5-2019

Exploring The Potential Yield Of Prenatal Testing By Evaluating A Postnatal Population With Structural Abnormalities

Peyton Busby

EXPLORING THE POTENTIAL YIELD OF PRENATAL TESTING BY EVALUATING A POSTNATAL POPULATION WITH STRUCTURAL ABNORMALITIES

by

Peyton Bree Busby, BS

APPROVED:

_____
Blair K. Stevens, MS, CGC
Advisory Professor

_____
Myla Ashfaq, MS, CGC

_____
Laura S. Farach, MD, FACMG

_____
S. Shahrukh Hashmi, MD, MPH, PhD

_____
Anthony Johnson, DO

_____
Claire N. Singletary, MS, CGC

APPROVED:

_____

Dean, The University of Texas
MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences

EXPLORING THE POTENTIAL YIELD OF PRENATAL TESTING BY EVALUATING A POSTNATAL POPULATION WITH STRUCTURAL ABNORMALITIES

A

THESIS

Presented to the Faculty of

The University of Texas

MD Anderson Cancer Center UTHealth

Graduate School of Biomedical Sciences

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

by

Peyton Bree Busby, BS

Houston, Texas

May, 2019

Acknowledgements

To my thesis chair, Blair Stevens, thank you for all of her help and support from the beginning of this process, as this project would not have even started without you. To my thesis committee members, thank you for your input and support over the past year and a half. Although not on the committee, thank you Dr. Eleazar E. Soto-Torres for lending your expertise in ultrasound findings. Thank you Memorial Hermann – Texas Medical Center for allowing me to use the EMR system. I would also like to thank the University of Texas MD Anderson UTHealth Graduate School of Biomedical Sciences Genetic Counseling Program for support and the opportunity to pursue this topic. Lastly, my wonderful family and friends have been through it all as I decided to pursue this degree and have been so supportive throughout this process, thank you.

EXPLORING THE POTENTIAL YIELD OF PRENATAL TESTING BY EVALUATING A POSTNATAL POPULATION WITH STRUCTURAL ABNORMALITIES

Peyton Bree Busby, BS

Advisory Professor: Blair K. Stevens, MS, CGC

After identification of one or more structural abnormalities in a fetus, pregnant women are offered a host of different testing options to identify a possible genetic cause for the structural abnormality(ies). When considering what type of test to undertake, there is limited information on the diagnostic yield of the varying testing options. Some women may miss an opportunity to gain the information they are seeking or make a less informed decision when they choose a testing option after identification of a structural abnormality due to this lack of information. This study aimed to identify the potential diagnostic yield of all currently available prenatal testing options in the presence of a structural abnormality through a retrospective chart review of a postnatal population of infants with structural abnormalities. Of 791 patients with at least one structural abnormality, 691 patients underwent genetic testing and 222 had a genetic aberration that explained their phenotype. Chromosomal microarray had the highest potential diagnostic yield across the entire cohort and among individuals with multiple structural abnormalities, 26.8% (95% CI: 23.5 - 30.3) and 29.0% (95% CI: 25.3 - 33.3) respectively, which reached significance ($p < 0.001$, $p = 0.029$) compared to all of the other prenatal screening and diagnostic options. In the isolated cohort, whole exome sequencing had a higher potential diagnostic yield of causative pathogenic aberrations, followed by chromosome microarray. Expanded non-invasive prenatal testing (NIPT with microdeletions and whole genome NIPT) had a higher potential yield than traditional NIPT. Whole genome NIPT also had a comparable yield as a karyotype, although this did not reach statistical significance. While interesting, it is important to consider the

limited data available on expanded NIPT panels compared to the robust studies of traditional NIPT and how this might affect these results and post-test counseling regarding positive screening results. This study provides further evidence for the use of chromosomal microarray for the highest potential diagnostic yield in genetic testing after identification of one or more structural abnormalities.

Table of Contents

Approval Page i
Title page ii
Acknowledgements iii
Abstract iv
Table of Contents vi
List of Illustrations vii
List of Tables viii
Abbreviations ix
Introduction 1
Methods 4
Results 10
Discussion 27
Conclusions 31
Appendix 33
Bibliography 39
Vita 44

List of Illustrations

Figure 1: Breakdown of study cohort 11

Figure 2: Etiology of structural abnormalities 12

Figure 3: Potential diagnostic yield of prenatal testing options for entire cohort 18

Figure 4: Breakdown of isolated cohort 19

Figure 5: Breakdown of MSA cohort 20

Figure 6: Potential diagnostic yield of prenatal testing options in isolated and MSA cohorts 26

List of Tables

Table 1: Non-invasive prenatal screening options included in this study…..……6

Table 2: Detection rates for prenatal screening tests……9

Table 3: Clinical diagnoses……13

Table 4: Non-causative clinically relevant findings……14

Table 5: Potential diagnostic yield of prenatal testing for entire cohort……16

Table 6: p-values of prenatal testing for entire cohort……..……..……17

Table 7: Potential diagnostic yield of prenatal testing in individuals with an isolated structural abnormality……..……...22

Table 8: p-values of prenatal testing in individuals with an isolated structural abnormality…..23

Table 9: Potential diagnostic yield of prenatal testing in individuals with MSA…….……24

Table 10: p-values of prenatal testing in individuals with MSA……..……..25

Supplemental table 1: Presumed causative pathogenic findings……...33

Supplemental table 2: Structural abnormalities included in study…….……...38

Abbreviations

CI	Confidence Interval
CMA	Chromosomal microarray
CNVs	Copy number variants
CVS	Chorionic villus sampling
DY	Diagnostic yield
DRT	Detection rate of the test
FISH	Fluorescence in situ hybridization
MSA	Multiple structural abnormalities
NIPT	Non-invasive prenatal testing
NGS	Next-generation sequencing
PPV	Positive predictive value
SCAs	Sex chromosome aneuploidies
SGD	Single gene disorder
VUS(es)	Variant(s) of uncertain significance
WES	Whole exome sequencing

Introduction

Structural abnormalities occur in 3-5% of all pregnancies and have a wide range of prognoses and etiologies [1]. Structural abnormalities (birth defects) are differences in fetal development that can affect any organ in the body [1]. Understanding the etiology of a structural abnormality allows for more accurate counseling regarding prognosis, pregnancy and neonatal management, and recurrence risks for future children. Common genetic etiologies include aneuploidy such as Down syndrome, copy number variants (CNVs) such as 22q11 deletion syndrome, and single gene disorders such as Noonan syndrome. Due to the association between genetic conditions and structural abnormalities, women are offered a host of different genetic testing options upon identification of an ultrasound abnormality. Testing options can include diagnostic testing via chorionic villus sampling (CVS) or amniocentesis, or for those who decline diagnostic testing, a variety of non-invasive prenatal testing (NIPT) options.

Diagnostic testing is clearly superior to screening tests when evaluating for genetic abnormalities as it allows for more comprehensive and more accurate testing, but these benefits must be weighed against procedure related risks.

When a diagnostic procedure is performed, a multitude of genetic tests can be ordered, such as karyotype, chromosomal microarray (CMA), and next-generation sequencing for single gene disorders. When offering prenatal testing after identification of a structural abnormality, there is limited information available on the diagnostic yield of each testing option to aid providers and patients in counseling and decision-making. Benn et al. found that when the indication for prenatal testing is a structural anomaly, a karyotype will detect an abnormality in 16.7% of pregnancies that undergo diagnostic testing [3]. Donnelly et al. found in karyotypically normal pregnancies, chromosome microarray detected an abnormality in an additional 5.6% of cases with one structural anomaly and 13.0% of cases with more than one structural anomaly [4]. There have been multiple studies (with differing inclusion criteria and testing strategies) looking at

the potential yield of prenatal WES, producing a wide diagnostic yield range from 6.2-80% that generally falls within the 15-45% range [5-8]. Although these studies provide some information about the yield of diagnostic testing options, many are limited regarding the specific type of structural abnormality, and do not include any comparison to the rapidly expanding NIPT options [5] [9].

The detection rate of NIPT for common aneuploidies (13, 18, 21, X, and Y) range between 91.7%-99.9% [11]. In addition to common aneuploidy, expanded NIPT panels are currently clinically available that include options such as screening for other chromosome aneuploidies, select microdeletions, genome-wide deletions or duplications greater than 7Mb and certain autosomal dominant single gene disorders [12-14]. The detection rates for these additional conditions are lower, ranging between 60-85% for specific CNVs and 43-99% for select single gene disorders [12-14]. However, peer-reviewed publications on the accuracy of NIPT for CNVs and single gene disorders is limited and peer reviewed data has yet to be published.

Many studies on expanded NIPT (NIPT for common microdeletions, whole genome NIPT, and NIPT for single gene disorders) do not have outcome data for all pregnancies tested, and often have a small number of positive results with multiple false positives [13-17]. This leads to vastly different quoted detection rates and confidence intervals for conditions within a specific tests and across different testing platforms [12-17].

Screening tests such as NIPT are desirable to patients because they do not pose a risk for miscarriage to the pregnancy, but they are not diagnostic and only screen for a limited number of conditions. Therefore, the American College of Obstetrics and Gynecology does not recommend the use of cell-free fetal DNA screening tests after the identification of a structural abnormality on ultrasound [18].

Despite these recommendations, many women decline diagnostic testing and opt for NIPT. Since the introduction of NIPT, the rate of invasive testing for all indications

has decreased significantly, by as much as 53% and 77% for amniocentesis and CVS respectively [19]. The rate of invasive testing is predicted to continue to decline by as much as 91% as predicted by some models [20]. Many women indicate their choice of NIPT is influenced by its high detection rates [21]. However, data has shown that patients overestimate the accuracy of NIPT for Down syndrome and overestimate the number of conditions NIPT can screen for [22]. It is also unclear if women who have a fetus with a structural abnormality also use this same reasoning when electing NIPT, as there have not been extensive studies in this particular patient population.

Few studies have evaluated the utility of NIPT in the presence of congenital anomalies. One retrospective study evaluated 251 pregnancies with NIPT results and abnormal ultrasound findings, including multiple anomalies, isolated anomalies, increased nuchal translucency (>3.5mm), soft markers, and growth restriction [23]. NIPT identified 26 genetic aberrations in this population. Thirty three of the 224 patients with negative NIPT underwent diagnostic testing postnatally, which identified an additional 7 aberrations missed by NIPT [23]. Over half of the study population had increased nuchal translucency and soft signs, which are associated mostly with aneuploidy and therefore more likely to be detected by NIPT [23]. Another study by Sotiriadis et al. [24] evaluated the potential yield of NIPT for common aneuploidies on prenatal CMA samples from pregnancies identified as having one or more structural abnormalities. Only 7 of the 22 aberrations identified on CMA would have been picked up by NIPT for common aneuploidies, and only one of the fifteen other aberrations would have potentially been picked up by NIPT plus common microdeletions [24].

