a carrier of the Fragile X syndrome. After exclusion of this diagnosis in the fetus in her second pregnancy by chorion villus sampling, an eventual diagnosis of the autosomal recessive Pena Shokeir syndrome type 1 was made. The third pregnancy was found to be affected by the 18th week.

The recurrence risk of the remaining four genetic syndromes was counseled to be sporadic (no.18), 20% (no.19) or autosomal recessive (nos 20 and 21).

Finally, infant 30 resulted from a first pregnancy of a consanguineous couple (first cousins) suggesting an autosomal recessive mode of inheritance although the infant showed sonographic signs of a possible viral infection during pregnancy (echodense bowel; oligohydramnios).

The complex situations described above demonstrate the need of careful follow up studies, post mortem investigation and genetic counseling. All parents were counseled prenatally based on the available information at that time. They were informed about the importance of postnatal evaluation which included determination of the recurrence risk of fetal pathology. It is striking that only 57% (12/21) gave permission for post-mortem investigation and also that only 60% consulted a clinical geneticist. This may be explained by different cultural backgrounds.

When microcephaly is part of a syndrome or sequence, prenatal diagnosis in a subsequent pregnancy should not be based on the detection of microcephaly because in many cases this feature will only become apparent during the late second or third trimester. A syndrome or sequence including microcephaly should therefore rather be based on other features, such as central nervous system, facial, cardiac and renal anomalies, which can be recognized in the late first or early second trimester of pregnancy. Unfortunately, a recurrence of isolated microcephaly is not always likely to be detected before fetal viability due to the relative late slow down in fetal head growth and variable expression of this anomaly.

The perinatal mortality rate was 70% (21/30), half of which representing the neonatal period. Of the 10 infants which remained alive, four presented with isolated microcephaly.

In summary, in the present study approximately half of the pregnancies were referred for reasons other than suspected microcephaly, thus clearly representing a selected subset of microcephalic infants. Perinatal mortality was determined by the severity of associated anomalies rather than by microcephaly alone. In chromosomally normal cases the recurrence risk of microcephaly varies depending on the presence of associated anomalies or syndromal pathology, the latter mostly following an autosomal recessive inheritance pattern.

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3.1.3 Prenatal diagnosis and confirmation of the Acrofacial Dysostosis Syndrome type Rodriguez

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Am J Med Genet, submitted

Abstract

The group of acrofacial dysostosis (AFD) syndromes is very heterogeneous and contains many different entities. In 1990, Rodriguez et al[1] described a new type of AFD characterized by severe mandibular hypoplasia, phocomelia and oligodactyly of the upper limbs, absence of fibulae, microtia, cleft palate, internal organ anomalies including arhinencephaly and abnormal lung lobulation, and early lethality. We describe here another case of AFD type Rodriguez, identified by prenatal ultrasonography at 25 weeks of gestation.

Introduction

The acrofacial dysostosis (AFD) syndromes are an aetiologically heterogeneous group of disorders with an ill-defined classification and inheritance. Traditionally, the AFD syndromes have been subdivided into the predominantly preaxial form (Nager syndrome) and the predominantly postaxial form (Genée-Wiedemann or Miller syndrome, also referred to as POADS)[2-4]. However, many other forms of AFD have been described although it is unclear whether or not all these different AFD anomalies represent distinct syndromes[3]. In 1990 Rodriguez et al[1] presented a previously unknown lethal AFD in three sibs with severe mandibular hypoplasia, upper limb phocomelia and oligodactyly. The lower limbs were of apparent normal length, but absence of the fibulae was a characteristic finding. Typically, two of the three sibs also showed internal organ anomalies including a congenital heart defect and arhinencephaly,

whereas abnormal lung segmentation was found in one of these two. Case 3 had less impressive symptoms and a clinical picture reminiscent of AFD type Nager. This led to the suggestion that some patients with Nager syndrome in fact had Rodriguez syndrome. Hecht et al[5] retrospectively diagnosed two earlier cases identified as Nager syndrome[6] as examples of this new AFD syndrome. However, Rodriguez et al[7] disagreed with this classification as internal anomalies were missing. Fryns and Kleckowska[8] suggested that one of their AFD cases with a Nager anomaly had Rodriguez syndrome, although many of the typical anomalies of the latter syndrome were absent. In 1992 Petit et al[9] presented a fetus with severe mandibulofacial dysostosis, tetraphocomelia and CNS, heart and renal anomalies. The authors considered this to be a case of AFD type Rodriguez because of the presence of severe internal malformations. Fryns[10] has suggested that an anatomical museum specimen described by Oostra et al[11] as having Nager anomaly, also might represent a case of Rodriguez syndrome, although Oostra et al[12] did not agree.

Here we report prenatal sonographic features, clinical description, skeletal X-rays and autopsy of an AFD fetus with abnormalities very similar to the cases described by Rodriguez et al[1].

Clinical report

A healthy 20-year-old primigravida of Asian origin was referred to our Division of Prenatal Diagnosis after a routine ultrasound scan elsewhere at 25 weeks of gestation had shown fetal anomalies. The family history of the woman was unknown as she was adopted, whereas the family history of her unrelated Dutch partner was noncontributory. Early pregnancy was uneventful and no teratogenic exposures were known. A detailed ultrasound scan at 25 weeks demonstrated severe micrognathia, severe phocomelia of the upper limbs with hands apparently attached to the trunk, an abnormal aspect of the hands with oligodactyly, and absence of both fibulae (Figure 1). Head and abdominal circumferences were normal for the gestational age.

Amniocentesis revealed a normal female karyotype without indication of premature centromere separation (making Robert's syndrome less probable) and a normal alpha-fetoprotein level. The combination of severe micrognathia with phocomelia was highly suggestive for an acrofacial dysostosis (AFD). Although no gross internal abnormalities were evident on ultrasonography, the combination of severe phocomelia and oligodactyly of the upper limbs with absence of the fibulae in lower limbs of apparent normal length suggested the diagnosis of AFD type Rodriguez. The couple opted for termination of the pregnancy which was carried out at 27 weeks and a female fetus of 718 g was stillborn.

