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# Chromosomal microarray in prenatal diagnosis – replacing traditional karyotyping

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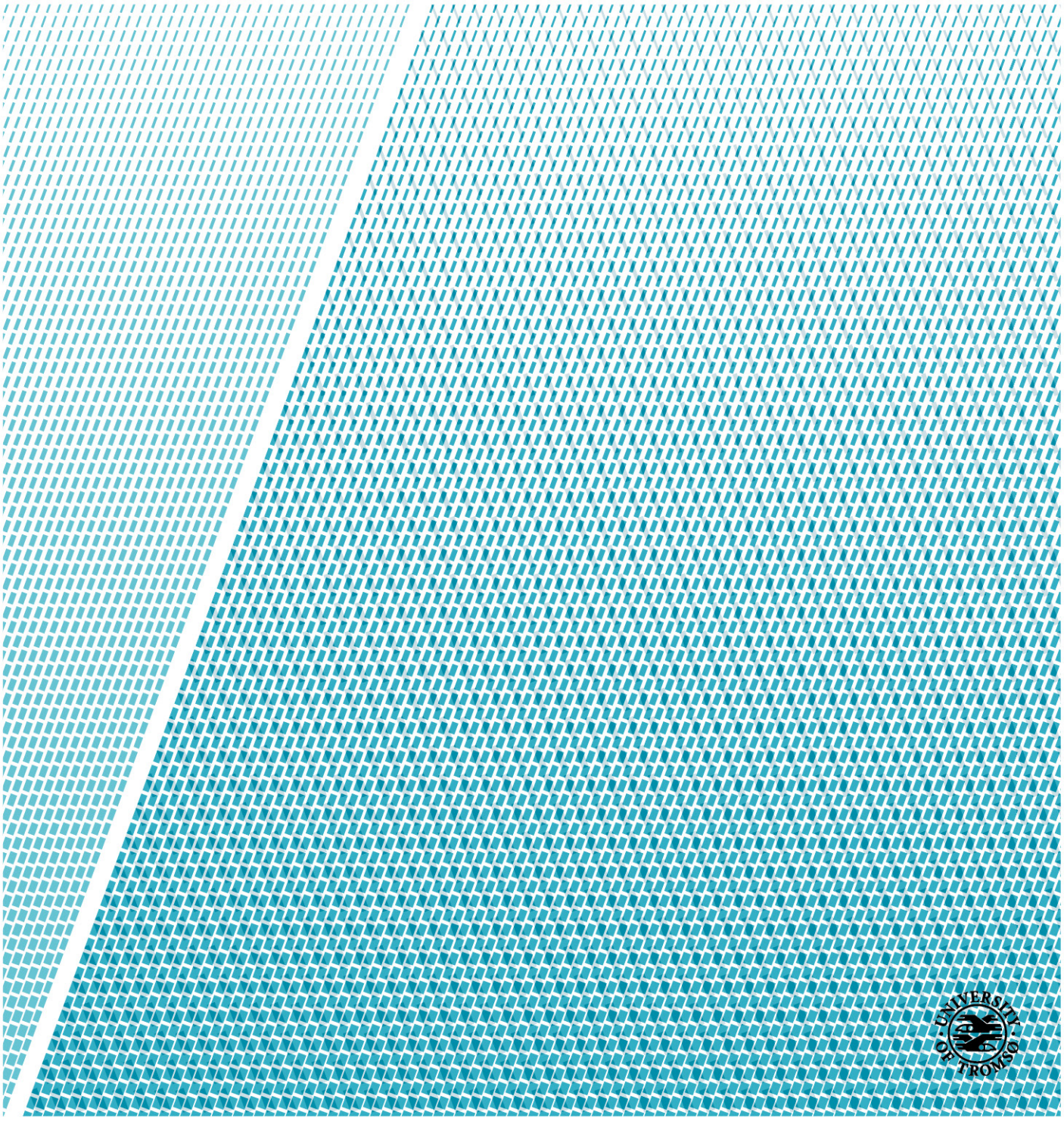
*Master's thesis in medicine MED-3950*



2. June 2019

**Preface**

The purpose of this thesis was to learn about chromosomal microarray (CMA) and its role in invasive prenatal testing and evaluate the findings in a selected North Norwegian population, all in the light of other published studies. In recent years, several papers concerning the developments in both non-invasive and invasive testing has been published. CMA offers higher resolution and detection of smaller copy number variations (CNVs) compared to conventional karyotyping (G-band analysis) but does not reveal all genomic aberrations. Fetal karyotyping was replaced by CMA in the Department of Medical Genetics at University hospital of North Norway (UNN) in 2017. In this study we compared karyotyping results (obtained from December 2015 until August 2017) with CMA results (obtained from August 2017 to December 2018). To my knowledge, this has not been investigated in a Norwegian population.

Ragnhild Glad helped define variables before data collection. Data was collected from the patients' medical records with help from Mona Nystad. I ran the statistical analyses and wrote the thesis with guidance from both supervisors.

I would like to thank my supervisors Mona Nystad and Ragnhild Glad for excellent guidance and support throughout the process. They are both great role models and inspire me to become a better medical physician for my patients. I would also like to thank the Department of Medical Genetics for giving me the opportunity to learn more about the advancing technology in genetic testing and the clinical challenges that prenatal diagnosis raises. At last I have to thank my boyfriend Bjørn-Eivind Kirsebom for support, love and amazing cooking in a period of intensive work.

Tromsø 02.06.19

Alise Marie Winther

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## Abstract

**Background:** In prenatal diagnosis, chromosomal microarray analysis (CMA) has not yet fully replaced conventional karyotyping. As CMA is able to detect smaller genomic imbalances compared to conventional karyotyping, it has become the first-tier test in pregnancies with ultrasound abnormalities.

**Objectives/aims:** The aim of the study was to learn about CMA and its appliance in invasive prenatal testing and evaluate the findings in a selected pregnant population. We intended to discuss diagnostic yield using quantitative fluorescence polymerase chain reaction (QF-PCR) prior to CMA instead of QF-PCR and karyotyping.

**Methods:** Data was collected at the University hospital of North Norway Department of Medical Genetics. The sample comprised 85 women aged 19 – 45 years (*M*=33.12, *SD*=6.6). Between December 2015 and august 2017, QF-PCR and karyotyping were performed in n=43 fetuses from women aged 19 - 44 (M=34.3, SD=6.4). Between September 2017 and December 2018 QF-PCR and CMA were performed in 41 fetuses from women aged 19 – 45 years (*M*=31.8, *SD*=6.7).

**Results:** In the Karyotyping group, 18.6 % of the fetuses had a results of clinical importance (trisomy, monosomy and mosaic trisomy). In the CMA group, 24.3 % of the patients had a copy number variant (CNV) which were either pathogenic (class 5), likely pathogenic (class 4) or a variant of uncertain significance (VOUS). Only a small fraction (4,8%) of the CNVs in the CMA group were classified as class 4-5 and reported to the patients. Only one of these CNVs would have been detected by karyotyping and only one was detected by QF-PCR.

**Conclusion:** As the purpose of this thesis was to learn about CMA and its role in invasive prenatal testing and evaluate the findings; we found CNVs that would not have been detected using karyotyping alone in the CMA group. Supporting the literature describing benefits for changing the invasive testing methods. However, findings of uncertain clinical significance challenge the genetic counselling. Therefore, a national collection of data concerning prenatal diagnosis in the Norwegian population should be considered.

## Abbreviations

aCGH – array comparative genomic hybridization

CMA – chromosomal microarray analysis

CNV – copy number variant

CUB – combined ultrasound and blood test

DD – developmental delay

DNA – deoxyribonucleic acid

ID – intellectual disability

LCSH – long continuously stretches of homozygosity

LOH – loss of heterozygosity

NGS - next-generation sequencing

NT – nuclear translucency

OMIM – Online Mendelian Inheritance in Man

QF-PCR – Quantitative fluorescent Polymerase Chain Reaction

PAPP-A – Pregnancy associated plasma protein A

SL – susceptibility loci

SNP – single nucleotide polymorphism

TAU – transabdominal ultrasound

TVU – transvaginal ultrasound

T21 – trisomy 21

T18 – trisomy 18

T13 – trisomy 13

UPD - uniparental disomy

VOUS – variant of uncertain significance

WES - whole exome sequencing

β-hCG – beta human choriongonadotropin

# 1 Background/ Introduction

## 1.1 Norwegian biotechnology law and indication of prenatal diagnosis

The biotechnology law in Norway defines prenatal diagnosis as investigation of the fetus, fetal cells, or the mother, with the intention to gather information of the fetus genetic characteristics. Ultrasound is therefore seen as part of prenatal diagnosis only if its performed with the intention of detecting or excluding disease or aberrations in fetal development (2). Approximately 4 % of Norwegian children are born with organ anomalies (3). According to the national birth register in Norway, in 2017 there were 219 of 57 930 fetuses that had a chromosomal abnormality, which accounted for about 0.4 % (3). Some of these may be caused by rare conditions or syndromes due to underlying aberrations in the DNA of the fetus. The aim of prenatal diagnosis is to identify these pregnancies in order to map out the risk factors, foresee and consider prognosis, and find the best way to aid the couple or mother throughout the pregnancy, during labor and after birth. This includes treatments when possible. In Norway all women are offered ultrasound scanning from gestational week 17 – 19 (2). First trimester ultrasound scan is considered prenatal diagnostics in Norway and therefore only offered patients with known risk factors such as advanced age, exposure to teratogens, or having known genetic diseases or risks in their family. Other indications for a first trimester scan may be challenging circumstances in life that complicate having a child with great disability. Anxiety alone is not an indication for prenatal diagnosis, but may be an indication of a first trimester ultrasound examination. For this reasons, today only a small group (about 11 %) of Norwegian women are given the choice of prenatal screening testing (2). The main indication for testing in 2017 was maternal age above 38 years (48 %) and abnormal ultrasound findings counting for 22 % of cases (4).

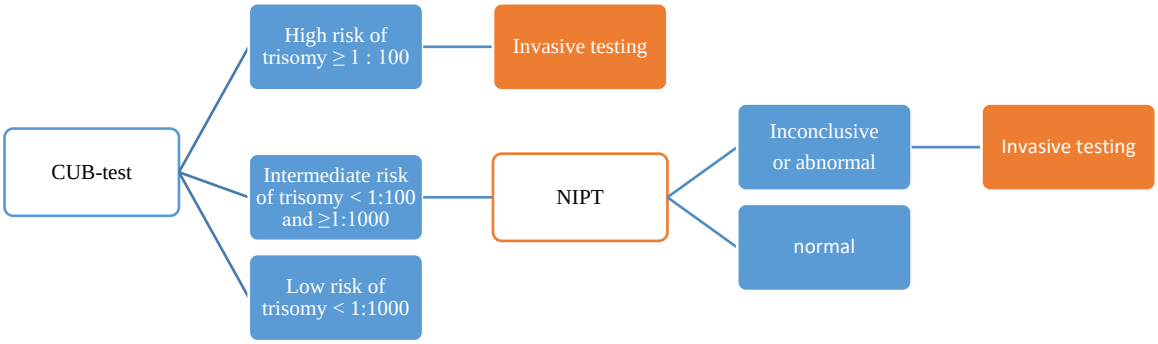


**Figure 1 Schematic representation of the order of the screening tests.** Indications for invasive testing are depicted. The CUB-test are initially offered to patients with known risk factors as advanced maternal age, exposure to teratogens ect. The screening is performed in the first trimester.

In prenatal diagnosis both non-invasive and invasive techniques are used. The non-invasive techniques are a combination of ultrasound biometrics and biochemical serum markers. Combined Ultrasound Blood (CUB)-test consist of the biochemical serum markers pregnancy-associated plasma protein A (PAPP-A) and free β-human choriogonadotropin (β-hCG) in combination with ultrasound biometrics. Together these values calculate a score of risk. In addition, a maternal blood test collecting cell free fetal deoxyribonucleic acid (cffDNA) fragments known as a non-invasive prenatal test (NIPT) are offered. The test is used as screening and the high risk (differently defined) group is offered invasive testing (*figure 1*). Invasive tests involves gathering material from the placenta or amniocentesis, and constitutes a 2 % risk of pregnancy loss (5).

## 1.2 Ultrasound in prenatal diagnosis

While ultrasound does not provide genetic information about the fetus (5), it is recognized as a safe screening tool for the detection of fetal malformations and useful in prenatal phenotyping. The technique is non-invasive, and there is no known associated risk of miscarriage. Early in pregnancy it is preferable to use transvaginal ultrasound (TVU) for examinations of the fetus, while transabdominal ultrasound (TAU) scanning is more commonly used in second trimester. The limitations attached to ultrasound screening are important to note, owing in part to the access of visibility which may be impeded by maternal high body mass index or how the fetus is situated in the uterus. In addition, one cannot be certain that an observed abnormality is isolated from ultrasound examinations, no matter how experienced the operator is (6).

Abnormalities may also develop with time and therefore repeated examinations may be required for making an accurate diagnosis. The detection rate of anomalies found in ultrasound scans depend on several factors. The type of anomaly may present different detection rates according to the nature of the specific anomaly. Some structural abnormalities are easy to diagnose due to their prominent visibility; others are more difficult. Garne *et al*., 2010 showed that detection rate of a hypoplastic left heart was 65 % and gastroschisis 85 % (7, 8). Timing is another important factor, because gestational week of pregnancy determine what to investigate during the ultrasound examination (9). Some "soft markers", like the lack of nasal bone or nuchal translucency (NT) thickness (10), should be considered in the first trimester, while other signs are rarely visible before the second trimester. Microcephaly and agenesia /hypoplasia of the corpus callosum are hardly ever detectable in the first trimester, while reference values for NT is limited to examinations from week 10 to 14 (11). Nuchal fold increases with gestational age at about 17 % and disappears when the subcutaneous tissue becomes more echogenic. Consequently, a normal first trimester scan does not exclude the possibility of abnormal findings in the second trimester, and parents should therefore be carefully counselled about the limitations of anatomy assessment in the first trimester (6). First trimester screening studies using TVU/TAU scanning for fetal abnormalities, show a detection rate in the range of 31 – 65 % in low risk population and 54 – 74 % in high risk pregnancies (8). The detection rate of combining both first, and second trimester scan, increase the detection rate of anomalies to as high as 97.4 % (8).

