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ORIGINAL ARTICLE

The clinical utility of genome-wide non invasive prenatal screening

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ABSTRACT

Objective In this study, we expanded conventional cell-free fetal DNA (cfDNA)-based non-invasive prenatal testing (NIPT) to cover the entire genome. We aimed to compare the performance of the two tests in a large general population of pregnant women, in order to assess the clinical utility of the genome-wide screening.

Method Genome-wide cfDNA analysis was offered to 12 114 pregnant women undergoing NIPT for common fetal aneuploidy. Sequencing data were analyzed using an algorithm optimized to identify aneuploidies and subchromosomal aberrations.

Results Genome-wide screening allowed detection of 12 (7.4%) potentially viable clinically relevant chromosomal abnormalities, which would have remained overlooked if only conventional NIPT had been performed. This resulted in a statistically significant higher sensitivity (100% vs 92.64%, $p < 0.001$) than did standard screening. This was achieved without sacrificing the specificity of the test, which resulted similar to that obtained with standard cfDNA testing (99.87% vs 99.77%, $p = 0.064$).

Conclusion Genome-wide cfDNA analysis represents an enhanced screening tool for prenatal detection of chromosomal abnormalities, allowing identification of clinically relevant imbalances that are not detectable by conventional cfDNA testing. The results of this study demonstrate the clinical utility of genome-wide cfDNA analysis. This level of screening provides a significant higher sensitivity compared to standard screening while maintaining a high specificity, with the potential to improve overall pregnancy management. © 2017 John Wiley & Sons, Ltd.

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INTRODUCTION

Since its introduction, noninvasive prenatal testing (NIPT) based on the analysis of circulating cell-free fetal DNA (cfDNA) in maternal plasma has had a significant impact on prenatal care. In only 4 years, NIPT has become integrated into clinical practice for detection of common fetal chromosomal aneuploidies.[1]

The high sensitivity and specificity resulting from multiple large-scale clinical trials[2–4] and updated meta-analyses,[5–7] and the endorsement of professional medical organizations,[8–12] have resulted in many institutions adopting NIPT for aneuploidy screening as standard option for high risk pregnancies.

Current cfDNA-based NIPT approaches focus on detection of a limited set of conditions, which typically include trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13), sex chromosome aneuploidies (SCAs) and selected microdeletions. Consequently, a large set (approximately 17%) of clinically relevant chromosomal abnormalities is currently not accessible to standard cfDNA testing and is neglected by such a restricted detection scheme.[13]

Genome-wide analysis of cfDNA would greatly expand the range of chromosomal rearrangements detectable by NIPT, because it extends screening to include also rare autosomal trisomies and structural chromosome anomalies throughout the fetal genome, specifically disease-causing copy-number variations (CNVs). Such level of testing has the potential to improve overall pregnancy management, providing a significant higher sensitivity compared to standard screening.

In principle, massively parallel sequencing (MPS) of cfDNA in maternal plasma for NIPT of common fetal aneuploidies can also be used for detecting other unbalanced chromosomal rearrangements prenatally. Previous proof-of-concept studies have shown the potential of extending conventional NIPT to detect fetal microdeletion syndromes from maternal plasma.[14–17] As a consequence, several commercial providers have expanded their NIPT platform to include a panel of common and well-characterized microdeletion syndromes.

Several groups have also demonstrated the feasibility to detect all fetal chromosomal aneuploidies and segmental imbalances by sequencing cfDNA from maternal plasma.[18–25] Recently, other studies showed how genome-wide cfDNA testing can contribute in lowering the incidence of false positive results generated by maternal copy number variants.[26,27]

Limited data are currently available on the clinical implementation of genome-wide cfDNA screening to detect rare autosomal trisomies or structural chromosome anomalies in the routine clinical setting. The lack of prospective clinical results in a large population of pregnant women makes it difficult to accurately determine the test performance parameters, which are crucial if this is to be implemented in clinical practice.

The clinical utility of expanding NIPT to include detection of these other rearrangements, particularly in low-risk pregnancies, is controversial because this could lead to a decrease of the specificity, potentially affecting to some degree one of the major perceived benefits of NIPT screening: the significantly reduced requirement for invasive testing. Therefore, any change to the standard cfDNA screening approaches that may increases the false-positive rate, leading to maternal anxiety, should be weighed against the possible benefits prior to routine implementation.