Autopsy of the fetus showed an asymmetric face with severe micrognathia, maxillary hypoplasia, hypertelorism with a high and broad nasal bridge,

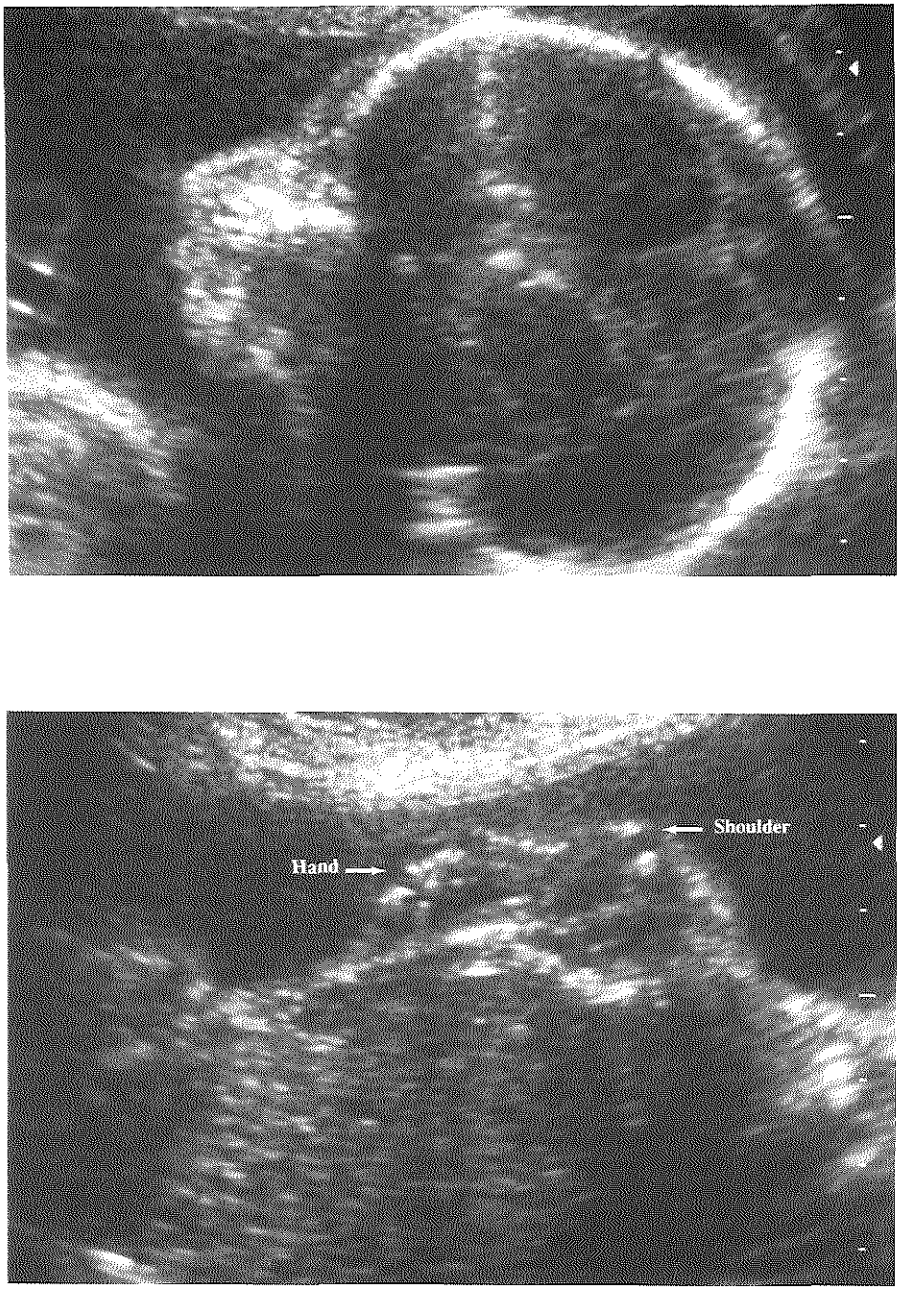

Figure 1. Prenatal ultrasound scan at 26 weeks of gestation showing severe micrognathia (above) and upper limb phocomelia with oligodactyly and only one bone present in the upper arm (below).

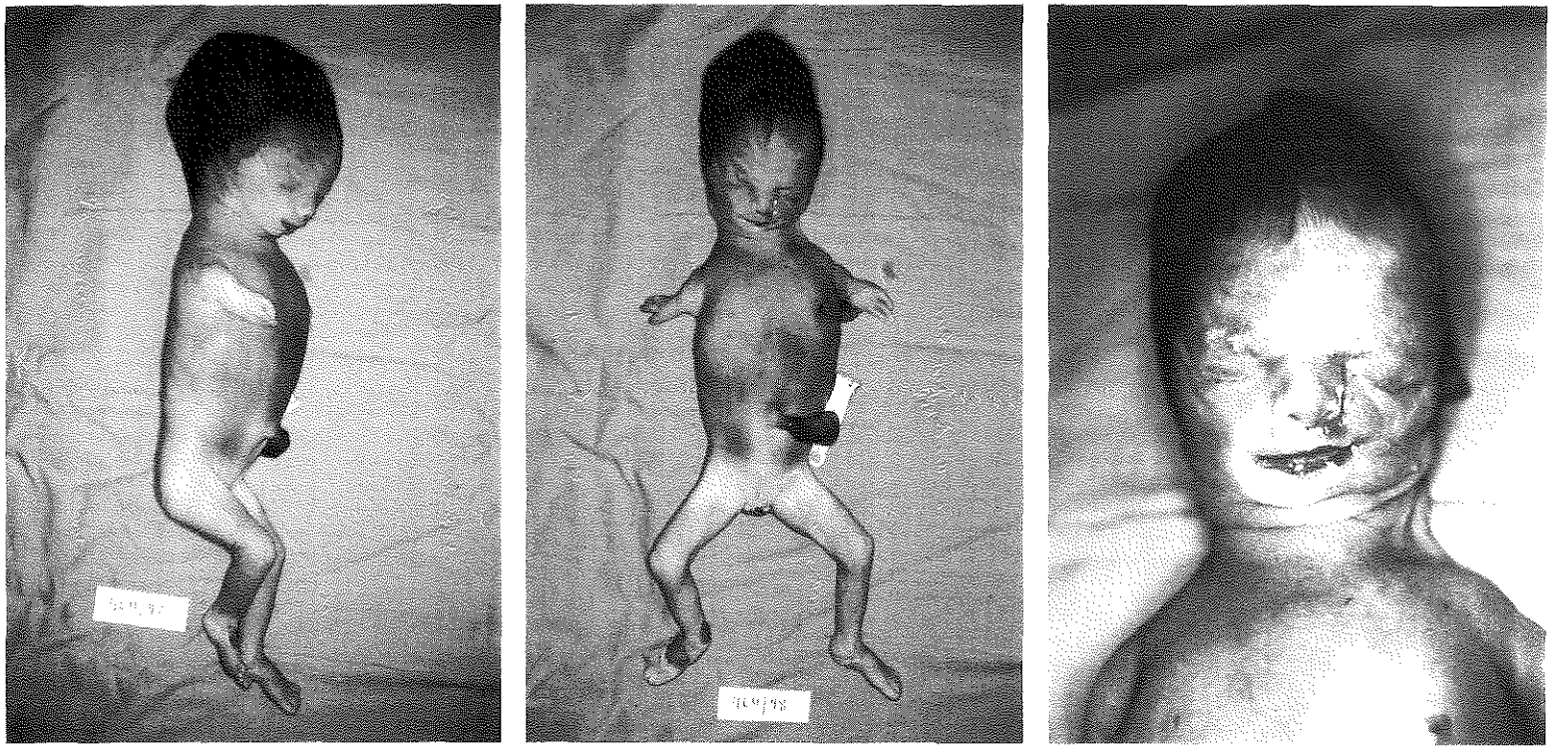

Figure 2. Fetus after termination of the pregnancy showing severe phocomelia of the upper extremities with apparently normal looking lower limbs (left and middle). Microtia is obvious. Oligodactyly is present, but it is unclear whether preaxial or postaxial rays are missing. The thorax and shoulder girdle are symmetric and hypoplastic (left and middle). Also the head is asymmetric and shows severe micrognathia with malar hypoplasia and a high nasal bridge (right).