This study aims to address the gap in the literature concerning the yield of prenatal testing after identification of specific structural abnormalities, particularly for newer tests such as cell-free DNA screening for single gene disorders and diagnostic sequencing tests. This information will allow for a better understanding of the likelihood of detecting an underlying genetic condition or an increased risk for a condition by each

prenatal test and ultimately facilitate more accurate genetic counseling and more informed decision making.

Methods

Inclusion and Exclusion Criteria

Individuals for this study were selected from a database of patients maintained by the Division of Medical Genetics in the Department of Pediatrics at McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth). The database consists of patients seen by the UTHealth Medical Genetics team since January of 2014. Protocol was submitted to the University of Texas Health Science Center Internal Review Board and Memorial Hermann Internal Review Board and was approved on June 12th, 2018 and September 11th, 2018 respectively (HSC-MS-18-0458).

Data from the clinical database was abstracted into a study database created in Qualtrics (Qualtrics, Provo, UT) and exported to Stata (v13.1, College Station, TX) for analysis.

Patients listed in the database were included in the study if, 1) they were initially seen by the genetics team as an inpatient consult at Children's Memorial Hermann Hospital, in Houston, Texas, 2) initial consult occurred from January 1st, 2014 through December 31st, 2017, 3) consulted within the first 6 months of life, 4) review of clinical records demonstrated evidence of at least one structural abnormality potentially detectable by ultrasound. Patients with only structural abnormalities that are not potentially detectable by prenatal ultrasound were excluded.

Once patients in the database satisfied inclusion criteria 1-3, their inpatient and outpatient medical records were reviewed from the corresponding electronic medical record systems (EMR). Only patients with a structural abnormality potentially detectable by ultrasound were included in the statistical analysis. Abnormalities not potentially detectable by prenatal ultrasound were excluded. Data on genetic testing results was

obtained from both the inpatient and outpatient EMR systems. When available, information regarding the reason for lack of testing was recorded.

Structural abnormalities

A list of structural abnormalities to be included in the analysis was generated from a previous study comparing CMA and karyotype in the presence of structural abnormalities [4] and from internal lists of structural abnormalities at the Children's Memorial Hermann Fetal Center. Structural abnormalities were determined to be either potentially detectable by ultrasound or not by two maternal fetal medicine specialists with a total of 37 years of experience in the field.

Determination of potential diagnostic yield of prenatal testing options

Appropriate Prenatal Test for Condition

The potential diagnostic yield of prenatal testing was determined first by evaluating whether the genetic conditions detected in our study population could have been detected by clinically available prenatal screening and diagnostic tests. Diagnostic testing options included in the analysis include aneuploidy fluorescence in situ hybridization (FISH), karyotype, chromosomal microarray (CMA), next-generation sequencing (NGS) panels, prenatal WES, methylation studies, and trinucleotide repeat analysis. Screening options included a variety of non-invasive prenatal testing (NIPT) screens, which are outlined in Table 1.

Table 1: Non-invasive prenatal screening options included in this study

Prenatal screening option	Conditions screened for
NIPT for common aneuploidies (NIPT)	Aneuploidy for chromosomes 13, 18, and 21
NIPT + sex chromosome abnormalities (NIPT+SCAs)	Aneuploidy for chromosomes 13, 18, 21, and X and Y
NIPT + common microdeletions/copy number variants (NIPT+CNVs)	Aneuploidy for chromosomes 13, 18, 21, X, and Y and common microdeletion syndromes 22q11.2 deletion syndrome (DiGeorge syndrome), 11q- (Jacobsen syndrome), 5p- (Cri-du-Chat), 8q24.1- (Langer-Giedion syndrome), 1p36 deletion syndrome, 4p- (Wolf-Hirschhorn syndrome),15q- (Prader-Willi syndrome; Angelman syndrome)
Whole genome NIPT	Identifies deletions or duplications 7Mb or greater across the entire genome, including aneuploidy for all 23 chromosomes and select copy number variants previously described for NIPT+ common microdeletions
NIPT for select single gene disorders (NIPT+ SGD)	Identifies *de novo* or paternally inherited mutations in the following genes: *BRAF, CBL, CDKL5, CHD7, COL1A1, COL1A2, FGFR2, FGFR3, HDAC8, HRAS, JAG1, KRAS, MAP2K1, MAP2K2, MECP2, NIPBL, NRAS, NSD1, PTPN11, RAD21, RAF1, RIT1, SHOC2, SMC1A, SMC3, SOS1, SOS2, SYNGAP1, TSC1,* and *TSC2*

For each genetic condition, a test was selected if it had the *potential* to detect the genetic aberration. When individuals had more than one identified genetic aberration, only the one that explained the phenotype was used for determination of potential diagnostic yield of prenatal testing.

Patient results were classified as negative or positive. Positive results were further broken down into benign, possible, and causative Individuals with a possible classification had a finding that could potentially explain their phenotype, but at the time of data collection there was not a decision or resolution of the uncertainty. These findings included variants of uncertain significance (VUS) in genes associated with the phenotype or in candidate genes, CNVs identified in individuals with similar findings or

normal phenotype, and likely diagnoses that needed further clinical correlation that could not be evaluated as the patient was lost to follow up.

When an individual had a positive finding or findings that did not explain their phenotype, they were included when determining the yield of that particular test in order to reflect the true positive rate for that test, but the individual was not counted as having a pathogenic variant causative of their structural abnormalities (causative genetic aberration).

Calculating Diagnostic Yield

To calculate the diagnostic yield for a test for causative genetic aberrations, we first calculated the number of patients in our dataset whose genetic aberration could have potentially been identified by that test (N_{ID}). This was done by dividing the number of patients with a causative genetic aberration that could have potentially been identified by the test (N_{POT}) by the sum of the number of patients in our dataset that had *any* causative genetic aberration identified (N_{CAU}) and those that only had a negative, benign or uncertain findings on appropriate testing (N_{NEG}). Appropriate testing was defined as the same test performed on the patient or a different test that would have been able to identify the same aberration.

The N_{ID} was then multiplied by the detection rate of the test (DRT). For the screening tests, the DRT was the sum of previously reported detection rates [11-14, 26, 27] of all the conditions that could have been identified by the test (Table 2), after taking into account the prevalence of that condition in our study cohort in order to account for the varying detection rates by condition for a particular test. Of note, the detection rate for triploidy was determined using two publications [19, 21], as well as the consideration of different rates of detection based on the origin of the extra genetic material. Considering 85% of triploid pregnancies are diandric and 15% are digynic, the detection rate for triploid pregnancy was estimated to be 97.8% using appropriate and available

screening methods (0.85*99%+0.15*91%=97.8%). For the diagnostic tests, the DRT were assumed to be 100%. Therefore, our formula for calculating the diagnostic yield (DY) for a test was as follows:

$$DY = \frac{N_{POT}}{N_{CAU} + N_{NEG}} \, x \, DRT$$

To determine the potential yield of testing, including individuals with benign findings, the same procedure was followed, except instead of calculating N_{POT}, we calculated N_{ALL}. N_{ALL} is the number of patients with a genetic aberration that could have potentially been identified by the test (N_{ALL}).

Table 2: Detection rates for prenatal screening tests by condition

Condition	Detection Rate	Source
Aneuploidies		
Trisomy 21	99.7% (99.1 – 99.9%)	[11]
Trisomy 18	97.9% (94.9 – 99.1%)	[11]
Trisomy 13	99.0% (65.8 – 100%)	[11]
Triploidy	97.80%	[19, 21]
Monosomy X (45, X)	95.8% (70.3 - 99.5%)	[11]
Other SCA	100% (83.6 – 100%)	[11]
Copy Number Variants		
22q11.2 deletion (DiGeorge) syndrome	53.9% (28-91%)	[14]
11q- (Jacobsen syndrome)	86.7% (57-99%)	[14]
5p- (Cri-du-Chat)	83.1% (48-96%)	[14]
8q24.1- (Langer-Giedion syndrome)	97.2% (80-99%)	[14]
1p36 deletion syndrome	50.7% (13-81%)	[14]
4p- (Wolf-Hirschhorn syndrome)	72.9% (37-91%)	[14]
15q- (Prader-Willi syndrome; Angelman syndrome)	59.2% (16-74%)	[14]
Whole Genome (>7 Mb)	95.9% (61-99%)	[14]
Single Gene Disorders		
BRAF	96%	[12]
CBL	86%	[12]
CDKL5	84%	[12]
CHD7	91%	[12]
COL1A1	92%	[12]
COL1A2	92%	[12]
FGFR2	96%	[12]
FGFR3	96%	[12]
HDAC8	66%	[12]
HRAS	92%	[12]
JAG1	79%	[12]
KRAS	96%	[12]
MAP2K1	96%	[12]
MAP2K2	96%	[12]
MECP2	78%	[12]
NIPBL	94%	[12]
NRAS	96%	[12]
NSD1	87%	[12]
PTPN11	96%	[12]
RAD21	43%	[12]
RAF1	96%	[12]
RIT1	96%	[12]
SHOC2	96%	[12]
SMC1A	96%	[12]
SMC3	96%	[12]
SOS1	96%	[12]
SOS2	96%	[12]
SYNGAP1	89%	[12]
TSC1	96%	[12]
TSC2	82%	[12]

Statistical Analysis

All data was analyzed using Stata (v13.1, College Station, TX). The categorical variables were reported as frequencies with percentages. Comparisons between categorical variables were performed using contingency tests (chi-square or Fisher exact). The diagnostic yields were described as proportions with 95% confidence intervals (CI) that were calculated as described by Wilson et al. [28]. These proportions were compared between groups using a two-sample proportion test. Statistical significance was assumed at a Type I error rate of 5%.

Results

Study Cohort

There were 931 records in the database maintained by the Medical Genetics Department at the University of Texas McGovern Medical School during the study period. Of these 931 subjects, 140 were excluded for not having a structural abnormality potentially detectable by ultrasound. Of the 791 remaining subjects, 100 individuals did not undergo genetic testing and were thus excluded from further analysis. There were a range of reasons for not pursuing genetic testing, including denial by insurance, loss to follow up, parental denial, lack of concern for a genetic condition, and in some cases, no results were available despite the indication that a patient underwent testing. This left 691 study that met inclusion criteria (Figure 1)

An etiology for structural abnormalities was identified for 323 individuals in our population with structural abnormalities, of which 222 individuals had an identified genetic condition presumed to cause their structural abnormalities (Figure 2). Additionally, twenty six individuals were given a clinical diagnosis (Table 3).