In this study, we expanded standard cfDNA testing to cover the entire genome. We aimed to compare the performance of the two test (standard vs genome-wide cfDNA screening) in a large general population of pregnant women in order to assess the clinical utility of the genome-wide screening.

METHODS

Study design

From December 2015 through May 2016, genome-wide cfDNA testing was offered to a consecutive nonselected series of pregnant women undergoing conventional cfDNA-based NIPT for common fetal aneuploidy. We aimed to compare the performance of the two tests in a general obstetrical population.

Patients underwent pre-test counseling, during which the issues that are encountered with both standard cfDNA screening and genome-wide cfDNA analysis were discussed. The patients who accepted evaluation by NIPT with both methodologies signed an informed consent form containing a summary of the testing process, potential benefits and limitations of testing, and possible testing outcomes, including the risk of obtaining results with unknown clinical significance. In addition, the possible risk of misdiagnosis was specified, and confirmatory prenatal diagnosis for any abnormal result was recommended. A post-test genetic counseling session was provided in all cases when a chromosomal abnormality was detected by any method. Routine prenatal care was provided to those with a negative NIPT result. Study inclusion required accessibility to pregnancy and delivery records, such as reports from laboratory screening, fetal ultrasonography, cytogenetic testing and newborn physical examinations.

The institutional review board at Genoma Laboratory approved the study.

Study population and sample collection

The indication for testing was one or more of the following: advanced maternal age (AMA—defined as maternal age 35 or greater at time of conception), previous positive prenatal screen (PPS), fetal ultrasound abnormality (FUA), prior pregnancy with fetal aneuploidy (PPFA) or parental anxiety (PA), that is patients younger than 35 years with no specific pregnancy risk. Women with a singleton pregnancy and a qualified blood sample were included in the study. All testing was performed on whole-blood samples (10 mL) received in cfDNA BCT™ tubes (Streck, Omaha, NE), collected from patients with a confirmed pregnancy greater than 10 weeks of gestation (mean 12.3 ± 2.1). Samples were received within five days of blood draw and accessioned with a complete test requisition form (TRF). Cell-free fetal DNA was extracted and processed at GENOMA Laboratory (Rome, Italy) for library preparation, sequencing and data analysis, following the protocol described in the next section.

Sample preparation

The blood samples were first centrifuged at 1600 g for 10 min at 4 °C to separate the plasma from peripheral blood cells. The plasma portion was then carefully transferred into a polypropylene tube and subjected to a second centrifugation at 16 000 g for 10 min at 4 °C, in order to remove residual cells. Cell-free DNA was extracted from 900 μL of maternal plasma using the QIAamp DNA Blood Mini Kit (Qiagen), following the manufacturer's protocol.

Sequencing and classification of the results

Sequencing libraries were prepared using TruSeq nano Kit (Illumina, San Diego, CA, USA) as reported elsewhere.[28] Samples were indexed during library preparation, and seven samples were pooled for multiplex sequencing on a NextSeq 550 (Illumina, San Diego, CA, USA), using the High Output v1.2 kit that generates 36 BP single-end reads.

To reduce noise and increase signal, sequencing depth was increased to target 30 million reads per sample, unambiguously mapping to a single genomic location.

A single sequencing run was performed for both conventional cfDNA screening and genome-wide analysis, followed by two different bioinformatic analyses, one limited to the common aneuploidies, the other involving testing for rare trisomies and segmental imbalances throughout the fetal genome.

The raw output from each run was analyzed as described elsewhere.[21] Briefly, sequencing reads were aligned to the reference genome hg19 using the Burrows–Wheeler aligner.[29] The genome was then partitioned into 50-kb bins, and the total number of reads for each bin was determined. The 50-kb bin count was then corrected with LOESS regression according to the bin GC content. Normalized bins were finally aggregated per 5 Mb windows, consisting of 100 subsequent 50 kb bins, where the 5 Mb windows are sliding by 50 kb.

