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**INCREASED FETAL NUCHAL TRANSLUCENCY IN THE
FIRST-TRIMESTER SCREENING:
PREGNANCY OUTCOMES AND A LONG-TERM FOLLOW-UP OF CHILDREN**

Outi Äyräs

Academic dissertation

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To my parents

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LIST OF ORIGINAL PUBLICATIONS

The following original publications serve as the basis of this thesis. They are referred to by their Roman numerals in the text, as indicated below:

I Äyräs O, Tikkanen M, Eronen M, Paavonen J & Stefanovic V 2013, “Increased nuchal translucency and pregnancy outcome: a retrospective study of 1063 consecutive singleton pregnancies in a single referral institution”, *Prenatal Diagnosis* vol. 33, no. 9, pp. 856-862. Erratum appears in *Prenatal Diagnosis* 2016 vol. 36, no. 2, pp. 199.

II Äyräs O, Eronen M, Tikkanen M, Rahkola-Soisalo P, Paavonen J & Stefanovic V 2015, ”Long-term neurodevelopmental outcome of children from euploid pregnancies with increased nuchal translucency in the first-trimester screening”, *Prenatal Diagnosis* vol. 35, no. 4, pp. 362-369.

III Äyräs O, Eronen M, Tikkanen M, Rahkola-Soisalo P, Paavonen J & Stefanovic V 2015, “The significance of gender in fetuses with increased nuchal translucency: pregnancy outcomes and long-term outcomes of children”, *Prenatal Diagnosis* vol. 35, no. 9, pp. 901-905.

IV Äyräs O, Eronen M, Tikkanen M, Rahkola-Soisalo P, Paavonen J & Stefanovic V 2016, “Long-term outcome in apparently healthy children with increased nuchal translucency in the first-trimester screening”, *Acta Obstetricia et Gynecologica Scandinavica* vol. 95, no. 5, pp. 541-546.

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ABBREVIATIONS

AC	Amniocentesis
aCGH	Array comparative genomic hybridization
β -hCG	Free human chorion gonadotropin β
bp	Base pair
CHD	Congenital heart defect
CI	Confidence interval
CNV	Copy number variant
CVS	Chorionic villus sampling
DFM	Department of Fetal Medicine
DNA	Deoxyribonucleic acid
FISH	Fluorescence in situ hybridization
HUH	Helsinki University Hospital
ICD	International classification of diseases
IUFD	Intrauterine fetal demise
LFD	Large for date
MC	Miscarriage
MoM	Multiples of median
NIPT	Non-invasive prenatal testing
NT	Nuchal translucency
OR	Odds ratio
PAPP-A	Pregnancy associated protein A
QF-PCR	Quantitative fluorescence polymerase chain reaction
THL	The National Institute of Health and Welfare
Tib	Thermal index bone
TOP	Termination of pregnancy
SFD	Small for date
SIIF	The Social Insurance Institute of Finland
US	Ultrasound

ABSTRACT

Background: The observation that fetuses with chromosomal abnormalities often have excess fluid at the back of their neck at gestational weeks 10 – 14 dates back to the early 1990s. Since then increased nuchal translucency (NT) has been used as a screening method for fetal aneuploidies. Increased NT is also associated with a wide variety of structural defects and genetic syndromes. Adverse outcomes become more likely by increasing NT. Long-term studies with a large cohort about the neurodevelopmental outcome of euploid children with increased fetal NT are sparse and the neurodevelopment is worth further study. There are controversial results about the effect of fetal gender in the NT.

Aims: The primary aim of this study is to collect data on the pregnancy outcomes, long-term neurodevelopmental and overall outcomes in a large cohort of fetuses with increased NT from singleton pregnancies in order to improve parental counselling. The secondary aim is to detect whether the gender of the fetus has an impact on the outcome when the NT is increased.

Subjects and methods: All singleton pregnancies referred to the Department of Fetal Medicine (DFM) at the Helsinki University Hospital (HUH) due to increased NT in the first-trimester screening during 2002 – 2007 were studied. The pregnancy outcome data were retrieved from hospital databases. Pregnancy outcome was defined adverse if there was an abnormal karyotype, structural defect, genetic syndrome, or if the pregnancy ended in miscarriage (MC), termination of pregnancy (TOP), or perinatal death. When assessing the pregnancy outcome, the health of the newborn was followed until discharge from the delivery hospital. In order to find out the long-term neurodevelopmental and long-term overall outcomes, data of the children born from this cohort were retrieved from several national registers. All data implicating health problems were checked from the individual medical charts. The gender impact on pregnancy outcomes and the long-term outcomes were analyzed.

Results: There were 1063 fetuses with increased NT in the first-trimester screening. Abnormal karyotype was observed in 224 fetuses (21%) and adverse pregnancy outcome occurred in 322 (30%). TOP was performed in 209 (20%) and there were 43 (4%) MCs (n = 36) and perinatal deaths (n = 7). Of the 834 euploid fetuses 74 (9%) had structural defects or genetic disorders. Favourable pregnancy outcome became less likely by increasing NT: favourable outcome occurred in 92% in the lowest NT group (95th percentile – 3.4 mm) and

in 18% in the highest NT group (≥ 6.5 mm). Of the euploid fetuses with normal second-trimester ultrasound (US) examination 96% were healthy at the discharge from the delivery hospital. Neurodevelopmental outcome was assessed in 691 euploid children. Neurodevelopmental impairment was detected in 29 (4.2%) and it was severe in 12 (1.7%). During the follow-up time (mean 6.5 years) of the euploid children with increased NT in the first-trimester screening but normal findings in the second-trimester US screening ($n=733$), major structural defects, severe neurodevelopmental impairment, or genetic disorders were detected in 54 cases (7%). There was no difference in the outcomes between the genders among the euploid fetuses. In the total cohort, male fetuses had better pregnancy outcomes than females ($p = 0.049$). This was due to the higher percentage of aneuploidies among the females compared to the males ($p = 0.04$).

Conclusions: One in five fetuses with increased NT ($\geq 95^{\text{th}}$ percentile) has chromosomal abnormalities and one in ten of the euploid fetuses has structural defects or genetic syndromes. Major health impairment (major structural defect, severe neurodevelopmental impairment, or genetic syndrome) is likely to be detected in 7% of euploid children with increased NT in the first-trimester screening but normal findings in the second-trimester screening. These results are helpful in counselling the parents when increased NT is detected.

1 INTRODUCTION

Two-dimensional US examination was introduced in obstetrics in the 1960s (Donald 1965). In the beginning, due to low resolution and the lack of grey scale image, this technique could only be used to estimate the number of fetuses, the presenting part, and the estimation of gestational age from the biparietal diameter. Since then the enormous development of the technique has led to the possibility of early prenatal diagnosis of even small structural defects.

Fetal screening for aneuploidies is a common practice in the developed countries. First-trimester US examination is part of the screening including NT thickness measurement of the fetus. Besides NT, the vitality, number, location, and gross anatomy of the fetus is determined. NT is an ultrasonographically visible translucent space at the back of the fetal neck (Figure 1) (Nicolaides et al. 1992; Wilson, Venir & Farquharson 1992). Some NT is visible in virtually all fetuses at 10 to 14 weeks of pregnancy, and after 14 gestational weeks it usually vanishes (Pajkrt et al. 1995). Increased NT is known to be associated with chromosomal and structural defects, genetic syndromes, and adverse pregnancy outcome (Kagan et al. 2006; Nicolaides, Brizot & Snijders 1994; Souka et al. 2001). Favourable pregnancy outcome becomes less likely by increasing NT (Bilardo et al. 2007; Souka et al. 2001).



Figure 1. Increased nuchal translucency of 3.1mm at the back of the fetal neck is visible as a translucent area.

Parental counselling follows a positive screening result. Parents are offered further diagnostic work-up to detect aneuploidies and structural defects. An abnormal finding in fetal screening raises anxiety in the parents (Chueh et al. 2007; Kleinveld et al. 2006; Kowalcek et al. 2003), although most of the screen positives will have a normal diagnostic result. Parents need to make difficult decisions based on the information they are offered. These decisions include invasive procedures chorionic villus sampling (CVS) or amniocentesis (AC) for karyotyping, carrying a small risk of MC, and if the fetus is diagnosed with chromosomal abnormalities, the decision of continuing or terminating the pregnancy. There is a need for accurate and clear counselling before the diagnostic work-up and after the result, even if it is normal. A prerequisite for adequate counselling is high-quality screening and subsequent studies about the outcomes after a positive screening result.

Although increased NT has been studied since the end of the 1980s, studies of the long-term neurodevelopmental or overall outcome of children born after increased fetal NT with a large cohort are sparse. Additionally, studies of fetal gender impact on NT have been controversial. The aim of this study is to gather high-quality data of Finnish pregnancies of a large number of fetuses with increased NT. By recording the pregnancy outcomes and the long-term outcomes of children born after increased fetal NT, we aim to produce additional data to improve parental counselling in such cases.

2 REVIEW OF THE LITERATURE

2.1 Screening

2.1.1 General

Screening refers to a systematic attempt to find, from an apparently healthy population, people at high enough risk of a specific condition to warrant further action. Most screening tests serve only to mark the condition and are not diagnostic as such. Screen positives are offered further diagnostic work-up, impossible to be offered to the whole population due to financial resources or the possible harmful effects, or both. The results of screening can be divided into four different groups: false and true positives and false and true negatives. In most cases those who have obtained positive markers in screening achieve normal test results in the further diagnostic work-up. This is the group of false positives. Those diagnosed with the condition screened for form the group of true positives. Those who failed to be marked in spite of the condition screened for are the false negatives; true negatives are those who do not have the condition screened for and obtain a negative result in the screening. The ability to correctly detect the individuals with the condition, i.e. the true positives, is called sensitivity. Specificity is the ability to correctly detect the individuals without the condition, i.e. the true negatives. Positive predictive value signifies the likelihood that an individual who obtains a positive screening result actually has the condition screened for (Figure 2).

	Condition +	Condition -
Test +	True positive A	False positive B
Test -	False negative C	True negative D

Figure 2. Sensitivity = $A/A+C$, Specificity = $D/B+D$, Positive predictive value = $A/A+B$

There are several general demands for a pathological condition to be suitable for screening. They have been stated by the World Health Organization in the 10 principles of screening (Wilson & Jungner 1968) and in the criteria for screening by the Danish Council of Ethics (The Danish Council of ethics 2000):

- The condition is significant both to an individual and society.
- It is possible to observe the condition by screening before it would naturally appear.
- The prevalence of the condition is (relatively) high and the condition is severe.
- The condition and its natural course are well known and it has a precursor or a pre-stage. There is an accepted treatment at this stage.
- It is clear how the screening test and the diagnostic work-up are interpreted and who is treated.
- There is a cost effective screening tool, which is fast, generally accepted, and does not carry a high risk or harm the participants.
- There is a diagnostic tool, which is available and generally accepted.
- There is a cure for the condition or the true positives benefit from the earlier diagnosis of the condition.
- The specificity and the sensitivity of the screening are high enough and the effectiveness of the screening is estimated.
- The social and ethical effects of the screening and the effects of a false positive and false negative test results have been evaluated.
- The costs of the screening are known and they are in proportion with the health care service resources.
- The screening organization is well described.
- Screening is continuous.

If the screening test is dichotomic (e.g. positive blood in feces), it is easy to define the result as positive or negative. If the screening test is a continuous variable, there is a cut-off point to separate the screen positives from the screen negatives. If the screening test is a combination of several independent tests, a mathematic model is needed for individual risk estimation. Several different issues are considered when the cut-off point between screen positives and screen negatives is defined. If the test is very sensitive, almost all individuals with the condition are detected. Unfortunately, usually when the sensitivity rises, the specificity decreases. This means that more individuals without the condition end up with

a positive screening result and more diagnostic tests are required. These false positives cause extra cost, raise unnecessary anxiety, and predispose healthy individuals to the risks of the diagnostic test. Thus the severity of the condition, the cost and the risk of the diagnostic test, and financial resources need to be considered when determining the cut-off point. Usually some kind of compromise is decided upon, as screening should produce enough health benefits to be justified from the perspective of public health.

Participating in screening is voluntary, and it is of great importance that the individuals attending screening understand the sensitivity and the specificity, what a positive or a negative screening result means, what the diagnostic tests are, and what possible treatments the condition screened for may need. This is not an easy task, since many screenings are complex and the information should be understandable to all possible participants. Different kinds of problems are encountered depending on the type of screening. On the one hand, preventing cervical cancer by pap-smear screening for example, is an almost perfect method for screening in many ways, but the main problem is how to make women attend the screening. On the other hand, fetal first-trimester screening for trisomy 21 is well attended, but it is difficult to make sure that women attending the screening understand the correct meaning of possibly getting a positive screening result and the decisions they need to make thereafter.

2.1.2 Fetal first-trimester screening

The main goal of first-trimester screening is to find fetuses with trisomy 21, which is the most common aneuploidy. The concept of screening for trisomy 21 was introduced in the late 1960s, when laboratory techniques for fetal karyotype determination were developed (Nadler 1968; Nadler & Gerbie 1970). Fetuses at a risk for other aneuploidies, some of which are phenotypically more severe than trisomy 21, are also detected by the same screening tests (Breathnach et al. 2007; Ekelund et al. 2011; Engelbrechtsen et al. 2013; Kagan et al. 2006; Loane et al. 2013; Topping et al. 2015). Structural defects are detected in the screening strategies including US examination (Souka & Nicolaides 1997, Whitlow et al. 1999). Several first-trimester screening strategies have been developed since the 1970s: screening by maternal age, screening by NT, combined screening, and non-invasive prenatal testing (NIPT). Each new screening strategy has a better performance compared to the older ones. The method of choice at present is still combined screening, although NIPT carries high expectations in the future but is still too expensive to be used as the general

method of screening. Different strategies of contingent screening have been introduced and are being studied. Since US examination is part of the combined and NT screening, the vitality, number, size, and gross anatomy of the fetus are determined in addition to the risk estimation for trisomy 21.

2.1.2.1 Screening by maternal age

The correlation of advancing maternal age and trisomy 21 was noted already in 1876, when a detailed description of 62 patients with Down syndrome was published (Fraser & Mitchell 1876). The incidence of trisomies increases by increasing maternal age (Antonarakis 1991; Hook 1981; Loane et al. 2013; Pandya et al. 1995; Savva, Walker & Morris 2010). Table 1 shows the risk of trisomy 21 by maternal age according to a recent Finnish study (Marttala et al. 2010). Offering karyotyping for mothers over certain age is the oldest fetal screening strategy. In the 1990s, it was estimated that if mothers over 37 years of age were offered karyotyping, the detection rate for trisomy 21 would be about 30% with a 5% invasive testing rate (Snijders et al. 1996). As the trend after the 1990s has been towards advancing maternal age, using 37 years as a cut-off would nowadays result in a higher intake than 5%, since at 2012 - 2013 already 19.7% of all deliveries in Finland were to mothers ≥ 35 years of age (THL 2014) and 11% of deliveries in Finland were to mothers ≥ 37 years of age (personal information from the birth register, THL). A similar trend can be verified in all Europe (Loane et al. 2013).

Table 1. The risk of trisomy 21 (terminations and newborns) in different maternal age groups according to a Finnish study (Marttala et al. 2010).

Maternal age (years)	Risk of trisomy 21
< 20	1:746
20 - 24	1:1140
25 - 29	1:862
30 - 34	1:566
35 - 39	1:172
40 - 44	1:49
≥ 45	1:23

2.1.2.2 Screening by nuchal translucency thickness

In the early 1990s NT was shown to be applicable as a screening tool for fetal aneuploidies (Nicolaidis et al. 1992; Savoldelli et al. 1993). At first fixed cut-off points, such as 3 mm

or 3.5 mm, were used. Soon, however, gestational age-dependent cut-off points were recommended, since the NT increases by increasing fetal size from nine to 14 gestational weeks (Pajkrt et al. 1995), and using such cut-off points reduces the number of false positive test results. It was also recommended that each center performing screening by NT measurement should have its own reference values (Pajkrt et al. 1995). Usually in NT screening information about maternal age is combined with the information about the NT thickness and an individual risk number is calculated. Fixed or gestational age dependent cut-off points are also used. NT screening detects about 75% of trisomic pregnancies with a 5% false positive rate (Gasiorek-Wiens et al. 2001; Pandya et al. 1995b; Schwarzler et al. 1999; Snijders et al. 1998; Zoppi et al. 2001), but the sensitivity of NT screening has varied from 62% (Taipale et al. 1997) up to 91% (Theodoropoulos et al. 1998).

2.1.2.3 Combined screening

In combined screening levels of pregnancy-associated protein A (PAPP-A) and free human chorion gonadotropin β unit (β -hCG) in the maternal serum, fetal NT, and maternal age are considered in a risk calculation. This method detects 80 - 90% of trisomy 21 fetuses with a false positive rate of 5% (Ekelund et al. 2015; Kagan et al. 2008; Kagan et al. 2009c; Nicolaides et al. 2005; Rozenberg et al. 2006; Spencer et al. 1999; Wald et al. 2003). In pregnancies affected by trisomy 21 the PAPP-A levels are lower and the β -hCG levels higher than in pregnancies with a normal fetus (Spencer et al. 1999). Several different strategies in producing combined screening have been developed. The serum tests can be taken before NT measurement, achieving the highest accuracy at 9-10 weeks of pregnancy (Kirkegaard et al. 2008; Wright et al. 2010), or at the same time as the NT measurement (one-stop combined screening OSCAR) (Kagan et al. 2008; Spencer et al. 2000a). Taking the serum tests earlier in the pregnancy gives slightly better screening performance compared to serum testing at the same time as NT measurement (detection rates 95% and 92%, respectively) (Ekelund et al. 2012), but as the detection rate of trisomy 21 from one-stop clinics has been reported to be as good as 90% with a 5% false positive rate (Bindra et al. 2002; Spencer et al. 2003) both strategies are used.

2.1.2.4 Noninvasive Prenatal Testing (NIPT)

Cell-free fetal deoxyribonucleic acid (DNA) is present in maternal plasma (Lo et al. 1997). The amount increases by increasing gestation and may be observed as early as seven weeks

of gestation (Lo et al. 1998). NIPT is a fairly new technique where cell-free fetal DNA is analyzed from the maternal blood sample. It is highly specific (99%) and highly sensitive (99.9%) in detecting trisomy 21, and the specificity for detecting trisomies 13 and 18 is also good: 91% and 96%, respectively (Gil et al. 2015). Most common trisomies and aberrations of sex-chromosomes can be screened by NIPT (Alberti et al. 2015; Liang et al. 2013; Porreco et al. 2014; Verweij et al. 2013; Zhang et al. 2015). The positive predictive value of NIPT in general population (81%) is markedly better than that of the combined screening (3%) (Norton et al. 2015). Unfortunately this technique is still rather expensive and thus not available for general screening (Conner, Gustafsson & Kublicas 2015; Cuckle, Benn & Pergament 2013). Since some false positive test results have been reported (Dugo et al. 2014), NIPT cannot serve as a diagnostic test and a positive NIPT result must be confirmed by invasive testing of the fetal karyotype by CVS or AC.

2.1.2.5 Contingent screening

Contingent screening combines different strategies of screening with maternal serum markers, US examination with different markers, and NIPT by offering further testing to only those with an intermediate (1:50 - 1:1000) and/or high risk ($\geq 1:50$) for aneuploidies. In the newest models, aiming at a better performance with low invasive testing rate and reasonable cost, karyotyping is offered to mothers at high risk and NIPT to women with an intermediate risk (Conner, Gustafsson & Kublicas 2015; Ekelund et al. 2012; Kagan et al. 2010; Munoz-Cortes et al. 2012; Nicolaides et al. 2013; Sahota et al. 2010).

2.1.2.6 Ultrasound markers for chromosomal abnormalities

There are several US markers in addition to NT at the time of the first-trimester scan connected with chromosomal abnormalities. Recent publications suggest that the detection rate may be significantly increased up to 92 - 100% by adding them to US screening. (Ghaffari et al. 2012; Hsiao et al. 2014; Karadzov-Orlic et al. 2012). These US markers include the assessment of fetal nasal bone and blood flow measurements in the ductus venosus and the tricuspid valve. The nasal bone is absent in approximately 60% of cases with trisomy 21 versus 2.6% of euploid fetuses (Kagan et al. 2009a & 2010) and tricuspid regurgitation and reverse a-wave in ductus venosus are present in 65% and 55% of fetuses with trisomy 21 versus 3.2 and 0.9% of euploid fetuses, respectively (Kagan et al. 2009b & 2010; Mula et al. 2015). The use of these US markers varies largely between the institutions.

2.1.3 Ethical aspects of fetal screening

There are several aspects in fetal screening that may lead to ethical problems (Committee on Ethics and Genetics ACOG 2008). Screening for aneuploidies does not fulfill all criteria for a good screening target. Aneuploidies are common enough and in most cases severe enough to justify screening, but there is no treatment for the condition. The diagnostic test carries a small risk of MC of a healthy fetus due to a false positive screening result. The parents receiving a positive screening result first need to decide about the invasive testing and secondly, in the case of positive diagnostic test, they need to decide whether to continue the pregnancy or terminate it. Decision of TOP is an extreme ethical challenge (Choi, van Riper & Thoyre 2012; Jotkowitz & Zivotofsky 2010; Learman et al. 2005; Natoli et al. 2012; Norton, Nakawaga & Kuppermann 2014). Several studies show that not all parents attending fetal screening are sufficiently aware of the method to make an informed decision whether to participate (Bangsgaard & Tabor 2013; Dahl et al. 2006; Farrell, Nutter & Agatisa 2011; Jaques, Halliday & Bell 2004; Santalahti et al. 1998; Ternby et al. 2015). Furthermore, many parents understand the screening as a part of routine antenatal care (Kobelka et al. 2009; Santalahti et al. 1999). Even if the screening result is positive, not all parents opt for invasive procedures (Kobelka et al 2009), which might reflect that the parents have not fully understood the information. High demands should be set to the information delivered to the parents before their decision to attend the screening.

Offering screening for chromosomal abnormalities and structural defects implicates that TOP for these reasons is accepted and cost effective. This may have social consequences to the attitudes towards disabled people. On the one hand, fetal screening can make the disabled feel not accepted in society and screening may even be understood as threatening to human rights. On the other hand, fetal screening improves the autonomy of the expecting parents (Caplan 2015). The risk of chromosomal abnormalities increases by maternal age. The possibility of fetal screening might encourage some women of advanced age to become pregnant.

Screening might reveal structural abnormalities, the clinical relevance of which is not known until the child is born. Such findings cause parental anxiety and might affect the pregnancy psychologically and disturb the emotional attachment to the unborn child. Chromosomal analysis might also reveal an abnormal result of which the prognosis and phenotype are difficult to interpret in the perinatal period, like mosaicisms, marker-

chromosomes, rare translocations, or variants of unknown significance if chromosomal microarray is used. False negative test results might cause bitter feelings in the parents. A negative screening test result is often falsely interpreted as a promise of a healthy child and the correct meaning of a negative screening test result is not understood (de Jong & de Wert 2015).

A positive screening result poses some problems in every screening. Expecting mothers are especially vulnerable and a positive screening result is likely to raise anxiety (Allison et al. 2011; Chueh et al. 2007; Kleinveld et al. 2006; Kobelka et al. 2009; Kowalcek et al. 2003; Marteau et al. 1992). The decision of whether to proceed with invasive testing carrying a risk of MC causes mental stress (Durand et al. 2010). However, the anxiety levels decrease after appropriate counselling (Tercyak et al. 2001) and return to normal after a normal diagnostic result (Allison et al. 2011; Chueh et al. 2007; Kleinveld et al. 2006). According to a recently published study, there is no additional residual anxiety after a positive screening test has been proven to be false-positive (Lou et al. 2015).

2.1.4 Screening and parental counselling

Prenatal counselling is very important for alleviating anxiety (Tercyak et al. 2001). Attending fetal screening is voluntary. Informed consent is requested and it should be based on an informed decision. Both pre- and post-screening counselling should provide objective, precise, and thorough information. In fetal screening for aneuploidies, the algorithm is complex and the methods are not easily understood, since the individual risk is calculated by a combination of background information (age) and test results (NT, maternal serum markers). Fetal screening is discussed among other issues about pregnancy at the antenatal clinics, and it is possible that parents assume that participating in fetal screening for chromosomal abnormalities is part of the routine antenatal care (Kobelka et al. 2009; Santalahti et al. 1999). It is of essential importance to inform the parents about the voluntary nature of the prenatal screening. Those who decide against it should be reassured about equal pregnancy care. The information should be easily understandable and include a description of the conditions that screening is supposed to reveal. Since the time frame in fetal screening is limited, it is possible that the information is not always well comprehended in time for scheduling the tests.

The aims of prenatal counselling about increased risk for a genetic condition are to provide pre-test information as well as psychosocial support and assistance in decision making (Cohn et al. 1996). Ideal counselling should be nondirective i.e. the genetic counselor must respect the client's right of autonomous decision. The goal of nondirectiveness is value-neutral communication. Providing nondirective counselling is challenging but it is a worthwhile goal. Parents' decisions are based on several different factors and it is important that these personal values and beliefs are listened to (Choi, van Riper & Thoyre 2012). More important than strict nondirectiveness or value-neutrality is that the facts are presented in a comprehensible and meaningful context to the parents (Anderson 1999; Kobelka et al 2009; Rentmeester 2001). The counselor has succeeded, if the parents reach a decision that is right for them and with which they feel satisfied.