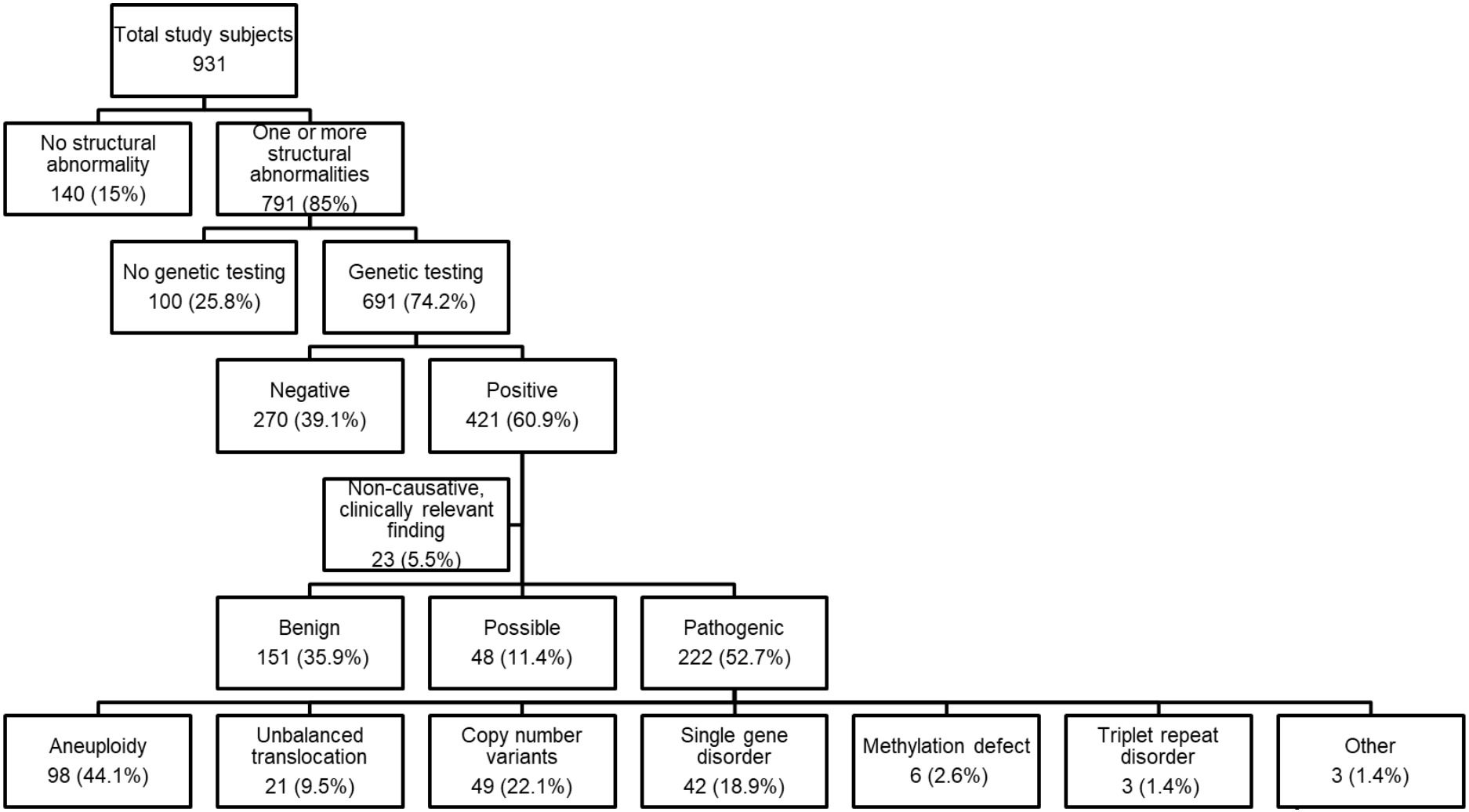

Figure 1: Breakdown of study cohort by testing and genetic aberration type

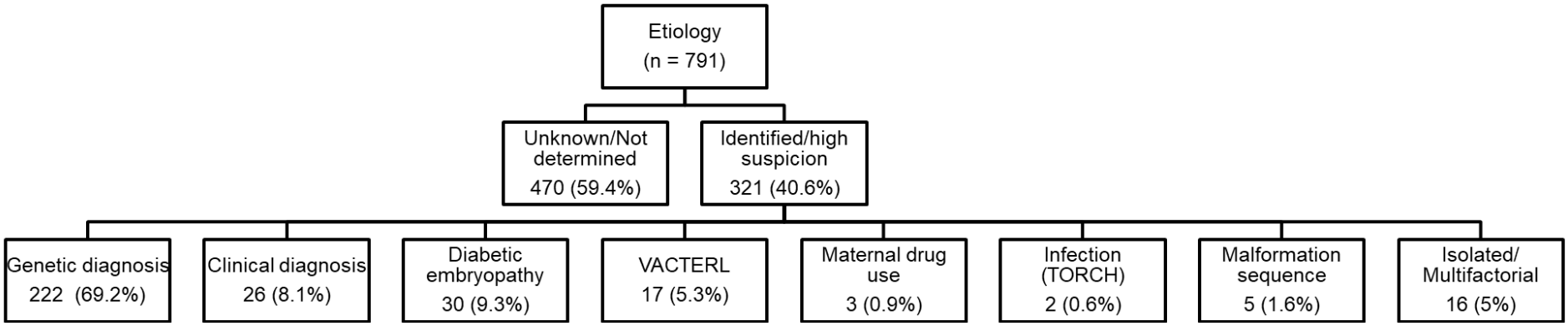

Figure 2: Etiology of structural abnormalities

Table 3: Clinical Diagnoses

Adams Oliver syndrome
Adrenal insufficiency
Beckwith-Wiedemann syndrome
Blue rubber bleb nevus syndrome (with rectal prolapse)
Campomelic dysplasia
Early infantile epileptic encephalopathy, type 5 (*SPTAN1* mutation)
Epidermolysis Bullosa
GLUT1 deficiency
Infantile cortical hyperostosis (Caffey's disease)
Kniest chondrodystrophy
Metaphyseal dysplasia
Non-syndromic autosomal dominant polydactyly (*x2*)
Oculo-Auriculo-Vertebral spectrum
Opitz GBBB syndrome type 2
Osteogenesis imperfecta
Popliteal Ptyergium Syndrome
Proximal focal femoral deficiency (PFFD)
Prune-Belly syndrome
Pseudohypoaldosteronism type 2
Septo optic dysplasia (*x2*)
Spondylothoracic dysostosis
Thanatophoric dysplasia (*x2*)
Tuberous sclerosis complex (TSC)

Of the 691 patients that underwent genetic testing, 270 (39.1%) had a negative result and 421 (60.9%) had a positive result. Of those with a positive result, 222 (52.7%) were pathogenic and attributed to the phenotype of the patient. There were 151 (35.9%) patients with one or more benign findings on genetic testing and 48 (11.4%) patients with one or more uncertain results (such as a variant of uncertain significance in one or more genes that could be related to phenotype), which were classified as “possible” (Figure 1). There were 23 (5.5%) patients with a clinically significant finding presumably unrelated to the structural abnormality on genetic testing (Table 4).

Table 4: Non-causative clinically relevant findings

Klinefelter syndrome *(x3 individuals; One mosaic individual)*
Hereditary Breast and Ovarian Cancer Syndrome (HBOC) *(x2: One patient had a deletion including BRCA2; the other had a point mutation in BRCA2)*
Disorder of Sexual Development /Discordant genitalia *(x2: one patient 46, XX with male genitalia; one patient 46, XY with female genitalia)*
48, XXYY syndrome
COL3A1 pathogenic variant, Vascular EDS (type IV EDS)
Mitochondrial disease of tRNA Ser (MELAS due to MT-TS1 mutation)
Biallelic HADB mutations (mitochondrial trifunctional protein deficiency)
Becker Muscular Dystrophy due to 0.145 Mb deletion at Xp21.1 encompassing dystrophin gene
Autosomal dominant polycystic kidney disease (ADPKD)
Axenfield-Rieger syndrome type 3
SPTAN1 mutation, Early infantile epileptic encephalopathy, type 5
Factor VIII deficiency
von Willebrand disease (from other workup)
Copy number loss at 1p31.1 (that father also has) associated with dilated cardiomyopathy
Alpha thalassemia trait
Additional *SHOX* gene due to gain at Xp22.33
Triple X (47, XXX)
9.399 Mb deletion of Xq27, increasing risk for developmental delay

In patients without an identified pathogenic mutation to explain their structural abnormalities and no other determined etiology, only 10.9% had comprehensive testing (CMA and WES). A majority of the patients (89.1%) at least underwent a CMA, with only 12.2% undergoing WES.

Potential Diagnostic Yield

The potential yield for each test was broken down in the following ways: first, the potential yield was calculated based on the number of individuals with a genetic aberration identifiable by the test, including causative and benign findings. Second, the diagnostic yield was refined for pathogenic variants that were described as causative of an individual's structural abnormalities.

When refined to pathogenic variants, CMA had the highest potential diagnostic yield of all the available prenatal testing and screening options included in this study [26.8% (95% CI: 23.5 -30.3)], followed by whole genome NIPT [21.2% (95% CI: 18.4 – 24.2)] karyotype [20.8% (95% CI: 17.8 – 24.0)], NIPT+CNVs [17.9% (95% CI: 15.3 - 20.9)], FISH [16.1% (95% CI: 13.5 - 19.1)], NIPT+ SCA [15.9% (95% CI: 13.4 - 18.9)], WES [15.1% (95% CI: 11.3 - 19.7)], NIPT [13.7% (95% CI: 11.3 - 16.6)], Methylation studies [2.4% (95% CI: 1.1 - 5.1)], NIPT+ SGD [2.3% (95% CI: 1.1 - 4.7)] and triplet repeat analysis [1.3% (95% CI: 0.4 - 3.8)], (Table 5, Figure 3).

In addition to having the highest diagnostic yield, CMA was also significantly more likely to identify a non-causative aberration compared to all other tests ($p < 0.001$) (Table 6), while WES was significantly more likely to identify a non-causative aberration compared to all other testing options except CMA ($p = 0.013$). Almost half (43%) of the findings identified by CMA were non-causative aberrations and 16% of the aberrations identified by WES were non-causative, compared to the 0-6% non-causative findings identified on all other tests.