Apart from calculating a *Z* score per chromosome, *Z* scores were also calculated per 5 Mb bins.

Quality control criteria

Samples were not included in the analyses if they did not pass one or more of the following quality control parameters:

(1) low fraction of fetal cfDNA (<2%);
(2) assay failure: that is, library concentration < 10 nM; number of unique sequence sites (i.e. sequence tags identified with unique sites in the genome) <25 000 000;

Fetal fraction measurement was performed as previously reported.[28] In samples from pregnancies carrying female fetuses, the FF was determined using the method described elsewhere,[30] with slight modifications consisting in the use of real time PCR technique.

Clinical outcomes

All patients were followed for pregnancy outcomes. Chromosomally abnormal results of cfDNA testing were confirmed performing a metaphase and/or array-CGH-based karyotyping after an invasive prenatal diagnostic procedure or from products of conception, in the case of a spontaneous miscarriage. Chromosomally normal results were confirmed by newborn physical examination and any genetic testing performed. In the absence of genetic testing, a newborn with a normal physical examination was considered to be euploid.

All pregnancy outcomes were recorded, including miscarriage, termination and delivery. Results of invasive prenatal diagnostic testing and testing of products of conception (i.e. miscarriages) were collected when available.

Follow-up information was obtained by telephone and recorded in an internal database. Telephone interviews were performed one month after the expected date of delivery to obtain information on neonatal outcome, newborn physical examination or any cytogenetic testing results. Karyotyping or clinical follow-up results were used as the gold standard to calculate sensitivity and specificity of NIPT in this population.

Statistical analysis

Statistical analyses were performed using Microsoft Excel® software statistical tools. We used the Clopper–Pearson method[31] to calculate the performance characteristics of the test (sensitivity, specificity, and positive and negative predictive values) and exact 95% confidence intervals.

We used Pearson chi-square test to evaluate the statistical significance of the comparison of the performance of the cfDNA approaches. A *p*-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

A total of 12 114/13 523 (89.6%) pregnant women agreed to undergo genome-wide cfDNA screening and were enrolled in the study. The patient demographic characteristics and indication for testing are summarized in Supplementary Table S1. The mean age of the pregnant women was 35.3 ± 4.1 years (range, 20–58 years), and gestational age ranged from 10 weeks to 29 weeks (mean 12.3 weeks ± 2.1 days).

Of the 12 114 samples received, 36 (0.3%) were excluded from the analysis because an assay failure. The remaining 12 078 (99.7%) samples were with a call (Table 1).

Among the samples with a result, 145 (1.2%) were cancelled because fetal cfDNA resulted below the limit of detection value (FF < 2%).[28]

A blood redraw was requested for samples with an assay failure and low FF. After reanalysis, all the above samples were classified as euploid, and the results were confirmed by newborn physical examination.

Clinically relevant chromosomal abnormalities were detected in 196 (1.6%) pregnancies and confirmed by metaphase karyotyping or array-CGH following invasive prenatal diagnosis in 169 (1.4%) cases, 151 of which involved common aneuploidies, 10 were rare autosomal trisomies and 8 were segmental imbalances (Supplementary Figure 7).

Trisomy 21, trisomy 18, trisomy 13 and sex chromosome aneuploidy detection

Among the 12 114 reportable samples following standard cfDNA screening, 89 were classified with T21, 16 with T18, 13 with T13, 48 with SCA and 11 766 as euploid. Out of 166 pregnancies, classified as chromosomally abnormal, 151 were confirmed by invasive prenatal diagnosis as true positives. Fifteen pregnancies, reported as T21 (1 patient), T18 (1 patient), T13, (1 patient), Monosomy X (10 patients), XXX (1 patient) and XXY (1 patient), respectively, resulted with a normal karyotype after amniocentesis (Table 2) and were then classified as false positives. No false negative cases have been reported.