2.1.5 Fetal screening in Finland

A working group on screenings was set in 2003 by the Ministry of Social Affairs and Health in Finland. One of its tasks is to re-evaluate the existing health-care screening programs and propose directions for the future.

The Health Care Act in Finland (Health Care Act 339/2011) states that local authorities are in charge of organizing screenings according to the national screening programs. The present statutory prenatal group of screenings was introduced in 2008 including general US examination during early pregnancy, screening for aneuploidies, and later US examination screening for major structural abnormalities.

The first-trimester screening for trisomy 21 is offered to all women attending the antenatal care clinics in Finland. Screening is voluntary and free of charge. The free-of-charge national antenatal clinics provide the information about the screening both orally and in written documents. At the HUH district, fetal first-trimester screening and second-trimester US screening were offered to all pregnant women registered at the antenatal clinics already prior to introduction of the Health Care Act. The current screening method at HUH is presented in Figure 3.

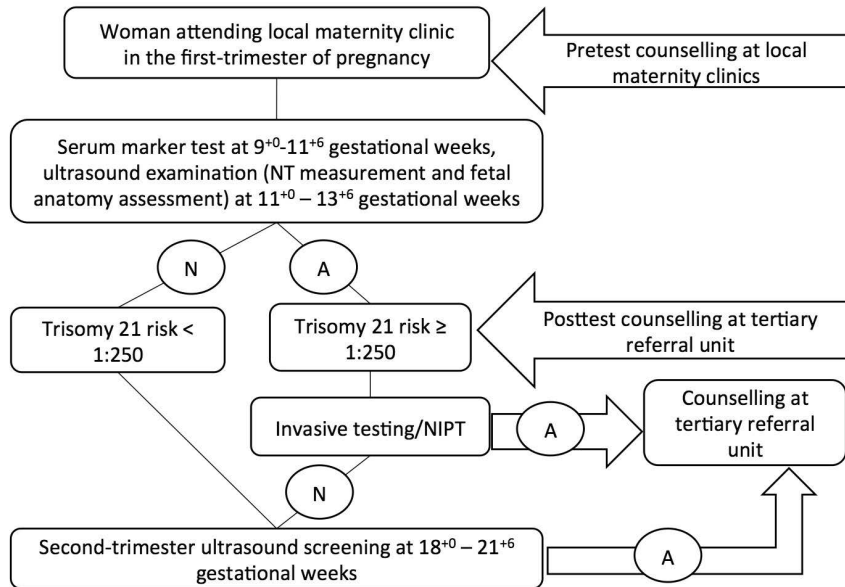


Figure 3. Current fetal screening protocol at Helsinki University Hospital -district. N, normal result; A, abnormal result.

TOP is legal in Finland under certain circumstances (Laki raskauden keskeyttämisestä 24.3.1970/239, 'The law of terminating pregnancy'). It is allowed up to 12⁺⁰ gestational weeks for social indications. This requires examination and consideration of two authorized physicians. From 12⁺¹ to 20⁺⁰ gestational weeks TOP is possible when there are exceptional social indications or there is a reason to believe that the fetus has or is going to develop a severe developmental handicap, illness, or defect. The permission must be applied for from the National Supervisory Authority of Welfare and Health. From 20⁺¹ to 24⁺⁰ gestational weeks TOP is possible only in cases with a definite diagnosis of severely affected fetus and it is applied from the same National Authority.

2.2 Chromosomal abnormalities

2.2.1 Definition and terminology

Human cells normally contain 46 chromosomes. Chromosomes are numbered, and each chromosome has a pair, of which one is inherited from the mother and the other from the father. Each cell has this diploid chromosomal count except for the gametes (the egg and the sperm), which are haploid and have 23 chromosomes. Sex chromosomes are XY in a male and XX in a female. A normal chromosome count is called euploid and marked either 46,XY or 46,XX. Full aneuploidy is a pathological condition, where the chromosome number is not normal. Trisomy is the most common aneuploidy where there is one excess chromosome. The most common trisomy in humans is 16-trisomy. These fetuses, however, are miscarried in the early pregnancy and thus not detected in screening (Hassold et al. 1995; Ljunger et al. 2005). The most common trisomies that survive to birth are trisomies 21, 18, and 13. The karyotype is marked for example 47,XY+21 in a male trisomy 21. In polyploidy there are more than two of the whole chromosome sets. In triploidy there are three instead of two whole chromosome sets, and it is marked e.g. 69XXX. In partial aneuploidy the number of chromosomes is normal, but the genetic material is either reduced or increased (deletions, duplications, and unbalanced translocations). Structural alterations are also possible when the number of chromosomes and the amount of genetic material are normal. They may influence the function of the genes (balanced translocations, inversions). Full aneuploidies are usually caused by nondisjunction during meiosis in the gametes. The frequency of full aneuploidies increases by increasing maternal age, since errors in meiosis occur more often in the ageing gametes (Antonarakis 1991). Partial aneuploidies are often caused by errors in the homologous recombination (the exchange of genetic material between the matching parts of the chromosomes) which happens in normal meiosis. Translocations can be balanced or unbalanced. Balanced translocations usually do not affect the phenotype, but in meiosis they can lead to the formation of an unbalanced gamete and cause an unbalanced translocation in the next generation. Mosaicism means that the same individual has both normal cell lines and abnormal cell lines.

Chromosomes contain genes, which are made of basepairs (bp). The whole human genome was mapped at 2001 (Mc Pherson et al. 2001). The size of the whole human genome is approximately 3 billion bps, one gene being from a few hundred bps to a few million bps.

A chromosome contains few hundred - few thousand genes and the size of a chromosome is 33 million - 250 million bps. (Gardner, Sutherland & Shaffer 2011; Richards & Hawley 2011; Sweet & Michaels 2011).

2.2.2 Prenatal chromosomal analysis

The most common ways to obtain fetal cells for chromosomal analysis are CVS and AC, which both require an invasive procedure and carry a small risk of MC. The first genetic AC was reported in 1956 (Fuchs & Riis 1956) and the first US guided CVS in 1984 (Smidt-Jensen & Hahnemann 1984). CVS can be taken from 10 gestational weeks and AC from 14 weeks on. Sometimes CVS is not technically possible, for example, in the case of posterior placenta in a retroverted uterus. Although the procedure-related risk of fetal loss has generally been considered to be approximately 0.5 - 1% (Alfirevic, Sundberg & Brigham 2003; Tabor et al. 1986), the latest publications show lower figures of 0.06 - 0.1% for AC and 0.2 % for CVS (Akolekar et al. 2015; Eddleman et al. 2006) or even no increased procedure-related risk (Wulff et al. 2016). It has been shown that experience reduces the procedure related loss rate (Tabor, Vestergaard & Lidegaard 2009; Verjaal, Leschot & Treffers 1981).

In CVS the specimen is placental tissue, which is a rich source of DNA since the cytotrophoblasts are rapidly duplicating cells and a substantial amount of DNA is received in the sampling. The CVS does not necessarily require culturing since the mitotic activity is high. In AC the cells are from the fetal skin, lungs, bladder, and the amnion. The mitotic activity is not as rapid as in the placental tissue. Cells need to be cultured before analysis, which takes approximately 14 days.

Conventional karyotyping with G-banding became the gold standard in detecting chromosomal abnormalities in the 1970s. In this method the cell cycle is stopped at mitosis, the chromosomes are stained and examined microscopically and the size, shape, and number of chromosomes are analyzed. Aneuploidies and large (5-10Mbp) deletions, duplications, translocations, and inversions are diagnosed (Caspersson et al. 1970; Steele & Breg 1966). It is possible to detect the most common trisomies rapidly, in one to two days without culturing by using the rapid fluorescence *in situ* hybridization (FISH) (Ward et al. 1993) or quantitative fluorescent polymerase chain reaction (QF-PCR) (Pertl et al. 1994) instead of conventional karyotyping (Hulten et al 2003). By using fluorescently labeled

probes in FISH or by measuring the quantities of chromosome specific short tandem repeats in QF-PCR, it is possible to diagnose 21-, 18-, 13- trisomies and differences in the number of sex chromosomes. The results are received faster, which is anxiety relieving for the parents (Leung et al. 2008), but other chromosomal abnormalities than trisomy 21, 18, 13, and sex-chromosome number variations are not detected by these rapid techniques (Caine et al. 2005; Gekas et al. 2011). The disadvantage of the CVS is the possibility of obtaining placental mosaicism as a result in 1-2% of samples (Hahnemann & Vejerslev 1997). In cases where placental mosaicism is suspected, the fetal karyotype is confirmed by AC, which usually contains cells of the fetus only.

Smaller (up to 400Kbp) submicroscopic, genetic changes are detected from the DNA by chromosomal microarray, which also has a short turn-around-time. There are two types of microarrays: array comparative genomic hybridization (aCGH) and single nucleotide polymorphism -array. Duplicated or deleted sections of DNA are called copy number variants (CNVs). They can be pathologic, benign, or of unknown significance. Another type of DNA alteration is single nucleotide polymorphism where only a single nucleotide is altered. Both microarray methods detect CNVs and trisomies, but aCGH is not capable of detecting triploidy or balanced translocations (Gardner, Sutherland & Shaffer 2011). In a large study of 4282 prenatal DNA samples, additional 6% of pathologic CNVs were detected by microarray compared to conventional karyotyping in fetuses with structural defects diagnosed by ultrasonography. In fetuses with no structural defects the microarray found 1.6% additional pathologic CNVs compared to conventional karyotyping (Wapner et al. 2012). Up to 20% of pathological CNVs were found when samples from mentally retarded persons with normal conventional karyotyping results were analyzed by aCGH (Siggberg et al. 2010). In the parental counselling CNVs of unknown significance are challenging (Mikhaelian et al. 2013), but the number of uncertain CNVs is decreasing due to accumulating clinical experience and international networking.

2.2.3 Trisomy 21

The vast majority, approximately 95%, of trisomy 21 cases (Down syndrome) are caused by nondisjunction in meiosis of the gametes (90% of maternal origin) and classified as regular or standard. The rest of 21 trisomies include isochromosome 21, unbalanced translocations involving the chromosome 21, or mosaics. If trisomy 21 is caused by parental

translocation, the translocation is usually balanced in the parent, but inherited as unbalanced to the child.

The incidence of trisomy 21 is approximately 22-27/10000 pregnancies (TOPs, MCs, intrauterine fetal demises (IUFDs), and liveborns) and 11-13/10000 liveborns (Loane et al. 2013; Marttala et al. 2010; Morris & Alberman 2009). There are more males than females with trisomy 21, with a ratio 1.23:1 (Gardner, Sutherland & Shaffer 2011).

Trisomy 21 is associated with fetal losses, congenital heart defects (CHDs), structural defects of the gastrointestinal organs and limb defects (Morris et al. 2014). Affected persons have typical dysmorphic features (broad flat face, slanting eyes, epicanthic eye folds, and short nose), short stature, their motor skills develop slowly, they are prone to infections, and have mental retardation of various degrees (Harper 2010).

2.2.4 Trisomy 18

The prevalence of trisomy 18 (Edward syndrome) is approximately 5-6/10000 pregnancies (TOPs, MCs, IUFDs, and liveborns) and 1- 2.7/10000 liveborns (Loane et al. 2013; Savva, Walker & Morris 2010; Tonks et al. 2013.). Common features are CHDs, spina bifida, structural anomalies of the central nervous system, omphalocele, and rocker bottom foot. Approximately 80% of trisomy 18 fetuses have increased NT in the first-trimester US screening (Sherod et al. 1997; Yang et al. 2005). Most of the pregnancies end in MC, TOP, or IUFD (Morris & Savva 2008). Fetuses are small for their gestational age and often born prematurely. Most of the liveborns die soon after birth and only few survive beyond one year of age. All affected are mentally severely retarded (Rasmussen et al. 2003).

2.2.5 Trisomy 13

The prevalence of trisomy 13 (Patau syndrome) is approximately 2/10000 pregnancies (TOPs, MCs, IUFDs, and liveborns) and 0.5-1.6 /10000 liveborns (Ekelund et al. 2011; Loane et al. 2013; Savva, Walker & Morris 2010; Tonks et al. 2013). Common features are holoprosencephaly, CHDs, gastrointestinal and vesicourinary malformations, facial clefts, microftalmy, and polydactyly. Approximately 70% of fetuses with trisomy 13 have increased NT in the first-trimester US screening (Snijders et al. 1999). Most pregnancies end in MC, TOP, or IUFD (Morris & Savva 2008). All affected rare survivors are mentally

retarded. The syndrome is usually lethal and liveborns die soon after birth (Rasmussen et al. 2003).

2.2.6 Other common chromosomal abnormalities

Sex-chromosome abnormalities are clinically less severe than those of other chromosomes. The commonest sex-chromosome abnormality in males is 47,XXY (Klinefelter syndrome). The prevalence among liveborn is approximately 17/10000 (Morris et al. 2008). Clinical features include reduced fertility, gynecomastia, small testicles, and tall stature (Giltay & Maiburg 2010). The commonest sex-chromosome abnormality in females is 45,X (Turner syndrome). It is the only monosomy of liveborns in humans. The prevalence among liveborns is approximately 3/10000 (Gravholt et al. 1996). Most, up to 99% (Hook 1978), of the fetuses having 45,X are miscarried or IUFD occurs later in pregnancy (Ranke & Saenger 2001). Fetuses with Turner syndrome often present with extremely thick NT or cystic hygroma (Pinsker 2012). Clinical features of the survivors are aortic coarctation, premature ovarian failure, thick neck, and shortness (Ranke & Saenger 2001).

2.3 Nuchal translucency

2.3.1 Background

NT is a fluid-filled collection at the back of the fetal neck. It is visible by US as a translucent area between the skin outline and the underlying soft tissues (Figure 1). In 1987 nuchal fold (thickening of the fetal neck in the late second trimester) was noticed to have an association with trisomy 21 in fetuses over 16 weeks of gestation (Benacerraf, Gelmon & Frigoletto 1987). In the early 1990s, first trimester CVS was becoming more popular in the UK among women over 35 years of age. Nicolaides et al. observed, while sampling the placenta, that some of the fetuses were hydropic and had an excess of fluid at the back of the neck. They called this accumulation of fluid NT. They reported that 35% of fetuses with NT of 3 - 8 mm had chromosomal abnormalities compared to 1%, when the NT was < 3mm (Nicolaides et al. 1992). At the same time other observations of the same kind were made (Wilson, Venir & Farquharson 1992).

Some NT is detected in all fetuses at 10 to 14 weeks of gestation. There is physiologic variation in NT thickness among normal fetuses and the thickness increases by increasing fetal size from 10 to 14 weeks (Braithwaite et al. 1996; Pajkrt et al. 1995; Wald et al. 2003). This is why it is important to use cut-off levels taking the fetal size into account rather than fixed cut-off levels. After 14 weeks the NT usually vanishes and in a fetus over 14 weeks nuchal fold is associated with an increased trisomy 21 risk (Agathokleus et al. 2013). About 72% of fetuses with trisomy 21 have NT thickness above the 95th percentile, compared to 4% of the euploid fetuses (Snijders et al 1998).

2.3.2 Etiology

The physiological background of the accumulation of extra fluid at the back of the fetal neck is not known. There are several hypotheses about the pathogenesis of this phenomenon and given the phenotypic heterogeneity of conditions associated with increased NT, a single cause is unlikely.

Possible etiologies include the following:

1. Lymph overproduction, failure or delayed maturation of the lymphatic system (Gittenberger-De Groot et al. 2004; Haak et al. 2002; van der Putte & van Limborgh 1980) which has been demonstrated among fetuses with Turner syndrome (von Kaisenberg, Nicolaides & Brand-Saberi 1999), other chromosomal abnormalities (Miyabara et al. 1989), and fetuses with altered movement (Hyett et al. 1997b).
2. Hemodynamic readjustment during cardiac development and cardiac dysfunction. Doppler studies of trisomic fetuses with increased NT have shown an abnormal flow in the ductus venosus and tricuspid regurgitation (Matias et al. 1999; Montenegro et al. 1997; Mula et al. 2015). Molecular studies of such fetuses' cardiac tissue have shown increased levels of atrial and B-type natriuretic peptide mRNA (Hyett et al. 1996b). In pathological examination of chromosomally normal fetuses with increased NT, abnormalities of the heart and/or great arteries were found in 19/21 of cases. The most common finding was narrowing of the aortic isthmus (Hyett et al. 1996a). These findings support the theory of cardiac dysfunction. However, in other studies of cardiac function

no difference between fetuses with increased and normal NT could be noticed (Haak et al. 2005; Huggon et al. 2004; Simpson & Sharland 2000). In one study signs of diastolic cardiac dysfunction were detected in fetuses with trisomy 21, but it could not be verified in those with isolated increased NT; this finding suggests that there are different etiologies causing the increased NT in different conditions (Mula et al. 2015).

3. Alteration in the composition of the extracellular matrix (von Kaisenberg et al. 1998a; von Kaisenberg et al. 1998b). CVS samples of euploid fetuses with increased NT showed underexpression of several genes including genes of embryonic development, extracellular matrix, vessel formation and differentiation (Farina et al. 2006).
4. Pressure and venous congestion in diaphragmatic hernia and skeletal dysplasias with narrowing of the chest (Sebire et al. 1997b; Spaggiari, Stirnemann & Ville 2012).
5. Accelerated growth of the fetus and delayed maturation of the cardiac system, which have been thought to be related to the thicker NT in normal male fetuses compared to the females (Prefumo, Venturini & de Biasio 2003; Timmerman, Pajkrt & Bilardo 2009).
6. Fetal anemia or hypoproteinemia (Lam et al. 1999).

Screening for perinatal infections is not warranted in pregnancies with increased NT (Sebire et al. 1997a).

2.3.3 Measurement

2.3.3.1 *Correct technique*

An accurate NT image acquisition and appropriate training of professionals involved in the first-trimester screening are essential for a good screening performance. NT is measured in the mid-sagittal section. The image is zoomed until the fetal head and upper trunk occupy at least 75% of the screen. Fetal skin and amnion are distinguished from each other by fetal movements (Nicolaidis 2004). The measurement is preferred at the fetal head in flexed position (Whitlow, Chatzipapas & Economides 1998), although the position does not make a big difference in the value (de Graaf et al. 2000). The calipers are placed correctly “on-

to-on” instead of “on-to-out” (Figure 4) (Herman et al. 2000). More than one measurement are taken and the largest is used in the risk assessment (Nicolaides et al. 1992).

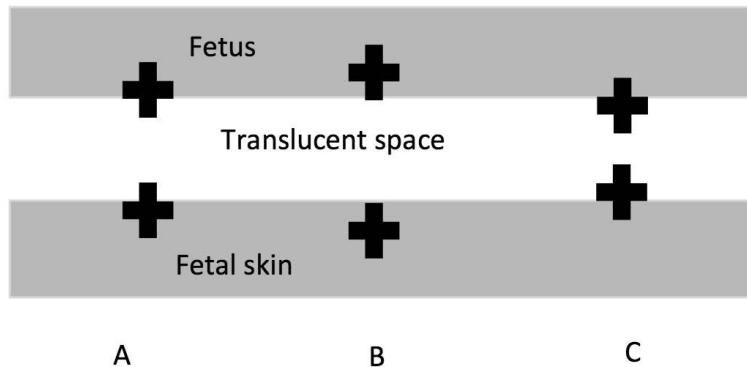


Figure 4. Correct placement of the calipers “on-to-on” (A), incorrect placement (B, C).

2.3.3.2 Timing

The NT-measurement should be performed at 10 to 14 weeks of gestation (fetal crown-rump length from 45 mm to 84 mm); before and after this time point the measurement fails more often. The recommended measurement time is at 12-13 weeks of gestation. At earlier gestation the fetal anatomical survey is not as accurate (6% success of anatomical visualization at 10 weeks vs. 96-98% at 12-13 weeks). Beyond 14 weeks the NT measurement becomes less accurate (98% success at 13 weeks vs 90% at 14 weeks). (Whitlow & Economides 1998; Mulvey et al. 2002).

2.3.3.3 Operator variability

Intra-observer variability in the first-trimester NT assessment has been shown to be < 0.70 mm and inter-observer variability < 0.88 mm in 95% of cases, and repeatability was not related to the NT thickness (Pajkrt et al. 2000; Pandya et al. 1995a). The caliper placement repeatability was < 0.58 mm in 95% of the cases and it was concluded that the intra- and interobserver variability could largely be caused by the variability in the caliper placement (Pandya et al. 1995a).

2.3.3.4 Education of sonographers

Any test is only as good as the persons performing the measurements, and therefore formal and structured education should be provided to screeners, as it gives the basis for a functional and reliable NT measurement. It has been demonstrated that the intra- and interobserver variability is higher among unexperienced sonographers compared to experienced professionals (Pajkrt et al. 2000). One study showed that the ability to obtain a good NT-measurement improved by experience of more than 400 NT measurements (Wald et al. 2003), and another demonstrated that good quality was reached after 80 - 100 measurements (Braithwaite, Morris & Economides 1996). Auditing the sonographers with personal feedback is compulsory in the quality control of NT measurements (Herman et al. 1998; Snijders et al. 2002).

2.3.3.5 Equipment

Obtaining satisfactory images is possible only with good-quality US equipment. The effect of the machine quality is independent of the experience of the screener (Wald et al. 2003). Both transabdominal and transvaginal probes are used in NT measurement. Transvaginal route is used whenever transabdominal screening does not yield satisfactory image quality (e.g. in obese patients). The need for transvaginal route decreases by increasing gestational age (100% at 10 weeks vs 11% at 14 weeks) (Whitlow & Economides 1998; Mulvey et al. 2002).

A study exploring different machine-probe combinations showed that the variability of different combinations was greater than the intraobserver variability, but no difference was found between the transabdominal and transvaginal probes (Axell et al. 2014). The study was performed using a phantom, which does not take maternal characteristics (obesity, tissue density, uterine tilt) into account. First-trimester anatomic survey was showed best performance using the combination of transabdominal and transvaginal probes (Rossi & Prefumo 2013).

2.3.3.6 Safety

Diagnostic US is a form of energy that has possible effects on tissues. The acoustic waves are transformed to thermal and mechanical energy (heating, cavitation, and radiation pressure). Cavitation is not an issue in obstetric US since the fetus does not contain any gas, which is needed for cavitation to occur. Thermal Index (TI) and mechanical index (MI) are

used to quantify the output of medical US systems. (Bigelow et al. 2011). Hyperthermia is considered teratogenic (Graham, Edwards & Edwards 1998) and thus tissue heating due to US examination and its possible effects in the fetus need to be discussed. The thermal index (TI) represents the reasonable worst-case estimate of the temperature rise resulting from exposure to US examination. The higher the TI, the higher the potential temperature rise. TIBone (Tib) should be used after 10 weeks of gestation. $Tib < 0.5$ can be used for extended time, Tib of 0.5 - 1.0 for 30 minutes, and $Tib \geq 2.5$ should be used only for less than one minute (Nelson et al. 2009). The Tib levels in US examination for NT-measurement without Doppler US were very low (0.2) (Sheiner & Abramowicz 2009). The TI is higher in Doppler US compared to B-mode sonography (Sheiner E & Abramowicz JS 2012). The “As Low as Reasonably Achievable” ALARA principle should be followed in all US examinations during pregnancy; this means that the total duration of scanning is minimized at the lowest possible acoustic output (American College of Obstetricians and Gynecologists 2009).

2.3.3.7 Expressing nuchal translucency

Fetal NT can be expressed in millimeters, but since the normal NT increases by increasing gestational age, it does not reflect the deviation from the normal median for a given crown-rump length measurement. There are two ways to quantify the deviation of the measured NT from the normal median NT: delta NT and NT Multiples of Median (MoM) (Biagiotti et al. 1997). Delta NT is calculated by subtracting the normal median NT for the CRL-value from the measured value and expressing the deviation in millimeters. NT MoM is calculated by dividing the measured NT by the normal median NT for the CRL-value (Schuchter et al. 1998). NT MoM 1.6 and delta NT 1 mm are approximately the same as the 95th centile NT value and NT MoM 2.0 and delta NT 1.5 mm are approximately the same as the 99th centile NT value (Maymon et al. 2004). The most recent modification is a mixture model for expressing fetal NT. The study that presented this model demonstrated that the NT of euploid fetuses increases by increasing gestational age in 95% of cases but only in 5% of fetuses with trisomy 21. The mixture model takes these gestation-dependent and gestation-independent distributions into account. (Wright et al. 2008).

2.3.4 Abnormal findings in fetuses with increased nuchal translucency

2.3.4.1 Chromosomal abnormalities

Increased NT is associated with chromosomal abnormalities, mostly trisomy 21. The sensitivity of NT-screening with 95th centile cut-off reaches 75% with a false-positive rate of 5%, and the prevalence of chromosomal abnormalities increases by increasing NT thickness (Kagan et al. 2006; Pandya et al. 1995; Snijders et al. 1998). The percentage of chromosomal abnormalities in fetuses with NT \geq 95th percentile – 3.5mm is 7% (Kagan et al. 2006) and in fetuses with NT \geq 6.5mm 65-70% (Kagan et al. 2006; Snijders et al. 1998). In general, approximately 20% of fetuses with NT \geq 95th percentile have an abnormal karyotype (Kagan et al. 2006; Mangione et al. 2001; Pandya et al. 1995), and half of the aneuploid fetuses with increased NT have trisomy 21. Other common chromosomal abnormalities among fetuses with increased NT are trisomies 13 and 18 and sex-chromosome abnormalities 45,X (Turner syndrome) and 47,XXY (Klinefelter syndrome), and triploidy (Kagan et al. 2006; Pandya et al. 1995). The distribution of NT is different for each type of abnormal karyotype: fetuses with trisomy 21 usually have NT < 4.5mm, whereas the majority of fetuses with trisomy 13 and 18 have NT 4.5 – 8.4 mm, and fetuses with Turner syndrome typically have very thick NT, \geq 8.5mm (Malone et al. 2005; Kagan et al. 2006; Wright et al. 2008).