Table 5: Potential diagnostic yield of prenatal testing for entire cohort

Test	Denominator	DRT*	All Findings		Causative, Pathogenic Findings	
			N_{ALL} ‡ ‡	DY**, % (95% CI†)	N_{POT} ‡ ‡	DY**, % (95% CI†)
Screening tests						
NIPT ‡	658	0.994	91	13.7 (11.3 - 16.6)	91	13.7 (11.3 - 16.6)
NIPT+ SCA ‡	658	0.989	111	16.7 (14.1 - 19.7)	106	15.9 (13.4 - 18.9)
NIPT +CNV ‡	655	0.911	134	18.6 (16.0 - 21.6)	129	17.9 (15.3 - 20.9)
Whole genome NIPT	655	0.918	157	22.0 (19.2 - 25.1)	151	21.2 (18.4 - 24.2)
NIPT + SGD‡	279	0.914	7	2.3 (1.1 - 4.7)	7	2.3 (1.1 - 4.7)
Diagnostic tests						
FISH ‡	658	1	111	16.9 (14.2 - 19.9)	106	16.1 (13.5 - 19.1)
Karyotype	655	1	145	22.1 (19.1 - 25.5)	136	20.8 (17.8 - 24.0)
CMA ‡	639	1	323	50.5 (46.7 - 54.4)	171	26.8 (23.5 - 30.3)
WES ‡	279	1	50	17.9 (13.9 - 22.9)	42	15.1 (11.3 - 19.7)
Methylation studies	250	1	6	2.4 (1.1 - 5.1)	6	2.4 (1.1 - 5.1)
Triplet repeat analysis	228	1	3	1.3 (0.4 - 3.8)	3	1.3 (0.4 - 3.8)

* DRT = Detection Rate of the test (For screening test: previously reported detection rate x prevalence of condition in cohort; For diagnostic tests: assumed to be 100%)

‡‡ N_{ALL} = number of patients with a genetic aberration that could have potentially been identified by the test ; N_{POT} = number of patients with a *causative* genetic aberration that could have potentially been identified by the test

** DY = Diagnostic yield, (Numerator/Denominator) x DR x 100

‡NIPT = Non-invasive prenatal test ; SCA= sex chromosome aneuploidy ; CNV = copy number variant ; SGD = single gene disorder; FISH = Fluorescent in situ hybridization; CMA = chromosomal microarray ; WES = whole exome sequencing ; CI = confidence interval

Table 6: p-values of prenatal testing for entire cohort										
	NIPT+ SCA	NIPT+ CNV	Whole genome NIPT	NIPT+ SGD	FISH	Karyotype	CMA	WES	Methylation studies	Repeat analysis
NIPT	0.261	**0.037**	**<0.001**	**<0.001**	0.222	**0.001**	**<0.001**	0.574	**<0.001**	**<0.001**
NIPT+ SCA		0.334	**0.014**	**<0.001**	0.921	**0.022**	**<0.001**	0.758	**<0.001**	**<0.001**
NIPT+ CNV			0.132	**<0.001**	0.385	0.184	**<0.001**	0.298	**<0.001**	**<0.001**
Whole genome NIPT				**<0.001**	**0.018**	0.859	**0.018**	**0.031**	**<0.001**	**<0.001**
NIPT +SGD					**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.940	0.406
FISH						**0.028**	**<0.001**	0.701	**<0.001**	**<0.001**
Karyotype							**0.011**	**0.043**	**<0.001**	**<0.001**
CMA								**<0.001**	**<0.001**	**<0.001**
WES									**<0.001**	**<0.001**
Methylation studies										0.376

Table 6: p-values of two-sample proportion test between the potential detection rates of each prenatal test among the entire study cohort. Statistical significance was assumed at a Type I error rate of 5%. Values bolded indicate statistical significance.

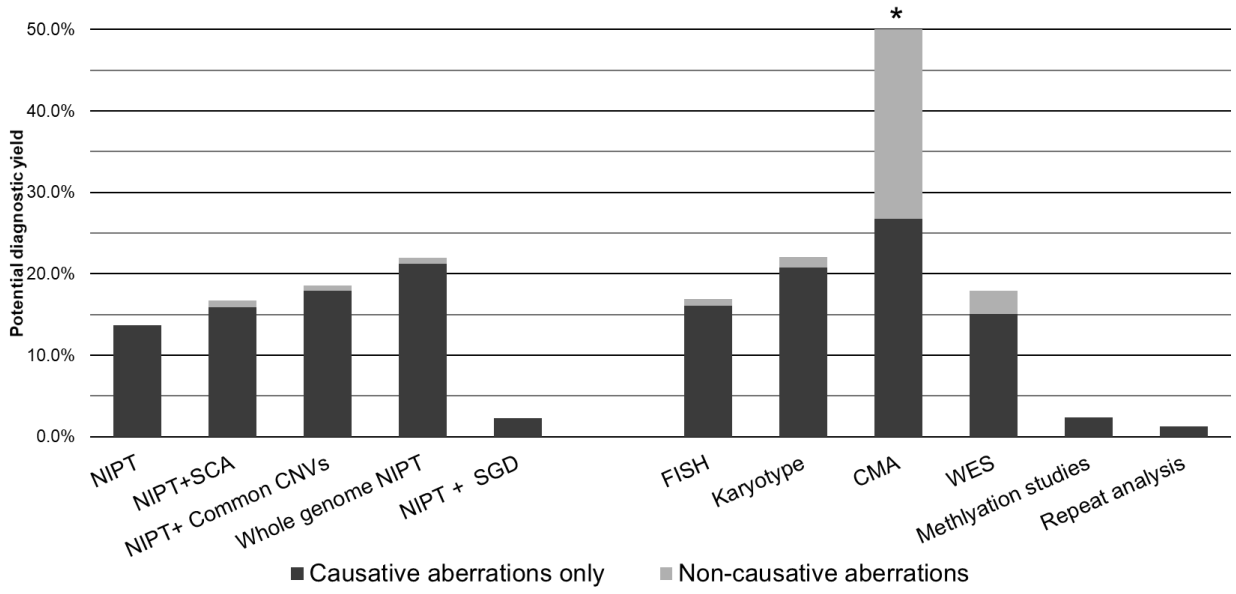

Figure 3: Potential diagnostic yield of prenatal testing options for entire cohort

The potential diagnostic yield of each prenatal testing option is depicted by the bar graph. Darker bars indicate the potential yield of pathogenic mutations presumed causative of an individual's structural abnormalities. The light bars indicate the additional yield of non-causative findings, which include benign findings, uncertain findings, and clinically significant findings presumed unrelated to the structural abnormalities. An asterisk (*) indicates a p-value less than 0.05 in two proportion comparison between CMA and every other test for both causative and non-causative aberrations potential yield.

Study Cohort: Isolated structural abnormality vs multiple structural abnormalities

Of the 791 individuals in the cohort who had one or more structural abnormalities, 143 (18.1%) had an isolated abnormality and 648 (81.9%) had multiple structural abnormalities (MSA) (two or more structural abnormalities). Of 143 the individuals with an isolated structural abnormality, 115 (80.4%) underwent genetic testing. Of these individuals 23 (20.0%) had a pathogenic result that explained their phenotype (Figure 4).

Of the 642 individuals with MSA, 576 (88.9%) underwent genetic testing. This was significantly greater than the 80.4% testing rate among individuals with an isolated structural abnormality (p=0.0057). Of these individuals, 199 (34.4%) had pathogenic results that explained their phenotype (Figure 5).