Table 3 summarizes the performance of the cfDNA testing for common aneuploidies. For T21, the sensitivity was 100% (95% confidence interval [CI], 95.89%–100%), and the specificity was 99.99% (95% CI, 99.95%–100%). For T18, the

Table 1 **Results of samples tested**

	Total
No. of patients analyzed	12 114
Samples with a call—no. (%)	12 078 (99.7)
Total cancellations—no. (%)	182 (1.5)
Samples with low FF—no. (%)	145 (1.2)
Samples with assay failure—no. (%)	36 (0.3)
Samples with a conclusive result—no. (%)	11 932 (98.5)
Samples with a conclusive result after reanalysis of samples with a cancellation—no. (%)	12 114 (100)
Chromosomally abnormal results	
Genome-wide cfDNA screening—no. (%)	196 (1.6)
Conventional cfDNA screening—no. (%)	166 (1.4)
Pregnancies confirmed as chromosomally abnormal—no. (%)	
Genome-wide cfDNA screening—no. (%)	169 (1.4)
Conventional cfDNA screening—no. (%)	151 (1.2)

FF, fetal fraction.

Table 2 **Clinically relevant chromosomal abnormalities detected by genome-wide cfDNA analysis and clinical outcome**

Chromosomal abnormality	Total number detected	Follow-up invasive testing		Follow-up miscarriages[a]	
		Confirmed	Not confirmed	Confirmed	Not confirmed
Common aneuploidies	166	151	15	—	—
• Trisomy 21	89	88	1	—	—
• Trisomy 18	16	15	1	—	—
• Trisomy 13	13	12	1	—	—
• Monosomy X	24	14	10	—	—
• Triple X Syndrome (XXX)	7	6	1	—	—
• Klinefelter Syndrome (XXY)	15	14	1	—	—
• Jacobs Syndrome (XYY)	2	2	0	—	—
Rare autosomal trisomies	17	3	7	7	0
• Trisomy 7	4	1[c]	3	—	—
• Trisomy 9	1	1[c]	0	—	—
• Trisomy 12	1	0	1	—	—
• Trisomy 14	1	0	1	—	—
• Trisomy 15	4	0	1[c]	3	0
• Trisomy 16	1	0	1	—	—
• Trisomy 22	5	1[c]	0	4	0
Structural abnormalities—CNV	13	8	5	—	—
• del5p15.32p13.2	1	1	0	—	—
• del5q14.3q32	1	0	1	—	—
• del7q21.11q31.1	1	0	1	—	—
• dup7p22.3p21.1/dup9p24.1q31.3	1	1	0	—	—
• dup8p23.3q13.3	1	0	1	—	—
• dup9p24.3p13.1	1	0	1	—	—
• dup11p15.1p15.4	1	1	0	—	—
• del13q33.1q34/dup20q13.33	1	1	0	—	—
• del18p11.32p11.31 – del18q21.32q23	1	1	0	—	—
• dup18p11.32p11.21	1	1	0	—	—
• del20q11.21q13.32	1	0	1	—	—
• del22q11.21	1	1	0	—	—
• del Xp22.33p11.1	1	1	0	—	—

[a]Karyotype from product of conception samples.
[b]Fetal mosaicism.
[c]Follow-up invasive testing resulted in a diploid fetus with UPD of chromosome 15.

sensitivity was 100% (95% CI, 78.20%–100%), and the specificity was 99.99% (95% CI, 99.95%–100%). For T13, the sensitivity was 100% (95% CI, 73.54%–100%), and the specificity was 99.99% (95% CI, 99.95%–100%). The sensitivity for SCA was 100% (95% CI, 90.26%–100%) with a specificity of 99.9% (95% CI, 99.82%–99.95%).

Overall, common aneuploidies were detected with a combined clinical sensitivity and specificity of 100% (95% CI, 97.59%–100%) and 99.87% (95% CI, 99.79%–99.93%), respectively.

Genome-wide detection of copy number variants

Among the 12 114 samples reportable following genome-wide cfDNA analysis, there were 30 pregnancies that had positive results for a variety of CNV aberrations other than common aneuploidies, including both segmental chromosomal imbalances and rare autosomal trisomies (Table 2). The details of the CNVs detected are shown in Supplementary Table S2. Several examples of NIPT-detected CNVs, confirmed by invasive prenatal diagnosis, are shown in Figures 1 and 2.