### 1.3 Biochemical serum markers in prenatal diagnosis

Serum markers that may be measured in prenatal diagnosis are PAPP-A, β-hCG, estriol, α-fetalprotein and NIPT. The measured concentrations of either PAPP-A or free β-hCG are converted into the multiples of median (MoM) appropriate to the gestational age of each pregnancy (12). PAPPA-A is a large glycoprotein produced by the placental syncytiotrophoblast and decidua. In a healthy pregnancy the maternal serum PAPP-A increase exponentially (11). Its function is thought to be multiple as it plays a role in several mechanisms concerning prevention of recognition of the fetus by the maternal immune system, matrix mineralization, and angiogenesis. If the value of the PAPP-A protein is low, <0.4 MoM, it may indicate a poor early placentation, which in turn may lead to other complications such as IUGR, preeclampsia or still birth. (13). The concentration of PAPP-A may be different in fetuses with genetic conditions, but is a poor predictor alone, since it has a high false positive rate. We therefore use it in combination with β-hCG values and NT measurements. β-hCG is a

glycoprotein hormone normally found in blood and urine only during pregnancy. Its function is to initiate and maintain pregnancy by influencing and mediate multiple placental, uterine and fetal functions. In a healthy pregnancy the value drops after an initial increase between week 10 to 14. A value above 1.5 MoM is considered to be associated with T21. One recent study in Poland showed that free β-hCG MoM over 1.5 was seen in 85% of cases with T21, but only 53 % had PAPP-A MoM below 0.5. In most of the cases with high β-hCG and normal PAPP-A value, the NT measurement was increased. This shows that combining ultrasound soft markers, like NT, with serum-markers, enhance the probability of detecting specific chromosomal abnormalities as trisomies (11, 13) and certain other genetic syndromes (14-17). Sensitivity for detection of T21 by combining first trimester scan, double test (free β-hCG and PAPP-A), maternal age over 35 years together with NT varies from 85 – 95 %, and is possible to implement from weeks 8 – 14 (10). Also, it is possible to detect short fetal DNA sequences released from the placentas apoptotic syntiotrophoblast cells in maternal blood. This method is called NIPT, as mentioned earlier, and is an expensive test. Test result using NIPT may be difficult to interpret in conditions like high maternal body mass index, twin pregnancy, or mosaicism. The NIPT test is therefore not a diagnostic test, but a screening test with improved quality. One study from the United States (US) showed a high positive predictive value overall (87.2 %) concerning sex-chromosomal aneuploidies, microdeletions, and trisomy 13, 18 and 21 (18).

### 1.4 Genetic testing in prenatal diagnosis

There are different methods of performing genomic testing. Array comparative genomic hybridization analysis (aCGH) and single nucleotide polymorphism (SNP)-array are both used for detection of deletions and duplications among other gross chromosome abnormalities. While gene mutations are detected through analysis by next generation sequence analysis (NGS), which enables many genes to be sequenced together in a single test. Genome exons represent 1 – 2 %, and consist of protein coding genes. Mutations in exons may therefore be of interest concerning genetic disorders. Gene-panels, all exons (exomes or whole exome sequencing (WES)) or whole genome sequencing (WGS) approaches may be used. However, in the prenatal setting, Norway performs NGS only where the fetus has ultrasound findings like with e.g. skeletal dysplasia where NGS-panels with skeletal gene panels are run on prenatal samples.

### 1.4.1 Quantitative fluorescens polymerase chain reaction

Quantitative fluorescens polymerase chain reaction (QF-PCR) is used for detection of specific aneuploidies in prenatal diagnosis. It is performed from the amniocentesis or CVS and gives the patient a rapid aneuploidy detection. The test detects chromosome copy number by amplification techniques of the most common aneuploidies trisomy 13, -18, -21 and sex chromosomes. Also, QF-PCR may be useful if time is an issue, e.g. if the mother is developing complications during pregnancy, like preeclampsia.

### 1.4.2 Karyotyping

Conventional karyotyping (G-banding) is a method used to investigate fetal cells obtained from invasive procedures. It is a visual technique where chromosomes are isolated, labelled by Giemsa stain and characterized in the microscope. Homologous chromosomes are paired according to size, banding patterns and centromere location to reveal the structural features of each chromosome (10). The process of the karyotyping method depends on dividing cells for isolation of metaphase chromosomes. Karyotyping is limited by the resolution of the light microscope and cannot detect duplications or deletions less than 5 megabases (Mb) (10-12) in size and also by the subjective evaluation of the technician. The fact that karyotyping also depends on cultured cells to grow, makes it a time consuming method (1) as well as labour intensive, with the technician being able to handle only about 250 samples per year (11). The benefits of G-banding is that it may detect balanced translocations, which provides important information regarding recurrence risk in future pregnancies. It may also identify mosaicism.

## 1.5 Chromosomal Microarray analysis in prenatal diagnosis

Chromosomal microarray analysis (CMA) is a form of molecular cytogenomic technique where we look at the DNA of the fetus in a submicroscopic level, enabling detection of aberrations as low as 0.7 kilobases (Kb) (19, 20). This form of higher resolution is able to detect copy number variants (CNVs) in the genome in form of deletions (one copy) and duplications (three copies or more) from thousand base pairs to several Mb in length (21-24). These changes are less correlated with maternal age (25, 26). Fetal material suitable for CMA are chorionic villi, amniotic fluid, fetal blood, fetal pleural effusion or fetal urine. There are two main types of CMA currently performed: array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) microarray (1). The benefits of using CMA is a high sensitivity value, and a faster turn-around time due to the possibility of using uncultured, or even non-dividing material. CMA can also detect early onset disorders such as Duchenne muscular

dystrophy, and mosaics down to about 30 % (27, 28). Balanced chromosomal aberrations and localization of extra chromosomal material, will not be detected by CMA. Neither can all small CNVs and point mutations be detected.

### 1.5.1 The difference between aCGH and SNP method

Array comparative genomic hybridization (aCGH) analysis compares the patient's DNA-sample to a normal reference DNA-sample reference. Both samples are fluorescently labelled with two different colors and hybridized to a chip containing the genome (*figure 3*). By measuring the fluorescent signals with a scanner, a plot generates from this hybridized microarray where the ratio between the sample and the reference DNA can be seen. An excess will imply a duplication, whereas a deficiency of test DNA will imply a deletion. The disadvantage of the aCGH is that it cannot identify triploidies because the chromosome which is redundant is simultaneously increased. The SNP array method do not use a comparative reference DNA sample, but instead determine the genotype of the highly polymorphic regions of the DNA between individuals, which are called different SNPs (*figure 2 and 4*).

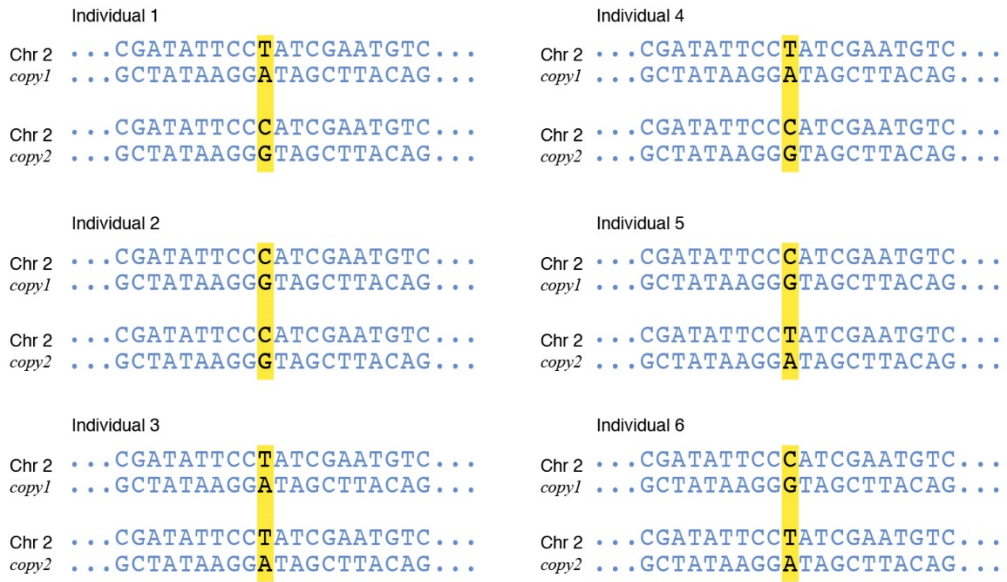


**Figure 2 Schematic representation of different SNPs in different individuals.** SNP are a type of polymorphism in the genome involving variation of a single base pair and can be correlated with risk of disease in regions of interest in the genome. Here we see different individuals with their chromosome 2 homologs and how they differ in single base pair. Courtesy: National Human Genome Research Institute (29).

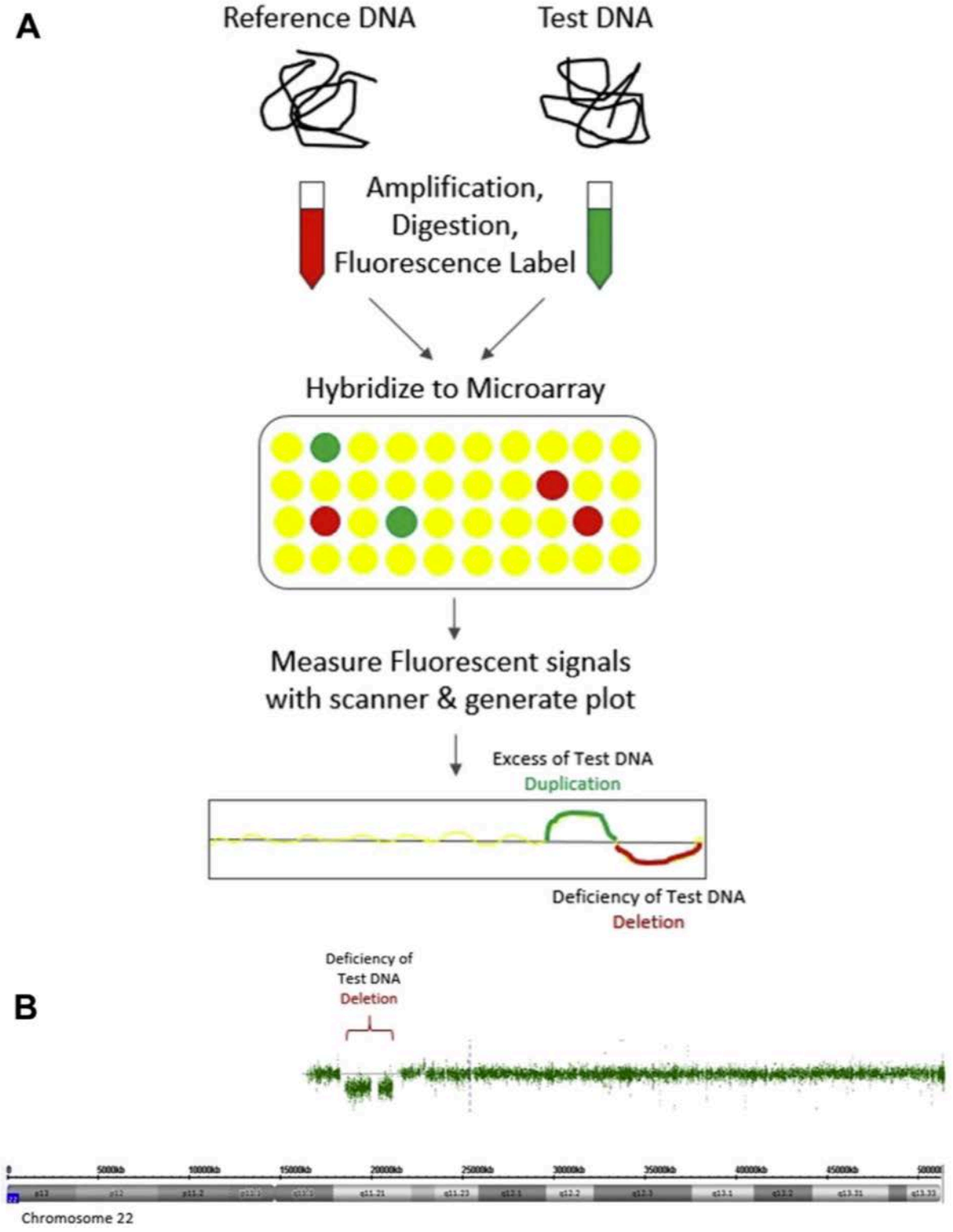


**Figure 3 Array CGH analysis** (1).
(A) After amplification, digestion and labeling processes the reference DNA and test DNA both hybridize to the array. A scanner generates a plot of the fluorescence signals which determinate the ratio of reference to test DNA at each fragment on the array. Red shows a deletion, green a duplication. (B) A plot from an aCGH analysis of a patient with DiGeorge deletion syndrome.

These areas of polymorphisms must occur with an allele frequency of at least 1 % in the population to be defined as SNPs. These germline point mutations, are naturally and statically occurring in the course of evolution (19). In a single human DNA there will be around 4 to 5 million SNPs, occurring almost once in every 1000 nucleotide on average. Most of the time a single SNP gives us sparse information about a person. In order to find an association between SNPs and disease, one has to look at multiple SNPs across the DNA. Each combination of SNPs is called a haplotype. Of all the possible haplotypes there are usually only a few of the combinations that actually exist in the population, and all humans have a pair, one from each parent. The haplotype pair can be seen as an individual's own SNP profile, as shown in *figure 2*. The principles of Affymetrix SNP array technology are shown in *figure 4*. First DNA is digested to fragments of varying lengths by restriction enzymes. Subsequently, the fragments of the DNA are ligated to adapters to enable a one-primer PCR to produce even smaller fragments of selected size (200-1100 bp) (19). After the fragments are labeled with fluorochrome and hybridized to the microarray, the DNA fragments from the sample can find the probe containing its perfect match of nucleotides forming a unique haplotype, or a SNP profile. There are two different types of probes analyzing test DNA; one which identifies SNPs and the other identifies CNVs (up to eight copies). Unlike aCGH the SNP arrays are therefore able to identify triploidies, homozygosity areas in the genome, or even areas of uniparental disomi (UPD) (1, 19). In the case of UPD, the fetus inherits both chromosomes from either of the parents (having a chromosome of two identical haplotypes/SNP profiles), instead of getting one from each, which may lead to known syndromes like Angelman or Prader-Willi.