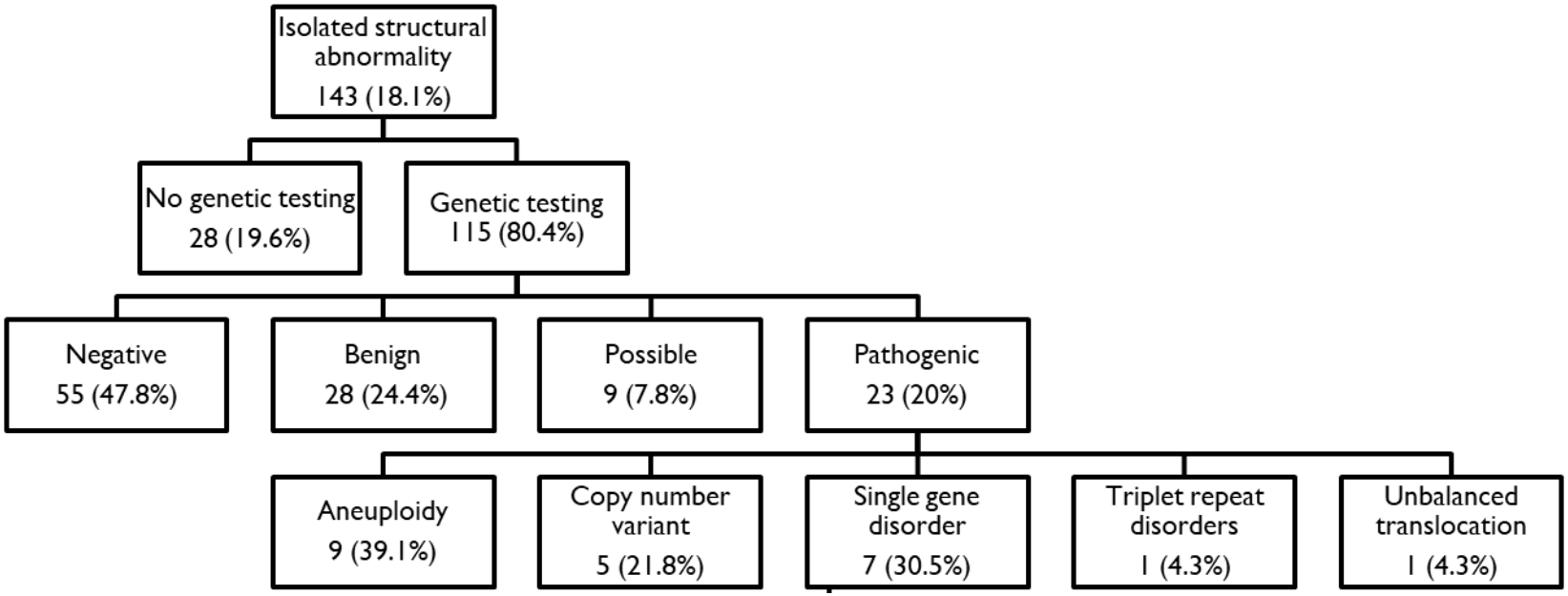

Figure 4: Breakdown of isolated cohort by testing and type of genetic aberration

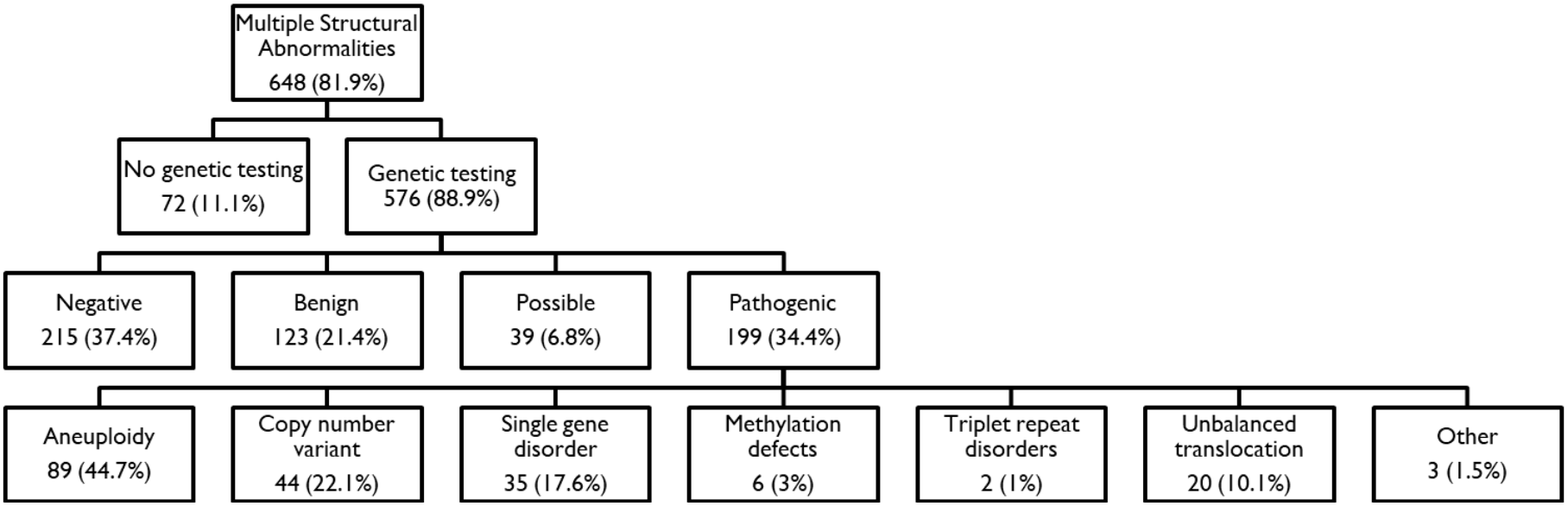

Figure 5: Breakdown of MSA cohort by testing and type of genetic aberration

Potential Diagnostic Yield:

The potential diagnostic yield for each test was broken down in the same manner as for the entire cohort. In the isolated cohort, WES had the highest potential diagnostic yield for causative aberrations 25.9% (95% CI: 13.2 - 44.7), followed by CMA 14.9% (95% CI: 9.2 - 23.1), (Table 7, Table 8). In the MSA cohort, CMA had the highest potential diagnostic yield of 29.0% (95% CI: 25.3 – 33.0), followed by whole genome NIPT 23.2% (95% CI: 21.8 – 29.0).

In addition to having the highest diagnostic yield, CMA was also significantly more likely to identify a non-causative aberration compared to all other tests ($p < 0.001$). WES was also significantly more likely to identify a non-causative aberration compared to all other testing options except CMA ($p = 0.001$). Similar to the entire cohort, almost half (45%) of the findings identified by CMA were non-causative aberrations and 19% of the aberrations identified by WES were non-causative, compared to the 0-5% of non-causative findings identified by all other tests (Table 9, Table 10).

Table 7: Potential diagnostic yield of prenatal testing in individuals with an isolated structural abnormality

Test	Denominator	DRT*	All Findings		Causative, Pathogenic Findings	
			N_{ALL} ‖	DY**, % (95% CI†)	N_{POT} ‖	DY**, % (95% CI†)
Screening tests						
NIPT†	105	0.997	7	6.6 (3.3 – 13.1)	7	6.6 (3.3 – 13.1)
NIPT+ SCA†	105	0.988	10	9.4 (5.2 – 16.7)	9	8.5 (4.6 - 15.5)
NIPT +CNV†	104	0.907	12	10.5 (6.7 – 19.1)	11	9.6 (6.0 – 18.0)
Whole genome NIPT	104	0.911	13	11.4 (7.5-20.2)	12	10.5 (6.7 -19.1)
NIPT + SGD†	27	0.820	1	3.0 (0.7 - 18.3)	1	3.0 (0.7 - 18.3)
Diagnostic tests						
FISH†	105	1.000	11	10.5 (6.0-17.8)	10	9.5 (5.3 – 16.6)
Karyotype	104	1.000	14	13.5 (8.2 - 21.3)	11	10.6 (6.0 – 16.6)
CMA†	101	1.000	43	42.6 (33.4 – 52.3)	15	14.9 (9.2 – 23.1)
WES†	27	1.000	7	25.9 (13.1 - 44.7)	7	25.9 (13.2 - 44.7)
Methylation studies	27	1.000	0	0.0 (0 - 0)	0	0.0 (0 - 0)
Triplet repeat analysis	23	1.000	1	4.3 (0.8 -21.0)	1	4.3 (0.8 – 21.0)

* DRT = Detection Rate of the test (For screening test: previously reported detection rate x prevalence of condition in cohort; For diagnostic tests: assumed to be 100%)

‖ N_{ALL} = number of patients with a genetic aberration that could have potentially been identified by the test ; N_{POT} = number of patients with a *causative* genetic aberration that could have potentially been identified by the test

** DY = Diagnostic yield, (Numerator/Denominator) x DR x 100

†NIPT = Non-invasive prenatal test ; SCA= sex chromosome aneuploidy ; CNV = copy number variant ; SGD = single gene disorder; FISH = Fluorescent in situ hybridization; CMA = chromosomal microarray ; WES = whole exome sequencing ; CI = confidence interval

Table 8: p-values of prenatal testing in individuals with an isolated structural abnormality

	NIPT+ SCA	NIPT+ CNV	Whole genome NIPT	NIPT+ SGD	FISH	Karyotype	CMA	WES	Methylation studies	Repeat analysis
NIPT	0.602	0.427	0.313	0.478	0.440	0.302	0.054	**0.004**	0.170	0.678
NIPT+SCA		0.782	0.622	0.329	0.800	0.605	0.152	**0.013**	0.117	0.495
NIPT+CNV			0.829	0.266	0.980	0.811	0.247	**0.025**	0.094	0.413
Whole genome NIPT				0.224	0.810	0.981	0.344	**0.038**	0.079	0.356
NIPT + SGD					0.271	0.220	0.096	**0.017**	0.365	0.806
FISH						0.791	0.236	**0.023**	0.096	0.420
Karyotype							0.356	**0.040**	0.077	0.350
CMA								0.179	**0.033**	0.172
WES									**0.005**	**0.038**
Methylation studies										0.277

Table 8: p-values of two-sample proportion test between the potential detection rates of each prenatal test among the isolated study cohort. Statistical significance was assumed at a Type I error rate of 5%. Values bolded indicate statistical significance.

Table 9: Potential diagnostic yield of prenatal testing in individuals with MSA

Test	Denominator	DRT*	All Findings		Causative Findings	
			N_{ALL} ⇈	DY**, % (95% CI[ǀ])	N_{POT} ⇈	DY**, % (95% CI[ǀ])
Screening tests						
NIPT[†]	553	0.994	84	15.1 (12.4 - 18.4)	84	15.1 (12.4 - 18.4)
NIPT+ SCA[†]	553	0.989	101	18.1 (15.3 - 21.7)	97	17.54(14.6 - 21.0)
NIPT +CNV[†]	551	0.911	122	20.2 (18.9 - 25.9)	118	19.5 (18.2 - 25.0)
Whole genome NIPT	551	0.919	144	24.0 (22.6 - 30.0)	139	23.2 (21.8 - 29.0)
NIPT + SGD[†]	252	0.930	6	2.2 (1.1 -5.1)	5	2.2 (1.1 -5.1)
Diagnostic tests						
FISH[†]	553	1	100	18.1 (15.1 - 21.5)	96	17.4 (14.4 - 20.7)
Karyotype	551	1	131	23.8 (20.4 - 27.5)	125	22.7 (19.4 - 26.4)
CMA[†]	538	1	808	52.0 (47.8 - 56.2)	156	29.0 (25.3 - 33.0)
WES[†]	252	1	43	17.1 (12.9 - 22.2)	35	13.9 (10.2 - 18.7)
Methylation studies	223	1	6	2.7 (1.2 - 5.7)	6	2.7 (1.2 - 5.7)
Triplet repeat analysis	205	1	2	1.0 (0.3 - 3.5)	2	1.0 (0.3 - 3.5)

* DRT = Detection Rate of the test (For screening test: previously reported detection rate x prevalence of condition in cohort; For diagnostic tests: assumed to be 100%)

⇈ N_{ALL} = number of patients with a genetic aberration that could have potentially been identified by the test ; N_{POT} = number of patients with a *causative* genetic aberration that could have potentially been identified by the test

** DY = Diagnostic yield, (Numerator/Denominator) x DR x 100

[†] NIPT = Non-invasive prenatal test ; SCA= sex chromosome aneuploidy ; CNV = copy number variant ; SGD = single gene disorder; FISH = Fluorescent in situ hybridization; CMA = chromosomal microarray ; WES = whole exome sequencing ; CI = confidence interval
MSA = multiple structural abnormalities, two or more structural abnormalities

Table 10: p-values of prenatal testing in individuals with MSA										
	NIPT+ SCA	NIPT+ CNV	Whole genome NIPT	NIPT+ SGD	FISH	Karyotype	CMA	WES	Methylation studies	Repeat analysis
NIPT	0.300	0.053	**0.001**	**<0.001**	0.300	**0.001**	**<0.001**	0.656	**<0.001**	**<0.001**
NIPT+ SCA		0.368	**0.016**	**<0.001**	1.000	**0.028**	**<0.001**	0.213	**<0.001**	**<0.001**
NIPT+ CNV			0.134	**<0.001**	0.368	0.193	**<0.001**	0.054	**<0.001**	**<0.001**
Whole genome NIPT				**<0.001**	**0.017**	0.844	**0.029**	**0.002**	**<0.001**	**<0.001**
NIPT+ SGD					**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.724	0.318
FISH						**0.028**	**<0.001**	0.213	**<0.001**	**<0.001**
Karyotype							**<0.001**	0.213	**<0.001**	**<0.001**
CMA								**<0.001**	**<0.001**	**<0.001**
WES									**<0.001**	**<0.001**
Methylation studies										0.197

Table 10: p-values of two-sample proportion test between the potential detection rates of each prenatal test among the MSA study cohort. Statistical significance was assumed at a Type I error rate of 5%. Values bolded indicate statistical significance.