2.3.4.2 Structural defects

Structural defects among euploid fetuses with increased NT have been verified in approximately 4% at birth in large studies (Pandya et al. 1995; Souka et al. 2001). However, the prevalence of such anomalies during pregnancy is higher, approximately 7 - 13% due to TOPs, MCs, and IUFDs (Bilardo et al. 1998; Souka et al. 2001; van Vugt, Tinnemans & van Zalen-Sprock 1998). The incidence of structural defects increases by increasing NT (Baer et al. 2014; Bilardo et al. 2007; Souka et al. 1998; Souka et al. 2001). CHDs comprise the majority of the structural defects associated with increased NT, followed by diaphragmatic hernia, pulmonary, genitourinary, musculoskeletal, and mid-line defects (e.g. exomphalos, cleft lip and palatum) (Baer et al. 2014; Pandya et al. 1995; Sebire et al. 1997b; Souka et al. 1998 & 2001; Timmerman et al. 2010). The detection rates of different structural defects at the time of first-trimester screening varies from 100% (acrania, anencephaly, ectopia cordis, encephalocele) to 0% (e.g. corpus callosum agenesis, bladder

exstrophy, cerebellar hypoplasia, duodenal atresia) and the detection rates of many structural defects fall in between these figures (e.g. CHDs, omphalocele, limb reduction, spina bifida, athrogyposis). In general, approximately half of major structural defects can be detected at the time of first-trimester US screening (Rossi & Prefumo 2013).

2.3.4.3 Congenital heart defects

Fetuses with increased NT have a higher prevalence of CHDs than the general population and the prevalence of CHDs increases by increasing NT (Bahado-Singh et al. 2005; Bilardo et al. 1998; Clur et al. 2008; Galindo et al. 2003; Ghi et al. 2001; Hafner E et al. 2003; Hyett et al. 1997a, Hyett et al. 1999; Makrydimas, Sotiriadis & Ioannidis 2003; Mavrides et al. 2001; McAuliffe et al. 2004; Michailidis & Economides 2001; Orvos et al. 2002; Zosmer et al. 1999). The prevalence of CHDs in the general population is 3.7 - 6/1000 (Ferencz et al. 1985; Hoffman & Kaplan 2002). Among fetuses with increased NT ≥ 3.5 mm, the prevalence of CHDs varies from 23.3/1000 (Bahado-Singh et al. 2005) to 107/1000 (McAuliffe et al. 2004) in different studies. All types of CHDs are detected after increased NT (Atzei et al. 2005; Clur et al. 2008; Simpson & Sharland 2000). These observations rose high expectations in using increased NT as a screening tool for CHDs. Unfortunately, NT is only moderately effective as a screening tool for CHDs, the detection rates ranging from 4.2 - 15% (Jouannic et al. 2011; Mavrides et al. 2001; Muller et al. 2007) to 40 - 50% (Hyett et al. 1999; Orvos et al. 2002; Sananes et al. 2010) for the cut-off of 3.5 mm. Pooled data showed 37.5% detection rate of CHDs with a false-positive rate of 4.9% with a cut-off of 95th percentile/2.5mm (Hyett 2004). In a meta-analysis focusing on those CHDs which would benefit from prenatal screening the estimated detection rate was 52% with a 5% false-positive rate using a cut-off of 1.7 MoM. (95% CI 42-71) (Wald et al. 2008). A recent review concluded a sensitivity value of 44% and a specificity of 94% with 95th percentile cut-off and sensitivity of 20% and specificity of 99% with 99th percentile cut-off using increased NT as a screening method for major CHDs (Sotiriadis et al. 2013). Increased NT in combination with abnormal ductus venosus flow and tricuspid regurgitation is associated with CHDs even in euploid fetuses (Clur, Ottenkamp & Bilardo 2009). In experienced centres, an early fetal echocardiography at < 16 weeks of gestation is 78.5% sensitive and there is a 74.5% concordance between the early and mid-trimester echocardiogram. However, an early diagnosis often needs to be confirmed later in the pregnancy and some

CHDs become evident later in gestation (e.g. ventricular septal defect, aortic coarctation) (Clur & Bilardo 2014; Huggon et al. 2002; Lopes et al. 2003).

2.3.4.4 Genetic syndromes

Increased NT implies numerous genetic syndromes (Souka et al. 2005). Of these Noonan syndrome has the strongest association with increased NT (Pergament et al. 2011). Congenital adrenal hyperplasia, 22q11 deletion syndrome (DiGeorge syndrome), spinal muscular atrophy, Stickler syndrome, Jarcho-Levin syndrome, arthrogyrosis, fetal akinesia deformation sequence, body stalk anomaly, Zellweger syndrome, and Smith-Lemli-Opiz syndrome have been noticed together with increased NT (Hyett et al. 1997b; Souka et al. 1998 & 2005). Since the prevalence of these syndromes generally is very low, it is very difficult to prove the correlation with statistical methods.

2.3.4.5 Cystic hygroma

Cystic hygroma is defined as an enlarged NT space extending along the entire length of the fetus. Septations are clearly visible in cystic hygroma. The outcome of fetuses with cystic hygroma is adverse: 50 - 60% are aneuploid (Graesslin et al. 2007; Kharrat et al. 2006; Malone et al. 2005; Scholl et al. 2012) and of the euploid, up to 29-34% have major structural defects (Malone et al. 2005; Scholl et al. 2012). Normal pediatric outcome has been reported in only 15-17% (Kharrat et al. 2006; Malone et al. 2005; Scholl et al. 2012). Turner syndrome is found in 12% of fetuses having cystic hygroma (Scholl et al. 2012). CHDs are more common among fetuses with cystic hygroma compared to those with increased NT without it (Sananes et al. 2010).

2.3.5 Pregnancy outcomes

Favourable pregnancy outcome of euploid fetuses with increased NT decreases by increasing NT. The outcome is favourable in approximately 90% of the fetuses with NT \geq 95th percentile – 3.5mm (Bilardo et al. 2007) and 20-30% in fetuses with NT \geq 6.5mm (Bilardo et al. 2007; Souka et al. 2001). This can be explained by the increasing rate of MCs, IUFDS, and structural defects by increasing NT (Bilardo et al. 2007; Michailidis & Economides 2001, Souka et al. 2001). The pregnancy outcome of euploid fetuses with increased NT followed by normal second-trimester screening is favourable in 96-98%

(Bilardo et al. 2007; Souka et al. 2001). However, in cases of persisting nuchal edema at the time of second-trimester screening adverse outcome is observed in up to 18% of the euploid fetuses (Souka et al. 2001). Screening for maternal infection (Cytomegalovirus, Parvovirus, and Syphilis) is warranted in such cases (Sebire et al. 1997a).

2.3.6 Neurodevelopmental outcome

Concern has been expressed about the later neurodevelopmental outcome of fetuses with increased NT. Data interpretation about this subject is difficult for several reasons:

- There is no generally accepted definition for neurodevelopmental disorder.
- Malfunction of the brain causes neurodevelopmental disorders that manifest as problems of cognition, behavior, and communication at a later age. For this reason, a long follow-up time is required.
- Children are flexible and the learning processes are individual. A child having a neurodevelopmental problem at a younger age may not present such problems later.
- Diagnosis of a neurodevelopmental disorder usually requires repeated examinations and follow-up in special health care.
- Parental questionnaires may be unreliable as they may give biased information.

Several studies report on the neurodevelopmental outcome of euploid children after increased NT in the first-trimester screening (Bilardo et al. 2007; Brady et al. 1998; Hiippala et al. 2001; Miltoft et al. 2012; Mula et al. 2012; Schou et al. 2009; Senat et al. 2002; Senat et al. 2007) with significant heterogeneity among studies in the NT cut-off levels, follow-up times, and methodology. The rate of neurodevelopmental impairment has varied from 0% (Maymon et al. 1998) to 7.4% (Senat et al. 2002). According to the most recent reports, the neurodevelopmental outcome of euploid children with increased NT in the first-trimester screening is favourable at the age of two years (Miltoft et al. 2012; Mula et al. 2012). In the studies with control groups no differences in the neurodevelopmental outcomes between those with increased NT and those with normal NT could be detected (Brady et al. 1998; Miltoft et al. 2012; Senat et al. 2007).

2.3.7 The significance of fetal gender

The results of previous research about the impact of fetal gender in NT thickness are not conclusive. Several studies with large cohorts (n = 2637-12189) have shown that male fetuses with normal outcome have thicker NT than female fetuses (the female NT being 0.98 MoM of the male NT) (Lam et al. 2001; Larsen et al. 2002; Rode et al. 2011; Spencer et al. 2000b; Timmerman, Pajkrt & Bilardo 2009). However, one study with a smaller cohort (n = 1325) failed to show this difference (Yaron et al. 2001). In one study the NT thicknesses of fetuses with trisomy 21 did not differ by gender (Cowans et al. 2009), while another study with a smaller cohort reported on a similar gender difference among healthy fetuses (Spencer et al. 2000b). The reason for the difference between the genders is not known, but accelerated growth and later maturation of male fetuses, especially of the cardiac system (Clur et al. 2011; Prefumo, Venturini & de Biasio 2003) have been thought to affect the NT thickness. The difference of the NT thickness between the genders is small (0.05-0.1mm) and thus the clinical implication is limited, especially because of intraobserver variation and limitations in the US resolution have larger impact on the measurement. At 12 weeks the accuracy of fetal gender determination by US examination is 87% (Hsiao et al. 2008), and therefore the impact of the gender difference on parental counselling is very limited. The only study about the relationship of gender and increased NT is from the Netherlands from 2009 (Timmerman, Pajkrt & Bilardo 2009). A comparison of fetuses (n= 7072) with normal NT to those with increased NT (n=636) gave a better outcome for males compared to females in the group of increased NT (RR for adverse outcome 0.72 for male gender and even 0.47 for male gender when NT was $\geq 95^{\text{th}}$ percentile and $< 99^{\text{th}}$ percentile). A small degree of NT enlargement in a male fetus was taken to be a normal finding in their study.

3 AIMS OF THE STUDY

The principal goal of this study is to provide data for high-quality parental counselling when the NT is increased in the first-trimester screening. In order to achieve this goal, three different aspects are considered with specific aims:

1. To find out the pregnancy outcomes after increased fetal NT in the first-trimester screening (Study I).
2. To detect the long-term neurodevelopmental outcome and the long-term overall outcome of euploid children with increased NT in the first-trimester screening and a normal findings in the second-trimester screening US (Studies II and IV).
3. To estimate the impact of fetal gender on the pregnancy outcome and on the long-term outcomes of children born after increased first-trimester fetal NT (Study III).

4 MATERIALS AND METHODS

This is a retrospective cohort study performed in a single referral institution. It includes all singleton pregnancies with increased NT in the first-trimester screening referred to the DFM in HUH during the period between January 2002 and December 2007. DFM is a tertiary referral centre providing health care for approximately 1.5 million inhabitants of the area. Until 1.3.2004 the institutional cut-off level for increased NT in HUH was 3.0 mm, thereafter the institutional 95th percentile cut-off levels based on internal material were used (Table 2).

Table 2. Fetal crown-rump length (CRL) dependent 95th percentile cut-off levels of nuchal translucency (NT) thickness used at Helsinki University Hospital during 2004 – 2007.

CRL (mm)	NT ≥ mm	CRL (mm)	NT ≥ mm
34-36	1.7	51-54	2.3
37-39	1.8	55-58	2.4
40-41	1.9	59-63	2.5
42-44	2.0	64-69	2.6
45-47	2.1	70-80	2.7
48-50	2.2		

During the study period first-trimester screening based on the NT measurement without maternal biochemistry for trisomy 21 was offered to all pregnant women booked at the local antenatal clinics. In Finland more than 99% of pregnant women attend the national antenatal clinics where care and screening are free of charge. Scans were performed by trained and experienced midwives or physicians working at the maternity units. The highest NT value was used in the risk assessment as recommended (Nicolaidis, Heath & Cicero 2002). Fetuses with cystic hygroma and hydrops were included in the study, since the same diagnostic work-up procedures were offered in these cases.

After an abnormal screening result parents were offered counselling and an option of invasive karyotyping by CVS or AC at the DFM. Further counselling by a perinatologist

and a geneticist was offered after an abnormal karyotyping result or if structural defects were diagnosed in the US examination.

Second-trimester US screening for structural defects was offered to all ongoing pregnancies at 18 – 22 gestational weeks. If the karyotype was normal and the initial NT < 3.0 mm, the second US screening was offered within the normal screening protocol and it was performed by a trained midwife. If the initial NT was ≥ 3.0 mm, the second screening was performed by a perinatologist at the DFM. Further examination by a pediatric cardiologist was available whenever there was any suspicion of a CHD.

All liveborns were examined by a pediatrician before discharge from the delivery hospital; this is the normal policy for all children in Finland. If prenatal karyotyping was not performed, the karyotype was assumed to be normal, if the newborn showed no dysmorphic features and was apparently healthy. The follow-up time of the children was counted in full years, from the year of birth until the end of year 2012, or emigration abroad, or death in some cases.

MC was defined as a spontaneous fetal loss at < 22 + 0 gestational weeks and stillbirth as a spontaneous fetal loss later during the pregnancy. Stillbirth and neonatal death during the first week of life were registered as perinatal deaths according to the Finnish Birth Register guidelines.

The study materials of the four studies are presented in Table 3.

Table 3. Study materials, data sources, follow-up periods, and definition of adverse outcomes in Studies I – IV.

	Study I	Study II	Study III	Study IV
Included subjects	1063 fetuses with increased NT in the first-trimester screening	691 euploid children from pregnancies with increased NT in the first-trimester screening and normal findings in the second-trimester US screening	1011 fetuses with increased NT in the first-trimester screening and normal sex-chromosomes	736 children with increased fetal NT in the first-trimester screening, normal second-trimester US screening and apparently healthy at discharge from the delivery hospital
Data sources	Hospital databases, individual medical charts	Hospital databases, individual medical charts, national registers*	As in Study II	As in Study II
Follow-up period	To the end of pregnancy (adverse outcome) or until discharge from the delivery hospital	From birth until the end of the year 2012	To the end of pregnancy (adverse outcome) or until the end of the year 2012	As in Study II
Study parameters	NT thickness, gestational age at screening, maternal age, prenatal karyotyping result, pregnancy outcome, health condition of liveborn children	NT thickness, gestational age at screening, maternal age. Register data: ICD-10 diagnoses of groups F and G, disability allowances, cases of death and their causes	NT thickness, gestational age at screening, maternal age, pregnancy outcome, long-term outcome, gender	NT thickness, gestational age at screening, maternal age. Register data: all ICD-10 diagnoses and cases of death and their causes

Adverse outcome parameters	Miscarriage, termination of pregnancy, perinatal death, delivery of a child with structural defects or genetic syndromes	Abnormal neurodevelopment during the follow-up: ICD-10 code from group F or G and an allowance for the impairment from the Social Insurance Institution of Finland	Adverse outcome in study I or II	Structural defect or genetic disorder diagnosed after discharge from the delivery hospital
Research question	Pregnancy outcome after increased NT	Neurodevelopmental outcome of euploid children with increased fetal NT in the first-trimester and normal findings in the second-trimester US screening	Significance of fetal gender on pregnancy outcome and on the long-term outcome after increased NT	Long-term overall outcome after increased fetal NT in the first-trimester screening and normal findings in the second-trimester US screening

*for details about the registers, see Table 4.
ICD, International classification of diseases; NT, nuchal translucency; US, ultrasound

Table 4. Different national registers used in the Studies II-IV.

	Medical Birth Register	Register of Congenital Malformations	Hospital Discharge Register	Allowance Register	Causes of Death Statistic
Register keeper	National Institute for Health and Welfare (THL)	THL	THL	Social Insurance Institution of Finland (SIIF)	Statistics Finland
Data	Until seven days of age: gestational age at birth, birth weight, 5-minute Apgar-score, pH-value of the umbilical artery, ICD-10 diagnoses	Until one year of age: All congenital structural defects	ICD-10 diagnoses of all in- and out-patient visits to all special health care units	Disability allowances granted from SIIF (Three rates: basic, middle and high)	All cases of death and their causes

A register search until the end of the year 2012 was performed from three different independent national sources: National Institute for Health and Welfare (THL), the Social Insurance Institute of Finland, Insurance of Medical Unit (SIIF) and Causes of Death Statistics. The data collected from the registers is described in Table 4. All health care units in Finland are obligated to report certain information to THL, which keeps several registers. The statistics are reliable as only less than 0.1% of births are missing from the Medical Birth Register. The Register of Congenital Malformations actively collects information about the malformations from Medical Birth Register, Hospital Discharge Register, Causes of Death Statistics, hospitals, cytogenetic laboratories, and health care professionals.

Every disabled Finnish child is entitled to a disability allowance from the SIIF, if the disability is severe enough. The allowance must be applied for by a standard form filled in by the physician responsible for the treatment of the child. The allowance is granted if the SIIF's physician agrees with the referring physician. Three different rates of disability allowance are used: basic, middle, and high. The rate depends on the strain the child's impairment causes on the family. Basic allowance is payable for a child whose impairment needs weekly treatment or rehabilitation. Middle allowance is payable when the impairment causes a more severe strain on the family, and highest allowance when the treatment and rehabilitation causes an extreme, around the clock, need of care and strain on the family.

All cases of death are reported to the Causes of Death Statistics in Finland, and recorded by Statistics Finland. We received data about all cases of death in the population under study. A child was considered to have neurodevelopmental impairment if there was an International Classification of Diseases (ICD-10) diagnosis of the group F (mental and behavioural diseases) or G (diseases of the neurological system) and a disability allowance from the SIIF was granted. The neurodevelopmental impairment was considered severe, if the disability allowance rate was medium or high; it was considered mild, if the rate was basic. All ICD-10 diagnoses attained from the registers were checked from the individual medical charts by the author. The data received from the Medical Birth Register was used to exclude children with confounding factors considering the neurodevelopment in Study II: prematurity ($< 37^{+0}$ weeks), asphyxia (umbilical artery pH ≤ 7.05 or ICD-10 diagnosis of asphyxia), small for date (SFD) or large for date (LFD) children (birth weight under or over two standard deviations for gestational age), or prenatally missed CHD (transposition of great arteries) with postnatal low oxygenation.

Structural defects were classified as major or minor using the classification of the European surveillance of congenital anomalies (Eurocat 2013; Eurocat 2014). Children with health problems not likely to be related to the increased NT (asthma, allergy, malignancies, diabetes mellitus, epilepsy, migraine, juvenile polyarthritis) were counted together with the healthy ones. Children with a minimal structural defect (ankyloglossia, preauricular fistula not needing operation, subluxation of the hip not needing any treatment, naevus flammeus without any anomalies of the vascular system) were also classified as healthy.

The study was approved by HUH Ethical Committee (Dnro 421/E9/07, 10 January 2008 and 79/13/03/03/2013, 27 February 2013) and by THL (Dnro THL/1715/5.05.00/2012, 21 March 2013), SIIF (Kela 69/522/2012, 15 May 2013), and Statistics Finland (TK-53-1474-12, 7 January 2013).

4.1 Statistical analyses

Continuous variables were presented as mean (range, \pm standard deviation), or n (%) with odds ratio (OR) and 95% confidence interval (95% CI). Continuous variables were analysed by Mann Whitney U-test. The Chi-square test was used in exploring the relationship of categorical variables. The tests were carried out by using Predictive Analysis Software 19.0 for Windows (SPSS Inc, Chigaco, IL, USA). The level of statistical significance was defined as $p < 0.05$.

5 RESULTS

We were able to identify altogether 1110 pregnancies with increased NT during the study period. In 47 (4%) cases the pregnancy data were insufficient and these had to be excluded. Pregnancy outcome data was available for 1063 pregnancies and the long-term outcome data for 763 euploid children. The mean NT thickness in the first-trimester US screening was 3.7 mm (1.9 – 14.7, \pm 1.7), with mean gestational age 12⁺⁵ weeks (10 – 14, \pm 6 days) (Figure 5). The mean maternal age was 31 years (17 – 47, \pm 5.5).

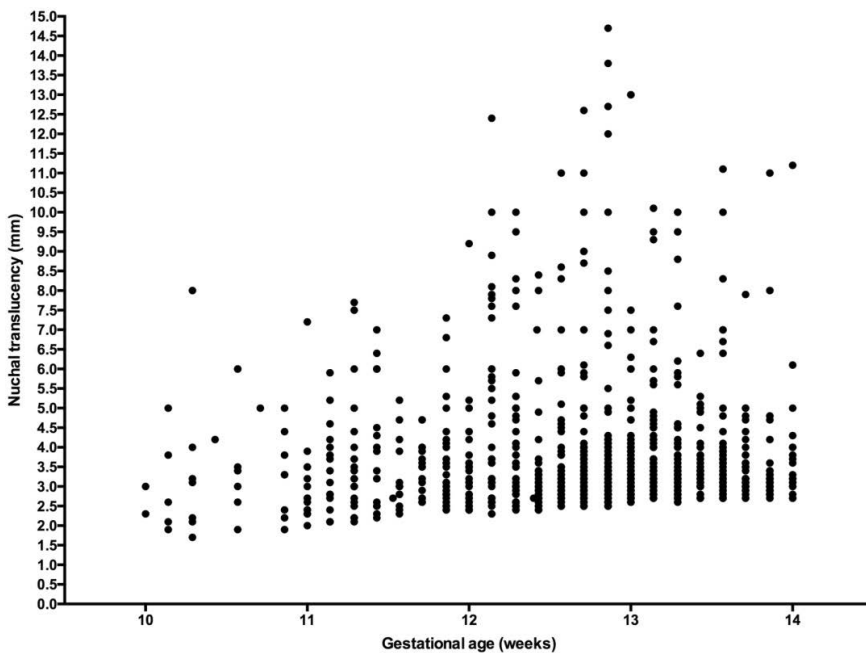


Figure 5. Nuchal translucency (NT) thickness (mm) and gestational age in a cohort of 1063 singleton pregnancies with increased NT in the first-trimester screening.

5.1 Pregnancy outcomes

Of the 1063 pregnancies, 209 (20%) ended in TOP, 36 (3%) in MC, four (0.4%) in stillbirth and three (0.3%) infants died in the early neonatal period. There were 811 (76%) children born alive and surviving the perinatal period. Of these, 740 (91%) were healthy at the time of discharge from the delivery hospital, whereas 71 (9%) had health problems; of these 44 had a normal karyotype. (Figure 6)

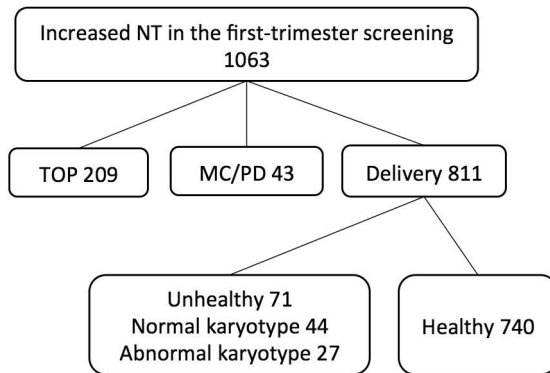


Figure 6. Pregnancy outcomes after increased nuchal translucency (NT). TOP, termination of pregnancy; MC, miscarriage; PD perinatal death.

Among the 209 pregnancies ending in TOP, 31 (15%) fetuses were euploid, 176 (84%) had chromosomal abnormalities, and the karyotyping failed in two (1%). A structural defect or syndrome was observed in 25/31 of the euploid fetuses, in six fetuses hydrops was the only abnormal finding after TOP in pathological examination. The cases with structural defects and syndromes are included in Tables 5-7. The mean maternal age in the TOP group was 34 years (17 – 47, ± 5.5), mean NT was 5.2 mm (2.1 – 13.8, ± 2.6), mean gestational age at the first-trimester US screening was 12⁺⁴ weeks (10 – 14, ± 6 days), and the mean gestation at the TOP was 15 weeks (11 – 22, ± 2.0). TOP was performed without prenatal karyotyping in 34 cases. In this group, 19 chromosomal abnormalities were detected, and in 13 the karyotype was normal and in two the karyotyping failed. There were severe structural defects in 11 of the euploid fetuses.

There were 771 euploid fetuses with normal findings in the second-trimester screening US: one was stillborn (no reason other than asphyxia was found in autopsy), three had an antenatal diagnosis after the second-trimester screening US (one of each: multiple

ventricular septal defects, multicystic kidney, and supraventricular tachycardia), and 27 had health problems before discharge from the delivery hospital (Tables 5-7, Figure 6). At the time of discharge from the delivery hospital 96% (740/771) of euploid fetuses with increased NT in the first-trimester screening and normal findings in the second-trimester US screening were healthy (Figure 7).