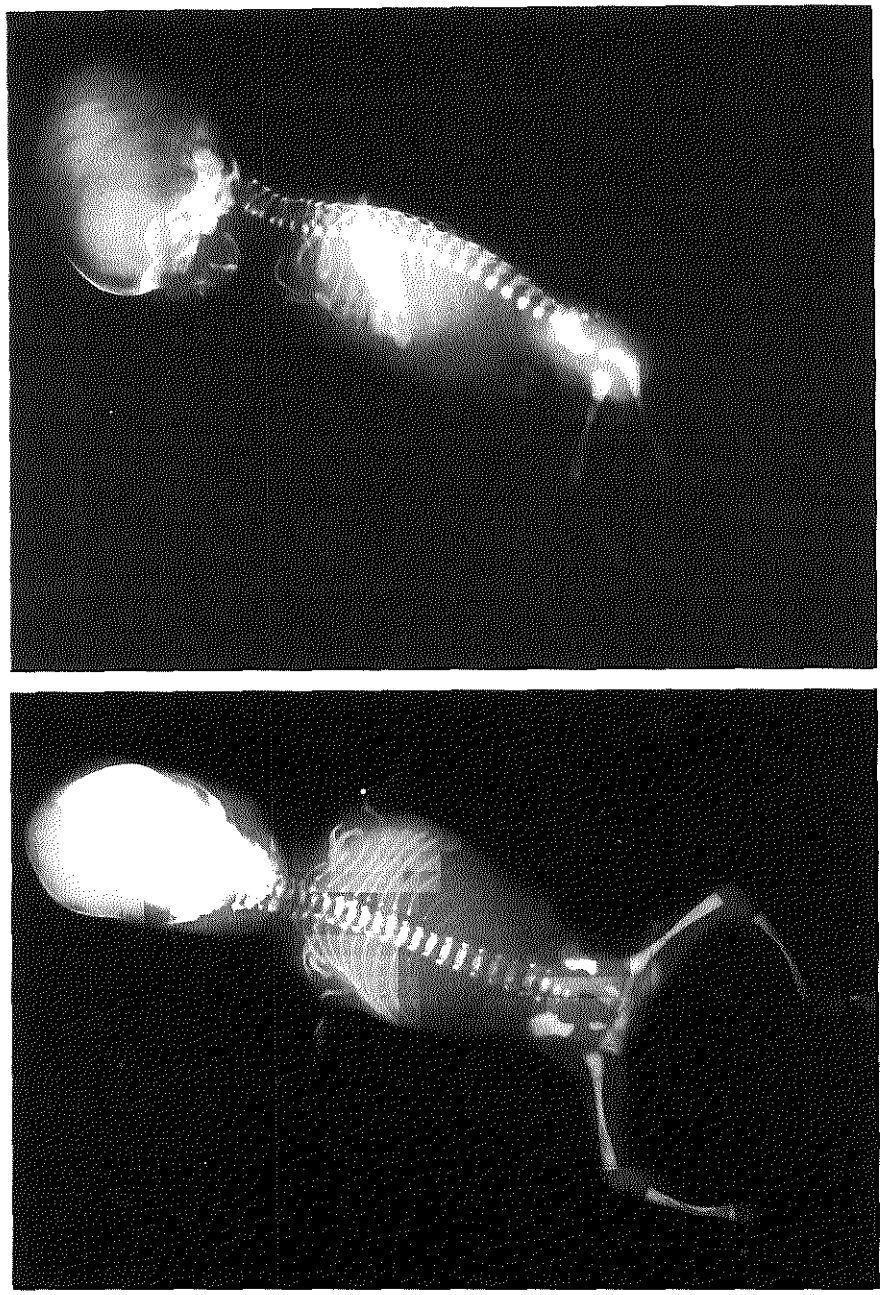

Figure 3. Radiographics of the head show micrognathia, malar hypoplasia and brachycephaly. One pair of ribs and both fibulae are missing whereas both scapulae and ischii are hypoplastic. Severe phocomelia of the upper limbs is present with the humerus, ulna and radius replaced by a single, angulated bone located in the shoulder girdle. Oligodactyly of the upper limbs is present. These radiologic abnormalities are very reminiscent of those of AFD cases described by Rodriguez et al[1].

bilateral cleft palate, and bilateral severe microtia without discernable auditory canals (Figure 2). There was severe phocomelia of both upper extremities with the hands attached directly to the shoulders. The right hand had four digits with the tiny fourth ray showing partial syndactyly with the third finger. The left hand only had three fingers and a thumb could not be identified. There existed mild shortening of both lower limbs, especially on the right side, with anterior bowing of the tibiae. Both feet were small, and showed a calcaneo-varus deformity, especially on the right side. There was partial syndactyly of the fourth and fifth toes. Radiographs showed a normal axial skeleton and skull (Figure 3). The upper limbs had no recognizable humeri, ulnae or radii. Instead a single, small and angulated bone was present between the hands and the shoulders. This bone was positioned perpendicularly to the metacarpal bones. It was unclear if this bone represented a synostosis of two different bones. The scapulae and ischii were hypoplastic and only 11 ribs were present on both sides. The lower limbs showed virtually normal femora, slightly shortened and bowed tibiae and complete absence of both fibulae.

Autopsy showed hypoplastic and hypolobated lungs (one lobe on the left side and two lobes on the right side). The thymus was asymmetrical and the left kidney was small and situated in the pelvis. The olfactory nerves could not be identified by neuropathological examination (arhinencephaly). Histologic study of the growth plate of the long bones demonstrated normal, orderly enchondral ossification with fairly narrow cartilaginous columns.