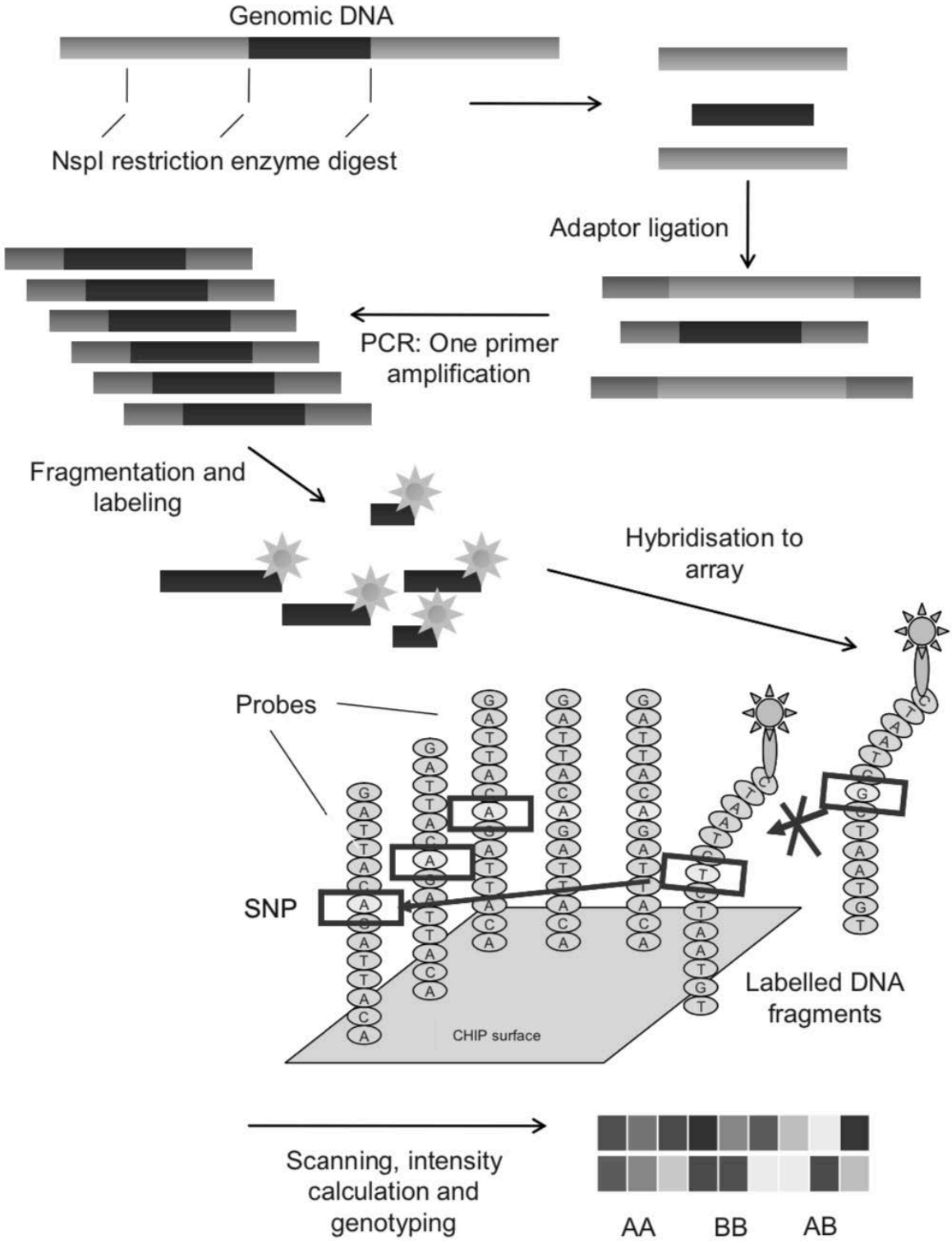


**Figure 4 Principle of Affymetrix SNP array technology.** Restriction enzymes digest test DNA to fragments of varying lengths, and they are next subjected to ligation to adapters. PCR primers then produce fragments of selected size which are labelled with fluorocrome so that the DNA fragments can be hybridized on the array. In the array the SNPs in the test DNA find their complementary match in the probes. After washing, the hybridized array is then scanned by a laser. The raw data can then be calculated into intensity of fluorescent signals in form of the DNA copy number and determination of SNP alleles to form a genotype (19).

### 1.5.2 Indications of performing CMA

At the University hospital of Northern Norway, CMA is used when non-invasive screening procedures indicates invasive testing. Indications for invasive testing are structural malformations (also isolated malformations) detected by ultrasound scans, known risk of genetic disease from family history, fetal intra uterine growth restriction (IUGR), or positive risk assessment results from CUB-testing or NIPT.

### 1.5.3 Detection rates of CMA in published literature

The prevalence of CNVs using CMA, and its relation to specific ultrasound anomalies varies between studies. The phenotype links to CNVs are mostly studied in postnatal settings, which makes phenotyping in prenatal diagnosis challenging. The most common anomalies associated with CNVs occur in the cardiac, skeletal, urogenital, renal and central nervous system (CNS) (28). Stosic *et al.*, 2017 summarized in a review article that CMA may detect CNVs with well-defined clinical significance in up to 1.7% with a normal ultrasound and karyotype (30). If abnormalities are detected by ultrasound, the detection rate rises up to 6 % (1). However, the percentage is reduced to 5.6 % if the ultrasound finding is isolated. The lowest prevalence is shown for isolated NT (3.1 %), indicating that this is a soft sign with low specificity. Similarly, in a systematic review, De Wit *et al*, 2014 summarizes that the chance of carrying a causative submicroscopic CNV, when an ultrasound anomaly is present, varies from 3.1 – 7.9 %, depending on the anatomical system affected. This increases to 9.1% for fetuses with multiple anomalies (31). Oneda *et al.*, 2017 discuss findings showing incremental yields of using CMA in fetuses with different types of ultrasound anomalies when karyotype is normal (28). In fetuses with congenital heart disease (CHD) with normal karyotype, CMA has shown to yield about 7 % additional clinically valuable information (32). As CHD is a common birth defect (0.5 – 0.7 %), findings concerning the yield of CMA for this defect is more reliable than less common defects. A small cohort comprising only 46 fetuses with CNS anomalies, identified pathogenic CNVs in 5 of 46 cases (10.9 %) (33). However, the sample size was relatively small and CNS anomalies are a largely heterogeneous group. Thus, additional confirmation in larger cohorts are needed to establish rates of incremental yield for CNS anomalies. Vogel *et al.,* 2018 recently published data showing a significantly higher detection rate using CMA compared to karyotyping in a Danish population with increased risk using first trimester screening tests (34). Therefore, Denmark also perform prenatal diagnosis on fetuses with high risk estimated by screening tests. In summary, the literature seems to support an increased diagnostic yield using

CMA in prenatal diagnosis, especially when ultrasound findings are present. However, one should carefully consider the type of anomaly found and the relation to the CNV detected.

### 1.6 Genetic counselling and ethical considerations in prenatal genomic testing

The array detects more clinically relevant anomalies including early onset disorders, not related to the indication and more genetic anomalies of yet unquantifiable risk, so called susceptibility loci (SL) for mainly neurodevelopmental disorders (35). These inconclusive findings challenge counselling about risk, clinical significance and possible considerations for the pregnant couple. However, by exploring the couple's desires regarding the level of information they want concerning their fetus health, coupled with their practical, medical, emotional and ethical views can help aid the parents arrive at a decision that is best suited for them. Sometimes this requires several posttest genetic counselling sessions, and may challenging due to time constraints. However, the benefits of prenatal genetic testing are many. This includes providing reassurance when results are normal, identifying disorders where *in utero* treatment may be needed, and optimizing neonatal outcomes. However, prenatal diagnosis also provides the option of pregnancy termination for individual families when it is the right choice for them. Consequently, the choice of prenatal testing is up to the patient alone with help from the individuals chosen to be included in the decision.

### 1.7 Aim of the study

The purpose of this thesis was to learn about chromosomal microarray (CMA) and its role in invasive prenatal testing and evaluate the findings in a selected North Norwegian population, all in the light of published studies. In recent years, several papers concerning the developments in both non-invasive and invasive testing has been published. Clearly, like other fields of medicine, the advances of genomic medicine are impacting prenatal diagnosis,. If the non-invasive screening test indicate higher risk of trisomy's or fetal abnormalities, the couple is offered invasive diagnostics testing. Until August 2017, conventional karyotyping was the choice of invasive diagnostic testing at the Medical Genetic department at University Hospital in Tromsø. However, karyotyping has now been replaced by the CMA method.

# 2 Material and Methods

## 2.1 Study design and data

This study is a historical cohort study comparing two group of patients by descriptive data, and the genetic findings. The patients in 2016 were offered karyotyping as invasive prenatal testing, and the patients in 2017 were offered CMA as invasive testing. Both groups underwent QF-PCR. Data was gained retrospectively by going through patients' medical records in "distribuent informasjons og Pasientdatasystem i sykehus" (DIPS), biometrics in the service application Partus and the lab results in MedGen-datasystem.

## 2.2 Karyotyping

Standard G-band analysis were performed (36). According to the labs routine, 11 cells with metaphase chromosomes were analyzed. In cases of suspected mosaicism, 30 cells were analyzed.

## 2.3 Quantitative fluorescence PCR analysis

Quantitative fluorescence PCR (QF-PCR) were done using Elucigene kits (37) which consist of 22 short tandem repeat (STR) markers for chromosomes X, Y, 13, 18 and 21. The method allows for prenatal diagnosis of the most common aneuploidies in a few hours, and is routinely used along with CMA or karyotyping techniques. Also, it is inexpensive and one single operator may perform up to 40 samples a day (37). The disadvantage is that it may be contaminated with maternal cells and therefore be a source of false positive results. Purified DNA from amniocentesis or chorionic villous samples were examined by semi-quantitative methods which included allele discrimination.

## 2.4 CMA analysis

In Tromsø, the CMA chip used is the Cytoscan HD from Thermo Fisher Scientific (earlier Affymetrix). It detects loss or deletions above 30 probes and gain/duplications above 90 probes. With the settings of choice (loss 30 probes, gain 90 probes, 5 Mb and 50 SNP markers for areas of homozygosity) one may detect unbalanced chromosomal aberrations down to 12 Kb (loss) and 36 Kb (gain), and areas of homozygosity larger than 5 Mb. The computer programs used in the process of interpretation is Chromosome Analysis Suite (ChAS) from Thermo Fisher Scientific and Cartagenia Bench Lab CNV from Agilent Technologies. In Cartegenia, class 3 – 5 CNV variants are shared with Haukeland University Hospital.

## 2.5 Nomenclature of variants

For all patients included in the study, variants were described using an international system for nomenclature as shown in the guidebook "An International system for Human Cytogenomic Nomenclature" (ISCN 2016) (38). If the results of the array are normal the nomenclature for a male would be presented as: arr(1-22)x2,(X,Y)x1. There is no space between the "arr" and the opening parenthesis, and the sex chromosomes are expressed followed by the autosomes. If the array shows an abnormal result one should list only the aberrations. The specific genome built, e.g. Genome Reference Consortium (GRCh38) synonymous with hg19, are named in the brackets followed by the description of the aberration. The aberration is expressed in the order of the chromosome sequence from the lowest to the highest chromosome, regardless of whether it is a deletion or duplication. The aberrant nucleotides are written in order from pter to qter. One may use commas or underscore between the nucleotides if they are multiple. An example, is the partly 7q trisomy we found in one patient named: arr[hg19] 7q11.23q36.3(77,000,129-159,119,707)x3. This means that the microarray analysis shows a gain in form of three copies (shown as x3) in the long arm of chromosome 7 at the bands 7q11.23 trough 7q36.3 in molecular position 77,000,129-159,119,707 of chromosome 7. This is a very large duplication of 82 120 Kb consisting of 532 genes, and is very rare (*table 2*).

## 2.6 Classifications and interpretation of CMA results

Abnormal test results are only given to the patients if the CNV findings are related to known disease. As much as 12 % of the human genome exhibits CNVs in normal individuals (39). Regularly updated online databases catalogue CMA results from normal individuals (39). It is not uncommon that a CNV interpretation of clinical significance can be complex. We therefore classified CNV findings in different classes (1). Tromsø use three different software programs for analysis and registration of findings: Chromosome analysis Suite (ChAS) for molecular karyotyping and visualization of findings, MedGen in-house registration system for registration of findings and reporting and Cartagenia which contains links to databases of interpretation of findings. If there is a CNV finding in class 3 or above, two clinical laboratory geneticists will start searching their home database which they share with Haukeland university hospital in Bergen. Then they will proceed to investigate the number of genes included in the CNV. If the gene in the region is related to disease, known as an Online Mendelian Inheritance in Man (OMIM)-morbid gene, it is more likely to be of clinical relevance. Subsequently, the clinical laboratory genetics will search the Human Genome Mutation Database Professional (HGMDp) for similar findings reported worldwide, and investigate if there are other publications in

PubMed, the book “Catalogue of unbalanced chromosome aberrations in man” by Schinzel or in other published literature related to the findings (40). The CNV is classified by both of the laboratory geneticists independently, before sending it to a medical genetic specialist who will conclude by evaluating the classified CNV and the clinical relevance it may hold, and whether or not to report the finding.

### 2.6.1 Benign and normal results

Many patients have CNVs that has no clinical consequence. Class 1 and 2 are CNV that are benign, and likely benign findings, respectively. Databases of normal variants as (aDGV) aids in this process. Benign and likely benign results are given out directly from the bioengineer as normal results in MedGen for the medical genetic specialist to convey to the patients in consultations.