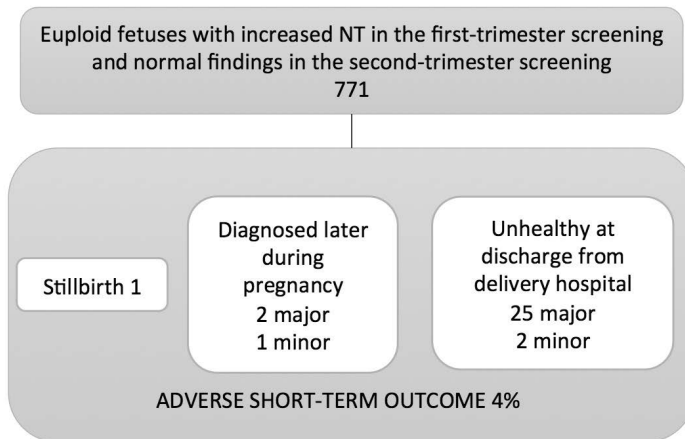


Figure 7. Adverse short-term outcomes of euploid fetuses with increased nuchal translucency (NT) in the first-trimester screening and normal findings in the second-trimester ultrasound screening.

5.2 Structural defects

Structural defects were detected in 87 of euploid fetuses or children: there were 34 CHDs (Table 5) and 53 other structural defects (Table 6). Major structural defects were detected in 7.2% (60/834) of the euploid fetuses. The rate of CHDs among the euploid fetuses was 4.1%. Of the 223 euploid fetuses with NT \geq 3.5 mm, 14 (6.3%) had a CHD.

5.3 Syndromes and genetic disorders

Syndromes or genetic disorders were diagnosed in 34 (4.1%) of the euploid fetuses (Table 7). Chromosomal microarray was not used in prenatal diagnosis at the time of the study. Later, during the follow-up of the children, chromosomal microarray was performed in a few cases with neurodevelopmental impairment and/or suspected genetic syndromes (Table 8). Targeted DNA testing revealed abnormal findings in five children with clinically suspected syndromes/diseases (Beckwith-Wiedemann syndrome, Mulibrey-Nanism, Noonan syndrome, Duchenne muscular dystrophy, CHARGE syndrome) after a normal conventional karyotype analysis.

5.4 Chromosomal abnormalities

There were 831 (78%) fetuses in the whole cohort of 1063 with normal karyotype, 227 (21%) had chromosomal abnormalities, of which three minor abnormalities were detected later during the follow-up when further diagnostic work-up was performed due to neurodevelopmental impairment and/or other health problems (118-p deletion mosaicism, microdeletion 1p36, microdeletion 12q21.31q22). The karyotype was unknown due to failed analysis after MC or TOP in five (1%) cases. CVS or AC was performed in 926 (87%) of patients. The percentage of fetuses with normal karyotype decreased by increasing NT: 93% among fetuses with NT 95th percentile – 2.9 mm had normal karyotype compared to 25% of fetuses with NT \geq 6.0 mm (Table 9). The mean NT for fetuses with normal karyotype was 3.3 mm (1.9 -12.4, \pm 1.0), for those with trisomy 21 4.7 mm (2.4 – 13.8, \pm 2.1), trisomy 18 5.3 mm (2.5-11.2, \pm 2.3), trisomy 13 5.2 mm (2.6 - 11.0, \pm 2.7), 45,X 7.8 mm (2.1 – 14.7, \pm 3.3), and for those with other chromosomal abnormalities 4.2 mm (2.4 – 10.1, \pm 1.8) (Figure 8).

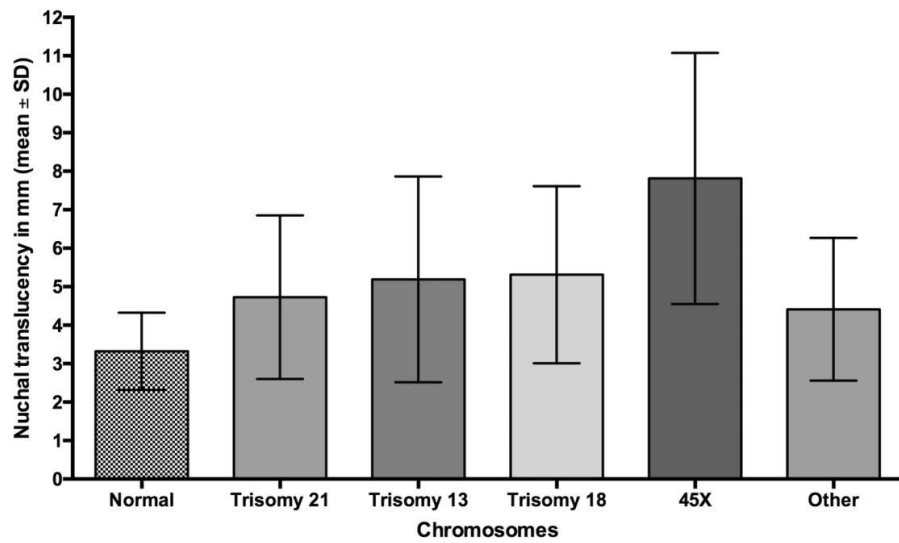


Figure 8. Nuchal translucency (NT) thicknesses in fetuses with increased NT in the first-trimester screening: chromosomally normal fetuses and fetuses with different chromosomal abnormalities.

Table 5. Congenital heart defects and outcome of 34 euploid fetuses with increased nuchal translucency (NT).

Defect	n	NT (mm)	Outcome
Defects diagnosed at second-trimester screening ultrasound or earlier			
HLHS	4	4.0 (mean)	TOP x 2, LB, surgery x 2 (f-u x 1, exitus at three months x 1)
TGA, pulmonary atresia, VSD, ASD secundum	1	3.2	LB surgery, f-u
Truncus arteriosus	2	6.0/4.7	MCx1, stillbirth x1
Aortic stenosis	1	3.1	LB surgery, f-u not available
VSD, ASD secundum	1	5.5	LB surgery, f-u
VSD	1	5.0	TOP
Minor tricuspid regurgitation	1	6.0	LB, f-u (mild tricuspid regurgitation persisting during f-u)
Defects diagnosed prenatally after second screening ultrasound			
Multiple VSD, ASD secundum, left vena cava superior	1	3.7	LB surgery, facio-auriculo-vertebral spectrum syndrome
WPW syndrome, intrauterine SVT	1	3.3	LB, medication, f-u
Defects diagnosed before discharge from the delivery hospital			
TOF, AVSD, hypoplastic right side	1	4.0	LB surgery, exitus at two months
TGA	1	2.7	LB, surgery, f-u. Neurodevelopmental impairment
AC, bicuspid aortic valve	1	2.5	LB surgery, f-u
TOF	1	2.7	LB, surgery, f-u
VSD	9	3.4 (mean)	LB x 9. Multibrey-Nanism x 1 and microdeletion (1)(p36) x 1
Minor PS	1	8.4	LB, clinical diagnosis of Noonan syndrome during f-u
Minor PS, WPW syndrome	1	2.4	LB, f-u
Defects diagnosed after discharge from the delivery hospital			
VSD	1	3.3	Dx 1 month, f-u
Minor AC	1	3.6	Dx 3 years, f-u

Bicuspid aortic valve, mild AS and mild aortic regurgitation	1	4.1	Age of dx not available, f-u
Patent ductus arteriosus	2	3.0/ 3.2	Dx 11 months and 4 years, catheterization x 2
ASD secundum	1	2.6	Dx 9 months, catheterization

AC, aortic coarctation; ASD, atrial septal defect; AVSD atrioventricular septal defect; Dx, diagnosis; f-u, follow-up; HLHS, hypoplastic left heart syndrome; LB, live born; MC, miscarriage; PS, pulmonary stenosis; SVT, supraventricular tachycardia; TGA, transposition of great arteries; TOF, Tetralogy of Fallot; TOP, termination of pregnancy; VSD, ventricular septal defect; WPW, Wolff-Parkinson-White.

Table 6. Structural defects, outcome, and severity of the defect according to Eurocat classification among euploid fetuses with increased nuchal translucency (NT).

Defect	n	NT (mm)	Outcome, time of diagnosis, treatment	Severity
Defects diagnosed at the second-trimester ultrasound screening or earlier				
Omphalocele	2	8.0/3.7	TOP/MC	Severe
Omphalocele, ectopia cordis	1	7.0	MC	Severe
Pentalogy of Cantrell	1	3.1	LB, operation	Severe
Thoracogastroschisis	1	5.0	TOP	Severe
Gastroschisis	1	4.7	TOP	Severe
Renal agenesis	1	3.2	TOP	Severe
Unilateral multicystic kidney	1	3.5	LB, f-u	Severe
Vesicoureteral reflux/hydronephrosis	1	2.8	LB, f-u	Severe
Hydrocephalus	1	3.1	TOP	Severe
Dandy-Walker variant	1	4.0	TOP	Severe
Spina bifida	1	2.8	TOP	Severe
Vesicorectal fistula, megacolon causing cardiac insufficiency due to pressure against thoracic cavity. Polysplenia	1	3.7	TOP	Severe
Diaphragmatic hernia	2	3.3/3.0	TOP, perinatal death	Severe
Defects diagnosed prenatally after second screening ultrasound				
Unilateral multicystic kidney	1	3.1	LB, operation, severe neurodevelopmental impairment	Severe

Defects diagnosed before discharge from the delivery hospital				
Diaphragmatic hernia	1	3.5	LB, operation, f-u	Severe
Sacral lipomeningocele	1	3.3	LB, operation	Severe
Cleft lip or palatum	3	3.0/3.3/4.0	LB, operation	Severe
Unilateral club foot	1	3.1	LB	Severe
Double thumb	1	3.0	LB	Severe
Bilateral popliteal subluxation	1	3.0	LB, f-u**	Mild
Anterior anus	1	2.9	LB, f-u	Mild
Facial asymmetry, mimical asymmetry	1	2.7	LB, f-u, mild neurodevelopmental impairment	Mild
Defects diagnosed after discharge from the delivery hospital				
High lying left kidney	1	5.0	Dx at 2 years. Diagnostic laparoscopy*	Mild
Vesicoureteral reflux/hydronephrosis	3	2.8/3.4/3.4	Dx at 1 - 10 months, f-u	Mild 2 Severe 1
Pyloric stenosis	1	3.1	Dx at 1 month. Operation	Mild
Left-sided hemihypertrophy	1	5.0	Dx at 1 month, Lymph therapy**	Mild
Bilateral camptodactylyia of fingers V	1	3.4	Dx at 1 month, f-u	Mild
Preauricular fistula	1	3.2	Dx at 3 years	Mild
Stenosis of the lacrimal duct	4	3.1 (mean)	Dx at 1 month - 1 year. Dilatation of the lacrimal duct	Mild
Corporal hemangioma	1	3.1	Dx at 1 month. Laser treatment	Severe
Nevus flammeus	1	3.7	Dx at 1 month. Laser treatment	Mild
Inguinal or umbilical hernia	7	3.4 (mean)	Dx at 1 month - 5 years. Operation	Mild
Testicular retention	4	4.1 (mean)	Dx at 1-7 years. Operation	Mild
Persistent hyperplastic vitreus	1	3.2	Dx at 2 weeks. Operation	Severe
Spondylocostal dysostosis, scoliosis	1	3.4	Dx at 4 years. Operation**	Severe

*Kidney visible at chest x-ray and was mistakenly diagnosed as pneumonia several times and as diaphragmatic herniation before operation. ** No specific syndrome diagnosed in the work-up. Dx, diagnosis; f-u, follow-up; LB, live birth; MC, miscarriage; TOP, termination of pregnancy.

Table 7. Syndromes and genetic disorders and outcome of 34 euploid fetuses with increased nuchal translucency (NT).

Diagnosis	n	NT (mm)	Outcome/time of diagnosis
Diagnosis at the second-trimester ultrasound screening or earlier			
VACTERL-H	1	4.7	Termination of pregnancy
Arthrogryposis	2	7.5/3.3	Termination of pregnancy
Achondrogenesis	1	2.7	Termination of pregnancy
Bone dysplasia	1	7.0	Termination of pregnancy
Osteochondrodysplasia, pulmonary hypoplasia, cleft palatum	1	3.9	Perinatal death
Meckel-Gruber syndrome	1	2.6	Termination of pregnancy
Hydrolethalus & hydrolethalus variant	3	4.2/7.5/7.5	Termination of pregnancy
Polycystic kidney disease	1	3.3	Termination of pregnancy
Body stalk anomaly/amniotic band	4	4.0 (mean)	Termination of pregnancy x3, live birth x1, follow-up
Non specified syndrome: pulmonary stenosis, left kidney agenesis, coloboma of the eye	1	3.8	Live birth, follow-up
Diastrophic dysplasia	1	5.0	Live birth, follow-up
Neurofibromatosis type 1	1	2.7	Live birth, follow-up
Diagnosis before discharge from the delivery hospital			
CHARGE-syndrome	1	5.5	Live birth, severe neurodevelopmental impairment
Spherocytosis	1	3.2	Live birth, splenectomy
Diagnosis after discharge from the delivery hospital			
Beckwith-Wiedemann syndrome	1	3.3	Dx at 2 months, follow-up.
Noonan syndrome	3	4.0/6.4/8.4	1x minor pulmonary stenosis dx before discharge. Dx of syndrome at 1-4 years. 1x minor neurodevelopmental impairment.
118-p deletion mosaicism	1	3.6	Dx at 2 years, follow-up. neurodevelopment normal.
Microdeletion (1)(p36)	1	2.5	VSD before discharge, dx of deletion at 2 years, severe neurodevelopmental impairment.

Facio-auriculo-vertebral syndrome, multiple VSD, ASD sec.	1	3.0	Heart dx antenatally (After II-US), syndrome dx at 2 years. Operation (heart), follow-up (syndrome).
Mulibrey-Nanism	1	3.0	VSD dx before discharge, Mulibrey-Nanism dx at follow-up, exitus at 3 years.
Septo-Optic dysplasia	1	3.4	Dx at 3 months. Severe neurodevelopmental impairment.
Del12q21.31q22	1	2.9	Dx at 4 years. Minor neurodevelopmental impairment.
Familial hypercholesterolemia, Helsinki mutation	1	3.2	Dx at 1.5 years. Medication.
Polycystic kidney disease	1	2.9	Dx at 3 years. Follow-up.
Duchenne muscular dystrophy	1	3.6	Dx at 6 months. Severe neurodevelopmental impairment.
Non specified syndrome	1	4.0	Dx at 3 months. Severe neurodevelopmental impairment.

ASD, atrial septal defect; Dx, diagnosis; US, ultrasound; VSD, ventricular septal defect.

Table 8. Cases with chromosomal microarray taken postnatally because of a clinical suspicion of a syndrome.

Diagnosis	NT (mm)	Age at microarray	Test indication and result
Beckwith-Wiedemann syndrome	3.3	6 months	Clinical suspicion of Beckwith-Wiedemann syndrome at 3 months, microarray revealed hypermethylation in H19 gene.
1p36 monosomy	2.5	2 years	VSD at birth, SFD. Neurodevelopmental impairment and restricted postnatal growth. Deletion in chromosome 1.
Multibrey-Nanism	8	9 months	VSD at birth, SFD. Clinical Multibrey-Nanism suspicion, mutation in MUL-gene. Exitus at 3 years.
Noonan/cardio-facio-cutaneous syndrome	4	4 years	Neurodevelopmental impairment and clinical suspicion of Noonan syndrome. PTPN11 gene normal, mutation in SOS1 gene
CHARGE syndrome	5.5	8 months	Clinical suspicion of CHARGE syndrome after birth. Mutation in CHD7 gene.
Duchenne muscular dystrophy	3.6	6 months	Suspicion of a syndrome for not reaching developmental milestones. Mutation in DMD gene.
Deletion in chromosome 12	2.9	3 years	Mild neurodevelopmental impairment.
Noonan syndrome	6.4	4 years	Clinical diagnosis after birth. PTPN11 gene normal, no other genetic testing performed.
Facio-Auriculo-Vertebral spectrum syndrome	3.7	10 years	Clinical diagnosis during the first year of life. Same spectrum syndrome affecting little sister, but no diagnostic changes in the microarray analysis in the offspring or the parents.
Noonan syndrome suspicion	8.4	4 months and 2 years	Clinical suspicion of Noonan syndrome due to mild pulmonary stenosis, unilateral hydronephrosis, epicanthus, hypotelorism. DNA testing for PTPN11 at 4 months and a wide genetic testing at 2 years, both normal.

DNA, deoxyribonucleic acid; SFD, small for date; VSD, ventricular septal defect

Table 9. Chromosomal abnormalities of 1063 fetuses n (%) with increased nuchal translucency (NT) in different NT thickness groups.

NT (mm)	Total	Normal	Abnormal	Trisomy 21	Trisomy 18	Trisomy 13	45 X	Other*	Unknown
	1063	831 (78.5)	227 (21.5)	114 (11.0)	39 (3.5)	17 (1.5)	30 (2.5)	27 (2.5)	5 (0.5)
95 th ** - 2.9	329 (31)	303 (93)	24 (7)	13	2	2	2	5	2
3.0 – 3.9	482 (45)	411 (85)	70 (15)	43	12	4	1	10	1
4.0 – 4.9	112 (11)	70 (63)	41 (37)	20	7	6	3	5	1
5.0 – 5.9	49 (5)	24 (49)	24 (49)	12	4	1	3	4	1
≥ 6.0	91 (8)	23 (25)	68 (75)	26	14	4	21	3	0

*Triploidy (n=4), 47,XXY (n=3), 48,XXY,+21 (n=2), 46,XX,del(18)(p11.2) (n=2), 47,XXX (n=2), 46,XX,i(18)(q10) (n=2), unbalanced translocation (n=3), chromosomal mosaicism (n=3), 47,XYY (n=1), 47,XX,+i(12p) (n=1), ring chromosome 6 (n=1), 118-p deletion mosaicism (n=1), microdeletion (1)(p36) (n=1), del12q21.31q22 (n=1) ** 95th percentile

5.5 Long-term outcomes

From the 771 euploid children with normal findings in the second-trimester US screening, one was stillborn and seven were lost for follow-up. Complete data were available for 763 children. The mean follow-up time was 6.5 years (0.2 – 10, \pm 1.8).

5.5.1 Long-term neurodevelopmental outcome

When assessing the long-term neurodevelopmental outcome of euploid children with normal findings in the second-trimester US screening, children with neurodevelopmental confounding factors (asphyxia, SFD, LFD, prematurity, CHD with low oxygenation postnatally) were excluded. Of the 70 (9%) excluded cases, eight (11%) had genetic syndromes, minor chromosomal abnormalities, or neurodevelopmental disorders (Table 10). Two cases were excluded due to death not related to the increased NT at two months' age (sudden infant death syndrome and carbon monoxide poisoning). The mean follow-up time of the 691 children remaining in the cohort after exclusions was 6.5 years (4-10, \pm 0.6). Of these, 29 (4.2%) had neurodevelopmental impairment, and in 12 (1.7%) children it was severe (Table 11). The mean NT of children with severe neurodevelopmental impairment was 3.9 mm (2.8 - 8.0, \pm 1.5). This was higher than the mean NT of healthy children and those with only minor neurodevelopmental problems, 3.2 mm (1.7 - 8.4 \pm 0.7) (p = 0.03) (Figure 9). The NT thicknesses of healthy children compared to those having any neurodevelopmental problems did not differ (p = 0.6). The neurodevelopmental outcome among children having fetal NT < 3.5 mm and those with NT \geq 3.5 mm did not differ (p = 0.7 for all neurodevelopmental disorders and p = 0.2 for severe neurodevelopmental disorders).

Table 10. Children with confounding neurodevelopmental factors and increased fetal nuchal translucency (NT) ruled out from the analysis of long-term neurodevelopmental outcome.

NT (mm)	Reason for exclusion (gestation/birth weight/Apgar score)	Diagnosis Disability allowance	Age at diagnosis
3.3	LFD (36 ⁺⁶ /3520g/9)	Beckwith-Wiedemann syndrome Middle allowance	2 months
6.4	LFD (37 ⁺⁵ /4290g/10)	Noonan syndrome. No allowance	3 years
2.7	Prematurity (31 ⁺⁶ /1800g/9)	Mixed developmental disorders Middle allowance	4 years
3.6	Asphyxia (UA pH 7.00) (41 ⁺⁶ /3810g/8)	18-p deletion mosaicism. No allowance	1 year
2.5	SFD, asphyxia (UA pH 7.00) (37 ⁺⁰ /2220g/1)	Microdeletion 1p36, VSD Middle allowance	VSD at birth, microdeletion at 2 years
3.7	SFD, asphyxia (38 ⁺² /2015g/1)	Facio-auriculo-vertebral-spectrum syndrome, VSD multiplex, ASD secundum Basic allowance	VSD at birth, syndrome at 3 years
8.0	SFD (40 ⁺² /2255g/9)	Mulibrey-Nanism, exitus at 3 years No allowance	VSD at birth, M-N at 9 months
2.7	CHD, low oxygenation postnatally	TGA and expressive language disorder Basic allowance	TGA at birth, language disorder at 5 years

ASD, atrial septal defect; UA, umbilical artery; CHD, congenital heart defect; LFD large for date; SFD, small for date; M-N, Mulibrey-Nanism; TGA, transposition of great arteries; VSD, ventricular septal defect

Table 11. Diagnoses of children with neurodevelopmental (ND) impairment diagnosed during the 6.5 year (mean) follow-up time with increased fetal nuchal translucency (NT) in the first-trimester screening..

Diagnosis (ICD-10 coding)	NT (mm)	Time of diagnosis	Severity
F84.9 Pervasive developmental disorder, autistic features	3.1	3 years	Severe
F80.2 Respective language disorder			
F80.2 Respective language disorder	3.1	5 years	Severe
F70.1 Moderate mental retardation	3.4	3 months	Severe
Q04.4 Septo-optic dysplasia			
F80.1 Expressive language disorder	3.1	5 years	Severe
F90.0 Hyperkinetic disorder			
F72 Severe mental retardation	3.6	6 months	Severe
F80.1 Expressive language disorder			
G71.06 Duchenne muscular dystrophy			
F82 Developmental disorder of motor function	2.8	3 years	Severe
F80.2 Respective language disorder			
F84.0 Childhood autism	8.0	2 years	Severe
F79 Unspecified mental retardation	4.0	3 months	Severe
Q89.7 Unknown syndrome			
F70.0 Mild mental retardation	5.5	CHARGE at birth, ND 8 months	Severe
Q87.27 CHARGE syndrome			
F80.9 Developmental disorders of speech and language	3.2	3 years	Severe
F83 Mixed specific developmental disorders	3.5	4 years	Severe
F80.2 Respective language disorder	3.1	Kidney at 31 gestational weeks, ND 6 years	Severe
Q61.3 Polycystic kidney			
F81.8 Developmental disorders of scholastic skills	3.3	5 years	Mild
F80.1 Expressive language disorder, facial asymmetry	2.7	Face at birth, ND at 3 years	Mild
F83 Mixed specific developmental disorders	2.6	Not available	Mild
F83 Mixed specific developmental disorders	3.4	3 years	Mild
F83 Mixed specific developmental disorders	2.9	ND 2 months, deletion 4 years	Mild
Q99.8 Del12q21.31q22			
F83 Mixed specific developmental disorders	3.4	3 years	Mild
F92.8 Mixed disorders of conduct and emotions	3.0	Not available	Mild

F98.0 Nonorganic enuresis			
F82 Developmental disorder of motor function	3.9	5 years	Mild
F90.0 Disturbance of activity and attention	2.7	7 years	Mild
F80.9 Developmental disorder of speech and language	3.0	3 years	Mild
F80.1 Expressive language disorder	3.8	4 years	Mild
F90 Disturbance of activity and attention	2.8	6 years	Mild
F92 Mixed disorders of conduct and emotions			
F80.1 Expressive language disorder	2.7	3 years	Mild
F81.8 Developmental disorders of scholastic skills	4.0	3 years	Mild
Q87.14 Noonan syndrome			
F80.1 Expressive language disorder	3.1	2 years	Mild
F90.0 Disturbance of activity and attention			
F80.1 Expressive language disorder	3.2	2 years	Mild
F80.2 Respective language disorder	3.7	6 years	Mild
F82 Developmental disorder of motor function			
F93.89 Childhood emotional disorder			

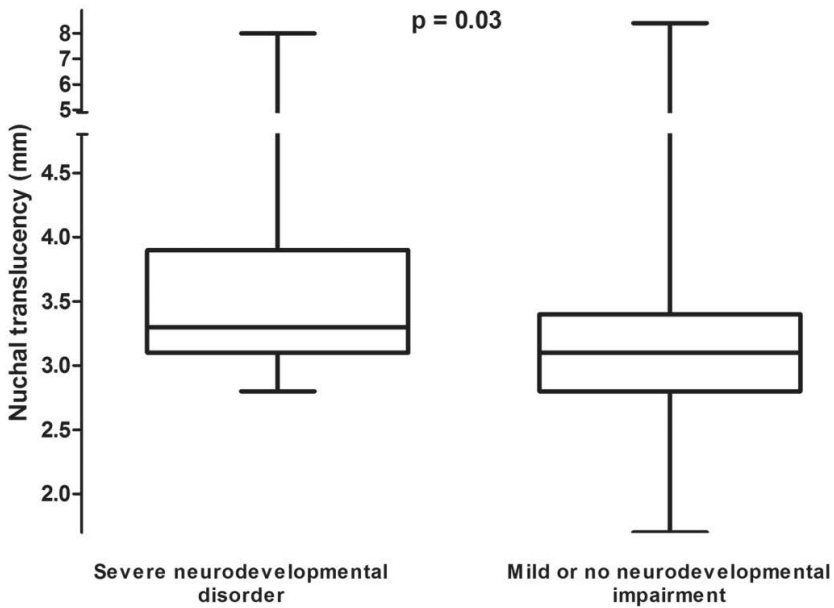


Figure 9. Nuchal translucency (NT) thickness of euploid children with increased fetal NT in the first-trimester screening: NT thickness of children with severe neurodevelopmental impairment and mild or no impairment.