Discussion

The association of a mandibulofacial dysostosis with limb defects is called an acrofacial dysostosis (AFD). This is a purely descriptive term often misused as a diagnosis. In fact, AFD is present in many different conditions including chromosomal anomalies and a large number of ill-defined anomalies including Nager and Genée-Wiedemann syndrome. The seminal paper by Opitz et al[3] has delineated the AFD syndromes from a clinical point of view, but the subdivisions will await a molecular classification.

The fetus reported here had a severe form of acrofacial dysostosis (AFD) with impressive micrognathia and phocomelia of the upper limbs. Oligodactyly, microtia with atretic external auditory canals, absence of fibulae, abnormal lung lobulation and arhinencephaly were also present. The combination of severe micrognathia and phocomelia can be present occasionally in Nager and Genée-Wiedemann syndrome. The combination of severe phocomelia of the upper limbs with lower limbs of nearly normal length but absent fibulae and arhinencephaly might be typical for the AFD syndrome first reported by Rodriguez et al[1] Also the replacement of the three long tubular bones of the upper limbs by a single angulated bone (Figure 3) might be a characteristic finding in Rodriguez syndrome (Table 1).

Table 1. Characteristic Symptoms in Acrofacial Dysostosis type Rodriguez

Anomaly	Our case	Rodriguez et al[1]		
		case 1	case 2	case 3
Radiological features				
Phocomelia upper limbs	+	+	+	-
lower limbs	-	-	-	-
Oligodactyly hands	+	+	+	-
feet	-	-	+	-
Absent/hypoplastic fibulae	+		+	-
Absent/hypoplastic ischii	+		+	-
Synostosis bones upper limb	+	+	+	-
Eleven ribs	+	+		+
Face				
Severe micrognathia	+	+	+	+
Malar hypoplasia	+	+	+	+
Microtia/atretic ear canal	+	+	+	+
High nasal bridge	+	+	+	+
Cleft palate	+	+	+	-
Internal signs				
Arhinencephaly	+	+	+	-
Abnormal lung lobulation	+	-	+	-
Cardiac malformation	-	+	+	-
Early lethality	aborted	+	+	+

The fetus presented here and the cases described by Rodriguez et al[1] lack coloboma of the eyelids and accessory nipples, typical characteristics of the Genée-Wiedemann syndrome[3]. Our case is also less likely an extreme phenotype of the Nager anomaly as extreme microtia, absence of the fibulae and arhinencephaly are rarely seen with the latter anomaly[3]. However, the third sib in the family described by Rodriguez et al[1] presented with a much less severe and Nager-like AFD phenotype without internal malformations, indicating clinical variability of Rodriguez syndrome. This opened the discussion about the possible reclassification of severe Nager anomaly cases as examples of Rodriguez syndrome[5,8-11]. However, none of these cases had the combination of symptoms typical for Rodriguez syndrome, including severe phocomelia of the upper limbs, a typical angulated bone in the shoulder, aplasia of the fibulae of the lower limbs, girdle and internal anomalies such as abnormal lung lobulation and arhinencephaly.

The three sibs described by Rodriguez et al[1] were all male, whereas their parents were nonconsanguineous. This is compatible with both an X-linked recessive or autosomal recessive mode of inheritance. As our case is female, it is likely that Rodriguez syndrome is an autosomal recessive condition.

Acknowledgement
We are imdebted to Tom de Vries Lentsch for photographic work.

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3.1.4 Prenatal diagnosis of type A1 brachydactyly

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Ultrasound Obstet Gynecol 2001;17:529-530

Abstract

Brachydactyly can occur as an isolated malformation or as part of numerous syndromes.

Prenatal assessment of brachydactyly may especially be helpful in multiple anomaly syndromes associated with hand and/or finger anomalies.

In isolated type A1 brachydactyly, which is an autosomal dominant disorder, all middle phalanges of the fingers and toes are affected. We present a fetus with type A1 brachydactyly inherited from the mother and grandmother.

Introduction

Brachydactyly can occur as an isolated malformation or as part of numerous syndromes, such as bone dysplasias. Bell classified brachydactyly into seven types: A1, A2, A3, B, C, D and E[1]. Type A3, clinodactyly of the fifth finger, and type D, 'stub thumb', are common and can be considered normal variations. The other types are rare. In the type A1 brachydactyly all middle phalanges of fingers and toes are affected. The middle phalanges are either absent, rudimentary or fused with the terminal phalanges. In the isolated form, type A1 brachydactyly is an autosomal dominant disorder. Detection of brachydactyly by prenatal ultrasound may especially be relevant in pregnancies at risk for syndromes associated with type A1 brachydactyly.

We report the prenatal diagnosis of a fetus with type A1 brachydactyly, whose mother and grandmother were affected by the same disorder.

Case report

A 32-year old gravida 2, para 1 with autosomal dominant type A1 brachydactyly was referred for ultrasound examination at 19 weeks of gestation to verify the

fetal hand development. The first child was born at term and has normal hands. The patient's mother is also affected with type A1 brachydactyly (Figure 1). The family history was otherwise unremarkable.

Ultrasound examination (ATL HDI 3000, Advanced Technical Laboratories, WA, USA) demonstrated fetal biometry consistent with dates and normal amniotic fluid volume. All fingers (Figure 1) and toes appeared short and the phalanges were not clearly discernable. No other (skeletal) anomalies were observed. A diagnosis of type A1 brachydactyly was made.

It is local policy that in the presence of a parental structural anomaly, amniocentesis for fetal karyotyping at 16 weeks of gestation is offered. A normal karyotype and normal alphafetoprotein level was established.

Pregnancy progressed uneventfully. At term a female infant of 3760 g was born. The brachydactyly of hands and feet was confirmed; there were no other anomalies.

Discussion

Bronshtein et al[2] were the first to report on the prenatal ultrasound diagnosis of fetal finger abnormalities between 13-17 weeks of gestation. In the 20 000 fetuses that were studied, 24 (0.12%) abnormalities were observed: overlapping fingers, polydactyly, syndactyly, cleft hand, adactyly, aphalangia and clasped thumbs. In 15 (62.5%) fetuses there were associated malformations and/or an abnormal karyotype. They concluded that reliable observation of all fingers and phalanges is possible as from 12-13 weeks of gestation. In early pregnancy the fetus tends to keep its hands open with the fingers extended. In the second trimester the fingers are more often flexed which reduces the possibility to examin the fetal fingers by ultrasound.

Reiss et al[3] published a prospective study on examination of the fetal hands during a prenatal scan between 13 and 39 weeks of gestation. The majority of the fetuses were scanned during the second trimester or later. Both hands were visualized in 188 out of 215 (87 %) fetuses. Four (2.1 %) hand abnormalities (syndactyly, clenched hands, wrist contractures with clenched hands, and polydactyly) were diagnosed prenatally whilst eight hand abnormalities were present at birth.

Both studies included pregnancies at high or low-risk for fetal anomalies. The various rates of hand abnormalities (0.12% and 2,1%) may be related to the number of patients included and differences in inclusion criteria.