Comparison of isolated cohort to MSA cohort

The potential diagnostic yield was significantly higher for all test types in individuals with MSA compared to individuals with an isolated strucutral abnormality except NIPT+SGD, WES, methylation studies and repeat analysis (Figure 6). For these tests, there were no significant differences in diagnostic yield based on isolated or multiple structural abnormalities.

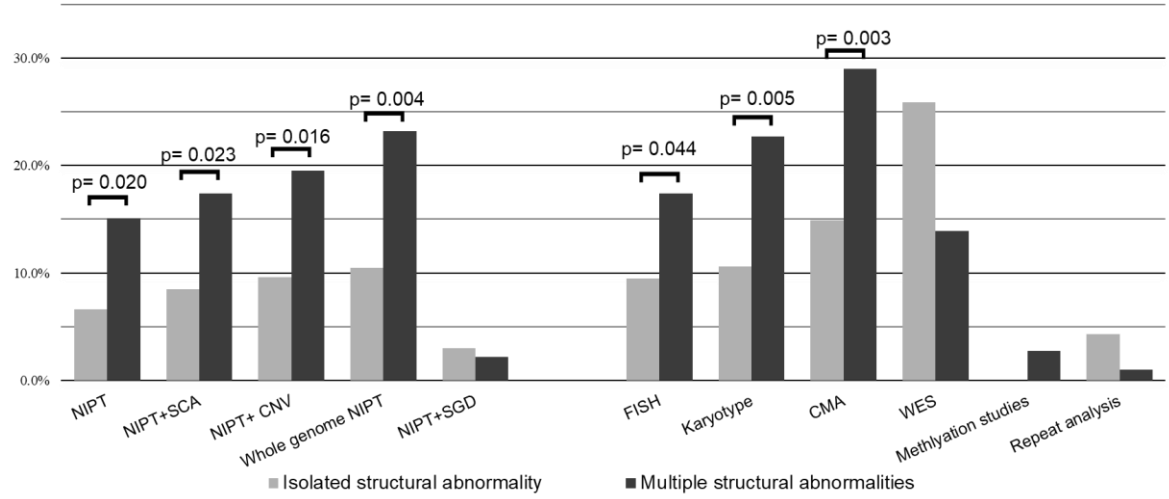

Figure 6: Potential diagnostic yield of prenatal testing options in isolated and MSA cohorts

The potential yield of each prenatal testing option is depicted by the bars. The light grey bars indicate the yield of a test in the isolated structural abnormalities cohort and the dark grey bars indicate the same in the multiple structural abnormalities cohort. Listed p-values are significant differences in the potential yield of testing modalities between the MSA and isolated cohorts. Those not listed did not reach significance.

Discussion

Chromosome Abnormalities

Of the 222 patients with a causative genetic aberration identified in our study, 164 (74%) were diagnosed with a chromosome abnormality or microdeletion or duplication (aneuploidy, unbalanced translocations, or copy number variants). Therefore, CMA had the highest potential diagnostic yield across the entire cohort compared to other prenatal screening and testing options. CMA also had a significantly higher diagnostic yield among individuals with MSA compared to an isolated structural abnormality ($p = 0.003$), indicating a high incidence of chromosome abnormalities in the presence of MSA. This is consistent with previous studies comparing CMA and karyotype, studies comparing CMA to NIPT, and studies comparing CMA in pregnancies with isolated vs. multiple anomalies [4, 17, 24].

Not surprisingly, the potential diagnostic yield of CMA was significantly greater than all NIPT screening options, including whole genome NIPT ($p =0.018$), further supporting the recommendation to use diagnostic testing over screening methods after identification of an ultrasound abnormality [2, 18]. Assuming data on detection rates for whole genome are accurate, there is potentially a loss of 5.6% of prenatal diagnoses if whole genome NIPT is used over CMA after identification of one or more structural abnormalities. This is important to discuss when reviewing test options, as the difference in diagnostic yield might influence whether a patient to chooses an invasive procedure over a screening procedure.

Due to the high rate of chromosome abnormalities and microdeletions and microduplications detected in our cohort, whole genome NIPT had the second highest potential yield in this study and karyotype had the third highest potential yield, but these were not significantly different from each other ($p=0.845$). From this data, one could extrapolate that whole genome NIPT provides an overall yield comparable to a karyotype. However, until sufficient data is published in peer reviewed journals

supporting high sensitivity and specificity claimed in the current literature for whole genome NIPT one may wish to proceed with caution at equivocating the two [13].

Single gene disorders

Forty-two patients were found to have a single gene disorder in our cohort (18.9%) that could be detected by a sequencing test, such as whole exome sequencing, but would not be found on a CMA or whole genome NIPT. The potential diagnostic yield of prenatal WES across our isolated and MSA, corresponds with the previously reported prenatal WES yield of 6.2-80% [5-8]. The predicted diagnostic yield from this study could potentially be lower in practice in a prenatal population, as the patients were evaluated after delivery and thus could have had additional clinical indications to suggest a single gene disorder that would not have been detected on a prenatal ultrasound.

WES findings were detected in 35 different genes, of which only 7 could have been screened by clinically available NIPT+SGD. This was reflected in the significant differences in potential diagnostic yield for prenatal WES and NIPT+SGD in both the isolated structural abnormality cohort [25.9% vs 3.0%], and MSA cohort [13.9% vs 2.2%]. While NIPT+SGD provides another avenue to identify individuals with a single gene disorder, the use of this test is limited to the specific genes on the panel and conditions that are de novo or paternally inherited. In addition, the only data available on this clinically available test is a single white paper and the detection rates quoted range from 43-99% [16]. These are important limitations to stress during pre-test counseling.

Due to the challenge of obtaining insurance coverage for prenatal whole exome sequencing, we sought to identify sequencing panels that could identify the genetic aberration in our single gene diagnosis category. We were able to identify available prenatal sequencing panels for 22 of the 35 different genes, which could have potentially provided a diagnosis for 29 out of the 42 (69.0%) individuals with an identified single gene disorder. The remaining 31% of these individuals could only have been identified

by prenatal WES. This demonstrates the utility of prenatal WES and the need for insurance coverage of prenatal WES after identification of one or more structural abnormalities on ultrasound.

Incidental and Uncertain Findings

In addition to the highest potential diagnostic yield, CMA had the highest rate of benign or uncertain results compared to all screening counterparts and other diagnostic tests ($p<0.001$). This is an important component of pre-test counseling to ensure there is a full consent to the testing type and the possibility of identifying a result that is either not causative of the identified structural abnormalities, an incidental finding, or variant of uncertain significance that could potentially provide an answer in the future or not.

WES had the second highest rate of benign or uncertain findings compared to other screening and diagnostic tests ($p = 0.013$). The higher rate of incidental findings on WES in our study cohort might be lower in a prenatal population, as the reporting is slightly different. Prenatal WES reports are typically focused on genes known to cause abnormalities noted in the clinical indication. Reports can include variants of uncertain significance and secondary findings. Our study cohort had 8 individuals with a finding potentially identifiable on prenatal WES that were not causative aberrations. Prenatal reporting of these findings would depend upon the performing laboratory and patient preferences.

Uncertain or incidental findings may also be identified through NIPT but due to variable reporting practices by NIPT laboratories we are unable to quantify how often these incidental findings may be detected and reported by a screening test.

Utilization of diagnostic yield in clinical settings

Discussions about prenatal testing options after the identification of one or more structural abnormalities should include a discussion of the risks, benefits and limitations

of genetic testing to allow for well-informed and autonomous patient decision-making. The diagnostic yields described in this study should be used as a baseline for this pre-test counseling. There are many other factors that should be considered in addition to potential diagnostic yield, including the differential diagnosis, patient desire for information, cost of testing/insurance coverage compared to the increase in yield, the potential for uncertain, incidental, or secondary findings, and the positive predictive value of testing. In addition, clinicians should also integrate relevant information such age, family history, and abnormalities identified to help direct testing recommendations.

Strengths and Limitations

Our study included a large population of infants who were seen by board certified medical geneticists in a large tertiary care, academic medical center. This setting allowed for a large study cohort of patients that underwent accurate postnatal assessments, but as in any retrospective chart review, ours was limited by information recorded in the electronic medical record. The patients in this study were first seen by the medical genetics team between January 2014 and December 2017, which should have allowed adequate time for full genetic workup by the time of data collection in fall 2018.

However, not every individual received the recommended workup due to insurance denials or loss to follow up. Furthermore, testing strategies utilized by healthcare providers are influenced by the clinical presentation, family history, cost considerations (including insurance), patient follow-up, and results of any other testing done. Since our data on the yield of the tests relies on which tests were or were not performed in our cohort, factors that influence testing might act as potential confounders and/or effect modifiers in our analysis. This is highlighted by the 77% (n=306, 95% CI: 73.10 – 81.31) who did not have a comprehensive workup (CMA & WES)

Additionally, the diagnostic yield experienced in a prenatal testing setting may not be equivalent due to postnatal ascertainment bias. Structural abnormalities were included in this study if they had the *potential* to be detected by prenatal ultrasound. We did not confirm that all structural abnormalities were in fact detected prenatally. Some structural abnormalities may not be detected on routine ultrasound or by all ultrasound centers and thus the classification of a patients as having an isolated or multiple structural abnormalities may differ between institutions, and thus the potential diagnostic yield of testing will vary as a function of the skills of the sonographers and the nature of the defects.

In order to evaluate diagnostic yields reflective of screening tests, prenatal screening test sensitivities were determined using current literature, which leads to some limitations. For some of the conditions on these screens, such as Down syndrome, robust data exists from which we were able to obtain well supported detection rates. For conditions that have been added to screening tests more recently, such as microdeletion syndromes and select autosomal dominant single gene disorders, data regarding the sensitivity of testing is not as widely available nor is it nearly as well established.

Despite these limitations, this study includes the most prenatal screening and diagnostic options available in clinical settings than any other previous study, providing a more robust look at the potential diagnostic yield of all prenatal testing options available.