### 2.6.2 Uncertain results

A class 3 finding is often called a variant of uncertain significance (VOUS). These CNVs can be categorized as likely benign (3-) or likely pathogenic (3+). In these cases, parental genetic information can be important. The lab always asks for parental genetic samples, but they only run the genetic analysis of the parents if there are CNV in class 3-5. This is mostly due to economic concerns, as the analysis is expensive and this therefore saves unnecessary costs for the laboratory. More importantly, if an apparently healthy parent has the same genotype its more likely to be benign.

### 2.6.3 Pathogenic results

CNVs that overlap genes, or larger regions that are known as critical regions of clinical significance, are likely to be pathogenic. Class 4 is likely of clinical relevance, and a class 5 finding is always pathogenic. Class 4 findings may be a CNV within a genotype that is known to be related to disease, but the phenotypic expressivity may vary (known as variants of vulnerability). An example of a CNV with extreme phenotypic diversity in forms of variable expressivity and phenotypic heterogeneity, is 16p11.2 microdeletion. When it comes to class 4 findings, at least two former patients should have been described with phenotypical features that explain the findings in the patient, and the CNV size and placement should be in accordance with the cases. When it comes to class 5, the policy is that at least 3 patient cases should be described in PubMed with the phenotypical features conformable of the clinical findings, and the aberration should have the same CNV and be overlapping in the same area of the known syndrome.

## 2.7 Population of the study

Before engaging in the study, the regional committee of medical and health research ethics (REK), 2018/1959/REK nord, evaluated the study to be a quality assurance study since this was a retrospectively collected sample of data with no new interventions. For the present study, we selected all women who had indications for invasive testing in prenatal diagnosis in Northern Norway between December 2015 and December 2018. This is a heterogeneous group comprising women who were candidates for the first trimester ultrasound because of a high risk pregnancy and also patients who were admitted for broad testing due to ultrasound abnormalities found in the routine scan in the second trimester. This makes the included sample a mix of both high risk patients, and women in the general population with anomalies findings on ultrasound discovered on their first routine scan. The sample comprised n=84 women aged 19 – 45 years (*M*=33,12, *SD*=6.6). Between December 2015 and august 2017, invasive tests comprised PCR and karyotyping from n=43 women, aged 19 - 44 (*M*=34.3, *SD*=6.4). Between September 2017 and December 2018, invasive tests comprised PCR and CMA from n=41 women, aged 19 – 45 years (*M*=31.8, *SD*=6.7).

## 2.8 Definition of variables

The definitions of variables in the dataset were determined in cooperation between the student and the supervisors prior to the data collection process. We wanted to collect results from both the non-invasive and the invasive testing methods. For the purposes of this study, we opted to collect as much as possible of the available data materials associated with prenatal testing. This included CUB-test results in the high risk pregnancies, structural findings on ultrasound, maternal age, QF-PCR results, karyotyping, and CMA results. The definitions of structural anomalies on ultrasound included both soft markes, intra uterine growth restriction (IUGR), structural anatomical defects, single umbilical artery (SUA), and placenta abnormalities. We only collected CMA results from classes 3 through 5, since these would be the CNVs of clinical interest. The data was plotted in a spreadsheet format in December 2018 by the student with the help from both supervisors.

## 2.9 Data analysis

Findings using karypotyping and CMA were analyzed descriptively and reported casewise in separate tables for each group. Within and between-groups results were compared and discussed. In addition, total number of reported findings with known clinical relevance was compared between groups using a Chi-square test. Between-group differences in demographics

(maternal age and gestational age) were compared using independent sample t-tests. All statistical analyses were performed using the IBM statistical package for social sciences (SPSS) version 25.

# 3 Results

## 3.1 The Karyotyping group

In the karyotype group, n=8 (18.6 %) of the fetuses had abnormal findings. All of the abnormal karyotypes also had an abnormal QF-PCR test result. In this sample, n=18 (41.8 %) of the patients underwent screening with CUB-test (*figure 5*). None of these patients underwent NIPT, as the test was not approved in the time of testing. The mean week of pregnancy when ultrasound screening was performed in the karyotyping group was 15.5 weeks, in order of biparietal diameter (BPD) measurement. Most of the fetuses in the karyotyping group had a normal ultrasound (*figure 6*).

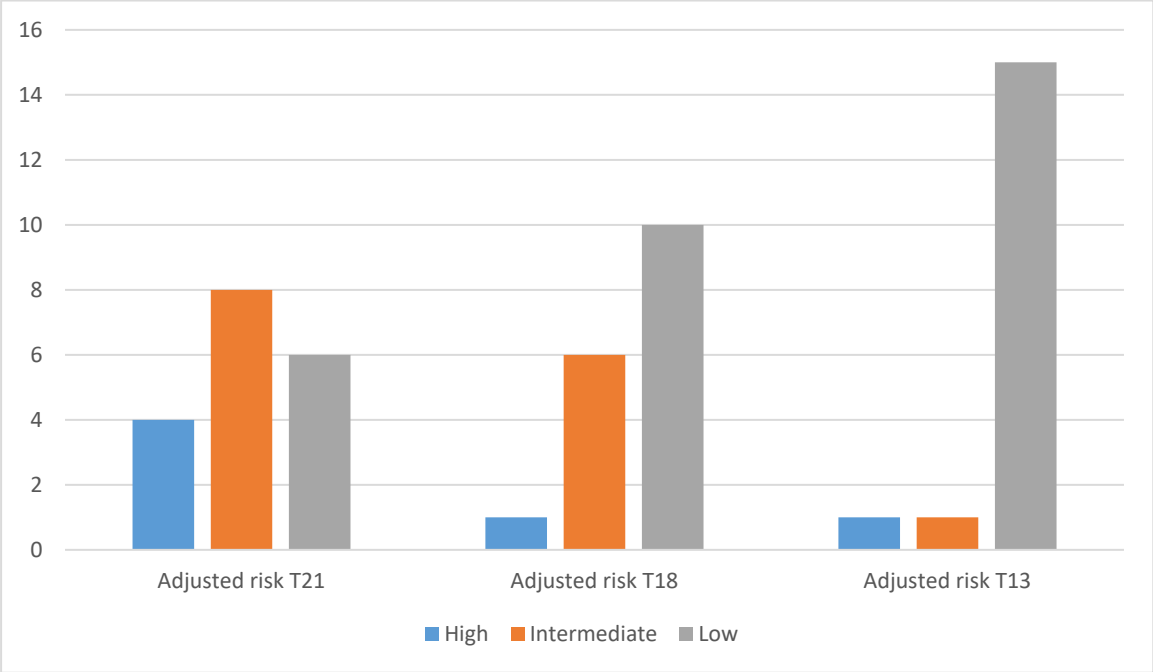


**Figure 5 KUB-test result in the screening process of the high risk pregnancies in the Karyotyping group.** A total of 18 patients had the KUB-test done. Only one patient had test results for trisomy 21 risk available in the journal. That is why there are only 17 in the adjusted risk column for T18 and T13. The Karyotyping group had more women with indication of early ultrasound.

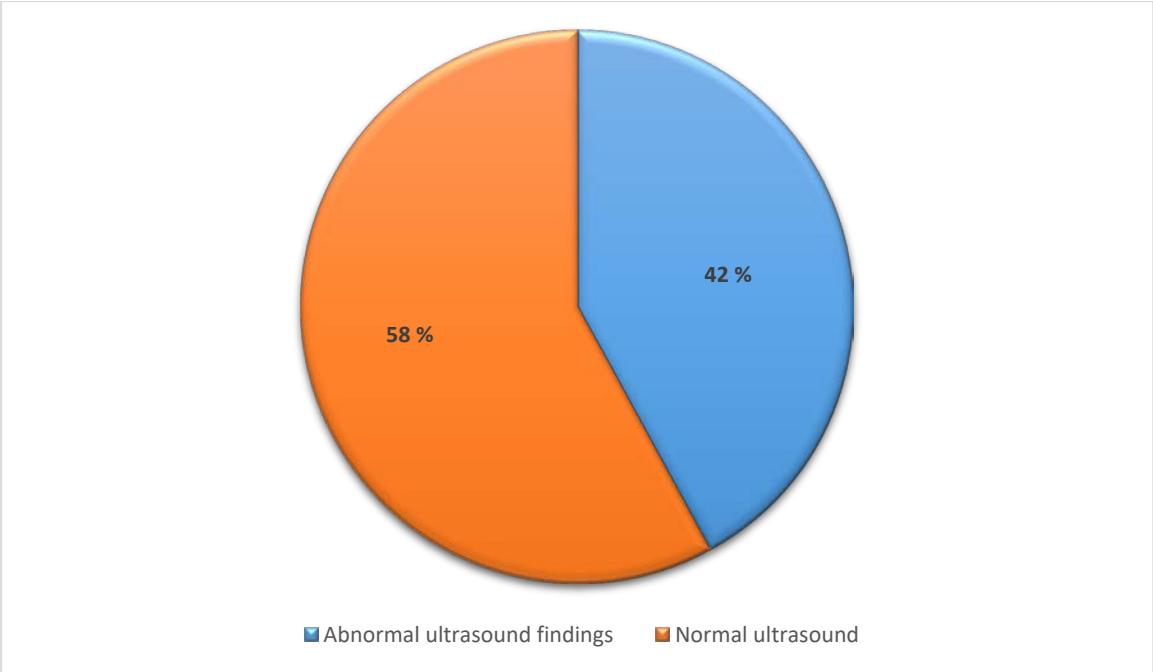


**Figure 6 Ultrasound anomalies in the karyotyping group.** 58 % of the fetuses in the karyotyping group had a normal ultrasound, while 42 % had ultrasound findings.

## 3.2 Genetic findings in the karyotype group

There were two cases of trisomy 21 (T21), three cases of trisomy 13 (T13) and one case of trisomy 18 (T18). Also, there were two cases of Turner syndrome: 45, X (*table 1*). All of the aneuploidies detected had abnormal ultrasound findings. Three of the aneuploidies had additional genetic chromosomal abnormalities (case 2, 4 and 6 in *table 1*). One of the T13 had a Robertsonian translocation. The T21 had the karyotype: mos 47, XX, + 21 [18]/48,XX,+10,21[12]. However, the mosaic in this patient seem to complicate the chromosomal aberration with a partly trisomy of chromosome 10 in addition to trisomy 21 in some of the cultured cells. This was considered to significantly contribute to the health aspects of the fetus. However, this fetus had a normal ultrasound scan, apart from the lack of nasal bone. Also, one of the fetuses with trisomy 13 had both Patau and Klinefelter syndrome, double trisomy, which is extremely rare (41). This fetus presented with the prenatal phenotype of omfalocele and hydronefrosis.

**Table 1** Descriptive findings in the karyotyping group.

| Case | Fetal karyotype | QF-PCR | Ultrasound findings | Major phenotypic features | Reporting of finding |
|---|---|---|---|---|---|
| 1 | 45, X | 45, X | Cystic hygroma Hypoplastic aorta Ductal regurgitation, hydronefrosis | Turner | 45, X |
| 2 | 46, XX, rob(13;14)(q10;q10)+ 13 Robertian translocation | Trisomy 13 | Enlargement of right atrium, ductal regurgitation, holoprosencephaly. hydronefrosis. talipes and enlargement of placenta. | Patau Syndrome | 46,XX, rob(13;14)(q10;q10)+ 13 |
| 3 | 46, XX, + 21 | Trisomy 21 | Nuclear Translucency > 3.5 mm | Down Syndrome | 46, XX, + 21 |
| 4 | 47, XXY, + 13 | Trisomy 13 and Klinefelter | Omfalocele, hydronefrosis | Patau syndrome and Klinefelter | 47, XXY, + 13 |
| 5 | 46, XX, + 18 | Trisomy 18 | Bilateral Plexus choroid cysts, VSD, possible double kidneys | Edwars syndrome | 46, XX, + 18 |
| 6 | mos 47, XX, + 21 [18]/48,XX,+10,21[12] | Trisomy 21 | No nasal bone present | Down syndrome | 47,XX+21 |
| 7 | 45, X | 45, X | Hydrops, nuchal fold, left ventricle anomaly. | Turner | 45, X |
| 8 | 46, XY, + 13 | Trisomy 13 | Omfalocele | Patau Syndrome | 46, XY, + 13 |

### 3.3 The CMA group

Although a total of 15/41 (36 %) patients in the CMA group had first trimester screening in weeks 10 – 13 and underwent invasive testing, only 11 of these underwent CUB screening (*figure 7*). While this may be due to missing test results in some of the patient's medical records, one patient had to be excluded because she was carrying twins. None of these patients underwent NIPT, as the test was not approved in the time of testing. The mean week of pregnancy when ultrasound screening was performed in the CMA group was 16,05 weeks, in order of biparietal diameter (BPD) measurement. A total of n=29 (71%) in the CMA group had abnormal ultrasound findings (*figure 8*). While there were no trisomy conditions, one case was found with a part aneuploidy on the X chromosome, discovered by QF-PCR. There were n=10

(24.3%) cases with CNVs uncovered (including VOUS). Some of the patients with CNV findings had more than one CNV, but these cases were counted in terms of the most severe CNV they had. All findings from class 3 – 5 are described in *table 2*. There were two patients (4.8 %) who had a class 4 – 5 CNV (*figure 9*), and they were therefore classified as pathogenic and reported as abnormal results to the parents (*table 2*).