5.5.2 Long-term overall outcome

Of the 771 euploid children with normal second-trimester US screening findings one was stillborn, 30 children were known to be unhealthy at the time of discharge from the delivery hospital, and seven were lost for follow-up. During the follow-up, additional structural defects, genetic disorders and syndromes, or neurodevelopmental impairment were diagnosed in 64 cases (see Tables 5 - 7, 10, 11). There were 14 children with a diagnosis of more than one category. The outcomes of euploid fetuses with normal second-trimester US screening result are presented in Figure 10.

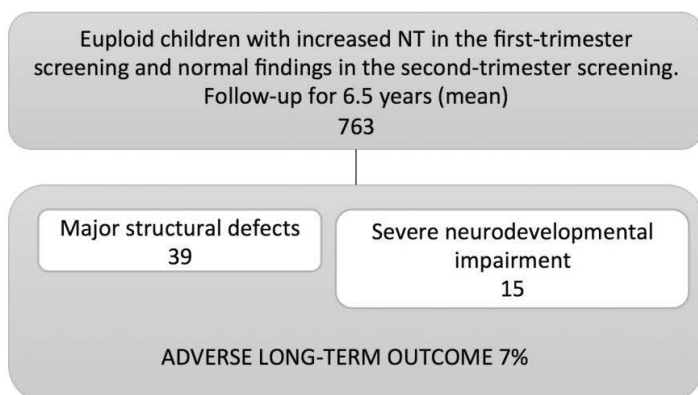


Figure 10. Long-term over-all outcome of euploid children with increased fetal nuchal translucency (NT) in the first-trimester screening and normal findings in the second-trimester ultrasound screening.

The percentage of favourable outcome after increased fetal NT and normal findings in the second-trimester US screening was 88%. When only major health impairment (major structural defects, genetic syndromes, and severe neurodevelopmental impairment) were considered, 54 (7%) of children were unhealthy and the long-term favourable outcome for euploid children with increased fetal NT and normal second-trimester US screening was 93%. The favourable outcome was 92-95% in different NT thickness groups up to 5.9 mm (Table 12). The NT thickness, mean 3.5 mm (2.4 - 8.4 ± 1.3), of the children with major health impairment did not differ from the healthy group and those with minor impairment, mean 3.2 mm (1.7 - 8.3 ± 0.7) ($p = 0.09$). Favourable outcome became less likely by increasing NT, the difference became significant at NT thickness ≥ 4.8 mm ($p = 0.048$, OR 3.1, 95% CI; 1.2–8.8).

Table 12. The long-term outcomes during the follow-up period of 6.5 years (mean) of euploid children with increased nuchal translucency (NT) thickness in the first-trimester screening and normal second-trimester ultrasound screening findings.

NT	n (%)	Favourable n (%)	Adverse* n (%)
95th** – 2.9 mm	291 (38)	277 (95)	14 (5)
3.0 - 3.9 mm	384 (51)	354 (92)	30 (8)
4.0 – 4.9 mm	63 (8)	58 (92)	5 (8)
5.0 - 5.9 mm	17 (2)	16 (94)	1 (6)
≥ 6.0 mm	8 (1)	4 (50)	4 (50)
All	763	709 (93)	54 (7)

*major structural defects, major neurodevelopmental impairment, syndromes and genetic disorders. **95th percentile

5.6 The effect of fetal gender

There were 1011 fetuses with known pregnancy outcome and normal sex-chromosomes. The mean NT was 3.6 mm (1.7 - 13.8, \pm 1.4), the mean gestation at the time of the screening was 12⁺⁶ weeks (10⁺⁰ - 14⁺⁰, \pm 6 days). There were 600 males and 411 females: the male to female ratio was 1.46:1. This ratio decreased by increasing NT: it was 1.6:1 when the NT was \geq 95th percentile – 3.9 mm and 1:1 when the NT was \geq 4.0 mm. The pregnancy outcomes according to gender in this cohort are presented in Table 13. The pregnancy outcome of males was better compared to females ($p = 0.049$). There were more aneuploidies among the females (21%) than among the males (16%) ($p = 0.04$, OR 1.4, 95% CI 1.03 – 2.0). Among euploid fetuses there was no difference between the genders in the pregnancy outcome ($p = 0.6$). The long-term outcome between the genders was equal ($p = 0.9$) (Table 14).

Table 13. Pregnancy outcome by gender in fetuses with increased nuchal translucency in the first-trimester screening.

	n	Favourable outcome n (%)	Adverse outcome n (%)
All	1011	740 (73)	271 (27)
All males	600	453 (75)*	147 (25)
All females	411	287 (70)*	124 (30)
All euploids	829	740 (89)	89 (11)
Euploid males	505	453 (90)	52 (10)
Euploid females	324	287 (89)	37 (11)

* $p = 0.049$

Table 14. Long-term outcome by gender in euploid children with increased nuchal translucency in the first-trimester screening and normal findings in the second-trimester screening.

	n (%)	Favourable outcome n (%)	Adverse outcome n (%)*
All	763 (100)	709 (93)	54 (7)
Males	465 (61)	431 (93)	34 (7)
Females	298 (39)	278 (93)	20 (7)

*Major structural defects, major neurodevelopmental impairment, genetic disorders and syndromes.

6 DISCUSSION

The results of this study showed that after a diagnosis of increased NT in the first-trimester screening the proportion of favourable pregnancy outcome decreased by increasing NT. In euploid fetuses with increased NT in the first-trimester screening and normal findings in the second-trimester US screening the pregnancy outcome was favourable in 96% and the long-term outcome in 93%. During the follow-up of 6.5 years (mean), severe neurodevelopmental impairment was detected in 1.7% of children. Adverse outcome (major structural defects, severe neurodevelopmental impairment, or genetic disorders) were detected in 7% of euploid children with increased fetal NT in the first trimester and normal findings in the second-trimester US screening. The pregnancy outcome of male fetuses was better compared to females with increased NT. This was explained by the higher incidence of chromosomal abnormalities among the female fetuses. Among euploid fetuses no gender difference could be verified in the pregnancy outcome or in the long-term outcome.

6.1 Pregnancy outcomes

Studies considering the pregnancy outcomes and increased NT are summarized in Table 15. There is substantial heterogeneity among the studies. Different NT cut-offs are used, study cohorts are derived from general population or from high-risk populations, and many cohorts are fairly small and only five studies have more than 500 cases. The overall adverse pregnancy outcome among euploid fetuses varied from 6% (Souka 1998) to 41% (Dane et al. 2008). In the largest studies the percentage of adverse outcome was 6 – 23% (Bilardo et al. 2007; Mangione et al. 2001; Michailidis and Economides 2001; Pandya et al. 1995; Senat et al. 2007; Souka et al. 1998; Souka et al. 2001; Study I). The 11% rate of adverse pregnancy outcome in our study is in line with these previous studies and gives a solid basis for parental counselling.

There are only three studies reporting on the outcome of euploid fetuses after increased NT and normal second-trimester US screening. Favourable outcome was recorded in 96-98% of cases (Bilardo et al. 2007; Souka et al. 2001; Study I). However, in the first study (Souka et al. 2001), the follow-up time of the children was not reported, and in the second study the follow-up time was only two years on average (Bilardo et al. 2007). In our first study

the follow-up time was until the discharge from the delivery hospital (Study I) and thus it did not allow most of the neurodevelopmental disorders to be included. Our more recent study (Study IV) of the overall outcome took into account the neurodevelopmental impairment and later diagnosed structural defects and genetic disorders and showed that during 6.5 years of follow-up, favorable outcome occurred in 93%. This fact made us conclude that the high rates of favourable outcome after normal second-trimester US screening in earlier studies are slightly misleading.

6.2 Structural defects

The 7.2% rate of major structural defects detected among the euploid fetuses in this study is in line with the previous large studies reporting rates of 3 – 11% (Bilardo et al. 2007; Mangione et al. 2001; Michailidis & Economides 2001; Pandya et al. 1995; Souka et al. 1998; Souka et al. 2001). An exceptionally high rate (30%) of structural defects was reported in one study (Senat et al. 2002). The NT cut-off in this study was higher than in the others, 4 mm, which might explain the difference. In our study the mean NT was 3.6 mm and most of the fetuses (76%) had a NT \leq 4mm. Most of the major structural defects in our cohort (Study I, Study IV) were CHDs, which is in keeping with previous studies (Bilardo et al. 2007; Souka et al. 1998; Souka et al. 1998).

Table 15. Summary of studies about pregnancy outcome after increased fetal nuchal translucency (NT) in the first-trimester screening.

Study	N	NT	Chromosomal abnormalities	Structural defects or genetic disorders*	Adverse outcome*	Other
Souka et al. 1998	4116	≥ 95 th percentile	Only euploid	4%	6%	94% born alive, no information about the health of the liveborn. Maternal age NA
Souka et al. 2001	1320	≥ 3.5 mm	Only euploid	15%	23%	Maternal age NA
Study I	1063	≥ 95 th percentile	22%	9%	11%	Mean maternal age 31 years
Pandya et al. 1995	1015	≥ 3mm	19%	4%	8%	Median maternal age 35 years Only outcome 565 euploid pregnancies reported
Bilardo et al. 2007	675	≥ 95 th percentile	33%	13%	19%	Maternal age NA
Mangione et al. 2001	252	≥ 3mm	20%	11%	16%	Maternal age NA
Senat et al. 2007	248	≥ 99 th percentile	26%	8%	18%	Median maternal age 31 years
Michailidis & Economides 2001	235	≥ 95 th percentile	Only euploid	5%	7%	Maternal age NA
Senat et al. 2002	160	≥ 4 mm	44%	30%	35%	Maternal age NA
Brady et al. 1998	89	≥ 3.5 mm	Only euploid	10%	10%	Maternal age NA
Maymon et al. 2000	78	≥ 95 th percentile	41%	9%	9%	Mean maternal age 31 years
Bilardo et al. 1998	74	≥ 3mm	34%	23%	32%	Maternal age NA
Van Vugt et al. 1998	63	≥ 3mm	Only euploid	13%	21%	Median maternal age 33 years
Cha 'ban et al. 1996	54	≥ 3mm	48%	32%	39%	Maternal age NA
Adekunte et al. 1999	53	≥ 4 mm	28%	18%	32%	Maternal age NA
Taipale et al. 2004	29	≥ 3mm	34%	16%	16%	Maternal age NA
Dane et al. 2008	27	≥ 3mm	33%	28%	41%	Maternal age NA

*chromosomally normal. NA not available

6.3 Congenital heart defects

The prevalence of CHDs in our study among all euploid fetuses was 41/1000 and 63/1000 for those with NT \geq 3.5 mm. This is in line with the previously reported prevalences (Bahado-Singh et al. 2005; Bilardo et al. 1998; Clur et al. 2008; Galindo et al. 2003; Ghi et al. 2001; Hafner et al. 2003; Hyett et al. 1997a & 1999; Makrydimas, Sotiriadis & Ioannidis 2003; Mavrides et al. 2001; McAuliffe et al. 2004; Michailidis & Economides 2001; Orvos et al. 2002; Zosmer et al. 1999). The definition of major CHD varies among studies since, unfortunately, there is no universal definition for major CHD. Whereas several studies classify major CHDs as those warranting interventions or those likely to be lethal without operative treatment, we relied on the Eurocat classification in defining the severity of anomalies. According to its criteria almost all CHDs are classified as major (Eurocat 2013) and this leads to overestimation of clinically relevant CHDs. If only those CHDs where intervention (operation, catheterization) were included, the rates would be 22/1000 (18/831) and 36/1000 (8/223), for all euploid fetuses and for those with NT \geq 3.5 mm, respectively. The prevalence of major CHDs in previous studies for fetuses with NT \geq 3.5 mm has varied between 23.3/1000 (Bahado-Singh et al. 2005) and 106.7/1000 (McAuliffe et al. 2004). Our results are near the lower rates of previous publications and reflect that our cohort was derived from general screening, as in the study of Bahado-Singh et al., and not from a selection with a high background risk. Although using the Eurocat classification may lead to overestimation in CHDs, its advantages of replicability and clarity make it feasible and recommendable. All types of CHDs are related to increased NT and this was also shown in our study. Of the clinically relevant CHDs warranting interventions 67% (10/15) were detected at the time of the second-trimester US screening. There were 18 CHDs requiring intervention, but three were not amenable for detection at the time of second-trimester US (two cases with patent ductus arteriosus and one case with secondary atrioventricular septal defect). It is important to carefully examine the heart of a fetus with a history of increased NT and specialized echocardiography by pediatric cardiologist should be performed with a low threshold.

6.4 Syndromes and genetic disorders

A wide variety of syndromes and genetic disorders were diagnosed in our cohort, and the results were in agreement with previous studies (Bilardo et al. 2010; Souka et al. 2005). Antenatal diagnosis was reached in 17/18 of those syndromes and genetic disorders that could have been diagnosed before discharge from the delivery hospital; only one case of CHARGE multianomaly syndrome passed undetected. However, during follow-up, severe syndromes (Mulibrey-nanism, Septo-optic dysplasia, Duchenne muscular dystrophy among other less severe syndromes) were diagnosed. These children did not show any distinct symptoms at the time of discharge from the delivery hospital. Antenatal diagnosis of these syndromes might be possible in the future as knowledge of the etiology and the use of chromosomal microarray is growing. A link between increased NT and Noonan syndrome has been shown (Pergament et al. 2011). Prenatal testing poses some problems, since several mutations are associated with Noonan syndrome, but not all are known yet. Three cases of Noonan syndrome were clinically diagnosed in our cohort as well. The genetic testing did not reveal this syndrome in two of the three children, but in one case the genetic mutation was verified. The clinical outcome of Noonan syndrome varies greatly and antenatal genetic testing for this syndrome raises ethical questions, since it is not possible to predict the severity of the disease in an affected fetus.

6.5 Chromosomal abnormalities

Two large studies about chromosomal abnormalities among fetuses with increased NT included 11315 (Kagan et al. 2006) and 1015 fetuses (Pandya et al. 1995) with NT cut-off of $\geq 95^{\text{th}}$ percentile and ≥ 3 mm, respectively. In both studies 19% of fetuses had abnormal karyotype. Our results with 22% of fetuses having abnormal karyotype are in keeping with these studies, as are the percentages in different NT thickness groups and the distributions of different chromosomal abnormalities.

A smaller percentage of chromosomal abnormalities, 10%, was reported in a study with 4767 fetuses with NT $\geq 95^{\text{th}}$ percentile (Snijders et al. 1998), and higher percentages 33-34% have been reported in smaller studies from the Netherlands with 74 (Bilardo et al. 1998) and 675 fetuses (Bilardo et al. 2007). There is no obvious reason (mean maternal age 31) for the smaller rate in the study by Snijders et al. The higher rates in the studies by

Bilardo et al. may be due to the population selection: there was no general screening program in the Netherlands at the time of the studies and thus the participating women had increased background risks (e.g. were older, had family history, a permanent disease) for an adverse outcome.

The results in different NT thickness groups in our study are also very similar to those of Kagan et al. who reported an aneuploidy rate ranging from 7%, when the NT was 95th centile – 3.4mm, reaching up to 75%, when the NT was ≥ 6.5 mm. The distribution of different chromosomal abnormalities in our study is in agreement with the previous reports: of the fetuses with chromosomal abnormalities approximately 50% had trisomy 21, 25% trisomy 13 or 18, and 10% Turner syndrome (Kagan et al. 2006; Pandya et al. 1995). The mean NTs of different chromosomal abnormalities in the present study were similar to those reported previously (Wright et al. 2008).

The uptake of invasive procedures was 87% in our study. The karyotypes of those with no invasive testing were examined after TOP, MC, or after the birth in children with clinical suspicion of chromosomal abnormality. If the child was apparently healthy, the karyotype was assumed to be normal and not examined. Since the follow-up time in our study is long and the information is gathered from several different sources, the assumption of a normal karyotype is likely to be true. The motives for not opting for invasive procedures are simplified in two categories: those proceeding to TOP because severe structural defects or fetal hydrops and those continuing the pregnancy and not daring to take any procedure-related risks (Kobelka et al. 2009). After TOP or MC the chromosomal analysis failed in five cases due to culture failure. This possibility should be taken into account when counselling the parents since chromosomal abnormality as an explanation for the US finding might affect the prognosis of a subsequent pregnancy. Thus, even in cases where TOP is opted for due to an US abnormality, CVS or AC should be considered prior to TOP to avoid the situation where the karyotype remains unknown.

There are several studies about the advantage of using chromosomal microarray after a normal QF-PCR result excluding the most common aneuploidies when the NT is ≥ 3.5 mm. Some studies did not show any pathologic CNVs (Huang et al. 2014; Schou et al. 2009) whereas in other studies 1-13% of fetuses had pathologic CNVs when analyzed with aCGH (Leung et al. 2011; Lund et al. 2015; Scott et al. 2013). Among fetuses with structural defects, 6% had pathologic CNVs compared to 0.4% among fetuses with false-positive trisomy 21 screening result but no structural defects (Wapner et al. 2012). Two studies about aCGH after increased NT ≥ 3.5 mm and normal QF-PCR result showed CNVs in 5.7 -13%

of fetuses (Lund et al. 2014; Pan et al. 2016). Chromosomal microarray was not used in our clinic at the time of this study. Retrospectively, we recorded whether chromosomal microarray had been performed after a syndrome had been clinically diagnosed/suspected or in the etiological work-up of neurodevelopmental impairment. The use of chromosomal microarray in HUH Children's hospital in such circumstances was lower than expected, since in only five out of 12 cases with severe neurodevelopmental impairment chromosomal microarray had been performed. Of the children excluded from the neurodevelopmental long-term analysis due to confounding factors (SFD, LFD, asphyxia, prematurity) 7% had abnormal findings on microarray (Mulibrey-Nanism, Beckwith-Wiedemann syndrome, microdeletion (1)(p36), deletion-mosaicism, Noonan syndrome). These children comprise a high-risk group and this should be taken into account if additional problems occur during the pregnancy or childbirth after increased NT. More studies about the use of chromosomal microarray in fetuses with increased NT are needed, but according to the available data, chromosomal microarray should be offered whenever there is an antenatal diagnosis of a structural defect.

6.6 Long-term outcomes

Studies reporting the neurodevelopmental outcome after increased NT are summarized in Table 16. To our best knowledge, our study has the longest follow-up time and one of the largest cohorts. There is a great deal of heterogeneity among previous studies in the cohort size, methodology, NT cut-off, follow-up time, and also in the definition of neurodevelopmental impairment, which is not simple. Many studies are performed at least partly by parental questionnaire or interview (Bilardo et al. 2007; Brady et al. 1998; Maymon et al. 2000; Schou et al. 2009; van Vugt, Tinnemans & van Zalen-Sprock 1998), which might bias the results since parents are not objective in assessing their child's development. Most studies did not have a control group and those containing a control group show no difference between the groups in their neurodevelopment (Brady et al. 1998; Miltoft et al. 2012; Senat et al. 2007). The rates of neurodevelopmental impairment in these studies varied from 0% to 13%. In our study the rate of neurodevelopmental impairment was 4.2% and of severe neurodevelopmental impairment 1.7 %. The prevalence of emotional, developmental, or behavioural disorders lasting for more than 12 months and warranting treatment or counselling among American children of ages 0 – 17 years is 5%

(Blanchard, Gurka & Blackman). Approximately 2% of Finnish children under 17 years of age receive disability allowance for neurodevelopmental disorders (Kelan vammaisetuustilasto 2014b, 'Disability allowance statistics Finland'). According to this we conclude that the neurodevelopmental outcome of euploid children after increased fetal NT does not differ from the background population, although a long-term follow-up study with a large cohort and a control group would be desirable.

Our study is the only one that excludes pregnancies from the final analysis with confounding factors (preterm delivery, perinatal asphyxia, SFD and LFD) that may influence the neurodevelopmental outcome from the final analysis. The overall rate of neurodevelopmental problems, genetic syndromes and minor chromosomal abnormalities was 10% among these children.

Only few major additional structural defects and genetic disorders were detected after an apparently healthy child was discharged from the delivery hospital (study IV). The policy of all newborns to be examined by a pediatrician before discharge from the delivery hospital in Finland seems to be appropriate and justified in detecting major defects.

Table 16. Studies about neurodevelopmental outcome of chromosomally normal children with increased nuchal translucency (NT) in the first-trimester screening during pregnancy.

Study	N	NT	Follow-up time	Follow-up method	Control group	Neurodevelopmental impairment
Souka et al. 2001	980*	≥ 3.5 mm	NA	Health care databases and parental interview.**	No	0.4%
Study II	691*	≥ 95th percentile	48 – 120	National registers and hospital charts	No	4.2%, severe 1.2%
Bilardo et al. 2007	375*	≥ 95th percentile	6 - 60	Questionnaire/ telephone interview/ hospital charts	No	1.1%
Senat et al. 2007	160*	≥ 99th percentile	24	Repeated clinical examination and ASQ	Yes	1.2%***
Mula et al. 2012	108	≥ 99th percentile	24	Repeated clinical examination or ASQ	No	3.7%
Brady et al. 1998	89	≥ 3.5 mm	6 – 42	Clinical examination or telephone interview	Yes	1.1%***
Miltoft et al. 2012	80	≥ 3.5 mm	24	ASQ	Yes	1.3%***
Schou et al. 2009	80	≥ 3.5 mm	8 - 33	Telephone interview and hospital charts	No	4%
Senat et al. 2002	54	≥ 4 mm	39	Repeated clinical examination and a structured questionnaire	No	7.4%
Hippala et al. 2001	50	≥ 3 mm	27 – 85	Clinical examination	No	2%
Maymon et al. 2000	36	≥ 95th percentile	12 – 36	Parental telephone interview	No	0%
Van Vugt et al. 1998	34	≥ 3 mm	7 - 75	Questionnaires or health care databases	No	2.9%
Adekunle et al. 1999	23	≥ 4 mm	12 – 38	Health care databases	No	13%

NA not available *fetuses with normal second-trimester ultrasonography **no definition for developmental delay ***no difference compared to the control group ASQ ages and stages questionnaire

6.7 The effect of fetal gender

Generally male fetuses seem to have poorer pregnancy outcome than female fetuses (Di Renzo et al. 2007), but a Dutch study reporting the outcome of fetuses with increased NT yielded contrary results with male fetuses having better prognosis compared to females. Results of this study implied that male fetuses with only slightly increased NT (95th percentile – 3.4 mm) had twice better chances of uneventful pregnancy outcome compared to females. The authors speculated that a slight increase in male fetuses' NT might be a normal phenomenon due to the accelerated growth of the fetus or the slower maturation of males compared to females (Timmerman, Pajkrt & Bilardo 2009). The Dutch study comprised 581 fetuses whereas ours is based on much larger data of 1011 fetuses (Study III). Our results do not support their explanation. We observed that the pregnancy outcome of males was better compared to females, but this was explained by the higher incidence of chromosomal abnormalities among the females. We suggest that the NT measurement might be a better screening tool for chromosomal abnormalities in female fetuses compared to males, although it was not possible to prove this in our material since we do not have the data of false negative cases in our screening. Determination of fetal gender is possible by US, chromosome analysis or by analysing the cell-free fetal DNA in the mother's blood sample (NIPT). Ultrasonographic accuracy for gender determination improves by increasing fetal age. At 12 weeks gender determination accuracy was 87% and at 13 weeks it was 96% (Hsiao et al. 2008). NT measurement performance is best before 13 weeks. Given the accuracy of 87% or less for gender determination at this point of time, it is not rational to determine the fetal gender by ultrasonography at the same time as the NT is measured, as the gender determination at the time of first-trimester US screening is possible but not definitive (Colmant et al. 2013; Hsiao et al. 2008). Our observation that the gender of euploid fetuses does not affect the outcome is reassuring. Fetal gender does not need to be taken into account in counselling the parents, the only exception being suspicion of Turner syndrome whenever a fetus has an extremely large NT.

6.8 Clinical relevance of nuchal translucency at present and in future

The methods of prenatal screening are being developed with the introduction of new screening strategies and techniques leading to the improvement of the previously used strategies. Introduction of NIPT created high expectations because of its better accuracy compared to other screening methods for aneuploidies, but the high cost of this technique prevents its use in general screening. Even if the price of NIPT decreased to a financially affordable level, which would make it a preferable screening method in general use, it would only screen for the chromosomal abnormalities and the advantages of the first-trimester US examination (location, number, viability, anatomy of the fetus) would be missed. The information from the first-trimester US examination is of great clinical importance as it has several obvious advantages: high risk pregnancies (e.g. of twins) receive adequate follow-up, TOP can be offered when fetuses with extremely severe structural defects (e.g. anencephaly) are diagnosed. Thus it is not likely, that the first-trimester US examination will be abandoned, even if NIPT is adopted for trisomy screening. Evidence shows that CHDs are more likely to be detected in high-risk compared to low-risk population (Rasiah et al. 2006). Since increased NT is associated with structural defects, especially CHDs and genetic disorders, its measurement has an important role in the selection of pregnancies with high-risk to whom expert anomaly scan and the most precise (and expensive) genetic analysis (aCGH) are offered (Figure 11).