Conclusions

The data presented here provide further evidence that CMA has the highest potential diagnostic yield among all current prenatal testing options after identification of one or more structural abnormalities on ultrasound. Additionally, CMA also had the highest rate of non-causative (benign, uncertain, or incidental) results. As expected, screening tests had a lower potential yield compared to CMA. Expanded NIPT (NIPT+CNVs and whole genome NIPT) had a higher potential yield than traditional NIPT

and whole genome NIPT had a comparable yield as a karyotype. While interesting, it is important to consider the limited data on expanded NIPT and how this might affect study results and post-test counseling regarding screening results. When deciding which testing options to pursue, patients should be counseled about the differences in potential yield of testing among diagnostic and screening tests, and be informed of the potential of obtaining a result that is uncertain or considered incidental. Further investigation into the potential yield of expanded NIPT panels and prenatal WES should be explored.

Appendix

Supplemental Table 1: Pathogenic findings presumed causative of structural abnormalities

Aneuploidies	n	Iso	MSA	Mos	FISH/Karyotype/CMA result
Monosomy X (Turner syndrome)	8	2	6	1	45, X ; 45,X/46, XX
Monosomy X (ring X)	2		2	2	45, X/46, X, +r
Monosomy X/ 46, XY	2		2	2	45, X/ 46, XY
Triploidy	1		1		69, XXX
Trisomy 13	3		3		47, XX, +13 (47, XY, +13)
Trisomy 16	1		1	1	47, XY, +16 /46, XY
Trisomy 18	11		11		47, XX, +18 (47, XY, +18)
Trisomy 21	68	6	62		47, XX, +21 (47, XY, +21)
49, XXXXY	2	1	1		49, XXXXY
Total	*98*	*8*	*89*		

Unbalanced translocation/chromosome rearrangements	n	Iso	MSA	Mos	Karyotype/CMA result
Monosomy X (Turner syndrome)	1		1		46, X, der(X)t(X;7)(q24;q22)
Monosomy X and isodicentric Y	1		1	1	45,X[26]/46,X, psu idic(Y)(q11.23)[4]
Tetrasomy isochromosome 12p (Pallister-Killian)	1		1	1	Tetrasomy 12p
Trisomy 13	1		1		46, XY, +13, der(13;14)(q10;q10)
Trisomy 18	1		1		46, XX, der(3)t(3;18)(p26.1;q11.2)
Trisomy 21	3		3	1	46, XX, +21, der(21;21)(q10;q10)
	1		1		46, XY, +21 der(21;21)(q10;q10)
	1	1			46, XX, +21, der(13;21)(q10;q10)
Complex chromosome 8 rearrangement	1		1		8p23.3p23.1(194617-8403434)x1, 8p23.1p12(12580104-33119221)x3, 8q24.12q24.3(121831416-146294241)x3
Derivative chromosome 12 with terminal duplication	1		1		12p13.33p13.32(189216-3454991)x1, 12p13.32p12.1(3523313-23666601)x3
1q deletion and 9p duplication	1		1		9p duplication, 1q deletion
4q terminal deletion and 8q terminal duplication	1		1		46,XX,der(4);t(4,8)(q35.2-24.3)

5p deletion and 9p duplication	1		1	5p15.33p15.2(113576-11410194)x1, 9p24.3p22.2(203861-18301446)x3
Partial trisomy 5p	1		1	46, XX, add 5p15.2
5 and 12	1		1	Unbalanced translocation between chromosomes 5 and 12
Partial 7q duplication	1		1	partial 7q duplication
9p deletion and 4q duplication	1		1	4q32.3q35.2(164780923-190791227)x3, 9p24.3p22.3(207454-14746829)x1
10q26.12 deletion and 3p26.3 duplication	1		1	46, XX, der(10)t(3;10)(p25.1;q26.12)
1q32-q41 and q41;qter duplication, and 10q terminal deletion	1		1	1q32-q41 duplication, 1q41;qter duplication, 10q terminal deletion
Total	*21*	*1*	*20*	

Copy Number Variants	n	Iso	MSA	Mos	CMA result	Size (range)
1p31.1-31.2 interstitial deletion	1		1		1p31.1p32.1 interstitial deletion	13.35 Mb
1p34 deletion	1		1		1p34.1p32.3 deletion (45748571-54527813)	8.779 Mb
1p36 deletion syndrome	2		2		1p36.3 deletion	3.4-9.736 Mb
1q43 deletion syndrome	3	1	2		1q43q44 deletion	4.88-12.1 Mb
2p23.3p25.1 deletion	1		1	1	2p23.3p25.1 deletion	15.8 Mb
2q22.1q33.2 deletion syndrome	1	1			2q33.1q33.2 deletion (199710981-204484143)	4.773 Mb
3q deletion syndrome	1		1		3q deletion	
3q26.32q29 duplication	1		1		3q26.31q29 duplication (174251329-197717518)	23.47 Mb
4q31.3 deletion syndrome	2		2		4q31.3 deletion	38-38.392 Mb
7q11.23 deletion (Williams syndrome)	1	1			7q11.23 deletion (72744494-74142327)	1.3 Mb
7q22.3 deletion	1		1		7p22.3 deletion (1-2759647)	2.760 Mb
7q36.2 deletion	1		1		7q36.2 deletion (153584506-153647972)	0.063 Mb
8q11.21q12.1 deletion	1		1		8q11.23q12.1 deletion (54871180-58883606)	4.012 Mb
9q34.3 deletion (Kleefstra Syndrome)	1		1		9q34.3 deletion (139876171-141213421)	1.337 Mb
13q12.3q13.2 deletion	1		1		13q12.3q13.2 deletion (28933097-35163380)	6.230 Mb
14q32.31 deletion	1		1		14q32.31 deletion	
15q11.2q13.3 deletion (Prader Willi syndrome)	1		1		15q11.2q13.3 deletion	10.75 Mb

15q21.2 deletion	1		1		15q21.2q22.2 deletion (50833347-61673964)	10.841 Mb
15q26.2-q26.3 deletion	1		1		15q26.2-q26.3 deletion (95462599-102354857)	7.096 Mb
16p13.3 deletion	1		1		16p13.3 deletion (3639643-4261338)	0.622 Mb
16q12.2 deletion	1		1		16q12.2q21 deletion (54514235-64397522)	9.883 Mb
17p13.3 deletion (Miller-Dieker syndrome)	1		1		17p13.3p13.2 deletion (1-5559951)	5.487 Mb
18q11.2 interstitial deletion	1		1		18q11.2 deletion	0.518 Mb
21q22.3 deletion	1		1		21q22.3 deletion (43619800-48157577)	4.5 Mb
22q11.21 deletion syndrome (DiGeorge syndrome)	19	2	17		22q11.21 deletion	2.762 - 5.747 Mb
22q13.31q13.33 deletion (Phelan McDermid)	1		1		22q13.31q13.33 deletion (47388907-51304566)	3.916 Mb
Xp11.4 deletion	1		1		Xp11.4 deletion (41589371-41599075)	0.010 Mb
Total	*49*	*5*	*44*			

Single gene disorders	n	Iso	MSA	Mos	Gene and coding change(s)
3-methylglutaconic aciduria type VII	1		1		*CLPB*: c.[1156+1G>A] ; [1156+1G>A]
Achondrogenesis type 2 or hypochondrogenesis	1		1		*COL2A1*: c.1587G>A (p.Gly513Ser)
Adams-Oliver syndrome	1		1		*NOTCH1*: c.166C>T (p.R56*)
Androgen insensitivity	1		1		*AR*: c.2659A>G (p.M887V)
Antley-Bixlar syndrome (POR deficiency)	1		1		*POR:* c.859G>C (p.Ala287Pro)
ARX related disorder	1		1		*ARX*: c.1295_ 1317dup23 (p.A440fs)
Autosomal Recessive Polycystic Kidney Disease (ARPKD)	1		1		*PKHD1*: c.[3761_3762delCCinsG] ; [5895dupA]
Beckwith-Wiedemann Syndrome (BWS)	1		1		*CDKN1C*: c.189C>G (p.Tyr63*)
Brain-Lung-Thyroid Syndrome	1		1		*NKX2-1:* c.390C>G (p.Y130X)
BRAT1 syndrome (Rigiddty and multifocal seizure syndrome, leathal neonatal)	1		1		*BRAT1*: c.[1710delG] ; [566dupG]
Campomelic dysplasia	2		2		*SOX9:*c.628_638dup11
					SOX9: c.55delT
CHARGE syndrome	2		2		*CHD7:* c.779_780delCC (p.P260fs)
					CHD7: c.3065_3066dupTT (p.A1023Lfs*20)
Congenital Adrenal Hyperplasia (CAH)	1		1		*CYP21*: In2G mutation and large gene conversion due to 30 kb deletion of *CYP21A1P* and *CYP21A2*

Congenital diaphragmatic hernia and heart defects, multiple 4	1		1	*NR2F2*: c.103_109delGGCGCCC (p.Gly35ArgfsTer75)
Cystic Fibrosis	2	2		*CFTR*: c.[1521_1523delCTT] ; [unknown] **+ sweat test
				CFTR: c. [1521_1523delCTT]; [1288insTA]
Desbuquois dysplasia type 2	1		1	*XYLT1*: c.[2560G>T] ; [2560G>T]
Dilated cardiomyopathy 1S	1	1		*MYH7* : c.602T>C (p.I201T)
Heterotaxy syndrome due to *NODAL* mutation	1		1	*NODAL*: c.778G>A (p.G260R)
Hypertrichotic osteochondrodysplasia (Cantu syndrome)	1		1	*ABCC9*: c.1664T>C (p.F555S)
Joubert syndrome type 10	1		1	*OFD1*: c.2668C>T (p.R890X)
Kabuki Syndrome	1		1	*KMT2D*: c.10938_10939delinsT (p.P3647fs)
Kniest dysplasia	1		1	*COL2A1* pathogenic mutation
Mandibulofacial Dysostosis, Guion-Almedia Type	1		1	*EFTUD2*: c.702+1G>T
Marfan syndrome	2		2	*FBN1*: c.3094T>C (p.Cys1032Arg)
				FBN1: c.4188delA (p.Gly1397Valfs*16)
Morquio Syndrome A (MPS 4)	2	1	1	*GALNS*: c. [633+1G>C]; [1558T>C] *sibs
Multiple Endocrine Neoplasia type 2 (MEN2)	1	1		*RET*: c.1144C>T (p.Gln382*)
Noonan syndrome	1		1	*PTPN11*: c.854T>C (p.F285S)
	1		1	*HRAS*: c.34G>A (p.G12S)
Osteogenesis Imperfecta type III	1		1	*COL1A2:* c.821G>A (p.Gly274Asp)
Pfeiffer syndrome type 3	1		1	*FGFR2*: c.870G>T (Pro250Arg)
Simpson-Golabi-Behmel syndrome	1		1	*GPC3*: c.760C>T (p.Arg254*)
Stickler syndrome	1		1	*COL2A1*: c.1587G>A (p.Gly513Ser)
TARP syndrome	1		1	*RBM10:* c.1473_1474delGT (p.S492Dfs*25)
Tuberous Sclerosis Complex (TSC)	1	1		*TSC2*: c.2590C>T (p.Gln864X)
X-Linked hydrocephalus	1		1	*L1CAM*: c.2014C>T (p.GLN672*)
X-linked Spinal Muscular Atrophy	1		1	*UBA1*: c.1731C>T (p.N577N)
ZIC3 mutation	1	1		*ZIC3*: c.75C>G (p.H25Q)
Total	*42*	*7*	*35*	