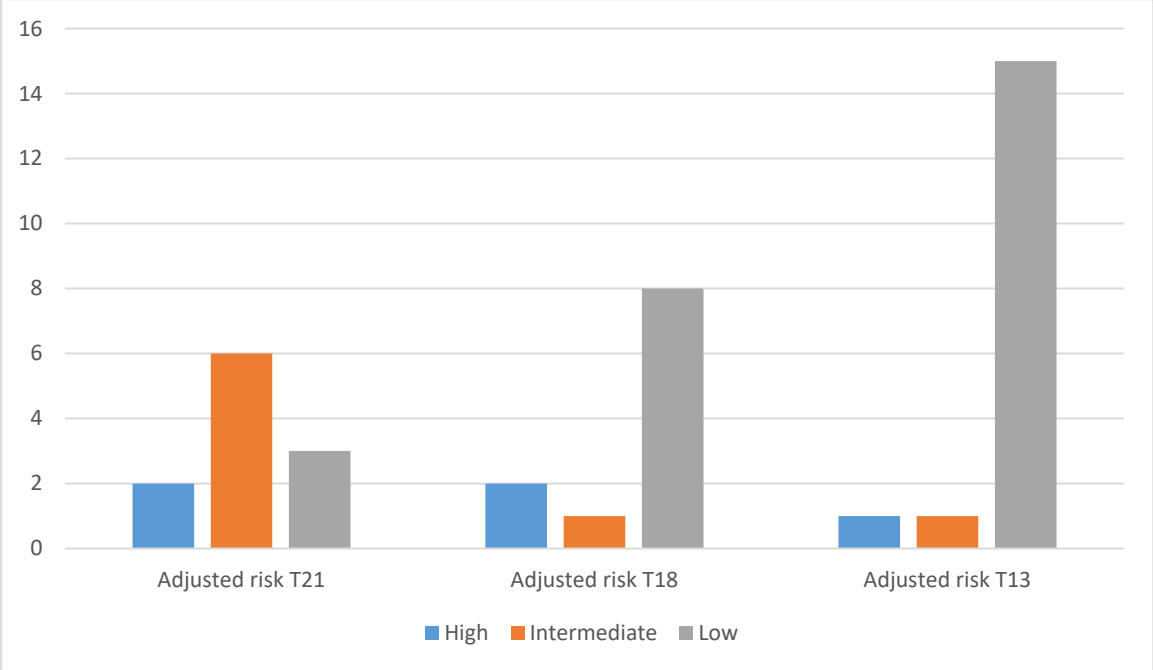


**Figure 7 CUB-test results in the CMA group.** Distribution of the adjusted risk of trisomies estimated using the CUB-test in screening before invasive testing in the CMA group. A total of 11 of the 41 patients had indication for first trimester screening. None of them had a fetus with a trisomy.

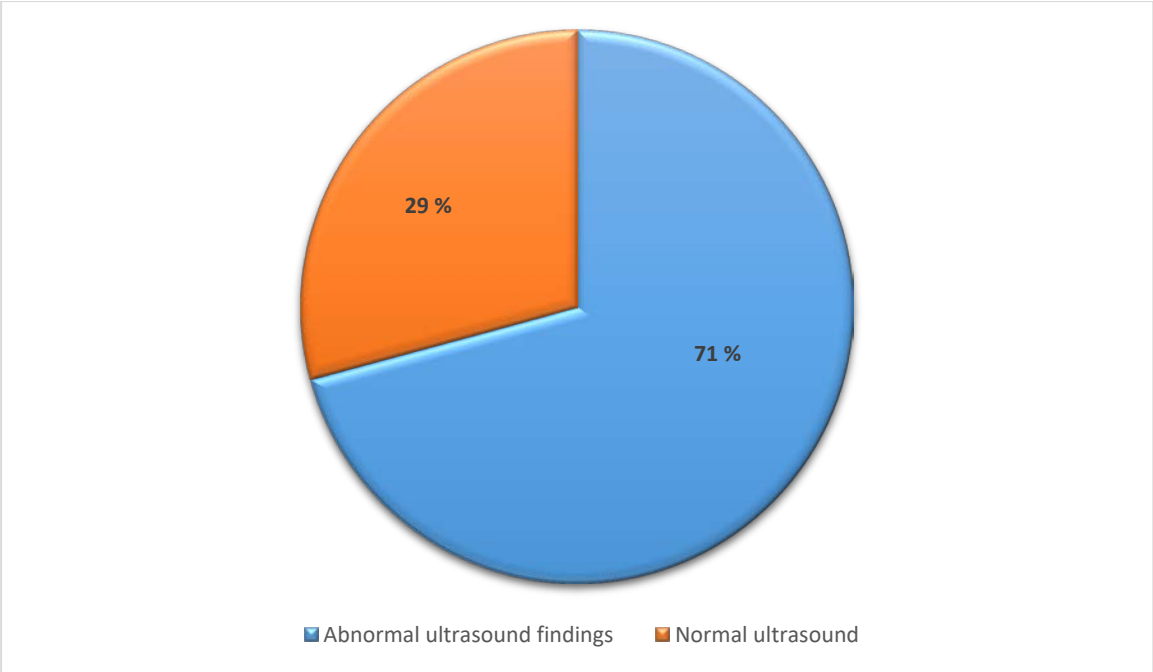


**Figure 8 Ultrasound anomalies in the CMA group.** This may explain why only 36 % of the patients (n=15) in the CMA group had a first trimester ultrasound scan from week 10 – 13. Most of the subjects in the CMA group were probably referred to invasive diagnostic testing due to structural findings on ultrasound screening during second trimester in relation to the national program for antenatal care.

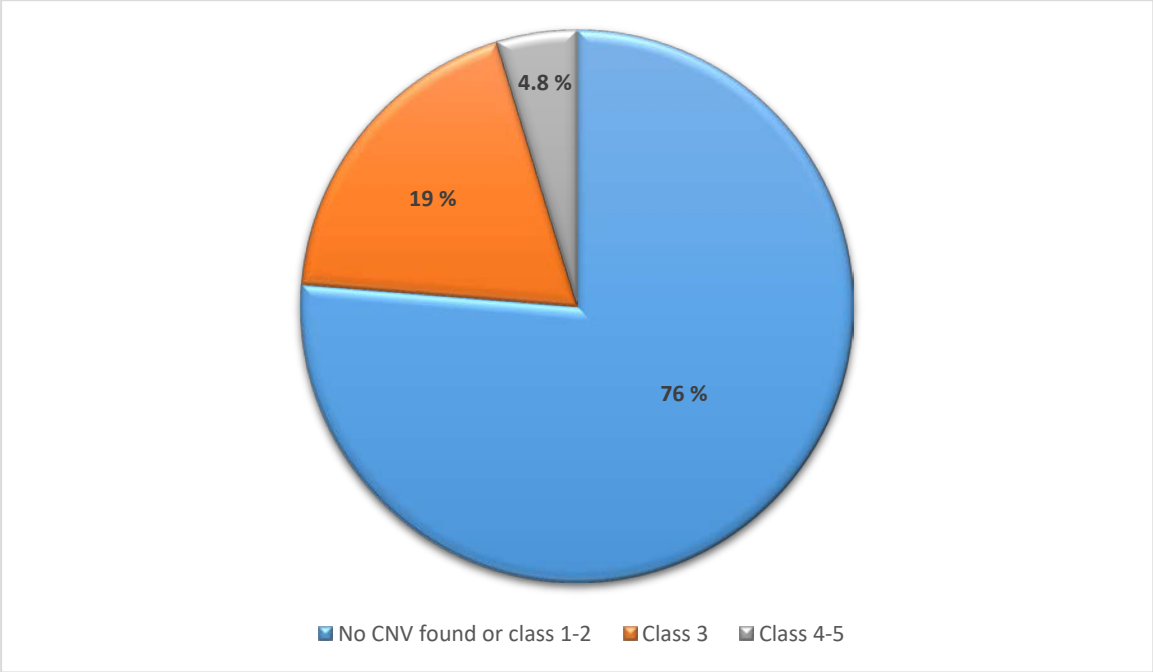


**Figure 9 CNV findings in the CMA group.** Portion of CNV findings in the CMA group.

## 3.4 Genetic findings in the CMA group

In total there were 14 CNVs distributed in 10 fetuses. Eleven of them where submicroscopic (<10 Mb). Therefore, CNVs were described that would not have been detected by using the karyotype method. Eleven of the CNVs were in class 3, classified as VOUS and thus reported

as normal. One class 4 finding (a 22q11.21 duplication) was reported (case 8, *table 2*) who also had a large class 5 finding. Fetuses with 22q11.21 duplication may be normal, have intellectual disabilities, experience developmental delay, growth retardation, or hypotonia. In this case, the class 4 finding was clinically subordinate to the class 5 finding, and both were reported to the parents. Because of the size of the class 5 CNV (the 7q11.23q36.3 duplication), counting for 82 Mb, it would also have been detected on karyotyping. It contained as much as 532 genes, and 73 of them where OMIM-morbid genes. In total there were three class 5 findings in our sample, with two of them occurring in the same fetus (case 9, *table 2*). Both were located on the same X chromosome. The largest was a Xp22.33p11.23 deletion, counting for 46.7 Mb, consisting of 185 genes, where 44 of them was OMIM-morbid genes. This type of deletion is often described in patients with a Turner phenotype. The other was a Xq25q28 duplication, counting for 27 Mb, consisting of 210 genes. 49 of these were OMIM-morbid genes, the MECP2 gene included. Being situated on the X chromosome, the clinical severity will vary between females and males. While females vary in terms of X-inactivation patterns, male fetuses are severely affected. Clinical features may include severe developmental disorder (42, 43). Xq28 is a type of duplication described in both male and females and may result in Xq28 duplication syndrome (44). In this patient QF-PCR was abnormal showing: 46, X, der(X). However, karyotyping was also performed in this patient and found to be normal, even though the size of the two class 5 findings was > 10 Mb.

### 3.4.1 Ultrasound findings and CMA test results

We found that 8 of the 10 patients with CNVs (class 3 – 5) had an ultrasound anomaly. Most were not isolated. These anomalies were only related to two of the CNVs found by CMA testing. The 7q11.23q36.3 duplication (case 8, *table 2*) was likely related to the brain and heart abnormalities as it has been described in other cases with this duplication (45). Also, the fetus had a 22q11.21 duplication which may have contributed to the IUGR. However, the 22q11.21 duplication has a more uncertain penetrance to phenotype, and the contribution to the IUGR is therefore less certain.

**Table 2** Descriptive findings in The CMA Group.

| Case | Class | Origin | Fetal findings Genomic localization (size) | Major phenotypic features described in literature | Ultrasound findings | Reporting of finding |
|---|---|---|---|---|---|---|
| 1 | 3 | Pat | 9p13.3 duplication *(171 Kb)* | | Talipes | Normal |
| 2 | 3 | Mat | Xp22.33 duplication *(871 Kb)* | | Talipes, Eccogenic focus in heart | Normal |
| 3 | 3 | Pat | 16p13.3 duplication *(182 Kb)* | Intermediate talassemia | Acrani | Normal |
| | 3 | Mat | Xp11.23 duplication *(118 Kb)* | | | Normal |
| 4 | 3 | Dn | 8p23.3p23.2 duplication *(1443 Kb)* | | Normal | Normal |
| 5 | 3 | Mat | Xp11.4 duplication *(163 Kb)* | | Myelomeningocele, Hydrocephalus | Normal |
| 6 | 3 | Mat | 3p26.3 duplication *(133 Kb)* | VOUS | SUA, Pulmonary stenosis, Double outlet right ventricle | Normal |
| | 3 | Mat | 3q28 deletion *(8 Kb)* | VOUS | | Normal |
| 7 | 3 | Mat | 2q37.1 duplication *(128 Kb)* | VOUS | Talipes | Normal |
| 8 | 5 | | 7q11.23q36.3 duplication *(82,2 Mb)* | Heart, brain and kidney anomalies. Intellectually; normal to ID. Speech problems, hypotonia, seizures, behavioral abnormalities, movement and walking problems | Aorta stenosis, Brain anomaly | Abnormal |
| | 4 | Pat | 22q11.21 duplication *(2,5 Mb)* | Growth retardation. Normal to ID or DD. Psychomotor developmental-disorder. | IUGR | Abnormal |
| | 3 | Mat | 2p16.3 deletion *(169 Kb)* | VOUS | | Normal |
| 9 | 5 | Dn | Xp22.33p11.23 deletion *(46,7 Mb)* | Turner phenotype | Normal | Abnormal |
| | 5 | | Xq25q28 duplication *(27 Mb)* | Different grades of DD, learning difficulties, distinctive facies. | | Abnormal |
| 10 | 3 | Mat | 16q23.1 duplication *(1950 Kb)* | VOUS | Hydrops | Normal |

*Note.* Abbreviations: ID = Intellectual disability, DD = Developmental delay (1, 44-46)

### 3.5 Comparing demographics and number of findings between groups

The maternal age in the karyotyping group were slightly higher (*M*=34.3, *SD*=6.4), as compared to the CMA group (*M*=31.8, *SD*=6.7). However, the difference did not reach the threshold of statistically significance (*t*=1.7, *p*=.09). While no differences in gestational age (as measured in weeks) between the karyotyping (*M*=15.5, *SD*=3.2) and CMA group (*M*=15.5, *SD*=3.2, *t*=0.7, p=n.s.) were demonstrated, some differences between the groups with regards to gestational age distributions were observed (*figure 10*). Although there were no statistically significant differences in number of reported findings in the karyotyping group (n=8) compared to the CMA group (n=10, $\chi^2$ = 2.06, *p* = n.s.), the small increase in reported findings using CMA may suggest a slightly higher detection rate.