There are several studies[4-6] reporting on individuals with brachydactyly type A1 in combination with other anomalies such as Klippel-Feil anomaly and facial anomalies[4], generalised skeletal anomalies (lumbar scoliosis and valgus deformities of the feet) together with nystagmus and squint[5] and the combination of abnormal menisci and scoliosis[6]. These observations prompted the question whether the extensively affected individuals represent the more

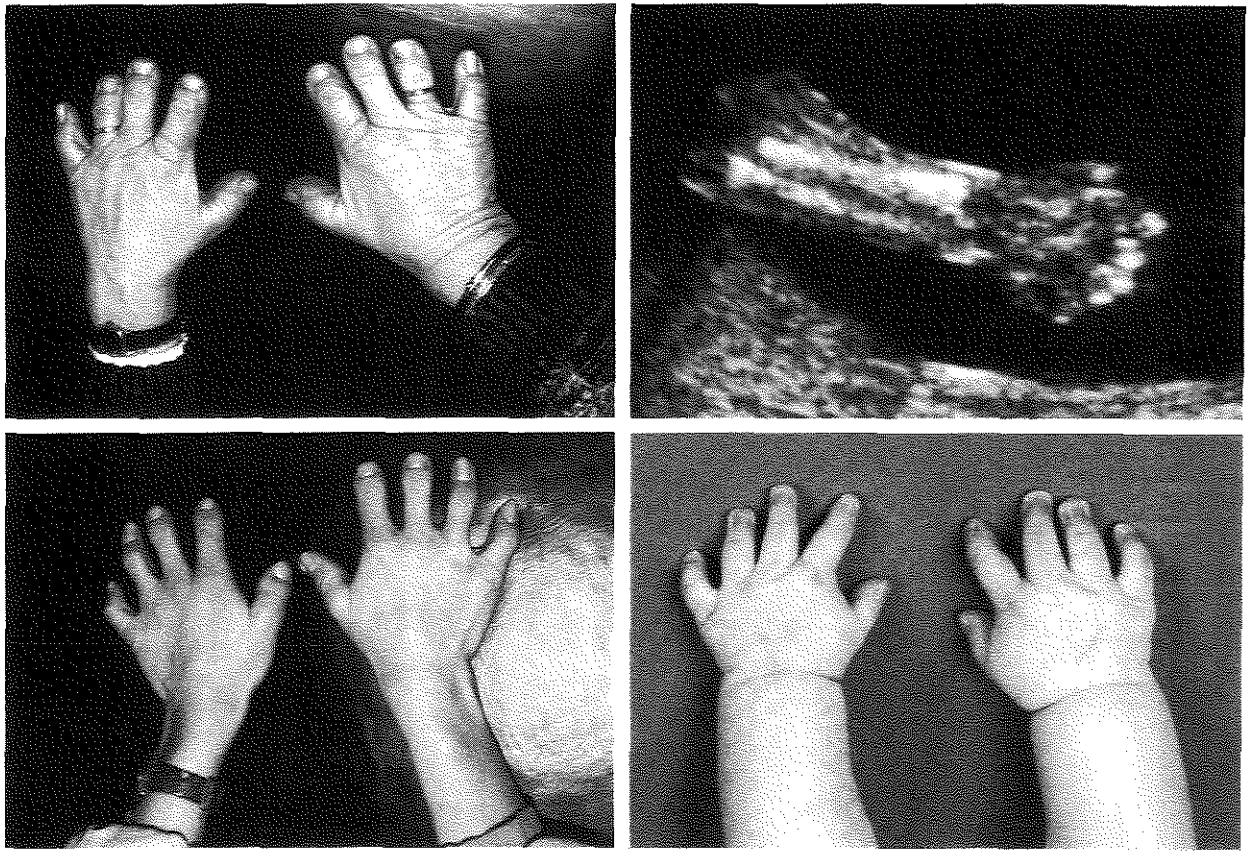

Figure 1. Photographs of the hands of the mother and grandmother of the fetus and an ultrasound picture of the fetal hand (the thumb and index finger are extended; the other fingers are somewhat bent), showing type A1 brachydactyly.

severe manifestations of the autosomal dominant gene for brachydactyly[5]. A candidate gene was unsuccessfully searched for in two families[7].

To the best of our knowledge this is the first report on the prenatal diagnosis of brachydactyly type A1. It shows the possibility of diagnosing this type of brachydactyly at 19 weeks of gestation. This may be relevant in pregnancies at risk for syndromes associated with type A1 brachydactyly. Possible variability of expression is to be considered in every individual syndrome.

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3.1.5 Clinomicrodactyly

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Ultrasound Obstet Gynecol 2000;16:204
(Picture of the month)

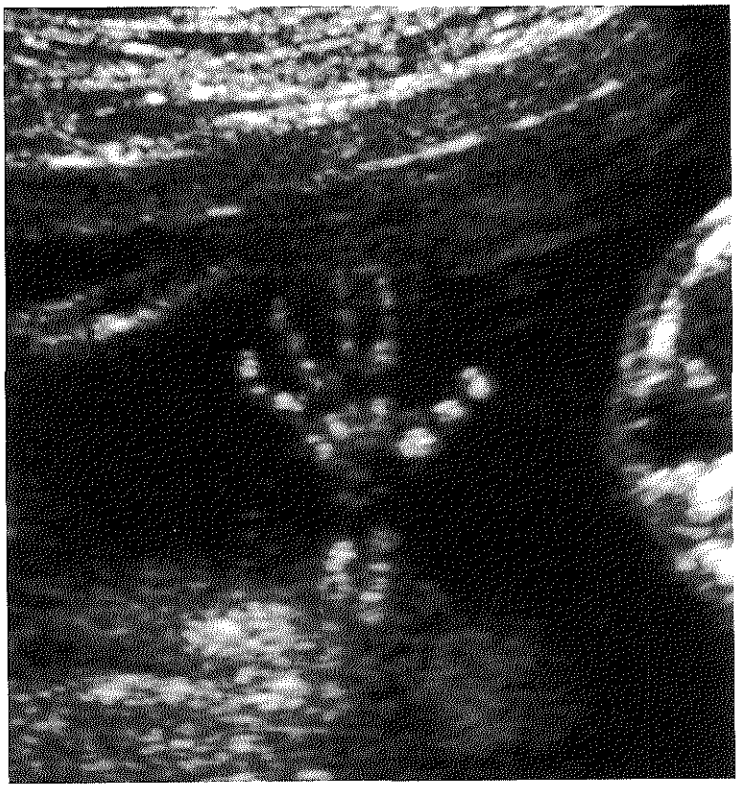

Figure 1. Fetal hand at 20 weeks of gestation showing clinodactyly of the fifth finger.