Rare autosomal trisomies were identified in 17 samples and confirmed in 10 pregnancies, three of which consisted in a low-grade fetal mosaicism and seven resulted in a spontaneous miscarriage (Supplementary Figures 5 and 6). In a pregnancy with a trisomy 15 detected, follow-up invasive testing resulted in a diploid fetus with uniparental disomy of chromosome 15, because of a trisomy rescue event.

Table 3 Performance of the genome-wide cfDNA screening approach

	Trisomy 21 (n = 12 114)	Trisomy 18 (n = 12 114)	Trisomy 13 (n = 12 114)	Sex chromosome aneuploidies (n = 12 114)	Rare aneuploidies (n = 12 114)	Segmental imbalances (n = 12 114)
False positive—no.	1	1	1	12	7	5
False negative—no.	0	0	0	0	0	0
True positive—no.	88	15	12	36	10	8
True negative—no.	12.025	12.098	12.101	12.066	12.097	12.101
Sensitivity (95% CI)—%	100.00% (95.89%–100.00%)	100.00% (78.20%–100.00%)	100.00% (73.54%–100.00%)	100.00% (90.26%–100.00%)	100.00% (69.15%–100.00%)	100.00% (63.06%–100.00%)
Specificity (95% CI)—%	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.90% (99.83%–99.95%)	99.94% (99.88%–99.98%)	99.96% (99.90%–99.99%)
Positive predictive value (95% CI)—%	98.88% (92.54% to 99.84%)	93.75% (67.88%–99.07%)	92.31% (62.83%–98.84%)	75.00% (63.02%–84.08%)	58.82% (40.52%–74.97%)	61.54% (39.98%–79.35%)
Negative predictive value (95% CI)—%	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100,00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)

Clinically relevant segmental chromosomal imbalances were detected in 13 pregnancies and confirmed by invasive prenatal diagnosis in eight cases (Figures 1 and 2; Supplementary Figures 1–4). It is worth noting that three out of eight structural chromosomal abnormalities occurred in low risk pregnancies. In addition, in two of these, a fetus with unbalanced translocations was identified, which was subsequently found to be inherited from the mother, who was unaware to be a balanced translocation carrier (Figure 2; Supplementary Figure 2).

Table 3 summarizes the performance of the cfDNA testing for chromosomal abnormalities other than common aneuploidies. For rare autosomal trisomies, the sensitivity was 100% (95% CI, 69.15%–100%), and the specificity was 99.94% (95% CI, 99.88%–99.98%). The sensitivity for segmental imbalances was 100% (95% CI, 63.06%–100%) with a specificity of 99.96% (95% CI, 99.90%–99.99%).

For the calculation of sensitivity of segmental imbalances, we assumed that all imbalances have been identified, although we cannot rule out that very small rearrangements may have remained unnoticed in the newborns.

Overall, genome-wide cfDNA screening provided a combined clinical sensitivity and specificity of 100% (95% CI, 97.84%–100%) and 99.77% (95% CI, 99.67%–99.85%), respectively.

Conventional versus genome-wide conventional cell-free fetal DNA screening

Genome-wide cfDNA screening allowed identification of 18/169 (10.7%) clinically relevant chromosomal abnormalities, not detected by conventional cfDNA screening (Supplementary Figures 8). Twelve (7.4%) of these (including also the UPD 15 occurrence), potentially resulting in the birth of babies with chromosomal anomalies, would have remained overlooked if only conventional NIPT had been performed. This resulted in a statistically significant higher sensitivity (100% vs 92.64%, $p < 0.001$) than did standard screening for the detection of common aneuploidies (Table 4). This was achieved without sacrificing the specificity of the test, that resulted very similar to that obtained with standard screening (99.87% vs 99.77%, $p = 0.064$).

DISCUSSION

In this study, we investigated the clinical performance of genome-wide cfDNA screening in a large general population of pregnant women and compared it with standard cfDNA screening. We aimed to assess the usefulness of offering genome-wide cfDNA analysis in NIPT on a routine basis, trying to address the following issues: (1) if genome-wide cfDNA testing is accurate in the detection of common and rare aneuploidies, as well as segmental chromosome abnormalities throughout the fetal genome; (2) if the approach improves the detection rate of genetic aberrations as compared with conventional cfDNA screening; and (3) if there is a statistically significant increase in false positive results that may substantially affect the specificity of genome-wide cfDNA analysis and cause difficulties in case management and parental anxiety.