6.9 Management of pregnancies with increased nuchal translucency

NT measurement is an essential part of combined screening. There is evidence that waiting for the serum marker results in pregnancies with $NT \geq 3\text{mm}$ does not bring any additional benefit in aneuploidy screening (Comstock et al. 2006). However, in cases with a NT between $\geq 95^{\text{th}}$ percentile and 3 mm, the serum markers define the individual risk and the need for further diagnostic work-up.

Fetuses with increased NT comprise a high-risk group. Since NIPT is more expensive than combined screening and aCGH is more expensive than QF-PCR or conventional

karyotyping, each society, with the locally given resources, has to make their own recommendations in patient selection to these tests.

Our proposal of pregnancy management after increased NT is shown in Figure 11. This is the current policy at the DFM in HUH. After any abnormal result information about different available options should be offered to the parents. Multidisciplinary counselling, in utero treatment when possible, pregnancy follow-up, and timing and delivery in the third-level prenatal centre with high quality neonatal care should be the standard procedure. Continuous education should be offered to the screeners and special attention should be paid to the examination of the fetal heart as its screening is particularly challenging due to the complex structure.

6.10 Strengths

The major strength of our study is the large cohort with a long follow-up time of 6.5 years (mean). The results are reliable since special attention was paid to confirm the short- and long-term diagnoses by searching the individual medical charts in all cases. Every diagnosis retrieved from the registers was double checked from the hospital databases. Additionally, several confounding factors were taken into account in the neurodevelopmental assessment. In order to ensure valid results in Study III, fetuses with abnormal sex-chromosomes were excluded.

6.11 Limitations

A control group would have given further strength to our study, but it was not technically possible to gather such a group for several reasons. Since many of the syndromes and structural defects diagnosed among fetuses with increased NT are extremely rare, the control group should have been very large to reveal any real difference. At the time of this study from 2002 to 2007 screening results were not computerized and thus it would not have been feasible to collect an adequate-sized and scientifically valid control group large enough with normal NT thicknesses. Even though many national registers are available in Finland, there is no national fetal screening register, which would ease the research.

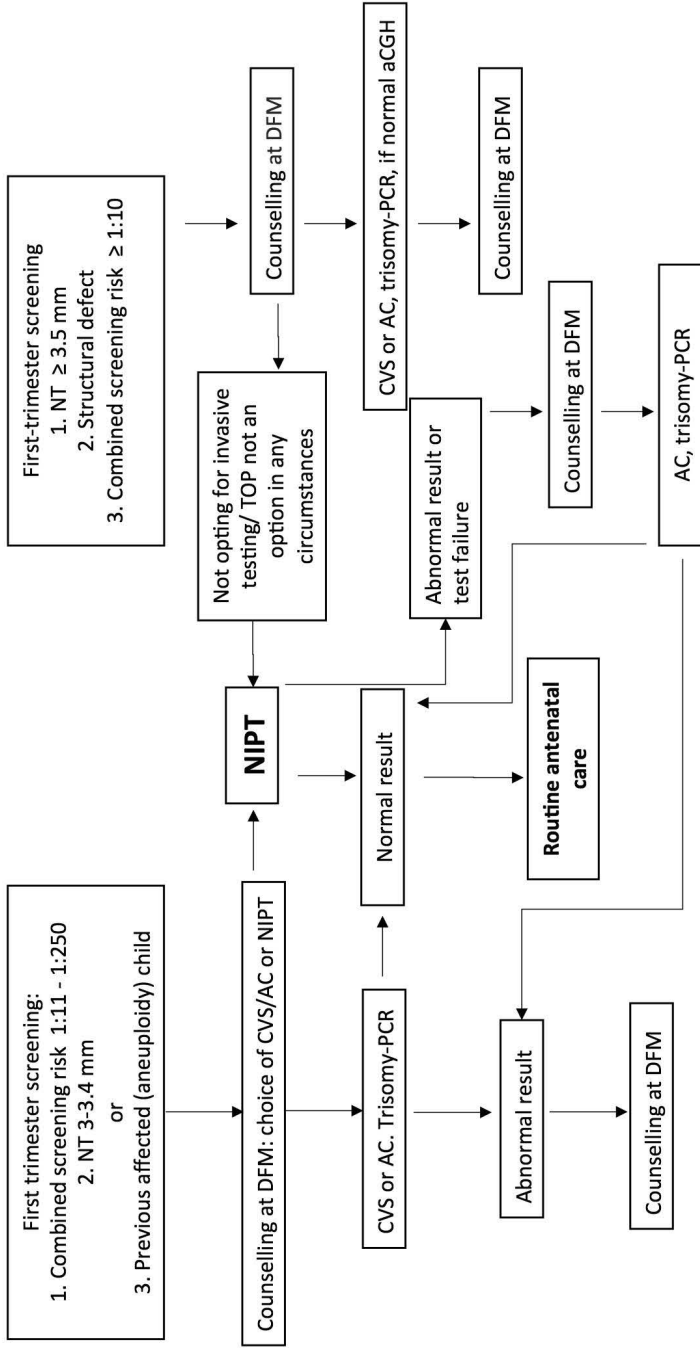


Figure 11. Proposal for management after increased nuchal translucency (NT) in the first-trimester screening and other high-risk pregnancies. AC, amniocentesis; aCGH array comparative genomic hybridization; CVS, chorionic villus sampling; NIPT non-invasive prenatal testing; PCR, quantitative fluorescence polymerase chain reaction.

7 CONCLUSIONS

Increased fetal NT is associated with chromosomal abnormalities, structural defects, genetic syndromes, and adverse pregnancy outcomes.

1. An adverse pregnancy outcome becomes more likely by increasing NT. One in five fetuses with increased NT in the first-trimester screening has chromosomal abnormalities, and structural defects or genetic syndromes are detected in one in ten of the euploid fetuses with increased NT.
2. The neurodevelopmental outcome of euploid children with increased fetal NT seems to be favourable. Severe neurodevelopmental impairment was detected in 1.2% of children with increased fetal NT with a follow-up time of 6.5 years. Major health impairment (major structural defects, severe neurodevelopmental impairment, or genetic syndromes) was detected in 7% of euploid children with increased fetal NT and normal findings in the second-trimester screening.
3. Male fetuses with increased NT have better pregnancy outcome than female fetuses, but there are more chromosomal abnormalities among the females. The pregnancy outcome or the long-term outcome of euploid fetuses do not differ by gender.

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A handwritten signature in black ink, appearing to read 'Outi Äyräs'. The signature is written in a cursive, flowing style.

Outi Äyräs

REFERENCES

- Adekunle O, Gopee A, el-Sayed M & Thilaganathan B 1999, "Increased first trimester nuchal translucency: pregnancy and infant outcomes after routine screening for Down's syndrome in an unselected antenatal population", *British Journal of Radiology*, vol. 72, no. 857, pp. 457-460.
- Agathokleous M, Chaveeva P, Poon LCY, Kosinski P & Nicolaides KH 2013, "Meta-analysis of second-trimester markers for trisomy 21", *Ultrasound in Obstetrics & Gynecology*, vol. 41, no. 3, pp. 247-261.
- Akolekar R, Beta J, Picciarelli G, Ogilvie C & D'Antonio F 2015, "Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis", *Ultrasound in Obstetrics & Gynecology*, vol. 45, no. 1, pp. 16-26.
- Alberti A, Salomon LJ, Le Lorc'h M, Couloux A, Bussieres L, Goupil S, Malan V, Pelletier E, Hyon C, Vialard F, Rozenberg P, Bouhanna P, Oury JF, Schmitz T, Romana S, Weissenbach J, Vekemans M & Ville Y 2015, "Non-invasive prenatal testing for trisomy 21 based on analysis of cell-free fetal DNA circulating in the maternal plasma", *Prenatal diagnosis*, vol. 35, no. 5, pp. 471-476.
- Alfirevic Z, Sundberg K & Brigham S 2003, "Amniocentesis and chorionic villus sampling for prenatal diagnosis", *Cochrane Database of Systematic Reviews*.
- Allison SJ, Stafford J & Anumba DO 2011, "The effect of stress and anxiety associated with maternal prenatal diagnosis on feto-maternal attachment", *BMC Women's Health*, vol. 11, pp. 33.
- American College of Obstetricians and Gynecologists 2009, "ACOG Practice Bulletin: Ultrasonography in Pregnancy", *Obstetrics & Gynecology*, vol. 113, no. 2, pp. 451-461.
- Anderson G 1999, "Nondirectiveness in prenatal genetics: patients read between the lines", *Nursing ethics*, vol. 6, no. 2, pp. 126-136.
- Antonarakis SE 1991, "Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. Down Syndrome Collaborative Group", *New England Journal of Medicine*, vol. 324, no. 13, pp. 872-876.
- Atzei A, Gajewska K, Huggon IC, Allan L & Nicolaides KH 2005, "Relationship between nuchal translucency thickness and prevalence of major cardiac defects in fetuses with normal karyotype", *Ultrasound in Obstetrics & Gynecology*, vol. 26, no. 2, pp. 154-157.
- Axell RG, Gillett A, Paupathe D, Chudleigh T, Brockelsby J, White PA & Lees CC 2014, "Accuracy of nuchal translucency measurement depends on equipment used and its calibration", *Ultrasound in Obstetrics & Gynecology*, vol. 44, no. 1, pp. 31-37.
- Baer RJ, Norton ME, Shaw GM, Flessel MC, Goldman S, Currier RJ & Jelliffe-Pawlowski LL 2014, "Risk of selected structural abnormalities in infants after increased nuchal translucency measurement", *American Journal of Obstetrics & Gynecology*, vol. 211, no. 6, pp. 675.e1-675.e19.
- Bahado-Singh RO, Wapner R, Thom E, Zachary J, Platt L, Mahoney MJ, Johnson A, Silver RK, Pergament E, Filkins K, Hogge WA, Wilson RD, Jackson LG & First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening Study Group 2005, "Elevated first-trimester nuchal translucency increases the risk of congenital heart defects", *American Journal of Obstetrics & Gynecology*, vol. 192, no. 5, pp. 1357-1361.
- Bangsgaard L & Tabor A 2013, "Do pregnant women and their partners make an informed choice about first trimester risk assessment for Down syndrome, and are they satisfied with the choice?", *Prenatal diagnosis*, vol. 33, no. 2, pp. 146-152.

Benacerraf BR, Gelman R & Frigoletto FD Jr 1987, "Sonographic identification of second-trimester fetuses with Down's syndrome", *The New England journal of medicine*, vol. 317, no. 22, pp. 1371-1376.

Biagiotti R, Periti E, Brizzi L, Vanzi E & Cariati E 1997, "Comparison between two methods of standardization for gestational age differences in fetal nuchal translucency measurement in first-trimester screening for trisomy 21", *Ultrasound in Obstetrics & Gynecology*, vol. 9, no. 4, pp. 248-252.

Bigelow TA, Church CC, Sandstrom K, Abbott JG, Ziskin MC, Edmonds PD, Herman B, Thomenius KE & Teo TJ 2011, "The thermal index: its strengths, weaknesses, and proposed improvements", *Journal of Ultrasound in Medicine*, vol 30, no. 5, pp. 714-734.

Bilardo CM, Pajkrt E, de Graaf I, Mol BW & Bleker OP 1998, "Outcome of fetuses with enlarged nuchal translucency and normal karyotype", *Ultrasound in Obstetrics & Gynecology*, vol. 11, no. 6, pp. 401-406.

Bilardo CM, Müller MA, Pajkrt E, Clur SA, van Zalen MM & Bijlsma EK 2007, "Increased nuchal translucency thickness and normal karyotype: time for parental reassurance", *Ultrasound in Obstetrics & Gynecology*, vol. 30, no. 1, pp. 11-18.

Bilardo CM, Timmerman E, Pajkrt E, & van Maarle M 2010, "Increased nuchal translucency in euploid fetuses-what should we be telling the parents?", *Prenatal Diagnosis*, vol. 30, no. 2, pp. 93-102.

Bindra R, Heath V, Liao A, Spencer K & Nicolaides KH 2002, "One-stop clinic for assessment of risk for trisomy 21 at 11-14 weeks: a prospective study of 15 030 pregnancies", *Ultrasound Obstetrics & Gynecology*, vol 20, no 3, pp. 219-225.

Blanchard LT, Gurka MJ & Blackman JA 2006, "Emotional, developmental, and behavioural health of American children and their families: a report from the 2003 National Survey of Children's Health", *Pediatrics*, no. 117, pp.1202-1212.

Brady AF, Pandya PP, Yuksel B, Greenough A, Patton MA & Nicolaides, KH 1998, "Outcome of chromosomally normal livebirths with increased fetal nuchal translucency at 10-14 weeks' gestation", *Journal of medical genetics*, vol. 35, no. 3, pp. 222-224.

Braithwaite JM, Kadir RA, Pepera TA, Morris RW, Thompson PJ & Economides DL 1996, "Nuchal translucency measurement: training of potential examiners", *Ultrasound in Obstetrics & Gynecology*, vol. 8, no. 3, pp. 192-195.

Braithwaite JM, Morris RW & Economides DL 1996, "Nuchal translucency measurements: frequency distribution and changes with gestation in a general population", *British Journal of Obstetrics & Gynaecology*, vol. 103, no. 12, pp. 1201-1204.

Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, Klugman S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Tripp T, Bianchi DW, D'Alton ME & First and Second Trimester Evaluation of Risk (FASTER) Research Consortium 2007, "First- and second-trimester screening: detection of aneuploidies other than Down syndrome", *Obstetrics & Gynecology*, vol. 110, no. 3, pp. 651-657.

Caine A, Maltby AE, Parkin CA, Waters JJ, Crolla JA & UK Association of Clinical Cytogeneticists (ACC) 2005, "Prenatal detection of Down's syndrome by rapid aneuploidy testing for chromosomes 13, 18, and 21 by FISH or PCR without a full karyotype: a cytogenetic risk assessment", *Lancet*, vol. 366, no. 9480, pp. 123-128.

Caplan AL 2015, "Chloe's Law: A Powerful Legislative Movement Challenging a Core Ethical Norm of Genetic Testing", *Plos Biology*, vol. 13, no. 8, pp. e1002219.

Caspersson T, Zech L, Johansson C & Modest EJ 1970, "Identification of human chromosomes by DNA-binding fluorescent agents", *Chromosoma*, vol. 30, no. 2, pp. 215-227.

Cha'ban FK, Van Splunder P, Los FJ & Wladimiroff JW 1996, "Fetal outcome in nuchal translucency with emphasis on normal fetal karyotype", *Prenatal diagnosis*, vol. 16, no. 6, pp. 537-541.

- Choi H, van Riper M & Thoyre S 2012, "Decision making following a prenatal diagnosis of Down syndrome: an integrative review", *Journal of midwifery & women's health*, vol. 57, no. 2, pp. 156-164.
- Chueh HY, Cheng PJ, Shaw SW, Lin CT, Hsu JJ & Hsieh TT 2007, "Maternal anxiety about first trimester nuchal translucency screening and impact of positive screening results", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 86, no. 12, pp. 1437-1441.
- Clur SA, Mathijssen IB, Pajkrt E, Cook A, Laurini RN, Ottenkamp J & Bilardo CM 2008, "Structural heart defects associated with an increased nuchal translucency: 9 years experience in a referral centre", *Prenatal diagnosis*, vol. 28, no. 4, pp. 347-354.
- Clur SA, Ottenkamp J & Bilardo CM 2009, "The nuchal translucency and the fetal heart: a literature review", *Prenatal diagnosis*, vol. 29, no. 8, pp. 739-748.
- Clur SA, Oude Rengerink K, Mol BW, Ottenkamp J & Bilardo CM 2011, "Is fetal cardiac function gender dependent?", *Prenatal diagnosis*, vol. 31, no. 6, pp. 536-542.
- Clur SA & Bilardo CM 2014, "Early detection of fetal cardiac abnormalities: how effective is it and how should we manage these patients?", *Prenatal Diagnosis*, vol. 34, no. 13, pp. 1235-1245.
- Cohn GM, Gould M, Miller RC, Habecker-Green J, Macri CJ & Gimovsky ML 1996, "The importance of genetic counseling before amniocentesis", *Journal of Perinatology*, vol. 16, no. 5, pp. 352-357.
- Colmant C, Morin-Surroca M, Fuchs F, Fernandez H & Senat MV 2013, "Non-invasive prenatal testing for fetal sex determination: is ultrasound still relevant?", *European Journal of Obstetrics, Gynecology, & Reproductive Biology*, vol. 171, no. 2, pp. 197-204.
- Committee on Ethics, American College of Obstetricians and Gynecologists & Committee on Genetics, American College of Obstetricians and Gynecologists 2008, "ACOG Committee Opinion No. 410: Ethical issues in genetic testing" *Obstetrics & Gynecology*, vol. 111, no. 6, pp. 1495-1502.
- Comstock CH, Malone FD, Ball RH, Nyberg DA, Saade GR, Berkowitz RL, Ferreira J, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Bianchi DW, D'Alton ME & FASTER Research Consortium 2006, "Is there a nuchal translucency millimeter measurement above which there is no added benefit from first trimester serum screening?", *American Journal of Obstetrics & Gynecology*, vol. 195, no. 3, pp. 843-847.
- Conner P, Gustafsson S & Kublickas M 2015, "First trimester contingent testing with either nuchal translucency or cell-free DNA. Cost efficiency and the role of ultrasound dating", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 94, no. 4, pp. 368-375.
- Cowans NJ, Stamatopoulou A, Maiz N, Spencer K & Nicolaides KH 2009, "The impact of fetal gender on first trimester nuchal translucency and maternal serum free beta-hCG and PAPP-A MoM in normal and trisomy 21 pregnancies", *Prenatal diagnosis*, vol. 29, no. 6, pp. 578-581.
- Cuckle H, Benn P & Pergament E 2013, "Maternal cfDNA screening for Down syndrome-a cost sensitivity analysis", *Prenatal diagnosis*, vol. 33, no. 7, pp. 636-642.
- Dahl K, Kesmodel U, Hvidman L & Olesen F 2006, "Informed consent: providing information about prenatal examinations", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 85, no. 12, pp. 1420-1425.
- Dane B, Dane C, Cetin A, Kiray M, Sivri D & Yayla M 2008, "Pregnancy outcome in fetuses with increased nuchal translucency", *Journal of Perinatology*, vol. 28, no. 6, pp. 400-404.
- de Graaf IM, Muller MA, van Zuylen-Vie AA, Bleker OP & Bilardo CM 2000, "The influence of fetal position on nuchal translucency thickness", *Ultrasound in Obstetrics & Gynecology*, vol. 15, no. 6, pp. 520-522.
- de Jong A & de Wert GM 2015, "Prenatal screening: an ethical agenda for the near future", *Bioethics*, vol. 29, no. 1, pp. 46-55.

Di Renzo GC, Rosati A, Sarti RD, Cruciani L & Cutuli AM 2007, "Does fetal sex affect pregnancy outcome?", *Gender Medicine*, vol. 4, no. 1, pp. 19-30.

Donald I 1965, "Ultrasonic echo sounding in obstetrical and gynecological diagnosis", *American Journal of Obstetrics and Gynecology*, vol. 93, no 7, pp. 935-941.

Dugo N, Padula F, Mobili L, Brizzi C, D'Emidio L, Cignini P, Mesoraca A, Bizzoco D, Cima A & Giorlandino C 2014, "Six consecutive false positive cases from cell-free fetal DNA testing in a single referring centre", *Journal of Prenatal Medicine*, vol. 8, no. 1-2, pp. 31-35.

Durand MA, Stiel M, Boivin J & Elwyn G 2010, "Information and decision support needs of parents considering amniocentesis: interviews with pregnant women and health professionals", *Health Expectations*, vol. 13, no. 2, pp. 125-138.

Eddleman KA, Malone FD, Sullivan L, Dukes K, Berkowitz RL, Kharbutli Y, Porter TF, Luthy DA, Comstock CH, Saade GR, Klugman S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM & D'Alton ME 2006, "Pregnancy loss rates after midtrimester amniocentesis", *Obstetrics & Gynecology*, vol. 108, no. 5, pp. 1067-1072.

Ekelund CK, Petersen OB, Skibsted L, Kjaergaard S, Vogel I, Tabor A & Danish Fetal Medicine Research Group 2011, "First-trimester screening for trisomy 21 in Denmark: implications for detection and birth rates of trisomy 18 and trisomy 13", *Ultrasound in Obstetrics & Gynecology*, vol. 38, no. 2, pp. 140-144.

Ekelund CK, Wright D, Kirkegaard I, Norgaard P, Sorensen S, Friis-Hansen L, Jorgensen FS, Torring N, Bech BH, Petersen OB & Tabor A 2012, "Prospective study evaluating performance of first-trimester combined screening for trisomy 21 using repeat sampling of maternal serum markers PAPP-A and free beta-hCG", *Ultrasound in Obstetrics & Gynecology* vol 40, no 3, pp. 276-281.

Ekelund CK, Petersen OB, Sundberg K, Pedersen FH, Vogel I & Tabor A 2012, "Screening performance for trisomy 21 comparing first trimester combined screening and a first trimester contingent screening protocol including ductus venosus and tricuspid flow", *Prenatal diagnosis*, vol. 32, no. 8, pp. 783-788.

Ekelund CK, Petersen OB, Jorgensen FS, Kjaergaard S, Larsen T, Olesen AW, Skibsted L, Skovbo P, Sommer S, Sperling L, Stavstrup B, Storup B, Zingenberg H, Ulbjerg N, Miltoft CB, Noergaard L, Wulff CB, Tabor A & Danish Fetal Medicine Research Group 2015, "The Danish Fetal Medicine Database: establishment, organization and quality assessment of the first-trimester screening program for trisomy 21 in Denmark 2008-2012", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 94, no. 6, pp. 577-583.

Engelbrechtsen L, Brondum-Nielsen K, Ekelund C, Tabor A, Skibsted L & Danish Fetal Medicine Study Group 2013, "Detection of triploidy at 11-14weeks' gestation: a cohort study of 198000 pregnant women", *Ultrasound in Obstetrics & Gynecology*, vol. 42, no. 5, pp. 530-535.

Eurocat: Detailed congenital anomaly coding guidelines 2013. Available from <<http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Section-3.5.pdf>>. [15 November 2015].

Eurocat guidelines: Minor anomalies for exclusion 2014a. Available from <<http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Section-3.5.pdf>>. [15 November 2015].

Farina A, Volinia S, Arcelli D, Francioso F, Desanctis P, Zucchini C, Pilu G, Carinci P, Morano D, Pittalis MC, Calderoni P, Vagnoni S & Rizzo N 2006, "Evidence of genetic underexpression in chorionic villi samples of euploid fetuses with increased nuchal translucency at 10-11 weeks' gestation", *Prenatal diagnosis*, vol. 26, no. 2, pp. 128-133.

Farrell RM, Nutter B & Agatista PK 2011, "Meeting patients' education and decision-making needs for first trimester prenatal aneuploidy screening", *Prenatal diagnosis*, vol. 31, no. 13, pp. 1222-1228.

Ferencz C, Rubin JD, McCarter RJ, Brenner JI, Neill CA, Perry LW, Hepner SI & Downing JW 1985, "Congenital heart disease: prevalence at livebirth. The Baltimore-Washington Infant Study", *American Journal of Epidemiology*, vol. 121, no. 1, pp. 31-36.

Fraser J & Mitchell A 1876, "Kalmuk idiocy: Report of a case with autopsy", *The British Journal of Psychiatry*, vol. 22, no. 98, pp. 169-79.

Fuchs F & Riis P 1956, "Antenatal sex determination", *Nature*, vol. 177, no. 18, pp. 330.

Galindo A, Comas C, Martinez JM, Gutierrez-Larraya F, Carrera JM, Puerto B, Borrell A, Mortera C & de la Fuente P 2003, "Cardiac defects in chromosomally normal fetuses with increased nuchal translucency at 10-14 weeks of gestation", *Journal of Maternal-Fetal & Neonatal Medicine*, vol. 13, no. 3, pp. 163-170.

Gardner R, Sutherland G & Shaffer L (eds) 2011, *Chromosome abnormalities and genetic counselling*, 4th edition, Oxford University Press, New York.

Gasiorek-Wiens A, Tercanli S, Kozlowski P, Kossakiewicz A, Minderer S, Meyberg H, Kamin G, Germer U, Bielicki M, Hackeloer BJ, Sarlay D, Kuhn P, Klapp J, Bahlmann F, Pruggmayer M, Schneider KT, Seefried W, Fritzer E, von Kaisenberg CS & German-Speaking Down Syndrome Screening Group 2001, "Screening for trisomy 21 by fetal nuchal translucency and maternal age: a multicenter project in Germany, Austria and Switzerland", *Ultrasound in Obstetrics & Gynecology*, vol. 18, no. 6, pp. 645-648.

Gekas J, van den Berg DG, Durand A, Vallee M, Wildschut HI, Bujold E, Forest JC, Rousseau F & Reinharz D 2011, "Rapid testing versus karyotyping in Down's syndrome screening: cost-effectiveness and detection of clinically significant chromosome abnormalities", *European Journal of Human Genetics*, vol. 19, no. 1, pp. 3-9.

Ghaffari SR, Tahmasebpour AR, Jamal A, Hantoushzadeh S, Eslamian L, Marsoosi V, Fattahi F, Rajaei M, Niroomanesh S, Borna S, Beigi A, Khazardoost S, Saleh-Gargari S, Rahimi-Sharbat F, Farrokhi B, Bayani N, Tehrani SE, Shahsavani K, Farzan S, Moossavi S, Ramezanzadeh F, Dastan J & Rafati M 2012, "First-trimester screening for chromosomal abnormalities by integrated application of nuchal translucency, nasal bone, tricuspid regurgitation and ductus venosus flow combined with maternal serum free beta-hCG and PAPP-A: a 5-year prospective study", *Ultrasound in Obstetrics & Gynecology*, vol. 39, no. 5, pp. 528-534.