Clinodactyly (Greec for 'bent finger') refers to curvature of any finger in a mediolateral plane either radial or ulnar. Clinomicrodactyly is clinodactyly of the fifth finger.

Bell[1] classified brachydactyly into seven types (A1, A2, A3, B, C, D, and E). In the type A brachydactylies variable abnormalities of the middle phalanx are observed. Clinomicrodactyly is type A3 brachydactyly and is characterized by shortening of the middle phalanx of the fifth finger. A single crease of the fifth finger indicates a short or absent middle phalanx. This type of brachydactyly is frequent both as an isolated malformation (autosomal dominant inheritance) and as part of syndromes. There are both sex and racial differences. Clinomicrodactyly must be differentiated from other types of crooked fingers, such as camptodactyly.

This fetus of a consanguineous couple presented with a nuchal translucency of 4 mm at 11 weeks. The karyotype was normal. An ultrasound scan at 20 weeks of gestation clearly demonstrated clinomicrodactyly in this fetus (Figure 1).

The first child of the consanguineous couple displayed finger and toe deformities after birth which were counseled as type A2 brachydactyly[1] with autosomal dominant inheritance. Characteristically, individuals affected with type A2 brachydactyly, have a triangular-shaped middle phalanx in the index fingers and second toes. Deformity of the second toe is a more consistent finding than deformity of the index finger. The big toe is often short and broad. Clinomicrodactyly is present in some degree in almost all cases. The parents and the second child do not show finger or toe deformities. Counseling will be reconsidered after birth of this infant.

Prenatal sonographic diagnosis of finger deformities may especially be relevant in pregnancies at risk for syndromes associated with finger deformities.

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CHAPTER 4
CONCLUDING REMARKS

Sonography is a method to investigate the *living* human embryo and fetus. With the introduction of high-resolution ultrasound technology and the possibility of transvaginal scanning, there has been increasing interest in normal and abnormal development of the *living* human embryo and fetus as early as 6-14 weeks of gestation. Sonographic identification of abnormal fetal development is determined by detailed knowledge of normal embryology and fetal sono-anatomy, understanding of the processes and chronological order of the development of specific malformations and extensive scanning experience of the sonographer.

Although transvaginal sonography provides better images of the embryo and fetus than transabdominal sonography, transvaginal sonography has a limitation of scanning planes and sometimes anatomical (uterus) and positional (fetus) limitations. Thus, the two methods are complementary.

An increasing number of structures can be visualized by sonography with advancing gestational age. The optimal gestational age for a *complete* anatomical survey and nuchal translucency measurement is at 13 weeks. The majority of structural and chromosomal anomalies can be diagnosed by experienced sonographers at this time. An extensive training program of the 11-14 week scan should be offered to sonographers, especially in tertiary centres.

A single ultrasound scan in pregnancy will not detect all fetal anomalies. Combining the 11-14 week scan and the standard 18-21 week scan, between 80-90% of the fetal anomalies will be detected. The remainder of the anomalies may be detected in the third trimester or not at all. Beside this, normal results on first or second trimester sonography do not rule out the diagnosis of, for example microcephaly, hydrocephaly or cardiomyopathy. These conditions may even occur as late as the neonatal period or in childhood.

Another pitfall is the variable phenotypic expression of anomalies and syndromes. An early manifestation in one pregnancy will not guarantee an early diagnosis in a next pregnancy.

In some patients prenatal ultrasound in following pregnancies will be secondary to or replaced by DNA mutation analysis as is demonstrated in this thesis (subchapters 2.2.2 and 2.2.3).

Combining dysmorphological evaluation, post mortem examination, cytogenetic/DNA studies and genetic counseling for future pregnancies is of considerable importance.

A diagnostic work up after a prenatal diagnosis, whether followed by pregnancy termination, stillbirth or birth of an affected infant should always include a comprehensive study of the affected fetus or neonate, the 'index patient'.

Paediatric, clinical genetic (dysmorphological) and post mortem evaluation by experienced clinicians in individual fields is a minimal standard to establish if there is a 'single' or 'syndromal' malformation and to allow an accurate diagnosis. Securing blood/tissue/skin specimen for cell culture or deep-frozen storage will remain an essential approach towards future studies such as mutation analysis which may have an impact on parents, siblings and other relatives.

The possibility and option of early diagnosis of a specific anomaly or associated anomalies at an 11-14 week scan by an experienced sonographer should be discussed with the parents. Although there is as yet no firm evidence that early termination of a pregnancy for fetal malformations is psychologically more acceptable for parents, to know that fetal development is normal as early as the late first to early second trimester of pregancy will be reassuring. This will be of particular advantage to parents who are at high risk of affected offspring.

Finally, 2-dimensional diagnostic ultrasound is still subject to further improvement. Moreover, transvaginal 3-dimensional ultrasound will open new avenues of research in embryonic and early fetal normal and abnormal development. The 'resolution' of prenatal ultrasound has equally advanced by the technology of ultrasound equipment as by its complementation by clinical genetic, cytogenetic, paediatric and pathological expertise.

Chapter 1

The most striking advances in human development occur in the first eight weeks following conception. The embryonic period is of particular importance because most congenital anomalies appear during that time.

Ultrasonography is a method to investigate the living human embryo and fetus. To be able to evaluate the abnormally developing early pregnancy, knowledge of the normal human embryologic development in vivo is essential. In chapter 1, the structures and functions that are detectable by high-frequency ultrasound at a given gestational age are described, based on several studies. An increasing number of structures can be visualized by sonography with advancing gestational age. Ultrasound images of normal and abnormal fetal anatomy are presented in the 'Appendix'. The optimal gestational age for a complete anatomical survey is at 13 weeks, which is also considered the optimal gestational age for nuchal translucency measurement.

The potential of late first trimester and early second trimester fetal anomaly scanning is dependent on the intricate relationship between high quality ultrasound scanning and expertise, detailed knowledge of exogenic and genetic syndromes (congenital anomalies) and associated inheritance patterns, and the availability of a well-qualified fetal pathology unit.

Chapter 2

In chapter 2 the validity and impact of early abnormal fetal sonoanatomy is discussed. Especially, the sonographer should understand the dynamic processes of the development of fetal anomalies and be aware of the chronological order of development of the specific malformations. Some anomalies may develop as a transitory finding in early pregnancy (enlarged nuchal translucency, hydronephrosis, etc) and may not be present later in gestation. Other anomalies may present at varying gestational age. However, the majority of fetal anomalies can be diagnosed in the late first to early second trimester of pregnancy. This was demonstrated by the prospective study of 101 fetuses at risk of congenital anomalies presented in this chapter.