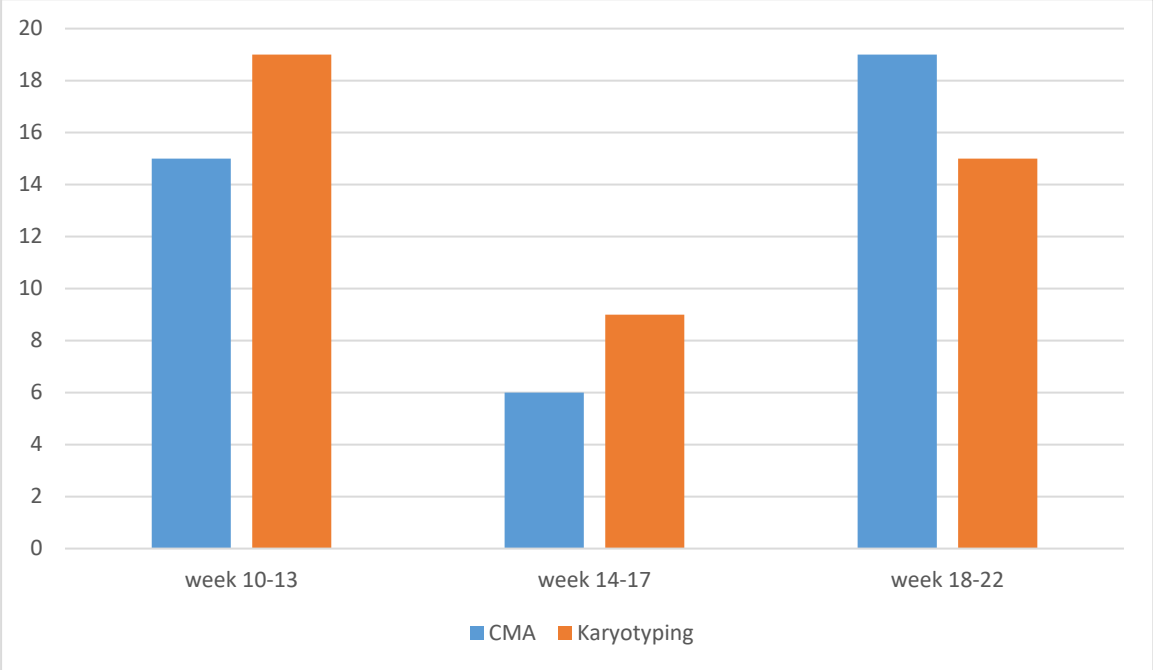


**Figure 10 Gestational age at diagnosis in the CMA and Karyotyping group.** Barchart showing the distributions of gestational age in the two groups.

# 4 Discussion

## 4.1 Important findings

### 4.1.1 Genetic findings in the karyotyping group and the CMA group

We found no statistically significant differences in number of aneuploidies in the karyotyping group compared to CNVs (in class 3 – 5) in the CMA group. However, when we look at the percentage we can see that there are some differences when we use CMA compared to karyotyping. The karyotyping group had 18.6% of aneuploidies in their group, all which would have been detected with QF-PCR or CMA alone. In contrast, the CMA group had 24.3% abnormal findings. That being said, one must be careful in drawing conclusions from these results, since the analyzing techniques were not performed in the same group. As submicroscopic CNVs are not detectable using karyotyping, we do not know how many CNVs could have been detected this group, and CMA analysis may have had added additional diagnostic value. Conversely, karyotyping allows for the detection of balanced translocations, that may be important regarding to the risk of recurrence, which CMA analysis is not able to uncover. If we sort the CNVs (from class 3 – 5) in the CMA group in order of size 11/14 (78.5%) are submicroscopic (<10Mb). However, only one of the class 5 CNVs (the 7q11.23q36.3 duplication, case 8, table 2) would have been detected with karyotyping due to the size of the duplication; being 82 Mb in size and a partly 7q trisomy. The other two class 5 CNVs (case 9, *table 2*) were not detected, even though their size was > 10 Mb. We know this, because karyotyping was performed and found to be normal in this fetus. This illustrates the limits of karyotyping in terms of being a visual technique. The giemsa stain may be situated in an area complicating detection of the chromosome anomaly, or simply bad quality of the chromosomes at examination. As both CNVs were located on the same X chromosome this may also have made the visualization of the chromosome challenging. If this patient would have been tested a year earlier, the class 5 findings would only have been detected as: “46, X, der(X)” on QF-PCR, and the genetic counselling may have been different. While this fetus had normal ultrasound findings, the indication of prenatal diagnosis was high maternal age. In addition to the Xp22.33p11.23 deletion, the Xq25q28 duplication consisted of 210 genes. 49 of these were OMIM-morbid genes, and the important MECP2 gene was included. MECP2 is associated with the MECP2 duplication syndrome. The clinical characteristics is severe neurodevelopmental disorder characterized by several features (infantile hypotonia, delayed psychomotor development, progressive spasticity ect.) (42). Severity of phenotypical deficits

will vary between males and females. While MECP2 duplication syndrome in males is 100 % penetrant, the clinical features in heterozygous females are more challenging to predict. Partly due to the course of X-inactivation patterns. As this fetus was female, the prognosis is difficult to predict. In contrast, a male fetus with these chromosome anomalies has a poor prognosis (42, 43). In addition, the Xq28 syndrome is described in both male and females with duplication in this area of the X-chromosome (44). However, looking at the overall picture of the descriptive findings in this study, all of the abnormal findings in the karyotyping group (except for the Robertsonian translocation), would also have been detected using the CMA technique.

### 4.1.2 Abnormal copy number variants compared to ultrasound findings

In several published reports from other cohorts, findings points to an increased detection of causative findings using SNP array technology in fetuses with ultrasound abnormalities (14, 15, 24). In our sample 29/41 (71 %) patients in the CMA group had an ultrasound anomaly, and as much as eight of the ten patients with CNVs (class 3 – 5) had ultrasound findings. This indicates that CMA is to be the recommended test of invasive testing in pregnancies with ultrasound abnormalities (28). Even though we are not able to draw conclusions saying CNVs are always related to ultrasound findings, we may argue that a such a finding should warrant performing an CMA analysis. Also, this points to the importance of further study of possible contributions to phenotypic anomalies, and the importance of publishing findings for improving knowledge in the field concerning prenatal phenotypes.

## 4.2 Results compared to relevant literature

As mentioned earlier, prevalence rates of CNV's and its relation to specific ultrasound anomalies vary between studies. Our study employed a relatively small sample size compared to larger cohorts (24). Also, it is challenging to compare our results directly to other published studies because of the heterogeneous group of individuals in our sample. Partly due to different indications for invasive of prenatal testing. We may however compare the fetuses in the CMA group with ultrasound finding to some numbers in the literature. For example, in a large cohort, Srebniak *et al*., 2015 showed a detection rate of 4.3 % (44/1033) CNVs explaining the abnormal fetal phenotype found on ultrasound (24). Stosic *et al.,* 2018, states that CMA has proven to detect CNVs with well-defined clinical significance in up to 6 % in cases with fetal abnormalities and a normal karyotype (1). However, in our study we had one 1/29 (3.4 %) fetus who had two CNVs that was related to the fetal phenotype, excluding all fetuses with normal ultrasound. The incremental yield of using CMA method over karyotyping varies in studies

from 1.2 – 2 %, with a incidence for VOUS being 0.3 – 2.7 % when there are no anomalies found on ultrasound (1). In our study there was only one CNV of well-defined clinical significance (class 4 – 5) that had a normal ultrasound scan. However, the QF-PCR was indeed abnormal in this patient (case 9, *table 2*). We found 11 class 3 findings described as VOUS in our sample, only one of these patients had a normal ultrasound. While only 1/29 (3.4 %) of the fetuses (case 8, table 2) in the CMA group with ultrasound findings had a submicroscopic CNV reported as pathogenic (class 4 – 5), Srebniak *et al.,* 2015 found 57/1033 (5.5 %) submicroscopic pathogenic CNVs in fetuses with ultrasound anomalies (24).

## 4.3 Benefits and Strengths of the study

### 4.3.1 First Norwegian data published

To our knowledge, no data from the use of CMA in the Norwegian population has been published. As CMA now has replaced conventional karyotyping as the standard invasive prenatal diagnostic test of choice in Norway, it is important to evaluate if the use of CMA is adequate compared to the conventional karyotyping method. This is especially important in the process of prenatal diagnosis, where the interpretation and reporting of findings to patients are intricate.

### 4.3.2 Limited knowledge of prenatal phenotype

Another important aspect of using CMA is that the more we use CMA SNP technology in prenatal diagnosis, the more experience and knowledge will thrive from it. The collection and evaluation of data generated from its use, will make diagnosis and prognosis more precise and easier to perform in the future. As mentioned earlier, currently we only have ultrasound to describe the phenotype in prenatal testing, and most of the phenotypes are described in postnatal tested patients/populations. Linking the phenotype to CNV findings is therefore challenging. Especially CNV related to neurodevelopmental disorders, since in prenatal setting, we cannot determine the neurodevelopmental phenotype, and the same CNV may also appear in asymptomatic carriers. Some clinics do not report these types of findings because of this reason. Even though there may be structural defects presented we can only by some degree of certainty link the findings to the CNV presented by CMA testing. There is still a lot of research that needs to be done in this field. Linking the type of ultrasound findings that may occur more frequently in fetuses with particular CNVs are important. In Denmark and France they have presented some studies who has shown that an increased NT appear in fetuses with CNVs (14, 15). Biochemical markers are also important (17). Since there are not many studies describing

phenotype links to CNVs, there is a possible bias in over-emphasizing the relevance of findings in published literature. Deletion and duplication syndromes that CNV findings can result in are mostly rare, which also makes the investigation of VOUS challenging as it requires larger datasets to establish potential clinical relevance of the findings. In most cases therefore large methodological epidemiological studies are often not possible. However, the descriptive and clinical findings offered by this study are of value as it highlights the differences with which karyotyping and CMA analysis offer, and it contributes to knowledge of the prenatal phenotypical presentation of the CNVs we found.

### 4.3.3 Reporting of genetic information

It has been shown that pregnant couples tend to prefer a maximum of information about the health of their unborn child and that parents highly appreciate individualized choices in their prenatal testing (28, 35, 46). In our study the termination of pregnancy did not significantly increase after CMA testing, which may indicate that ultrasound findings are more important in terms of making a decision of termination. Different medical centers have different strategies for reporting of CNV findings. In Tromsø, for example, we do not report VOUS found in the fetus to the parents. This conservative attitude is shared with the UK, where guidelines recommend that VOUS unable to be linked to a potential phenotype should not be reported. Australian guidelines highlight the importance of genetic counselling for disclosing abnormal VOUS results, and Netherland report all VOUS in genetic counselling. The present paper adds to the knowledge of currently available prenatal testing technology, which contribute to the goal of giving patients the support and assurance they need to make a choice that is right for them and their family.

## 4.4 Limitations and weakness of the study

CMA and karyotyping was not performed in the same group. Direct comparison of the methods was because of this not possible. We opted to compare findings in two independent, but demographically similar samples, drawn from a population with clearly defined risk parameters. However, chance dissimilarities between our samples may have arisen which could have led to biases influencing our results and possible conclusions drawn from them. For example, the span of age is very similar, ranging from 19 – 44 and 19 – 45 but the mean value of age in the CMA group is somewhat lower. High maternal age will affect the number of finding of aneuploidies in the group of investigation, but not CNV findings. This reflects the gestational age of testing as well. A higher age in the mother will result in more pregnancies

with indication for first trimester screening. *Figure 10* shows the karyotyping group had more first trimester ultrasound scans. As earlier mentioned, our study composed of both women with an indication of CMA mainly because of high maternal age, while others had an indication due to a structural defect in the fetus detected on their first ultrasound. This makes the group heterogeneous, and one should therefore be careful to draw any hasty conclusions out of the findings comparing the groups. Statistical analysis showed no significant difference in gestational age between the groups. The gestational age does not affect the invasive test result, but may matter when comparing number of findings between the groups. Here, the slightly higher age may explain why there are more aneuploidies in the karyotyping group. If there are more high risk pregnancies in the karyotyping group, there will be a higher probability of aneuploidies detected. While the ideal study design may have been to perform both karyotyping and CMA in the same group for comparison. To run both tests would have cost the lab extra money, and would have made the planning of the study more intricate.

### 4.5 Future perspectives of prenatal diagnosis

In retrospect of this thesis one should consider establishing a national collection of data concerning prenatal diagnosis in the Norwegian population. By collecting data of biochemical serum markers, phenotypical findings, and molecular genetic findings, this would contribute to the field of prenatal diagnosis. Also, following the fetuses prospectively from described prenatal phenotypical features, to postnatal, and neonatal settings may be of great clinical value. In the future one may also see prenatal diagnosis evolve in concern of being more accurate in the purpose of investigation. Different techniques used for genomic testing, like WES, may also be achievable in dedicated laboratories with the intention to investigate specific exons known to cause genetic disorders. Sanders and collages has performed WES in a neonatal setting where rapid diagnosis was performed in neonatal units (47). While these methods may contribute to customize the etiology of ultrasound findings, it may also help evolve the field of medical genetics. However, this is a medical field in great development and as we learn to understand, more data is needed to secure our knowledge.

# 5 Conclusion

The purpose of this thesis was to learn about chromosomal microarray (CMA) and its role in invasive prenatal testing, and evaluate the findings, as it recently replaced the karyotyping method. Using CMA, we discovered 14 CNVs distributed in 10 fetuses. As our reporting policy is conservative, only the CNVs of certain clinical importance was reported to the parents, and four CNVs were reported. As CMA was recently introduced to prenatal diagnosis in Norway, the evaluation of its use through the first year in Tromsø was of important value, but more study is needed. Thus, one should consider establishing a national cohort to evaluate the use of CMA in prenatal diagnosis in the Norwegian population.

# 6 GRADE-evaluation

**Referanse:**
Katarzyna Ziolkowska AUPDAUMSAUKT-WAUEWAUMP. The clinical usefulness of biochemical (free β-hCG, PAPP-A) and ultrasound (nuchal translucency) parameters in prenatal screening of trisomy 21 in the first trimester of pregnancy. The clinical usefulness of biochemical (free β-hCG, PAPP-A) and ultrasound (nuchal translucency) parameters in prenatal screening of trisomy 21 in the first trimester of pregnancy. 2019;90:161-6--6.