Ghi T, Huggon IC, Zosmer N & Nicolaides KH 2001, "Incidence of major structural cardiac defects associated with increased nuchal translucency but normal karyotype", *Ultrasound in Obstetrics & Gynecology*, vol. 18, no. 6, pp. 610-614.

Gil MM, Quezada MS, Revello R, Akolekar R & Nicolaides KH 2015, "Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis", *Ultrasound in Obstetrics & Gynecology*, vol. 45, no. 3, pp. 249-266.

Giltay JC & Maiburg MC 2010, "Klinefelter syndrome: clinical and molecular aspects", *Expert Review of Molecular Diagnostics*, vol. 10, no. 6, pp. 765-776.

Gittenberger-De Groot AC, Van Den Akker NM, Bartelings MM, Webb S, Van Vugt JM & Haak MC 2004, "Abnormal lymphatic development in trisomy 16 mouse embryos precedes nuchal edema", *Developmental Dynamics*, vol. 230, no. 2, pp. 378-384.

Graesslin O, Derniaux E, Alanio E, Gaillard D, Vitry F, Quéreux C & Ducarme G 2007, "Characteristics and outcome of fetal cystic hygroma diagnosed in the first trimester", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 86, no. 12, pp. 1442-1446.

Graham JM, Edwards MJ & Edwards MJ 1998, "Teratogen update: gestational effects of maternal hyperthermia due to febrile illnesses and resultant patterns of defects in humans", *Teratology*, vol. 58, no. 5, pp. 209-221.

Gravholt CH, Juul S, Naeraa RW & Hansen J 1996, "Prenatal and postnatal prevalence of Turner's syndrome: a registry study", *BMJ*, vol. 312, no. 7022, pp. 16-21.

Haak MC, Bartelings MM, Jackson DG, Webb S, van Vugt JM & Gittenberger-de Groot AC 2002, "Increased nuchal translucency is associated with jugular lymphatic distension", *Human Reproduction*, vol. 17, no. 4, pp. 1086-1092.

- Haak MC, Twisk JW, Bartelings MM, Gittenberger-de Groot AC & van Vugt JM 2005, "First-trimester fetuses with increased nuchal translucency do not show altered intracardiac flow velocities", *Ultrasound in Obstetrics & Gynecology*, vol. 25, no. 3, pp. 246-252.
- Hafner E, Schuller T, Metzgenbauer M, Schuchter K & Philipp K 2003, "Increased nuchal translucency and congenital heart defects in a low-risk population", *Prenatal diagnosis*, vol. 23, no. 12, pp. 985-989.
- Hahnemann JM & Vejerslev LO 1997, "Accuracy of cytogenetic findings on chorionic villus sampling (CVS)-diagnostic consequences of CVS mosaicism and non-mosaic discrepancy in centres contributing to EUCROMIC 1986-1992", *Prenatal Diagnosis*, vol. 17, no. 9, pp. 801-820.
- Harper, P.S. (ed) 2010, *Practical Genetic Counselling*, 7th edition, CRC Press & NA.
- Hassold T, Merrill M, Adkins K, Freeman S & Sherman S 1995, "Recombination and maternal age-dependent nondisjunction: molecular studies of trisomy 16", *American Journal of Human Genetics*, vol. 57, no. 4, pp. 867-874.
- Health Care Act 339/2011 (Finland). *Valtioneuvoston asetus seulonnoista*. <<http://www.finlex.fi/fi/laki/alkup/2011/20110339>>. [15 November 2015].
- Herman A, Maymon R, Dreazen E, Caspi E, Bukovsky I & Weinraub Z 1998, "Nuchal translucency audit: a novel image-scoring method", *Ultrasound in Obstetrics & Gynecology*, vol. 12, no. 6, pp. 398-403.
- Herman A, Dreazen E, Samandarov A, Bukovsky Y, Weinraub Z & Maymon R 2000, "On-to-on versus on-to-out nuchal translucency measurements", *Ultrasound in Obstetrics & Gynecology*, vol. 15, no. 2, pp. 126-130.
- Hiiippala A, Eronen M, Taipale P, Salonen R & Hiilesmaa V 2001, "Fetal nuchal translucency and normal chromosomes: a long-term follow-up study", *Ultrasound in Obstetrics & Gynecology*, vol. 18, no. 1, pp. 18-22.
- Hoffman JI & Kaplan S 2002, "The incidence of congenital heart disease", *Journal of the American College of Cardiology*, vol. 39, no. 12, pp. 1890-1900.
- Hook EB 1978, "Spontaneous deaths of fetuses with chromosomal abnormalities diagnosed prenatally" *New England Journal of Medicine*, vol. 299, pp. 1036-38.
- Hook, EB 1981, "Rates of chromosome abnormalities at different maternal ages", *Obstetrics & Gynecology*, vol. 58, no. 3, pp. 282-285.
- Hsiao CH, Wang HC, Hsieh CF & Hsu JJ 2008, "Fetal gender screening by ultrasound at 11 to 13(+6) weeks", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 87, no. 1, pp. 8-13.
- Hsiao CH, Cheng PJ, Shaw SW, Hsu JJ, Chen RC, Tseng YJ & Chu WC 2014, "Extended first-trimester screening using multiple sonographic markers and maternal serum biochemistry: a five-year prospective study", *Fetal Diagnosis & Therapy*, vol. 35, no. 4, pp. 296-301.
- Huang J, Poon LC, Akolekar R, Choy KW, Leung TY & Nicolaides KH 2014, "Is high fetal nuchal translucency associated with submicroscopic chromosomal abnormalities on array CGH?", *Ultrasound in Obstetrics & Gynecology*, vol. 43, no. 6, pp. 620-624.
- Huggon IC, Ghi T, Cook AC, Zosmer N, Allan LD & Nicolaides KH 2002, "Fetal cardiac abnormalities identified prior to 14 weeks' gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 20, no. 1, pp. 22-29.
- Huggon IC, Turan O & Allan LD 2004, "Doppler assessment of cardiac function at 11-14 weeks' gestation in fetuses with normal and increased nuchal translucency", *Ultrasound in Obstetrics & Gynecology*, vol. 24, no. 4, pp. 390-398.

- Hulten MA, Dhanjal S & Pertl B 2003, "Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF-PCR", *Reproduction*, vol. 126, no. 3, pp. 279-297.
- Hyett J, Moscoso G, Papapanagiotou G, Perdu M & Nicolaides KH 1996a, "Abnormalities of the heart and great arteries in chromosomally normal fetuses with increased nuchal translucency thickness at 11-13 weeks of gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 7, no. 4, pp. 245-250.
- Hyett JA, Brizot ML, Von Kaisenberg CS, McKie AT, Farzaneh F & Nicolaides KH 1996b, "Cardiac gene expression of atrial natriuretic peptide and brain natriuretic peptide in trisomic fetuses", *Obstetrics & Gynecology*, vol. 87, no. 4, pp. 506-510.
- Hyett JA, Perdu M, Sharland GK, Snijders RS & Nicolaides KH 1997a, "Increased nuchal translucency at 10-14 weeks of gestation as a marker for major cardiac defects", *Ultrasound in Obstetrics & Gynecology*, vol. 10, no. 4, pp. 242-246.
- Hyett J, Noble P, Sebire NJ, Snijders R & Nicolaides KH 1997b, "Lethal congenital arthrogryposis presents with increased nuchal translucency at 10-14 weeks of gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 9, no. 5, pp. 310-313.
- Hyett J, Perdu M, Sharland G, Snijders R & Nicolaides KH 1999, "Using fetal nuchal translucency to screen for major congenital cardiac defects at 10-14 weeks of gestation: population based cohort study", *BMJ*, vol. 318, no. 7176, pp. 81-85.
- Hyett, J 2004, "Does nuchal translucency have a role in fetal cardiac screening?", *Prenatal diagnosis*, vol. 24, no. 13, pp. 1130-1135.
- Jaques AM, Halliday JL & Bell RJ 2004, "Do women know that prenatal testing detects fetuses with Down syndrome?", *Journal of Obstetrics & Gynaecology*, vol. 24, no. 6, pp. 647-651.
- Jotkowitz A & Zivotofsky AZ 2010, "The ethics of abortions for fetuses with congenital abnormalities", *European Journal of Obstetrics, Gynecology, & Reproductive Biology*, vol. 152, no. 2, pp. 148-151.
- Jouannic JM, Thieulin AC, Bonnet D, Houyel L, Lelong N, Goffinet F & Khoshnood B 2011, "Measurement of nuchal translucency for prenatal screening of congenital heart defects: a population-based evaluation", *Prenatal diagnosis*, vol. 31, no. 13, pp. 1264-1269.
- Kagan KO, Avgidou K, Molina FS, Gajewska K & Nicolaides KH 2006, "Relation between increased fetal nuchal translucency thickness and chromosomal abnormalities", *Obstetrics & Gynecology*, vol. 107, no. 1, pp. 6-10.
- Kagan KO, Wright D, Baker A, Sahota D & Nicolaides KH 2008, "Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A", *Ultrasound in Obstetrics & Gynecology*, vol. 31, no. 6, pp. 618-624.
- Kagan KO, Cicero S, Staboulidou I, Wright D & Nicolaides KH 2009a, "Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 33, no. 3, pp. 259-264.
- Kagan KO, Valencia C, Livanos P, Wright D & Nicolaides KH 2009b, "Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 33, no. 1, pp. 18-22.
- Kagan KO, Etchegaray A, Zhou Y, Wright D & Nicolaides KH 2009c, "Prospective validation of first-trimester combined screening for trisomy 21", *Ultrasound in Obstetrics & Gynecology*, vol. 34, no. 1, pp. 14-18.
- Kagan KO, Staboulidou I, Cruz J, Wright D & Nicolaides KH 2010, "Two-stage first-trimester screening for trisomy 21 by ultrasound assessment and biochemical testing", *Ultrasound in Obstetrics & Gynecology*, vol. 36, no. 5, pp. 542-547.

Karadzov-Orlic N, Egic A, Milovanovic Z, Marinkovic M, Damnjanovic-Pazin B, Lukic R, Joksic I, Curkovic A & Mikovic Z 2012, "Improved diagnostic accuracy by using secondary ultrasound markers in the first-trimester screening for trisomies 21, 18 and 13 and Turner syndrome", *Prenatal diagnosis*, vol. 32, no. 7, pp. 638-643.

Kelan vammaisetuustilasto 2014b. Available from <http://www.kela.fi/documents/10180/1630858/Kelan_vammaisetuustilasto_2014.pdf>. [18 December 2015].

Kharrat R, Yamamoto M, Roume J, Couderc S, Vialard F, Hillion Y & Ville Y 2006, "Karyotype and outcome of fetuses diagnosed with cystic hygroma in the first trimester in relation to nuchal translucency thickness", *Prenatal diagnosis*, vol. 26, no. 4, pp. 369-372.

Kirkegaard I, Petersen OB, Uldbjerg N & Topping N 2008, "Improved performance of first-trimester combined screening for trisomy 21 with the double test taken before a gestational age of 10 weeks", *Prenatal diagnosis*, vol. 28, no. 9, pp. 839-844.

Kleinveld JH, Timmermans DR, de Smit DJ, Ader HJ, van der Wal G & ten Kate LP 2006, "Does prenatal screening influence anxiety levels of pregnant women? A longitudinal randomised controlled trial", *Prenatal diagnosis*, vol. 26, no. 4, pp. 354-361.

Kobelka C, Mattman A & Langlois S 2009, "An evaluation of the decision-making process regarding amniocentesis following a screen-positive maternal serum screen result", *Prenatal diagnosis*, vol. 29, no. 5, pp. 514-519.

Kowalcek I, Huber G, Lammers C, Brunk J, Bieniakiewicz I & Gembruch U 2003, "Anxiety scores before and after prenatal testing for congenital anomalies", *Archives of Gynecology & Obstetrics*, vol. 267, no. 3, pp. 126-129.

Laki raskauden keskeyttämisestä 24.3.1970/239. Available from <<http://www.finlex.fi/fi/laki/ajantasa/1970/19700239>>. [7 January 2016].

Lam YH, Tang MH, Lee CP & Tse HY 1999, "Nuchal translucency in fetuses affected by homozygous alpha-thalassemia-1 at 12-13 weeks of gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 13, no. 4, pp. 238-240.

Lam YH, Tang MH, Lee CP, Sin SY, Tang R, Wong HS & Wong SF 2001, "The effect of fetal gender on nuchal translucency at 10-14 weeks of gestation", *Prenatal diagnosis*, vol. 21, no. 8, pp. 627-629.

Larsen SO, Wojdemann KR, Shalmi AC, Sundberg K, Christiansen M & Tabor A 2002, "Gender impact on first trimester markers in Down syndrome screening", *Prenatal diagnosis*, vol. 22, no. 13, pp. 1207-1208.

Learman LA, Drey EA, Gates EA, Kang MS, Washington AE & Kuppermann M 2005, "Abortion attitudes of pregnant women in prenatal care", *American Journal of Obstetrics & Gynecology*, vol. 192, no. 6, pp. 1939-1945.

Leung WC, Lau ET, Ngai C, Lam H, Leung KY, Lee CP, Lao TT & Tang MH 2008, "A prospective study on the effect of rapid aneuploidy testing (amnio-PCR) on anxiety levels and quality of life measures in women and their partners with positive Down screening result", *Fetal Diagnosis & Therapy*, vol. 24, no. 3, pp. 165-169.

Leung TY, Vogel I, Lau TK, Chong W, Hyett JA, Petersen OB & Choy KW 2011, "Identification of submicroscopic chromosomal aberrations in fetuses with increased nuchal translucency and apparently normal karyotype", *Ultrasound in Obstetrics & Gynecology*, vol. 38, no. 3, pp. 314-319.

Liang D, Lv W, Wang H, Xu L, Liu J, Li H, Hu L, Peng Y & Wu L 2013, "Non-invasive prenatal testing of fetal whole chromosome aneuploidy by massively parallel sequencing", *Prenatal diagnosis*, vol. 33, no. 5, pp. 409-415.

- Ljunger E, Cnattingius S, Lundin C & Anneren G 2005, "Chromosomal anomalies in first-trimester miscarriages", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 84, no. 11, pp. 1103-1107.
- Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW & Wainscoat JS 1997, "Presence of fetal DNA in maternal plasma and serum", *Lancet*, vol. 350, no. 9076, pp. 485-487.
- Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, Wainscoat JS, Johnson PJ, Chang AM & Hjelm NM 1998, "Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis", *American Journal of Human Genetics*, vol. 62, no. 4, pp. 768-775.
- Loane M, Morris JK, Addor MC, Arriola L, Budd J, Doray B, Garne E, Gatt M, Haeusler M, Khoshnood B, Klungsoyr Melve K, Latos-Bielenska A, McDonnell B, Mullaney C, O'Mahony M, Queisser-Wahrendorf A, Rankin J, Rissmann A, Rounding C, Salvador J, Tucker D, Wellesley D, Yevtushok L & Dolk H 2013, "Twenty-year trends in the prevalence of Down syndrome and other trisomies in Europe: impact of maternal age and prenatal screening", *European Journal of Human Genetics*, vol. 21, no. 1, pp. 27-33.
- Lopes LM, Brizot ML, Lopes MA, Ayello VD, Schultz R & Zugaib M 2003, "Structural and functional cardiac abnormalities identified prior to 16 weeks' gestation in fetuses with increased nuchal translucency", *Ultrasound in Obstetrics & Gynecology*, vol. 22, no. 5, pp. 470-478.
- Lou S, Mikkelsen L, Hvidman L, Petersen OB & Nielsen CP 2015, "Does screening for Down's syndrome cause anxiety in pregnant women? A systematic review", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 94, no. 1, pp. 15-27.
- Lund IC, Christensen R, Petersen OB, Vogel I & Vestergaard EM 2015, "Chromosomal microarray in fetuses with increased nuchal translucency", *Ultrasound in Obstetrics & Gynecology*, vol. 45, no. 1, pp. 95-100.
- Makrydimas G, Sotiriadis A & Ioannidis JP 2003, "Screening performance of first-trimester nuchal translucency for major cardiac defects: a meta-analysis", *American Journal of Obstetrics & Gynecology*, vol. 189, no. 5, pp. 1330-1335.
- Malone FD, Ball RH, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, Gross SJ, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Dukes K, Canick JA, Bianchi DW, D'Alton ME & FASTER Trial Research, C. 2005, "First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome", *Obstetrics & Gynecology*, vol. 106, no. 2, pp. 288-294.
- Mangione R, Guyon F, Taine L, Wen ZQ, Roux D, Vergnaud A, Maugey-Laulom B, Horovitz J & Saura R 2001, "Pregnancy outcome and prognosis in fetuses with increased first-trimester nuchal translucency", *Fetal Diagnosis & Therapy*, vol. 16, no. 6, pp. 360-363.
- Marteau TM, Cook R, Kidd J, Michie S, Johnston M, Slack J & Shaw RW 1992, "The psychological effects of false-positive results in prenatal screening for fetal abnormality: a prospective study", *Prenatal diagnosis*, vol. 12, no. 3, pp. 205-214.
- Marttala J, Yliniemi O, Gissler M, Nieminen P & Ryynanen M 2010, "Prevalence of Down's syndrome in a pregnant population in Finland", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 89, no. 5, pp. 715-717.
- Matias A, Huggon I, Areias JC, Montenegro N & Nicolaidis KH 1999, "Cardiac defects in chromosomally normal fetuses with abnormal ductus venosus blood flow at 10-14 weeks", *Ultrasound in Obstetrics & Gynecology*, vol. 14, no. 5, pp. 307-310.
- Mavrides E, Cobian-Sanchez F, Tekay A, Moscoso G, Campbell S, Thilaganathan B & Carvalho JS 2001, "Limitations of using first-trimester nuchal translucency measurement in routine screening for major congenital heart defects", *Ultrasound in Obstetrics & Gynecology*, vol. 17, no. 2, pp. 106-110.
- Maymon R, Jauniaux E, Cohen O, Dreazen E, Weinraub Z & Herman A 2000, "Pregnancy outcome and infant follow-up of fetuses with abnormally increased first trimester nuchal translucency", *Human Reproduction*, vol. 15, no. 9, pp. 2023-2027.

- Maymon R, Tercanli S, Dreazen E, Sartorius G, Holzgreve W & Herman A 2004, "Comparison of pregnancy outcome of euploid fetuses with increased nuchal translucency (NT) expressed in NT MoM or delta-NT", *Ultrasound in Obstetrics & Gynecology*, vol. 23, no. 5, pp. 477-481.
- McAuliffe FM, Hornberger LK, Winsor S, Chitayat D, Chong K & Johnson JA 2004, "Fetal cardiac defects and increased nuchal translucency thickness: a prospective study", *American Journal of Obstetrics & Gynecology*, vol. 191, no. 4, pp. 1486-1490.
- McPherson JD, Marra M, Hillier L & Waterston, RH 2001 "A physical map of the human genome", *Nature*, vol. 409, no. 6822, pp. 934-941.
- Michailidis GD & Economides DL 2001, "Nuchal translucency measurement and pregnancy outcome in karyotypically normal fetuses", *Ultrasound in Obstetrics & Gynecology*, vol. 17, no. 2, pp. 102-105.
- Mikhaelian M, Veach PM, MacFarlane I, LeRoy BS & Bower M 2013, "Prenatal chromosomal microarray analysis: a survey of prenatal genetic counselors' experiences and attitudes", *Prenatal diagnosis*, vol. 33, no. 4, pp. 371-377.
- Miltoft CB, Ekelund CK, Hansen BM, Lando A, Petersen OB, Skovbo P, Jorgensen FS, Sperling L, Zingenberg H, Nikkila A, Shalmi AC, Stornes I, Ersbak V & Tabor A 2012, "Increased nuchal translucency, normal karyotype and infant development", *Ultrasound in Obstetrics & Gynecology*, vol. 39, no. 1, pp. 28-33.
- Miyabara S, Sugihara H, Maehara N, Shouno H, Tasaki H, Yoshida K, Saito N, Kayama F, Ibara S & Suzumori K 1989, "Significance of cardiovascular malformations in cystic hygroma: a new interpretation of the pathogenesis", *American Journal of Medical Genetics*, vol. 34, no. 4, pp. 489-501.
- Montenegro N, Matias A, Areias JC, Castedo S & Barros H 1997, "Increased fetal nuchal translucency: possible involvement of early cardiac failure", *Ultrasound in Obstetrics & Gynecology*, vol. 10, no. 4, pp. 265-268.
- Morris JK, Alberman E, Scott C & Jacobs P 2008, "Is the prevalence of Klinefelter syndrome increasing?", *European Journal of Human Genetics*, vol. 16, no. 2, pp. 163-170.
- Morris JK & Savva GM 2008, "The risk of fetal loss following a prenatal diagnosis of trisomy 13 or trisomy 18", *American Journal of Medical Genetics. Part A*, vol. 146A, no. 7, pp. 827-832.
- Morris JK & Alberman E 2009, "Trends in Down's syndrome live births and antenatal diagnoses in England and Wales from 1989 to 2008: analysis of data from the National Down Syndrome Cytogenetic Register", *BMJ*, vol. 339, pp. b3794.
- Morris JK, Garne E, Wellesley D, Addor MC, Arriola L, Barisic I, Beres J, Bianchi F, Budd J, Dias CM, Gatt M, Klungsoyr K, Khoshnood B, Latos-Bielenska A, Mullaney C, Nelen V, Neville AJ, O'Mahony M, Queisser-Luft A, Randrianaivo H, Rankin J, Rissmann A, Rounding C, Sipek A, Stoianova S, Tucker D, de Walle H, Yevtushok L, Loane M & Dolk H 2014, "Major congenital anomalies in babies born with Down syndrome: a EUROCAT population-based registry study", *American Journal of Medical Genetics. Part A*, vol. 164A, no. 12, pp. 2979-2986.
- Mula R, Gonce A, Bannasar M, Arigita M, Meler E, Nadal A, Sanchez A, Botet F & Borrell A 2012, "Increased nuchal translucency and normal karyotype: perinatal and pediatric outcomes at 2 years of age", *Ultrasound in Obstetrics & Gynecology*, vol. 39, no. 1, pp. 34-41.
- Mula R, Grande M, Bannasar M, Crispi F, Borobio V, Martinez JM, Gratacos E & Borrell A 2015, "Further insights into diastolic dysfunction in first-trimester trisomy-21 fetuses", *Ultrasound in Obstetrics & Gynecology*, vol. 45, no. 2, pp. 205-210.
- Muller MA, Clur SA, Timmerman E & Bilardo CM 2007, "Nuchal translucency measurement and congenital heart defects: modest association in low-risk pregnancies", *Prenatal diagnosis*, vol. 27, no. 2, pp. 164-169.

- Mulvey S, Baker L, Edwards A, Oldham J, Shekleton P & Wallace EM 2002, "Optimising the timing for nuchal translucency measurement", *Prenatal diagnosis*, vol. 22, no. 9, pp. 775-777.
- Munoz-Cortes M, Arigita M, Falguera G, Seres A, Guix D, Baldrich E, Acera A, Torrent A, Rodriguez-Veret A, Lopez-Quesada E, Garcia-Moreno R, Gonce A, Borobio V & Borrell A 2012, "Contingent screening for Down syndrome completed in the first trimester: a multicenter study", *Ultrasound in Obstetrics & Gynecology*, vol. 39, no. 4, pp. 396-400.
- Nadler HL 1968, "Antenatal detection of hereditary disorders", *Pediatrics*, vol. 42, no. 6, pp. 912-918.
- Nadler HL & Gerbie AB 1970, "Role of amniocentesis in the intrauterine detection of genetic disorders", *New England Journal of Medicine*, vol. 282, no. 11, pp. 596-599.
- Natoli JL, Ackerman DL, McDermott S & Edwards JG 2012, "Prenatal diagnosis of Down syndrome: a systematic review of termination rates (1995-2011)", *Prenatal diagnosis*, vol. 32, no. 2, pp. 142-153.
- Nelson TR, Fowlkes BJ, Abramowicz JS & Church CC 2009, "Ultrasound biosafety considerations for the practicing sonographer and sonologist", *Journal of Ultrasound in Medicine*, vol. 28, no. 2, pp. 139-150.
- Nicolaides KH, Azar G, Byrne D, Mansur C & Marks K 1992, "Fetal nuchal translucency: ultrasound screening for chromosomal abnormalities in first trimester of pregnancy", *BMJ*, vol. 304, no. 6831, pp. 867-869.
- Nicolaides KH, Brizot ML & Snijders RJM 1994, "Fetal nuchal translucency: ultrasound screening for fetal trisomy in the first trimester of pregnancy", *British Journal of Obstetrics & Gynaecology*, vol. 101, no. 9, pp. 782-786.
- Nicolaides KH, Heath V & Cicero S 2002, "Increased fetal nuchal translucency at 11-14 weeks", *Prenatal diagnosis*, vol. 22, no. 4, pp. 308-315.
- Nicolaides KH 2004, "Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities", *American Journal of Obstetrics & Gynecology*, vol. 191, no. 1, pp. 45-67.
- Nicolaides KH, Spencer K, Avgidou K, Faiola S & Falcon O 2005, "Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening", *Ultrasound in Obstetrics & Gynecology*, vol. 25, no. 3, pp. 221-226.
- Nicolaides KH, Wright D, Poon LC, Syngelaki A & Gil MM 2013, "First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing", *Ultrasound in Obstetrics & Gynecology*, vol. 42, no. 1, pp. 41-50.
- Norton ME, Nakagawa S & Kuppermann M 2014, "Women's Attitudes Regarding Prenatal Testing for a Range of Congenital Disorders of Varying Severity", *Journal of Clinical Medicine*, vol. 3, no. 1, pp. 144-152.
- Norton ME, Jacobsson B, Swamy GK, Laurent LC, Ranzini AC, Brar H, Tomlinson MW, Pereira L, Spitz JL, Holleman D, Cuckle H, Musci TJ & Wapner RJ 2015, "Cell-free DNA analysis for noninvasive examination of trisomy", *New England Journal of Medicine*, vol. 372, no. 17, pp. 1589-1597.
- Orvos H, Wayda K, Kozinszky Z, Katona M, Pál A & Szabó J 2002, "Increased nuchal translucency and congenital heart defects in euploid fetuses. The Szeged experience", *European Journal of Obstetrics, Gynecology, & Reproductive Biology*, vol. 101, no. 2, pp. 124-128.
- Pajkrt E, Bilardo CM, Van Lith JM, Mol BW & Bleker OP 1995, "Nuchal translucency measurement in normal fetuses", *Obstetrics & Gynecology*, vol. 86, no. 6, pp. 994-997.
- Pajkrt E, Mol BW, Boer K, Drogtróp AP, Bossuyt PM & Bilardo CM 2000, "Intra- and interoperator repeatability of the nuchal translucency measurement", *Ultrasound in Obstetrics & Gynecology*, vol. 15, no. 4, pp. 297-301.