Skeletal dysplasias are not easily detected in the first trimester of pregnancy. However, the early diagnosis by ultrasound of several skeletal dysplasias are reported in the literature and in this chapter. Variable phenotypic expression of skeletal dysplasias, as in Jeune syndrome, has to be taken into account. The recurrence of less severe skeletal dysplasias may be established in early pregnancy by the associated malformations, as in Beemer-Langer dysplasia.

The enlarged nuchal translucency may be based on several different aetiologies as it is associated with a wide range of chromosomal and structural anomalies and genetic syndromes. A case of an enlarged nuchal translucency associated with a metabolic disorder, mucopolysaccharidosis type VII, is presented in this chapter.

The validity and impact of the early pregnancy scan depends on the natural history of anomalies (detectability at a certain gestational age) and the variable phenotypic expression of anomalies/syndromes.

Chapter 3

Combining the 11-14 week scan and the standard 18-21 week scan, between 80-90% of the fetal anomalies will be detected. The remainder of the fetal anomalies may be recognized in the third trimester of pregnancy or not at all. Normal fetal biometry and normal atrium/ventricular diameter on a second trimester scan does not exclude the development of microcephaly and hydrocephaly in a fetus or infant. This is not surprising as both anomalies may even develop as late as the neonatal period or in childhood. In high-risk pregnancies serial observations are necessary.

In this chapter a retrospective study of fetuses with sonographically diagnosed ventriculomegaly and a retrospective study of fetuses with microcephaly are presented. Both hydrocephaly and microcephaly are conditions with a high percentage of poor outcome, such as mental disability. The possibility of a prenatal diagnosis will be determined by the presence of associated anomalies.

A combination of certain malformations may lead to the prenatal diagnosis of a rare syndrome as is reported by the fetus with acrofacial dysostosis syndrome in this chapter.

The careful observation of hand/foot malformations is not only useful to inform parents about a possible recurrence of their often dominantly transmitted disorder, such as type A1 brachydactyly, but may especially be helpful in a multitude of multiple anomaly syndromes associated with such anomalies. With the improving resolution of ultrasound equipment these anomalies might even become detectable as early as the late first to early second trimester of pregnancy.

After (still)birth or pregnancy termination, a precise diagnosis is essential, also for genetic counseling of parents and family. Extensive dysmorphological examination of the index patient or post mortem examination by an experienced paediatric/fetal pathologist are important in order to document and confirm the prenatally established anomalies and/or to determine the associations leading to a diagnosis. As post mortem examination is not always consented or performed or informative (in case of maceration), ultrasound findings may be invaluable in genetic counseling. The value of a detailed sonographic dysmorphology will

remain even when a slowly increasing number of syndromes becomes detectable at DNA-level.

Chapter 4

Conclusions:

- Sonographic identification of abnormal fetal development is determined by detailed knowledge of normal embryology and fetal sonoanatomy, understanding of the processes and chronological order of the development of specific malformations and extensive scanning experience of the sonographer.
- An increasing number of structures can be visualized by sonography with advancing gestational age. The optimal gestational age for a *complete* (early) anatomical survey and nuchal translucency measurement is at 13 weeks.
- A single ultrasound scan in pregnancy will not detect all fetal anomalies.
- Normal results on first or second trimester sonography do not rule out the diagnosis of, for example, microcephaly, hydrocephaly or cardiomyopathy. These conditions may even occur as late as the neonatal period or in childhood.
- The variable phenotypic expression of anomalies and syndromes can be a pitfall. An early manifestation in one pregnancy will not guarantee an early diagnosis in a next pregnancy.
- As the routine scanning at 11-14 weeks, as well as routine scanning at 18-21 weeks is still subject to debate in The Netherlands, a considerable percentage of pregnancies is referred to tertiary centres in the late second to third trimester of pregnancy with divergent fetal anomalies.
- In some patients prenatal ultrasound in following pregnancies will be secondary to or replaced by DNA mutation analysis.
- Combining dysmorphological evaluation, post mortem examination, cytogenetic/DNA studies and genetic counseling for future pregnancies is of considerable importance.
- The possibility and option of early diagnosis of a specific anomaly or associated anomalies at an 11-14 week scan by an experienced sonographer should be discussed with the parents.
- Although there is as yet no firm evidence that early termination of a pregnancy for fetal malformations is psychologically more acceptable for parents, to know that fetal development is normal as early as the late first to early second trimester of pregancy will be reassuring.

Hoofdstuk 1

De meest indrukwekkende vorderingen in de humane ontwikkeling vinden plaats in de eerste acht weken na de conceptie. De embryonale periode is van belang aangezien de meeste congenitale afwijkingen in die periode ontstaan.

Echoscopie is een methode om het levende, humane embryo en de foetus te onderzoeken. Om in de jonge zwangerschap de afwijkende ontwikkeling te kunnen beoordelen, is kennis van de normale, embryonale ontwikkeling in vivo essentieel. De embryonale structuren en functies, die zichtbaar zijn met behulp van echoscopisch onderzoek met hoge frequenties worden in dit hoofdstuk besproken. Een toenemend aantal structuren wordt zichtbaar met het vorderen van de zwangerschapsduur. De ideale zwangerschapsduur voor een complete, anatomische beoordeling van de foetus is 13 weken. Dan is ook het meten van de nekplooi het nauwkeurigst en informatief. Afbeeldingen van echoscopisch vastgelegde, normale en afwijkende foetale anatomie worden gepresenteerd in de Appendix van Hoofdstuk 1.

Het potentieel van eerste en vroeg tweede trimester foetale echoscopie wordt benut bij een combinatie van hoge kwaliteit van en ruime ervaring met geavanceerd ultrageluidonderzoek, gedetailleerde kennis van exogene en genetische syndromen (congenitale afwijkingen), de geassocieerde erfelijkheids-patronen en de samenwerking met een afdeling Pathologie met ervaring in de foetale pathologie.

Hoofdstuk 2

De waarde en de impact van vroegdiagnostiek van echoscopisch vastgestelde foetale afwijkingen worden besproken in dit hoofdstuk.

De echoscopist in het bijzonder moet op de hoogte zijn van de dynamiek en chronologie van het ontstaan van foetale afwijkingen en de daarmee samenhangende specifieke malformaties. Sommige afwijkingen, die vroeg in de zwangerschap bestaan, zijn van voorbijgaande aard en zijn later in de zwangerschap niet meer zichtbaar. Andere afwijkingen presenteren zich bij een variërende zwangerschapsduur. Echter, de meeste foetale afwijkingen kunnen in het eerste en late tweede trimester van de zwangerschap worden gediagnosticeerd. Dit wordt ook gedemonstreerd in de prospectieve studie van 101 foetus, in ons centrum onderzocht, met een risico op congenitale afwijkingen.