**Design: Kohort**

| Dokumentasjonsnivå | |
|---|---|
| Grade: | Moderat |

| Formål | Materiale og metode | Resultater | Diskusjon/kommentarer |
|---|---|---|---|
| Undersøke verdier av serummarkørene PAPP-A, β-hCG og NT verdier i uke 11-13 hos pasienter hos pasienter som hadde bekreftet T21 vha cytogenetiske tester.<br><br>**Konklusjon**<br>PAPP-A og β-hCG verdier oppgitt i MoM viste ingen korrelasjon med NT-mål, og er derfor uavhengige faktorer i diagnostisering av T21<br><br>**Land**<br>**Polen**<br>**År data innsamling**<br>2018 | **Populasjon**:<br>251 i første trimester med en økt risiko for trisomi 21 (>1:300) basert på dobbeltesten.<br><br>Fostervannsprøver ble tatt for å bestemme karyotypen til fostrene.<br><br>Delt inn i to subgrupper<br>1. normal karyotype (n = 217)<br>a. PAPP-A (MoM)<br>0.001-0.500<br>0.501-0.900<br>0.901-<br>b. B-hCG(MoM)<br>0.001-1.000<br>1.001-1.500<br>1.501-2.000<br>2.0-<br><br>2. Karyotype T21 (n = 34)<br>a. PAPP-A (MoM)<br>0.001-0.500<br>0.501-0.900<br>0.901-<br>b. B-hCG(MoM)<br>0.001-1.000<br>1.001-1.500<br>1.501-2.000<br>2.0-<br><br>NT mål ble kategorisert mm:<br>1.0-2.0<br>2.1-3.0<br>3.1-5.0<br>5.1-8.0<br>8.1 < | **Hovedfunn**<br>Median maternell alder 35.9 (18-46)<br>67.6% av fostrene som hadde T21 hadde en mor over 35 år.<br><br><u>1. Normal karyotype (n = 217)</u><br>**c. PAPP-A (MoM)**<br>0.001-0.500*(36.87%)*<br>0.501-0.900<br>0.901-<br>**d. B-hCG(MoM): (Mean MoM = 1.979)**<br>0.001-1.500 *(41.5%)*<br>1.501-2.000<br>2.0-<br><br><u>2. Karyotype T21 (n = 34)</u><br>**e. PAPP-A (MoM)**<br>0.001-0.500 *(52.94%)*<br>0.501-0.900<br>0.901-<br>**f. B-hCG(MoM): (Mean MoM =2.894)**<br>0.001-1.500 *(14.7%)*<br>1.501-2.000<br>2.0-<br><br>26.5% av fostrene som hadde T21 hadde NT over 3.1 mm hvis PAPP-A Mom var i området 0.001-0.500 *(52.94%)* | **Sjekkliste:**<br>**Er formålet klart formulert?**<br>**Foreligger det seleksjonsbias?**<br>**Er det tatt hensyn til bakgrunnsfaktorer?**<br>**Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon?**<br>**Er den som vurderte resultatet blindet for gruppetilhørighet?**<br>**Var studien prospektiv?** Ja.<br>**Ble mange nok personer i kohorten fulgt opp?**<br>**Er det fullført frafallsanalyser?**<br>**Var oppfølgningstiden lang nok til å påvise positive/negative utfall?** Ja.<br>**Er det tatt hensyn til viktige konfunderende faktorer?** Ikke tatt hensyn til: næring i svangerskapet, uoppdagede mutasjoner, polymorfisme og miljøfaktorer.<br>**Tror du på resultatene?**<br>**Kan resultatene overføres til den generelle befolkningen?**<br>**Annen litteratur som styrker eller svekker resultatene?**<br>**Hva betyr resultatene for endring av praksis?**<br><br>**Styrke**<br>• Bare 251 foster er undersøkt<br>• Alle er testet og tolket på samme senter<br>**Svakhet**<br>•<br>• |

**Referanse:**
Srebniak MI, Diderich KEM, Joosten M, et al. Prenatal SNP array testing in 1000 fetuses with ultrasound anomalies: causative, unexpected and susceptibility CNVs. *European Journal Of Human Genetics.* 2015;24:645.

**Design: Kohort**

| Dokumentasjonsnivå | |
|---|---|
| Grade: | Moderat |

| Formål | Materiale og metode | Resultater | Diskusjon/kommentarer |
|---|---|---|---|
| Evaluere diagnostisk verdi av SNP array analyse av foster som har ultralydfunn, samt undersøke prevalens og den genetiske naturen av de patogene funnene man fant.<br><br>**Konklusjon**<br>CMA burde benyttes for å undersøke for kromosom aberrasjoner med kausativ karakter hos foster hvor det foreligger ultralyd funn.<br><br>I tillegg ble det påvist uforutsette funn som kan være fordelaktig for pasienten å ha kunnskap om.<br><br>SL opptrådde oftere hos foster uten ultalydfunn, enn de med ultralydfunn. Disse burde få mer oppmerksomhet i klinikken.<br><br>**Land**<br>**Nederland**<br>**År data innsamling**<br>2009-2013 | **Populasjon**:<br>N = 1033<br><br>**Alle foster med ultralydfunn ble inkludert for CMA analyse:**<br>Myke markører, IUGR, polyhydramnios, strukturelle misdannelser, NT ≥ 3.5 mm. og de med IUFD (med eller uten ultralydfunn)<br><br>**Eksklusjon:**<br>• De med aneuploidier påvist på hurtigtest (76 stk)<br><br>Array klassifisering<br>1) Benigne<br>2) VOUS<br>3) Patogene<br>a. CAU (kausative funn)<br>b. Unexpected diagnoses (DU)<br>c. Susceptibility locus (SL)<br>d. Insident funn (IF)<br><br>Funnene ble også sortert etter størrelse<br>• Mikroskopiske<br>• Submikroskopiske (<10Mb)<br><br>Alle er funn er kjørt på samme array platform på samme senter<br><br>**Statistiske metoder**<br>Fisher exact | **Hovedfunn**<br><br>Til sammen 76/1099 (7.4%) patogene funn<br>• Mikroskopiske patogene 19/1033 (1.8%)<br>• Submikroskopiske patogene 57/1033 (5.5 %)<br><br>Array klassifisering av de patogene funn<br>• CAU (58%) n = 44<br>31 av 44 ved kausative funn var de novo, 13 var nedarvet.<br>• SL (35%) n = 27<br>6 var de novo og 13 var arvet fra tilsynelatende frisk forelder<br>• UD (6%) n = 5<br>Alle var de novo<br><br>58% (44/76) av CAU hadde en fenotype som samsvarte med arrayfunnet.<br>25% hadde blitt funnet ved bruk av karyotypering<br>75% kunne kun finnes ved bruk av CMA<br><br>Det ble funnet en signifikant høyere insident av SL hos foster med ultralydfunn, kontra de uten ultralydfunn. 2.6% (27/1033) versus 1.35% (18/1033), Odds ratio 1.056 med 95% KI 1.071, 3.572, P =0.01951. (Fisher exact) | **Sjekkliste:**<br>**Er formålet klart formulert?**<br>Ja, men kunne vært mer spisset.<br>**Foreligger det seleksjonsbias?** Kunne ha oppgitt distribusjon av feks maternell alder, hvor mange ultralydundersøkelser, gj.snittlig gestasjonsalder osv.<br>**Er det tatt hensyn til bakgrunnsfaktorer?**<br>Aneuploidier er ekskludert<br>**Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon?**<br>foster m/ ultralydfunn uten aneuploidier hos foster<br>**Er den som vurderte resultatet blindet for gruppetilhørighet?** Usikkert.<br>**Var studien prospektiv?** Ja.<br>**Ble mange nok personer i kohorten fulgt opp?**<br>Ja.<br>**Er det fullført frafallsanalyser?** Usikkert.<br>**Var oppfølgningstiden lang nok til å påvise positive/negative utfall?** Ja.<br>**Er det tatt hensyn til viktige konfunderende faktorer?** Ikke tatt hensyn til: næring i svangerskapet, uoppdagede mutasjoner, polymorfisme og miljøfaktorer.<br>**Tror du på resultatene?** Ja.<br>**Kan resultatene overføres til den generelle befolkningen?** Til gravide med ultralydfunn uten andre spesifikasjoner.<br>**Annen litteratur som styrker eller svekker resultatene?** Styrker.<br>**Hva betyr resultatene for endring av praksis?** At CMA er en god analyse for foster med ultralydfunn. At man vil få en del uventede funn når man gjør prenatal genomisk testing.<br><br>**Styrke**<br>• Høyt ant foster er med i studien, over 1000<br>• Viser ratio mellom kausative og uventede funn<br>• Alle er testet og tolket på samme senter<br>**Svakhet**<br>• Veldig mange ulike grupper av fenotyper er inkludert. Gir oss kun generell info.<br>• Lite maternell informasjon |

**Referanse:**
Egloff M, Hervé B, Quibel T, Jaillard S, Le Bouar G, Uguen K, et al. Diagnostic yield of chromosomal microarray analysis in fetuses with isolated increased nuchal translucency: a French multicenter study. Ultrasound in Obstetrics & Gynecology. 2018;52(6):715-21.

**Design: Historisk Kohort**

Grade: Moderat

## Formål

Avgjøre frekvensen og naturen av kopitallsfunn som identifiseres vha CMA i en populasjon av foster med økt med NT ≥ 3.5 mm.

## Konklusjon

CMA kan være en fordel å benytte når man søker årsaks diagnose hos foster med en isolert økt NT

Funn viser 2% har en cryptic patogen CNV. I tillegg fant man genomic imbalances (1q21.1 og 15q11.2) assosiert med medfødte hjertefeil.

## Land

**Frankrike**

## År data innsamling

April 2012-Desember 2015

## Materiale og metode

**Populasjon:**
720 foster med isolert NT ≥ 3.5 mm målt i gestasjonsalder uke 10 til 13+6

**Eksklusjon:**
- Foster med andre UL funn.
- De med aneuploidier funnet vha hurtigtester
- De med aneuploidier påvist av hurtigtester som PCR (121 stk av de 720 (16.8%))

**Metode**
- Prøvematerialet: AF(78%) og CVS (22%)
- Hurtigtester: MLPA, BoBs, PCR, eller direct CVS preparation avhengig av sentre.
- Blood-stained AF prøver ble i tillegg dyrket.
- Resultat av CMA ble tolket «på nytt» i retrospekt av to medisinske cytogenetikere.
- Det ble brukt offentlige databaser og publisert litteratur for å fasilitere tolkningen.
- Når det ikke ble gjort konvensjonell karyotypering i tillegg, ble det antatt at de over 10 Mb var synlig.
- Ved mistanke om placental mosaicisme ble det gjort karyotyping og/eller CMA fra dyrkede CVS og AF.

**CNV ble klassifisert i 5 kategorier:**
1. Benign CNV
2. VOUS
3. Klinisk relevant/patogen
4. Neurodevelopmental disorders (autisme spekteret, shizofreni, intellektuelle handikap, osv)
5. CNV ikke relatert til fenotype

## Resultater

**599 stk gjennomgikk CMA analyse**

Median maternell alder hos de 599 var 31 år (14-45).
Median gestasjons alder var 13 uker. Median NT tykkelse var 4.3 mm.

**53/599 (8.8%) hadde kopitallsfunn**
Av disse 53 var
1) 21/53 (3.5%) Benigne
2) 8/53 (1.3%) VOUS
3) 16/53 (2.7%) Patogen

7/53 korresponderte til genetiske tilstander med variabel penetrans og ekspressivitet.
1/53 insident funn

6 av CNVene korresponderte med kjente syndromer. Kun et av disse relatert til DiGeorge syndrom.

Distribusjon av ulike former for CNV relatert til NT tykkelse:

| Nuchal translucency | Pathogenic CNVs | Cryptic pathogenic CNVs | VUS* | Predisposing CNVs** |
|---|---|---|---|---|
| 3.5 – 4.4 mm | 2.0 % (n= 7 / 343) | 1.2 % (n= 4 / 343) | 0.6 % (n= 2 / 343) | 0.9 % (n= 3 / 343) |
| 4.5 – 5.4 mm | 2.4 % (n= 3 / 124) | 2.4 % (n= 3 / 124) | 2.4 % (n= 3 / 124) | 1.6 % (n= 2 / 124) |
| 5.5 – 6.4 mm | 6.8 % (n= 5 / 73) | 5.5 % (n= 4 / 73) | 1.4 % (n= 1 / 73) | 1.4 % (n= 1 / 73) |
| > 6.5 mm | 1.7 % (n= 1 / 59) | 0 % (n= 0 / 59) | 3.4 % (n= 2 / 59) | 1.7 % (n= 1 / 59) |

*Variant of Uncertain Significance
**CNVs predisposing to neurodevelopmental disorders, with incomplete penetrance and/or variable expressivity

**Vi ser at deteksjonsraten av patogene/kryptiske CNV korrelerer med verdien av NT målet.**
- Av de svangerskapene hvor man fant patogene CNVs valgte 12/16 (75%) kvinner å terminere svangerskapet.
- Av de 8 hvor man fant VUS valgte 3/8 (38%) å terminere svangerskapet.
- Av de 7 med predisposisjon til neurodevelopmental disorders valgte 3/7 (43%) å terminere svangerskapet.

I denne studien ble det funnet at de relevante CNV kunne vise til en et diagnostisk utbytte på kun 2.7% som er lavere enn 4% som er funnet i artikkelen Grande, et al.2015,
de Wit et al. reported that the rate of cryptic CNVs can reach 7% when increased NT is associated with at least one other ultrasound finding.
Det er altså forskjeller I studier.

## Diskusjon/kommentarer

**Sjekkliste:**
**Var gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer?** Vanskelig å svare på. Vi vet ikke enda nok om hva som gir nakkeoppklaring. Høy maternell alder? Teratogener? Miljø i uterus? Se eksl krit. Ifht hva man har antatt vil påvirke resultatet.
**Er gruppene rekruttert fra samme populasjon/befolkningsgruppe?** Ja.
**Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon?** Representativt for foster med økt NT. Median maternell alder oppgitt og median tykkelse på NT hvor det ikke foreligger aneuploydi.
**Var studien prospektiv?** Nei, retrospektiv.
**Ble eksposisjon og utfall målt likt og pålitelig i de to gruppene?** En gruppe.
**Ble mange nok personer i kohorten fulgt opp?** Godt ant. 720.
**Var oppfølgingstiden lang nok til å påvise positive og/eller negative utfall?** Ja
**Er det tatt hensyn til viktige konfunderende faktorer i design/gjennomføring?** Eksklusjonskr. Ja.
**Er den som vurderte resultatene (endepunktene) blindet gruppetilhørighet?** En gruppe.