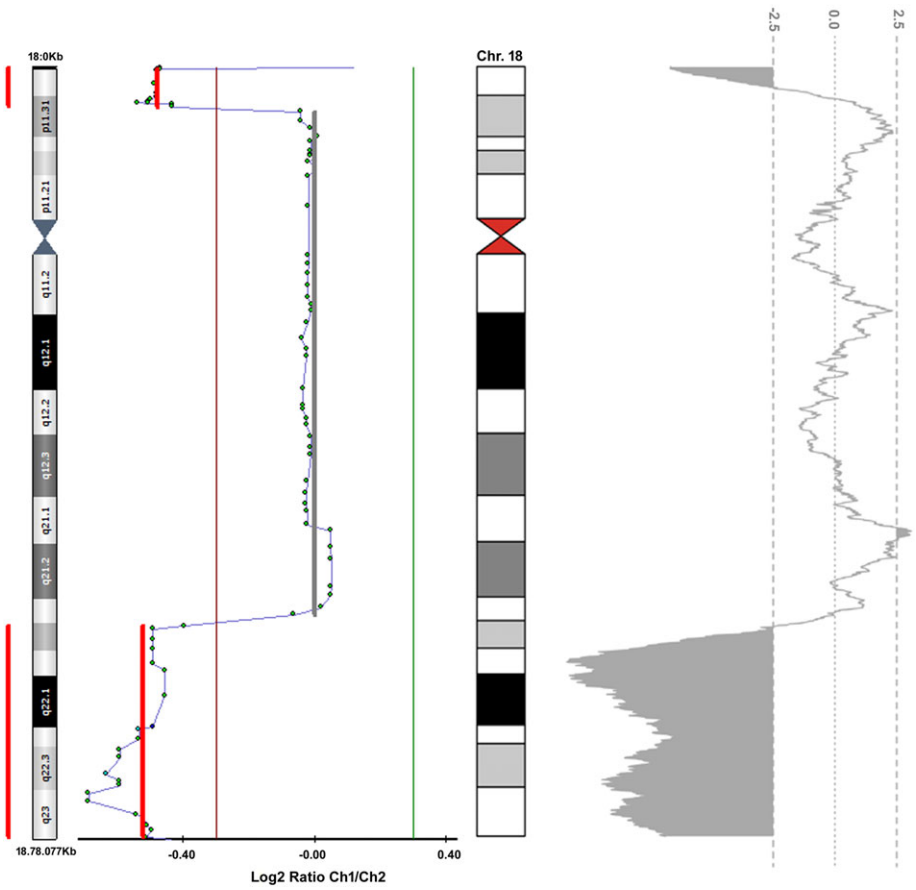

Figure 1 Clinically relevant fetal copy number variants (CNVs) detected in maternal plasma by genome-wide cfDNA screening, with results confirmed by invasive testing. Detection of a 3.9 Mb deletion at 18p11.32p11.31 and a 21.3 Mb deletion at 18q21.32q23 (Case 2). The right panel shows cfDNA sequencing results; *Z*-scores of 5 Mb sliding windows are plotted across the chromosome, dotted lines represent ±2.5 *z*-scores and the areas above or below these cutoff values, colored in gray, highlight the aberrations. The left panel shows invasive testing results by microarray analysis [Colour figure can be viewed at wileyonlinelibrary.com]

The results achieved from this prospective study demonstrated the effectiveness and benefits of NIPT performed by genome-wide cfDNA analysis compared to conventional cfDNA testing.

As expected, the use of genome-wide cfDNA screening resulted in a statistically significant higher sensitivity (100% vs 92.64%, $p < 0.001$), with the detection of 12 (7.4%) clinically relevant fetal chromosome anomalies, potentially resulting in the birth of chromosomally abnormal babies, that would have been missed if only conventional NIPT had been performed. This increased detection rate was achieved without sacrificing the specificity of the test, which resulted very similar to that obtained with standard screening (99.87% vs 99.77%, $p = 0.064$).