Methylation Disorders	n	Iso	MSA	Mos	Methylation analysis
Beckwith Wiedemann syndrome (BWS)	3		3		IC2 (LIT1) hypomethylation
Beckwith-Wiedemann syndrome (BWS)	1		1		Paternal UPD of 11p15
Russell Silver syndrome (RSS)	1		1		Loss of methylation at DMR1
Prader Willi syndrome (PWS)	1		1		Maternal UPD of 15q11.2-11.3
Total	*6*	*0*	*6*		

Triplet Repeat Disorders	n	Iso	MSA	Mos	Gene, triplet repeat, number of repeats
Congenital Myotonic Dystrophy (type 1)	2		2		*DMPK*: greater than 1500 CTG repeats
Congenital Central Hypoventilation Syndrome (CCHS)	1	1			*PHOX2B:* 27 and 20 polyalanine repeats
Total	*3*	*1*	*2*		

"Other" Disorders	n	Iso	MSA	Mos	Description of defect
Testicular Disorder of Sexual Development	1		1		46, XX (with male genitalia)
Mosaic Trisomy 21 and Trisomy 18	1		1	1	Mosaic Trisomy 21 [70%] and Trisomy 18 [30%]
Uniparental disomy of chromosome 20	1		1		Uniparental disomy of chromosome 20 (AOH of chromosome 20)
Total	*3*	*0*	*3*		

[†]Iso = isolated structural abnormality; MSA = multiple structural abnormalities (more than 1); Mos = Mosaic, FISH = Fluorescent in situ hybridization; CMA = chromosomal microarray

Supplemental Table 2: Structural abnormalities included in study

Organ system	n*	Isolated	MSA*
Cardiac	465	85	380
Central nervous system	102	5	97
Effusion	57	3	54
Face/ear	423	7	416
Gastrointestinal	185	11	174
Genital	89	4	85
Growth	180	13	167
Head shape	138	2	136
NT/nuchal fold/cystic hygroma	8	0	8
Placental	13	0	13
Prenatal	145	3	142
Renal	78	3	75
Skeletal	180	4	176
Spine	65	0	65
Thorax	40	2	38
Umbilical	41	0	41
Other	24	1	23
Total	2233	143	2090

n = number of individuals with a structural abnormality in specified organ system.

MSA = multiple structural abnormalities

*not mutually exclusive and therefore do not add up to 791 and 647 respectively

Bibliography

1. Hoyert DL, Mathews T, Menacker F, Strobino DM, Guyer B: Annual summary of vital statistics: 2004. *Pediatrics* 2006, 117(1):168-183.
2. Obstetricians ACo, Gynecologists: Microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology. ACOG Committee opinion no. 682. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2016, 128:e262-268.
3. Benn PA, Egan JF, Fang M, Smith-Bindman R: Changes in the utilization of prenatal diagnosis. *Obstetrics & Gynecology* 2004, 103(6):1255-1260.
4. Donnelly JC, Platt LD, Rebarber A, Zachary J, Grobman WA, Wapner RJ: Association of copy number variants with specific ultrasonographically detected fetal anomalies. *Obstetrics and gynecology* 2014, 124(1):83.
5. Carss KJ, Hillman SC, Parthiban V, McMullan DJ, Maher ER, Kilby MD, Hurles ME: Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. *Human molecular genetics* 2014, 23(12):3269-3277.
6. Fu F, Li R, Li Y, Nie ZQ, Lei Ty, Wang D, Yang X, Han J, Pan M, Zhen L: Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities. *Ultrasound in Obstetrics & Gynecology* 2018, 51(4):493-502.
7. Pangalos C, Hagnefelt B, Lilakos K, Konialis C: First applications of a targeted exome sequencing approach in fetuses with ultrasound abnormalities reveals an important fraction of cases with associated gene defects. *PeerJ* 2016, 4:e1955.
8. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, Spiegel E, Brennan K, Stong N, Jobanputra V: Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *The Lancet* 2019, 393(10173):758-767.

9. Westerfield LE, Stover SR, Mathur VS, Nassef SA, Carter TG, Yang Y, Eng CM, Van den Veyver IB: Reproductive genetic counseling challenges associated with diagnostic exome sequencing in a large academic private reproductive genetic counseling practice. *Prenatal diagnosis* 2015, 35(10):1022-1029.
10. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW: DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genetics in medicine* 2012, 14(3):296.
11. Gil M, Accurti V, Santacruz B, Plana M, Nicolaides K: Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound in Obstetrics & Gynecology* 2017, 50(3):302-314.
12. Baylor Genetics: Performance of Newly Developed Non-Invasive Prenatal Multi-Gene Sequencing Screen. 2016.
13. Lefkowitz RB, Tynan JA, Liu T, Wu Y, Mazloom AR, Almasri E, Hogg G, Angkachatchai V, Zhao C, Grosu DS: Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants. *American journal of obstetrics and gynecology* 2016, 215(2):227. e221-227. e216.
14. Tynan JA, Mazloom AR, Liu T, Lefkowitz RB, Wu Y, Hogg G, Tim R, O'Neal C, Qazi Z, Oeth P *et al*: Karyotype-level noninvasive prenatal testing by sequencing of circulating cell-free DNA from maternal plasma. In: *International Society for Prenatal Diagnosis: 2016; Berlin, Germany*; 2016.
15. Helgeson J, Wardrop J, Boomer T, Almasri E, Paxton W, Saldivar J, Dharajiya N, Monroe T, Farkas D, Grosu D: Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenatal diagnosis* 2015, 35(10):999-1004.

16. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, Braxton A, Van Den Veyver IB, Breman AM: Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. *American journal of obstetrics and gynecology* 2017, 217(6):691. e691-691. e696.

17. Wapner RJ BJ, Levy B, Stosic M, Zimmermann B, Sigurjonsson S, Wayham N, Ryan A, Banjevic M, Lacroute P, Hu J, Hall MP, Demko Z, Siddiqui A, Rabinowitz M, Gross SJ, Hill M, Benn P: Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *American Journal of Obstetrics and Gynecology* 2015, 213(2):254-255.

18. Obstetricians ACo, Gynecologists: Cell-free DNA screening for fetal aneuploidy. Committee Opinion No. 640. *Obstet Gynecol* 2015, 126(3):e31-e37.

19. Larion S, Warsof SL, Romary L, Mlynarczyk M, Peleg D, Abuhamad AZ: Uptake of noninvasive prenatal testing at a large academic referral center. *American journal of obstetrics and gynecology* 2014, 211(6):651. e651-651. e657.

20. Okun N, Teitelbaum M, Huang T, Dewa C, Hoch J: The price of performance: a cost and performance analysis of the implementation of cell-free fetal DNA testing for Down syndrome in Ontario, Canada. *Prenatal diagnosis* 2014, 34(4):350-356.

21. Chen A, Tenhunen H, Torkki P, Heinonen S, Lillrank P, Stefanovic V: Considering medical risk information and communicating values: A mixed-method study of women's choice in prenatal testing. *PloS one* 2017, 12(3):e0173669.

22. Wittman AT, Hashmi SS, Mendez-Figueroa H, Nassef S, Stevens B, Singletary CN: Patient perception of negative noninvasive prenatal testing results. *American Journal of Perinatology Reports* 2016, 6(04):e391-e406.

23. Beulen L, Faas BH, Feenstra I, van Vugt JM, Bekker MN: Clinical utility of non-invasive prenatal testing in pregnancies with ultrasound anomalies. *Ultrasound in Obstetrics & Gynecology* 2017, 49(6):721-728.

24. Sotiriadis A, Papoulidis I, Siomou E, Papageorgiou E, Eleftheriades M, Papadopoulos V, Alexiou M, Manolakos E, Athanasiadis A: Non-invasive prenatal screening versus prenatal diagnosis by array comparative genomic hybridization: a comparative retrospective study. *Prenatal diagnosis* 2017, 37(6):583-592.

25. StataCorp. 2013. Stata Statistical Software: Release 13. College Station TSL.

26. Curnow KJ, Wilkins-Haug L, Ryan A, Kırkızlar E, Stosic M, Hall MP, Sigurjonsson S, Demko Z, Rabinowitz M, Gross SJ: Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism–based noninvasive prenatal test. *American journal of obstetrics and gynecology* 2015, 212(1):79. e71-79. e79.

27. McKanna T, Ryan A, Krinshpun S, Kareht S, Marchand K, Grabarits C, Ali M, McElheny A, Gardiner K, LeChien K: Fetal fraction-based risk algorithm for non-invasive prenatal testing: screening for trisomies 13 and 18 and triploidy in women with low cell-free fetal DNA. *Ultrasound in Obstetrics & Gynecology* 2019, 53(1):73-79.

28. Wilson EB: Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association* 1927, 22(158):209-212.

29. Liang D, Cram DS, Tan H, Linpeng S, Liu Y, Sun H, Zhang Y, Tian F, Zhu H, Xu M: Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. *Genetics in Medicine* 2019:1.

30. Martin K, Iyengar S, Kalyan A, Lan C, Simon A, Stosic M, Kobara K, Ravi H, Truong T, Ryan A: Clinical experience with a single-nucleotide polymorphism-

based non-invasive prenatal test for five clinically significant microdeletions. *Clinical genetics* 2018, 93(2):293-300.

Vita

Peyton Bree Busby was born in Austin, Texas, the daughter of Barry Bryce Busby and Evelyn Carolan Busby. After completing her work at Round Rock High School, Round Rock, Texas in 2013, she entered the University of Texas in Austin, Texas. She received the degree of Bachelor of Science with a major in biology from UT in May, 2017. In August of 2017 she entered The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences.

Permanent address:

2109 Woodston Drive

Round Rock, Texas, 78681