- Pan M, Han J, Zhen L, Yang X, Li R, Liao C & Li D-Z 2016, "Prenatal diagnosis of fetuses with increased nuchal translucency using an approach based on quantitative fluorescent polymerase chain reaction and genomic microarray", *European Journal of Obstetrics, Gynecology & Reproductive Medicine*, vol. 197, pp. 164-167.
- Pandya PP, Altman DG, Brizot ML, Pettersen H & Nicolaides KH 1995a, "Repeatability of measurement of fetal nuchal translucency thickness", *Ultrasound in Obstetrics & Gynecology*, vol. 5, no. 5, pp. 334-337.
- Pandya PP, Snijders RJ, Johnson SP, De Lourdes Brizot M & Nicolaides KH 1995b, "Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation", *British Journal of Obstetrics & Gynaecology*, vol. 102, no. 12, pp. 957-962.
- Pandya PP, Kondylis A, Hilbert L, Snijders RJ & Nicolaides KH 1995, "Chromosomal abnormalities and outcome in 1015 fetuses with increased nuchal translucency", *Ultrasound in Obstetrics & Gynecology*, vol. 5, no. 1, pp. 15-19.
- Pergament E, Alamillo C, Sak K & Fiddler M 2011, "Genetic assessment following increased nuchal translucency and normal karyotype", *Prenatal diagnosis*, vol. 31, no. 3, pp. 307-310.
- Pertl B, Yau SC, Sherlock J, Davies AF, Mathew CG & Adinolfi M 1994, "Rapid molecular method for prenatal detection of Down's syndrome", *Lancet*, vol. 343, no. 8907, pp. 1197-1198.
- Pinsker JE 2012, "Clinical review: Turner syndrome: updating the paradigm of clinical care", *Journal of Clinical Endocrinology & Metabolism*, vol. 97, no. 6, pp. E994-1003.
- Porreco RP, Garite TJ, Maurel K, Marusiak B, Obstetrix Collaborative Research Network, Ehrich M, van den Boom D, Deciu C & Bombard A 2014, "Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA", *American Journal of Obstetrics & Gynecology*, vol. 211, no. 4, pp. 365.e1-365.12.
- Prefumo F, Venturini PL & de Biasio P 2003, "Effect of fetal gender on first-trimester ductus venosus blood flow", *Ultrasound in Obstetrics & Gynecology*, vol. 22, no. 3, pp. 268-270.
- Ranke MB & Saenger P 2001, "Turner's syndrome", *Lancet*, vol. 358, no. 9278, pp. 309-314.
- Rasiah SV, Publicover M & Ewer AK, "A systematic review of the accuracy of first trimester ultrasound examination for detecting major congenital heart disease", *Ultrasound Obstetrics & Gynecology*, vol. 28, no. 1, pp. 110-116.
- Rasmussen SA, Wong LY, Yang Q, May KM & Friedman JM 2003, "Population-based analyses of mortality in trisomy 13 and trisomy 18", *Pediatrics*, vol. 111, no. 4 Pt 1, pp. 777-784.
- Rentmeester CA 2001, "Value neutrality in genetic counseling: an unattained ideal", *Medicine, Health Care & Philosophy*, vol. 4, no. 1, pp. 47-51.
- Richards JE & Hawley SR 2011, "Mitosis and meiosis: How cells move your genes around" in *The Human genome*, ed. Richards Julia E, Hawley Scott R, Third edition, Elsevier, pp. 199-246.
- Rode L, Ekelund C, Pedersen NG, Wojdemann KR, Christiansen M, Sundberg K & Tabor A 2011, "Maternal smoking, obesity and male fetal sex predispose to a large nuchal translucency thickness in healthy fetuses", *Fetal Diagnosis & Therapy*, vol. 29, no. 3, pp. 201-207.
- Rossi AC & Prefumo F 2013, "Accuracy of ultrasonography at 11-14 weeks of gestation for detection of fetal structural anomalies: a systematic review", *Obstetrics & Gynecology*, vol. 122, no. 6, pp. 1160-1167.
- Rozenberg P, Bussieres L, Chevret S, Bernard JP, Malagrida L, Cuckle H, Chabry C, Durand-Zaleski I, Bidat L, Lacroix I, Moulis M, Roger M, Jacquemot MC, Bault JP, Boukobza P, Boccara P, Vialard F, Giudicelli Y & Ville Y 2006, "Screening for Down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population", *American Journal of Obstetrics & Gynecology*, vol. 195, no. 5, pp. 1379-1387.

Sahota DS, Leung TY, Chan LW, Law LW, Fung TY, Chen M & Lau TK 2010, "Comparison of first-trimester contingent screening strategies for Down syndrome", *Ultrasound in Obstetrics & Gynecology*, vol. 35, no. 3, pp. 286-291.

Sananes N, Guigue V, Kohler M, Bouffet N, Cancellier M, Hornecker F, Hunsinger MC, Kohler A, Mager C, Neumann M, Schmerber E, Tanghe M, Nisand I & Favre R 2010, "Nuchal translucency and cystic hygroma colli in screening for fetal major congenital heart defects in a series of 12,910 euploid pregnancies", *Ultrasound in Obstetrics & Gynecology*, vol. 35, no. 3, pp. 273-279.

Santalahahti P, Aro AR, Hemminki E, Helenius H & Ryynanen M 1998, "On what grounds do women participate in prenatal screening?", *Prenatal diagnosis*, vol. 18, no. 2, pp. 153-165.

Santalahahti P, Hemminki E, Aro AR, Helenius H & Ryynanen M 1999, "Participation in prenatal screening tests and intentions concerning selective termination in Finnish maternity care", *Fetal Diagnosis & Therapy*, vol. 14, no. 2, pp. 71-79.

Savoldelli G, Binkert F, Achermann J & Schmid W 1993, "Ultrasound screening for chromosomal anomalies in the first trimester of pregnancy", *Prenatal diagnosis*, vol. 13, no. 6, pp. 513-518.

Savva GM, Walker K & Morris JK 2010, "The maternal age-specific live birth prevalence of trisomies 13 and 18 compared to trisomy 21 (Down syndrome)", *Prenatal diagnosis*, vol. 30, no. 1, pp. 57-64.

Scholl J, Durfee SM, Russell MA, Heard AJ, Iyer C, Alammari R, Coletta J, Craigo SD, Fuchs KM, D'Alton M, House M, Jennings RW, Ecker J, Panda B, Tanner C, Wolfberg A & Benson CB 2012, "First-trimester cystic hygroma: relationship of nuchal translucency thickness and outcomes", *Obstetrics & Gynecology*, vol. 120, no. 3, pp. 551-559.

Schou KV, Kirchhoff M, Nygaard U, Jorgensen C & Sundberg K 2009, "Increased nuchal translucency with normal karyotype: a follow-up study of 100 cases supplemented with CGH and MLPA analyses", *Ultrasound in Obstetrics & Gynecology*, vol. 34, no. 6, pp. 618-622.

Schuchter K, Wald N, Hackshaw AK, Hafner E & Liebhart E 1998, "The distribution of nuchal translucency at 10-13 weeks of pregnancy", *Prenatal diagnosis*, vol. 18, no. 3, pp. 281-286.

Schwarzler P, Carvalho JS, Senat MV, Masroor T, Campbell S & Ville Y 1999, "Screening for fetal aneuploidies and fetal cardiac abnormalities by nuchal translucency thickness measurement at 10-14 weeks of gestation as part of routine antenatal care in an unselected population", *British Journal of Obstetrics & Gynaecology*, vol. 106, no. 10, pp. 1029-1034.

Scott F, Murphy K, Carey L, Greville W, Mansfield N, Barahona P, Robertson R & McLennan A 2013, "Prenatal diagnosis using combined quantitative fluorescent polymerase chain reaction and array comparative genomic hybridization analysis as a first-line test: results from over 1000 consecutive cases", *Ultrasound in Obstetrics & Gynecology*, vol. 41, no. 5, pp. 500-507.

Sebire NJ, Bianco D, Snijders RJ, Zuckerman M & Nicolaides KH 1997a, "Increased fetal nuchal translucency thickness at 10-14 weeks: is screening for maternal-fetal infection necessary?", *British Journal of Obstetrics & Gynaecology*, vol. 104, no. 2, pp. 212-215.

Sebire NJ, Snijders RJ, Davenport M, Greenough A & Nicolaides KH 1997b, "Fetal nuchal translucency thickness at 10-14 weeks' gestation and congenital diaphragmatic hernia", *Obstetrics & Gynecology*, vol. 90, no. 6, pp. 943-946.

Senat MV, De Keersmaecker B, Audibert F, Montcharmont G, Frydman R & Ville Y 2002, "Pregnancy outcome in fetuses with increased nuchal translucency and normal karyotype", *Prenatal diagnosis*, vol. 22, no. 5, pp. 345-349.

Senat MV, Bussieres L, Couderc S, Roume J, Rozenberg P, Bouyer J & Ville Y 2007, "Long-term outcome of children born after a first-trimester measurement of nuchal translucency at the 99th percentile or greater

with normal karyotype: a prospective study", *American Journal of Obstetrics & Gynecology*, vol. 196, no. 1, pp. 53.e1-53.e6.

Sheiner E & Abramowicz JS 2009, "Acoustic output as measured by thermal and mechanical indices during fetal nuchal translucency ultrasound examinations", *Fetal Diagnosis & Therapy*, vol. 25, no. 1, pp. 8-10.

Sheiner E & Abramowicz JS 2012, "A symposium on obstetrical ultrasound: is all this safe for the fetus?", *Clinical Obstetrics & Gynecology*, vol. 55, no. 1, pp. 188-198.

Sherod C, Sebire NJ, Soares W, Snijders RJ & Nicolaides KH 1997, "Prenatal diagnosis of trisomy 18 at the 10-14-week ultrasound scan", *Ultrasound in Obstetrics & Gynecology*, vol. 10, no. 6, pp. 387-390.

Siggberg L, Ala-Mello S, Jaakkola E, Kuusinen E, Schuit R, Kohlhase J, Bohm D, Ignatius J & Knuutila S 2010, "Array CGH in molecular diagnosis of mental retardation - A study of 150 Finnish patients", *American Journal of Medical Genetics.Part A*, vol. 152A, no. 6, pp. 1398-1410.

Sillanpää M & Airaksinen E 2013, *Lastenneurologia*, Kustannus oy Duodecim, Finland.

Simpson JM & Sharland GK 2000, "Nuchal translucency and congenital heart defects: heart failure or not?", *Ultrasound in Obstetrics & Gynecology*, vol. 16, no. 1, pp. 30-36.

Smidt-Jensen S & Hahnemann N 1984, "Transabdominal fine needle biopsy from chorionic villi in the first trimester", *Prenatal Diagnosis*, vol. 4, no. 3, pp. 163-169.

Snijders RJ, Johnson S, Sebire NJ, Noble PL & Nicolaides KH 1996, "First-trimester ultrasound screening for chromosomal abnormalities", *Ultrasound in Obstetrics & Gynecology*, vol. 7, no. 3, pp. 216-226.

Snijders RJ, Noble P, Sebire N, Souka A & Nicolaides KH 1998, "UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First-trimester screening Group", *Lancet*, vol. 352, no. 9125, pp. 343-346.

Snijders RJ, Sebire NJ, Nayar R, Souka A & Nicolaides KH 1999, "Increased nuchal translucency in trisomy 13 fetuses at 10-14 weeks of gestation", *American Journal of Medical Genetics*, vol. 86, no. 3, pp. 205-207.

Snijders RJ, Thom EA, Zachary JM, Platt LD, Greene N, Jackson LG, Sabbagha RE, Filkins K, Silver RK, Hogge WA, Ginsberg NA, Beverly S, Morgan P, Blum K, Chilis P, Hill LM, Hecker J & Wapner RJ 2002, "First-trimester trisomy screening: nuchal translucency measurement training and quality assurance to correct and unify technique", *Ultrasound in Obstetrics & Gynecology*, vol. 19, no. 4, pp. 353-359.

Sotiriadis A, Papatheodorou S, Eleftheriades M & Makrydimas G 2013, "Nuchal translucency and major congenital heart defects in fetuses with normal karyotype: a meta-analysis", *Ultrasound in Obstetrics & Gynecology*, vol. 42, no. 4, pp. 383-389.

Souka AP & Nicolaides KH 1997, "Diagnosis of fetal abnormalities at the 10-14-week scan", *Ultrasound in Obstetrics & Gynecology*, vol. 10, no. 6, pp. 429-442.

Souka AP, Snijders RJ, Novakov A, Soares W & Nicolaides KH 1998, "Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10-14 weeks of gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 11, no. 6, pp. 391-400.

Souka AP, Krampfl E, Bakalis S, Heath V & Nicolaides KH 2001, "Outcome of pregnancy in chromosomally normal fetuses with increased nuchal translucency in the first trimester", *Ultrasound in Obstetrics & Gynecology*, vol. 18, no. 1, pp. 9-17.

Souka AP, von Kaisenberg CS, Hyett JA, Sonek JD & Nicolaides KH 2005, "Increased nuchal translucency with normal karyotype", *American Journal of Obstetrics & Gynecology*, vol. 192, no. 4, pp. 1005-1021.

Spaggiari E, Stirnemann J & Ville Y 2012, "Outcome in fetuses with isolated congenital diaphragmatic hernia with increased nuchal translucency thickness in first trimester", *Prenatal diagnosis*, vol. 32, no. 3, pp. 268-271.

Spencer K, Souter V, Tul N, Snijders R & Nicolaides KH 1999, "A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A", *Ultrasound in Obstetrics & Gynecology*, vol. 13, no. 4, pp. 231-237.

Spencer K, Spencer CE, Power M, Moakes A & Nicolaides KH 2000a, "One stop clinic for assessment of risk for fetal anomalies: a report of the first year of prospective screening for chromosomal anomalies in the first trimester", *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 107, no. 10, pp. 1271-1275.

Spencer K, Ong CY, Liao AW, Papademetriou D & Nicolaides KH 2000b, "The influence of fetal sex in screening for trisomy 21 by fetal nuchal translucency, maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation", *Prenatal diagnosis*, vol. 20, no. 8, pp. 673-675.

Spencer K, Spencer CE, Power M, Dawson C & Nicolaides KH 2003, "Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience", *BJOG: An International Journal of Obstetrics & Gynecology*, vol. 110, no. 3, pp. 281-286.

Steele MW & Breg WR Jr 1966, "Chromosome analysis of human amniotic-fluid cells", *Lancet*, vol. 1, no. 7434, pp. 383-385.

Sweet KM & Michaels RC 2011, "Genetic variability provides the biochemical basis to our individuality, including differences in our susceptibility to many common diseases", in *The Busy Physician's Guide to Genetics, Genomics and Personalized medicine*, eds. KM Sweet & RC Michaels, Springer Science+Business Media BV, pp. 1-34.

Tabor A, Philip J, Madsen M, Bang J, Obel EB & Norgaard-Pedersen B 1986, "Randomised controlled trial of genetic amniocentesis in 4606 low-risk women", *Lancet*, vol. 1, no. 8493, pp. 1287-1293.

Tabor A, Vestergaard CH & Lidegaard O 2009, "Fetal loss rate after chorionic villus sampling and amniocentesis: an 11-year national registry study", *Ultrasound in Obstetrics & Gynecology*, vol. 34, no. 1, pp. 19-24.

Taipale P, Hiilesmaa V, Salonen R & Ylostalo P 1997, "Increased nuchal translucency as a marker for fetal chromosomal abnormalities", *New England Journal of Medicine*, vol. 337, no. 23, pp. 1654-1658.

Taipale P, Ämmälä P, Salonen R & Hiilesmaa V 2004, "Two-stage ultrasonography in screening for fetal anomalies at 13-14 and 18-22 weeks of gestation", *Acta Obstetrica & Gynecologica Scandinavica*, vol. 83, no. 12, pp. 1141-1146.

Tercyak KP, Johnson SB, Roberts SF & Cruz AC 2001, "Psychological response to prenatal genetic counseling and amniocentesis", *Patient Education & Counseling*, vol. 43, no. 1, pp. 73-84.

Ternby E, Ingvaldstad C, Anneren G, Lindgren P & Axelsson O 2015, "Information and knowledge about Down syndrome among women and partners after first trimester combined testing", *Acta Obstetrica et Gynecologica Scandinavica*, vol. 94, no. 3, pp. 329-332.

The Danish Council of ethics 2000, *Screening - a report*, The Danish Council of Ethics, Copenhagen.

Theodoropoulos P, Lolis D, Papageorgiou C, Papaioannou S, Plachouras N & Makrydimas G 1998, "Evaluation of first-trimester screening by fetal nuchal translucency and maternal age", *Prenatal diagnosis*, vol. 18, no. 2, pp. 133-137.

THL 2014, "Synnyttäjät ja synnytystoimenpiteet sairaaloittain 2012–2013" in *ISSN: 1798-0887; Tilastoraportti*.

Timmerman E, Pajkrt E & Bilardo CM 2009, "Male gender as a favorable prognostic factor in pregnancies with enlarged nuchal translucency", *Ultrasound in Obstetrics & Gynecology*, vol. 34, no. 4, pp. 373-378.

- Timmerman E, Pajkrt E, Maas SM & Bilardo CM 2010, "Enlarged nuchal translucency in chromosomally normal fetuses: strong association with orofacial clefts", *Ultrasound in Obstetrics & Gynecology*, vol. 36, no. 4, pp. 427-432.
- Tonks AM, Gornall AS, Larkins SA & Gardosi JO 2013, "Trisomies 18 and 13: trends in prevalence and prenatal diagnosis - population based study", *Prenatal diagnosis*, vol. 33, no. 8, pp. 742-750.
- Torring N, Petersen OB, Becher N, Vogel I, Ulbjerg N, Danish Fetal Medicine Study Group & Danish Clinical Genetics Study Group 2015, "First-trimester screening for other trisomies than trisomy 21, 18, and 13", *Prenatal diagnosis*, vol. 35, no. 6, pp. 612-619.
- van der Putte SC & van Limborgh J 1980, "The embryonic development of the main lymphatics in man", *Acta Morphologica Neerlandico-Scandinavica*, vol. 18, no. 4, pp. 323-335.
- van Vugt JM, Tinnemans BW & van Zalen-Sprock RM 1998, "Outcome and early childhood follow-up of chromosomally normal fetuses with increased nuchal translucency at 10-14 weeks' gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 11, no. 6, pp. 407-409.
- Verjaal M, Leschot NJ & Treffers PE 1981, "Risk of amniocentesis and laboratory findings in a series of 1500 prenatal diagnoses", *Prenatal diagnosis*, vol. 1, no. 3, pp. 173-181.
- Verweij EJ, Jacobsson B, van Scheltema PA, de Boer MA, Hoffer MJ, Hollemon D, Westgren M, Song K & Oepkes D 2013, "European non-invasive trisomy evaluation (EU-NITE) study: a multicenter prospective cohort study for non-invasive fetal trisomy 21 testing", *Prenatal diagnosis*, vol. 33, no. 10, pp. 996-1001.
- von Kaisenberg CS, Brand-Saberi B, Christ B, Vallian S, Farzaneh F & Nicolaides KH 1998a, "Collagen type VI gene expression in the skin of trisomy 21 fetuses", *Obstetrics & Gynecology*, vol. 91, no. 3, pp. 319-323.
- von Kaisenberg CS, Krenn V, Ludwig M, Nicolaides KH & Brand-Saberi B 1998b, "Morphological classification of nuchal skin in human fetuses with trisomy 21, 18, and 13 at 12-18 weeks and in a trisomy 16 mouse", *Anatomy & Embryology*, vol. 197, no. 2, pp. 105-124.
- von Kaisenberg CS, Nicolaides KH & Brand-Saberi B 1999, "Lymphatic vessel hypoplasia in fetuses with Turner syndrome", *Human Reproduction*, vol. 14, no. 3, pp. 823-826.
- Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L & Mackinson AM 2003, "First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS)", *Journal of medical screening*, vol. 10, no. 2, pp. 56-104.
- Wald NJ, Morris JK, Walker K & Simpson JM 2008, "Prenatal screening for serious congenital heart defects using nuchal translucency: a meta-analysis", *Prenatal diagnosis*, vol. 28, no. 12, pp. 1094-1104.
- Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG & Jackson L 2012, "Chromosomal microarray versus karyotyping for prenatal diagnosis", *New England Journal of Medicine*, vol. 367, no. 23, pp. 2175-2184.
- Ward BE, Gersen SL, Carelli MP, McGuire NM, Dackowski WR, Weinstein M, Sandlin C, Warren R & Klinger KW 1993, "Rapid prenatal diagnosis of chromosomal aneuploidies by fluorescence in situ hybridization: clinical experience with 4,500 specimens", *American Journal of Human Genetics*, vol. 52, no. 5, pp. 854-865.
- Whitlow BJ, Chatzipapas IK & Economides DL 1998, "The effect of fetal neck position on nuchal translucency measurement", *British Journal of Obstetrics & Gynaecology*, vol. 105, no. 8, pp. 872-876.
- Whitlow BJ & Economides DL 1998, "The optimal gestational age to examine fetal anatomy and measure nuchal translucency in the first trimester", *Ultrasound in Obstetrics & Gynecology*, vol. 11, no. 4, pp. 258-261.

Whitlow BJ, Chatzipapas IK, Lazanakis ML, Kadir RA & Economides DL 1999, "The value of sonography in early pregnancy for the detection of fetal abnormalities in an unselected population", *British Journal of Obstetrics & Gynaecology*, vol. 106, no. 9, pp. 929-936.

Wilson JMG, Jungner G (ed) 1968, *Principles and practice of screening for disease*, Geneva: World Health Organization.

Wilson RD, Venir N & Farquharson DF 1992, "Fetal nuchal fluid - physiological or pathological? In pregnancies less than 17 menstrual weeks", *Prenatal diagnosis*, vol. 12, no. 9, pp. 755-763.

Wright D, Kagan KO, Molina FS, Gazzoni A & Nicolaides KH 2008, "A mixture model of nuchal translucency thickness in screening for chromosomal abnormalities", *Ultrasound in Obstetrics & Gynecology*, vol. 31, no. 4, pp. 376-383.

Wright D, Spencer K, Kagan K, Topping N, Petersen OB, Christou A, Kallikas J & Nicolaides KH 2010, "First-trimester combined screening for trisomy 21 at 7-14 weeks' gestation", *Ultrasound in Obstetrics & Gynecology*, vol. 36, no. 4, pp. 404-411.

Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB & Tabor A 2016, "Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147 987 singleton pregnancies", *Ultrasound in Obstetrics & Gynecology*, vol. 47, no. 1, pp. 38-44.

Yang JH, Chung JH, Shin JS, Choi JS, Ryu HM & Kim MY 2005, "Prenatal diagnosis of trisomy 18: report of 30 cases", *Prenatal diagnosis*, vol. 25, no. 2, pp. 119-122.

Yaron Y, Wolman I, Kupferminc MJ, Ochshorn Y, Many A & Orr-Urtreger A 2001, "Effect of fetal gender on first trimester markers and on Down syndrome screening", *Prenatal diagnosis*, vol. 21, no. 12, pp. 1027-1030.

Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, Zhu Z, Lin M, Liu Q, Tian Z, Zhang H, Chen F, Lau TK, Zhao L, Yi X, Yin Y & Wang W 2015, "Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies", *Ultrasound in Obstetrics & Gynecology*, vol. 45, no. 5, pp. 530-538.

Zoppi MA, Ibba RM, Floris M & Monni G 2001, "Fetal nuchal translucency screening in 12495 pregnancies in Sardinia", *Ultrasound in Obstetrics & Gynecology*, vol. 18, no. 6, pp. 649-651.

Zosmer N, Souter VL, Chan CS, Huggon IC & Nicolaides KH 1999, "Early diagnosis of major cardiac defects in chromosomally normal fetuses with increased nuchal translucency", *British Journal of Obstetrics & Gynaecology*, vol. 106, no. 8, pp. 829-833.