A limitation of this study is related with the estimation of sensitivity of segmental imbalances. No live-born child resulted to have chromosomal abnormalities; consequentially, we reported 100% sensitivity for genome-wide screening assuming that all imbalances have been identified. However, we cannot rule out that very small rearrangements may have remained unnoticed in the newborns. At present, confidently excluding the presence of a pathogenic CNV requires chromosome microarray analysis (CMA) of genetic material obtained from the newborns. The current clinical study design did not include infant follow-up by CMA, so we were unable to determine whether negative genome-wide screening results were actual true negative, although the possibility of genome-wide analysis false results going undetected remained low.

Evidence regarding the increased detection yield of the genome-wide cfDNA testing approach with respect to conventional cfDNA screening makes its use attractive in a routine NIPT practice. However, the clinical utility of expanding NIPT to cover the entire genome is controversial, especially in low-risk pregnancies. In fact, it pertains a risk of overdiagnosis with a higher number of false positives because of chromosomal rearrangements which are confined to the placenta. It may lead to maternal anxiety and may potentially determine an increase in unnecessary invasive testing, in women for whom this would not normally be considered. The results of this study demonstrate that a high specificity may be maintained while extending the screen to all chromosomal abnormalities.

Genome-wide cfDNA screening allowed not only the detection of common chromosomal aneuploidies but also enabled the identification of rare autosomal trisomies. Extending the screen to all chromosomal aneuploidies may