Skeletdysplasieën zijn vaak moeilijk detecteerbaar in het eerste trimester van de zwangerschap. Vroegdiagnostiek is echter mogelijk van een aantal vormen, zoals elders en door ons (dit hoofdstuk) is aangetoond. Variatie in fenotypische expressie, d.w.z. verschillen tussen aangedane familieleden) komt frequent voor.

Geassocieerde misvormingen kunnen dan een belangrijke echoscopische aanwijzing voor herhaling zijn.

De 'verdikte nekplooi' heeft verschillende etiologieën omdat deze is geassocieerd met een spectrum van chromosomale en structurele afwijkingen en genetische syndromen. In dit hoofdstuk wordt een foetus gepresenteerd met een verdikte nekplooi, die berustte op een stofwisselingsstoornis (lysosomale stapelingsziekte), hetgeen de complexiteit van differentiaal diagnostiek in deze zwangerschapsperiode onderstreept.

De waarde en impact van echoscopisch onderzoek in de vroege zwangerschap is afhankelijk van de ontwikkelingsgeschiedenis van de afwijkingen (mogelijkheid van detectie bij een bepaalde zwangerschapsduur) en de variabele fenotypische expressie van zulke afwijkingen c.q. syndromen.

Hoofdstuk 3

Wanneer het echoscopisch onderzoek bij een zwangerschapsduur van 11-14 weken gecombineerd wordt met een echoscopisch onderzoek bij 18-20 weken, zal 80-90% van de foetale afwijkingen kunnen worden opgespoord. De overige foetale afwijkingen worden eventueel in het derde trimester vastgesteld of eerst postnataal. Micro- en hydrocephalie hebben een variabele prenatale (en postnatale) beginleeftijd en verloop en herhaalde waarneming in zwangerschappen met een verhoogd risico zijn dus nodig. Normale, foetale biometrie en anatomie en een normale atrium/ventrikel diameter bij een tweede trimester echo, sluiten deze afwijkingen bij de foetus of het kind niet uit.

Twee grote retrospectieve studies van foetus met echoscopisch vastgestelde hydrocephalie of microcephalie worden gepresenteerd in dit hoofdstuk. Beide aandoeningen kennen een hoge frequentie van 'slechte uitkomst', vooral van de verstandelijke ontwikkeling. Bijkomende afwijkingen, zoals bij een groot aantal micro/hydrocephalie syndromen, zijn van grote betekenis bij de vroege detectie.

Soms kan de kennis van een combinatie van bepaalde afwijkingen leiden tot de eerste prenatale diagnose van een zeldzame aandoening. De prenatale dysmorfologische evaluatie van een foetus met het acrofaciaal dysostosis syndroom (dit hoofdstuk) werd door pathologisch anatomisch onderzoek van o.a. de hersenen bevestigd.

Nauwkeurige observatie van hand/voetafwijkingen wordt niet alleen toegepast om ouders in te lichten over de herhaling van een, vaak dominant overervende afwijking, zoals brachydactylie type A1, maar is evenzeer van belang bij de talrijke syndromen, die geassocieerd zijn met dergelijke afwijkingen. De verbeterde resolutie van echo-apparatuur maakt vroege (eerste/tweede trimester) detectie van dergelijke extremiteitsafwijkingen mogelijk.

Na (dood)geboorte of zwangerschapsafbreking is een nauwkeurige bevestiging van de diagnose essentieel, ook voor erfelijkheidsadvies aan ouders en familie. Bij elke 'index patient' is zorgvuldig dysmorfologisch onderzoek en pathologisch anatomisch onderzoek door een ervaren kinderpatholoog nodig om de prenataal vastgestelde afwijkingen te bevestigen en nadere diagnosestelling mogelijk te maken, bijvoorbeeld op grond van geassocieerde afwijkingen. Aangezien een obductie vaak niet wordt toegestaan óf uitgevoerd óf informatief is (in geval van maceratie), kunnen prenataal echoscopische bevindingen de enige onderbouwing zijn voor het erfelijkheidsadvies. De waarde van gedetailleerde, echoscopische dysmorfologie zal blijven bestaan ondanks het langzaam toenemende aantal syndromen, dat met behulp van DNA-onderzoek kan worden vastgesteld.

Hoofdstuk 4

Conclusies:

- Echoscopische identificatie van de afwijkende, foetale ontwikkeling wordt bepaald door gedetailleerde kennis van de normale embryologische en foetale ontwikkeling, kennis van de chronologische volgorde van de ontwikkeling van specifieke malformaties en de (echoscopische) ervaring van de echoscopist.
- Een toenemend aantal structuren is echoscopisch zichtbaar met met het vorderen van de zwangerschapsduur. Een complete, anatomische beoordeling van de foetus is mogelijk bij 13 weken. Dit is ook de beste tijd voor het meten van de nekplooi.
- Met één enkel echoscopisch onderzoek tijdens de zwangerschap kunnen niet alle, detecteerbare, foetale afwijkingen worden opgespoord.
- Normale echoscopische resultaten in het eerste en tweede trimester van de zwangerschap, sluit de ontwikkeling van afwijkingen, bijvoorbeeld microcephalie, hydrocephalie of cardiomyopathie niet uit: deze aandoeningen kunnen ook nog ontstaan in de neonatale periode of in de kindertijd.
- Met variabele, fenotypische expressie van afwijkingen en syndromen moet rekening worden gehouden. Een vroege manifestatie in één zwangerschap, garandeert geen vroege diagnose van de afwijking in een volgende zwangerschap.
- Aangezien routine echoscopisch onderzoek bij een zwangerschap van 11-14 weken en bij 18-21 weken nog steeds een discussiepunt is in Nederland, zal een aanzienlijk percentage zwangerschappen pas in het late, tweede tot derde trimester worden verwezen naar tertiaire centra.
- Prenataal geavanceerd ultrageluidonderzoek zal secundair of vervangen worden door DNA mutatie analyse, als tijdig de beschikbaar komende technologie wordt toegepast.

- Voor toekomstige zwangerschappen is het combineren van dysmorfologische evaluatie, obductie, cytogenetische en DNA-studies en erfelijkheidsadvies van groot belang.
- Wanneer er een herhalingsrisico is op een kind met congenitale afwijkingen, dient de mogelijkheid en optie van vroegdiagnostiek naar een specifieke afwijking of geassocieerde afwijkingen met geavanceerd ultrageluidonderzoek bij een zwangerschapsduur tussen 11 en 14 weken door een ervaren echoscopist te worden besproken met de ouders.
- Hoewel er geen wetenschappelijk bewijs voor is dat vroege zwangerschapsafbreking in geval van foetale afwijkingen psychologisch gezien acceptabeler is voor ouders, blijkt het voor ouders zeer geruststellend te zijn al in het eerste of vroege, tweede trimester van de zwangerschap te weten dat de foetale ontwikkeling normaal verloopt.

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