**Styrke**
- Høyt antall foster innen gitte kriterier
- Man har ekskludert andre tilstander som kan gi NT hos et foster
- Retrospektiv analysering av resultater av CMA i tillegg til opprinnelig analysering som var foretatt i klinikken

**Svakhet**
- Ulike CMA maskiner er benyttet.
- Gjengir ikke tydelig samme kategorier i resultater som i metodedel.
- Oppgir ikke hvordan de regner ut KI

**Referanse:**
Vogel I, Petersen OB, Christensen R, Hyett J, Lou S, Vestergaard EM. Chromosomal microarray as a primary diagnostic genomic tool for pregnancies defined as being at increased risk within a population based combined first-trimester screening program. *Ultrasound in Obstetrics & Gynecology*.n/a-n/a.

**Design: Historisk Kohort**

Grade - kvalitet: Moderat

## Formål

Å evaluere bruk av CMA som diagnostisk test i undersøkelse av genetiske aberrasjoner hos foster hvor det foreligger økt risiko ved første trimester screening (cFTS) ≥ 1:300

## Konklusjon

CMA kan identifisere flere genomiske aberrasjoner i svangerskap med økt risiko definert fra cFTS.

Dersom man kun bruker CMA i de gruppene med høy risiko (1:100 eller 1:50), slik det er foreslått i modeller med gruppestrategi, til bruk av NIPT hos de > 1:50, så vil det føre til at en signifikant proporsjon av patogene CNV ikke blir oppdaget ved screening i første trimester. Dette fordi også mange blir fanget opp i grupper med lavere cFTS score.

## Land

Danmark

## År data innsamling

September 2015-september 2016.

## Materiale og metode

**Studiepopulasjon: N = 575**
svangerskap med forhøyet *combined first-trimester screeningtest* (cFTS) for aneuploidier i gestasjonsuke 8-20 uke og dermed vedgikk invasiv testing med CVS og amniocentese.
Alle med forhøyet risiko (≥ 1:300 for T21, ≥150 for 18/13) etter screeningtest basert på: maternell alder, Nuchal Translucecy (NT) tykkelse, og maternelt serum proteinene PAPP-A og β-HCG i kombinasjon. Samt andre trimester ultralyd screening ved uke 18-20 gestasjonsalder.

**Eksklusjon:**
Indikasjoner for invasiv testing som følge av
- NT > 3.5 mm
- NIPT
- IUGR
- Kjent CNV

Disse ble ekskludert fordi de allerede har vist å medvirke til en høyere deteksjonsrate ved bruk av CMA.

**CMA Matrise**
Alle CMA analyser ble gjort med CGH microarray 180K. Funn av CNV ble kategorisert i tre grupper: benign, patogen, eller VOUS. En subgruppe patogene CNV ble navngitt susceptibility variant.

561 stk. hadde en CVS, resten hadde fostervannsprøve.

**Statistisk analyse**
McNemar two tailed test Chi-square and McNemar two-tailed tests were usedfor statistical analysis and http://vassarstats.net/ was usedfor the calculation of CIs

## Resultater

**Table 1** Distribution of abnormal findings detected on chromosomal microarray analysis in 575 pregnancies that underwent invasive testing, according to risk on combined first-trimester screening (cFTS) for trisomy (T) 21, T18 and T13

| | | Pathogenic result | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Aneuploidy | | | | | | |
| *cFTS risk* | *Total* | *Total* | *T21, T13, or T18* | *SCA* | *Other* | *CNV pathogenic/ susceptibility** | *CNV probably pathogenic* | *VOUS* | *CNV incidental findings* |
| > 1 in 50 | 139 (24.2) | 23 (16.5) [45.1] | 18 (12.9) [81.8] | 2 (1.4) [33.3] | 2 (1.4) [25.0] | 0 (0.0) [0.0]/ 0 (0.0) | 1 (0.7) [50.0] | 0 (0.0) [0.0] | 1 (0.0) [25.0] |
| 1 in 50 to 1 in 100 | 135 (23.5) | 12 (8.9) [23.5] | 1 (0.7) [4.5] | 2 (1.5) [33.3] | 3 (2.2) [37.5] | 5 (3.7) [38.5]/ 1 (0.7) | 1 (0.7) [50.0] | 0 (0.0) [0.0] | 0 (0.0) [0.0] |
| 1 in 100 to 1 in 300 | 301 (52.3) | 16 (5.3) [31.4] | 3 (1.0) [13.6] | 2 (0.7) [33.3] | 3 (1.0) [37.5] | 8 (2.7) [61.5]/ 5 (1.7) | 0 [0.0] | 1 (0.3) [100.0] | 3 (1.0) [75.0] |
| > 1 in 300 | 575 (100.0) | 51 (8.9) [100.0] | 22 (3.8) [100.0] | 6 (1.0) [100.0] | 8 (1.4) [100.0] | 13 (2.3) [100.0]/ 6 (1.0) | 2 (0.3) [100.0] | 1 (0.2) [100.0] | 4 (0.7) [100.0] |

Data are reported as *n* (%). For each category of abnormal findings, data in square brackets are percentages calculated against total number of such findings in whole cohort. *Number of pathogenic copy number variants (CNVs) that are syndromes with variable phenotypic effects that do not always result in severe impairment and may be inherited from a parent with minimal or no clinical features such as 22q11 duplication syndrome[12]. All above findings were reported to patients, including two probably benign CNVs: 15q11 BP1-BP2 deletion. SCA, sex chromosome aneuploidy; VOUS, variants of unknown significance.

Median maternell alder var 33 år (21-47).

**Fordeling av risiko i studiepopulasjonen:**
**139 stk** (24%) av svangerskapene hadde risiko estimert til >1:50
**135 stk** (24%) –"— mellom 1:50 og 1:99
**301 stk** (52%) ---"--- mellom 1:100 og 1:300.

**Patogene funn i 51/575 (8.9%)**
22/575 trisomier (21, 18 og 13),
6/575 (1%) X aneuploidi
8/575 andre former for aneuploidi (1.4%).

15 CNV av sannsynlig patogen og patogen variant ble funnet.
3/15 CNV var > 10 Mb og hadde vært mulig å se på karyotypering
12/15 CNV var < 10 Mb og kun synlig på CMA

8.9% (95% CI, 6.8-11.5%) var sig høyere enn karyotyp 6.8% (95% CI, 5.0-9.1%)(P=0.0049).
**Deteksjonsraten for CMA samlet sett var signifikant høyere hvor 51/575 (8.9%), sammenliknet med estimerte funn av tradisjonelle cytogenetiske analyser hvor bare 39/575 (6.8%) hadde blitt oppdaget. (McNemar two tailed test).**

Hvis man slår sammen de to risikogruppene med minst risiko (altså fra 1:50-1:300) vil det bli 28 oppdagede patogene tilfeller. Av disse 28 ville kun 4 blitt fanget opp vha. NIPT som fokuserer på kromosom 21, 18 og 13.

## Diskusjon/kommentarer/sjekkliste

**Sjekkliste:**
**Er formålet klart formulert?** Ja
**Er gruppene rekruttert fra samme populasjon/befolkningsgruppe? (seleksjonsbias)** Samme gruppe.
**Var gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer?** Ja, fordi det er undersøkt i samme populasjon.
**Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon?** Ja
**Ble eksposisjon og utfall målt likt og pålitelig (validert) i de to gruppene? (classification bias)** Ikke relevant.
**Er den som vurderte resultatene (endepunkt-ene) blindet for gruppetilhørighet?** Ukjent.
**Var studien prospektiv?** Nei.
**Ble mange nok personen i kohorten fulgt opp?** Ja.
**Er det utført frafallsanalyser?** Nei.
**Var oppfølgingstiden lang nok til å kunne påvise positive eller negative utfall?** Ja.
**Er det tatt hensyn til viktige konfunderende faktorer i design/gjennomføring av analyser?** Ja. Man har ekskludert viktige kjente konfunderende faktorer som NT ≥ 3.5, IUGR .
**Tror du på resultatene?** Ja.
**Kan resultatene overføres til den generelle befolkningen?** Nei, dette er en selektert høyrisikogruppe.
**Annen litteratur som styrker/svekker resultatene?**
**Hva betyr resultatene for endring av praksis?**
At man ved å kategorisere risikogrupper ved bruk av screeningtesten må være forsiktig. Lavere risiko på screeningtestene utelukker ikke kopitalsfunn. NIPT burde ikke benyttes som diagnostisk test, da CMA i mye større grad er sensitiv for funn.
**Ble eksposisjon og utfall målt likt og pålitelig i de to gruppene?**
Alle CMA analyser ble utført av en SurePrint G3 Human CGH microarray 180 K
**Styrke**
Noen senter tilbyr NIPT istedenfor karyotypering til kvinner med en cFTS risiko mellom 1:50-1:000. Hvis en slik gruppestrategi hadde blitt gjort her til sammenlikning, ville hele 24 av de 51 abnormale resultatene blitt oversett.
**Svakhet** cFTS er designet til å oppdage trisomi 21, 18 og 13 – ikke generelle genomiske aberrasjoner.
Konfunderende faktorer som teratogener/eksposisjoner

**Referanse:**
Lund IC, Christensen R, Petersen OB, Vogel I, Vestergaard EM. Chromosomal microarray in fetuses with increased nuchal translucency. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology.* 2015;45(1):95-100.

| **Design: Pasientserie** | |
|---|---|
| Dokumentasjonsnivå | |
| Grade: | Lav |

## Formål

Å vurdere klinisk nytteverdi av å benytte CMA i prenatal diagnostikk av foster med økt nakkefold. Dette i en populasjon hvor individene ble screenet med screeningverktøy designet for å oppdage T21 i første trimester hvor bla NT-mål inngår.

## Konklusjon

CMA er en verdifull diagnostisk teknikk i svangerskap med isolert føtal NT ≥ 3.5mm.

Deteksjonsrate på 12.8% hos de med NT ≥ 3.5mm, mens det i gruppen med NT ≥ 4.5mm viste en deteksjonsrate så høy som 26.5%.

## Land

**Danmark**

## År data innsamling

Januar 2013 til Juli 2014. (18 måneder).

## Materiale og metode

**Populasjon:**
136 foster med NT mål ≥ 3.5 mm. Første trimester (11-13 uke).
95% gjennomgikk screeningtester

**Eksklusjon:**
4 tilfeller ble ekskludert da det forelå flere UL anomalier enn NT:
- myelomeningocele (n=1)
- omphalocele + talipes (n=1)
- talipes (n= 1)
- kardiell arrytmi + hydrops (n=1)

N = 132

**Prøvemateriale**
CVS

**Framgangsmåte Analyse**
- PCR av alle (n = 132)
- Karyotypering av alle abnormale PCR (n = 38)
- CMA av alle normale PCR (n = 94)

**CMA:**
SurePrint G3 Human CGH microarray 180K (Agilent Technologies Inc., Santa Clara, CA,USA)

**Array klassifisering:**
1. Benign
2. VOUS
3. Patogen

**Statistisk analyse**
Utført vha GraphPad Prism, versjon 4.03.
Mann-Whitney U-test for å undersøke forskjeller mellom gruppene.
Chi-square test for å teste for sig. Forskjell i forventet frekvens.
P<0.05 ble vurdert statistisk signifikant i en two-sided test.

**Viktige konfunderende faktorer**
Foreldreprøver ble kjørt dersom det forelå VOUS hos foster.

## Resultater

**Hovedfunn**
N = 132. Maternell alder varierte mellom 18-43. Median alder var 31. Median NT mål var 4.2 (3.5-13.8 mm). 96.2% hadde isolert NT. 3.8% hadde føtal hydrops i tillegg til økt nakkefold.

**Karyotypering**
- 38/132 (28.8%; KI 21.8-37.0%) hadde vanlige aneuploidier vist på QF-PCR og karyotypering.
  - 28/38 T21
  - 3/38 T18
  - 4/38 45,X
  - 1/38 47,XXX
  - 2/38 annen tripoidi

**CMA**
- 15/94 (16%; 95% KI 9.9-24.7%) som utførte CMA analyse hadde kopitallsfunn i form av VOUS eller patogen klassifikasjon. 11/15 var submikroskopiske og hadde ikke blitt sett på karyotypering.
  - Benign 5/94
  - VOUS 3/94 (3.2%;95% KI, 1.1-9.0%)
  - Patogene: 12/94 (12.8%; 95% KI, 7.5-21%)
    - Median alder hos mødrene med de patogene variantene av CMA funn var 30 år (21 - 35 år).

**NT størrelse**
- 75/132 (57.6%) var NT 3.5-4.4 mm. I de resterende 56 casene var NT > 4.5mm.
- De høyeste NT verdiene var målt hos de fostrene som hadde anormale funn på QF-PCR og CMA
  - Abnormale QF-PCR: aneuploidier (n=38) hadde median NT: 4.8 mm vs. 4.1 mm hos de med normal QF-PCR (n = 94), P = 0.01.
  - Abnormale CMA (n=15). Her hadde 9/15 NT mål over 4.5 mm

## Diskusjon/kommentarer

**Sjekkliste:**
- **Var formålet klart formulert?** Ja
- **Var studien basert på et tilfeldig utvalg fra en egnet pasientgruppe? (seleksjonsbias**) Ja.
- **Var inklusjonskriteriene klart definert?** Ja
- **Var alle pasientene i samme stadium av sykdommen?** Ja. Alle er i første trimester.
- **Er svarprosenten høy nok? (frafallsanalyser)** Noe lavt ant.
- **Ble objektive kriterier benyttet for å vurdere/validere endepunktene? (classfic bias)**
- **Ved sammenlikninger av pasientserier, er seriene tilstrekkelig beskrevet?** Ja
- **Er prognostiske/konfunderende faktorers beskrevet/tatt hensyn til i design og analyse?** Alle burde hatt isolert NT
- **Var registreringen av data prospektiv?** Ja.
- **Var oppfølgningen lang nok?** Ja
- **Var oppfølgningen tilstrekkelig for å nå endepunktene?** Ja
- **Stoler du på resultatene?** Ja
- **Kan resultatene overføres til praksis?** Ja.
- **Annen litteratur som støtter resultatene?** Ja

**Styrke**
Viser at det er nyttig å gjøre CMA hos foster med NT over 3.5 mm og at karyotypering ikke er tilstrekkelig alene.

**Svakhet**
Lavt ant foster.

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