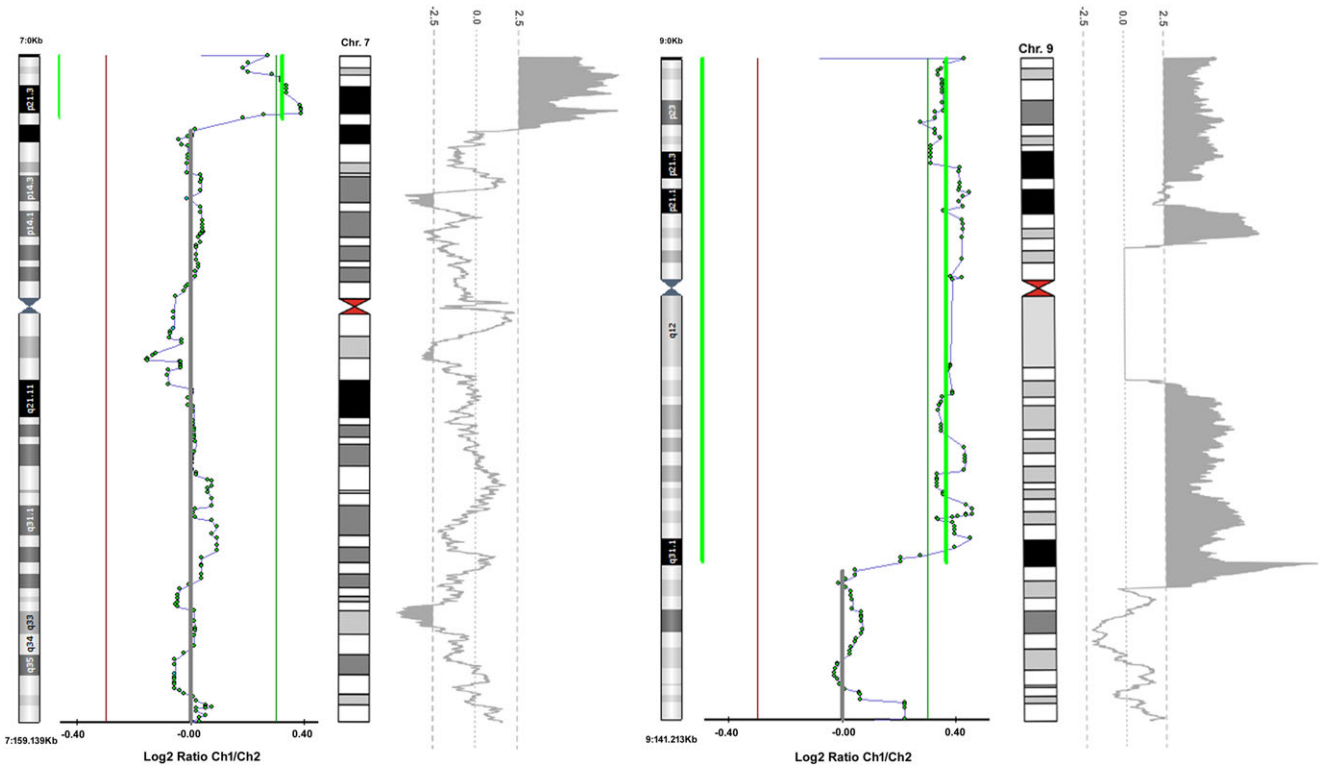

Figure 2 **Fetal unbalanced translocations detected in maternal plasma by genome-wide cfDNA screening, with results confirmed by invasive testing. Detection unbalanced translocation derivatives consisting of a 15.0 Mb duplication at 7p22.3p21.1 and a 107.5 Mb duplication at 9p24.1q31.3 (Case 4). The right panel shows cfDNA sequencing results; the left panel shows invasive testing results by microarray analysis [Colour figure can be viewed at wileyonlinelibrary.com]**

Table 4 **Performance of conventional cfDNA screening versus genome-wide analysis**

	Conventional cfDNA screening	Genome-wide cfDNA screening	*p*-Value[b]
No. of pregnancies assessed	12 114	12 114	
Clinical relevant chromosomal abnormalities detected—no.	166	196	
Pregnancies confirmed as chromosomally abnormal—no.	151	169	
False positive	15	27	
False negative	12[a]	0	
True positive	151	169	
True negative	11.936	11.918	
Sensitivity	92.64%	100.00%	<0.001
Specificity	99.87%	99.77%	0.064
Positive predictive value (PPV)	90.96%	86.22%	0.161
Negative predictive value (NPV)	99.90%	100.00%	<0.001

[a]Clinically relevant chromosomal abnormalities, not detected by conventional cfDNA screening, potentially resulting in the birth of babies with chromosomal anomalies, have been considered as false negative.
[b]A *p*-value of less than 0.05 was considered to indicate statistical significance.

be of questionable clinical utility, because it could determine a useless increase in invasive testing. However, most of the rare trisomies identified in this study are not viable and, therefore, may not require an invasive testing follow-up, thus limiting the risk of overdiagnosis. On the other hand, such additional data have important clinical implications and may be helpful in improving pregnancy management. In fact, it is known that placental mosaicism carries a small but significant risk for intrauterine growth restriction (IUGR), small-for-gestational-age infants and unfavorable pregnancy outcome (e.g. T16),[32–35] as well as a risk of mosaic fetal aneuploidy and/or fetal uniparental disomy (UPD), resulting from the loss of one chromosome following a trisomic conception because of a trisomy rescue event.[36] In our study, three pregnancies resulted with a fetal mosaicism of a rare trisomy. In addition, in a pregnancy with a trisomy 15 detected, follow-up invasive testing resulted in a diploid fetus with UPD of chromosome 15.

Hence, when rare autosomal aneuploidies are observed by genome-wide analysis, amniocentesis is indicated in some cases to confirm confined placental mosaicism (CPM) and to rule out fetal mosaicism. It is also advisable to undertake UPD testing, in particular where chromosomes 6, 7, 11, 14, 15 or 20 are involved because of the presence of known imprinting disorders. Serial ultrasound examinations to monitor fetal growth for IUGR are also warranted in such cases.

We believe that a genome-wide analysis can lead to an improved clinical management. However, at this initial stage,

the additional information on rare trisomies must be interpreted with caution in order to minimize increases in invasive tests because of aneuploidies which are probably confined to the placenta. The need to perform invasive testing in cases with rare trisomies should be discussed between laboratory specialists, medical geneticists and obstetricians, on a case-to-case base. The advice to the parents will depend on gestational age, type of chromosome abnormality, presence or absence of ultrasound findings and parental preferences. Further studies are warranted to evaluate the outcomes of such pregnancies which will eventually allow the development of rules for the best clinical follow-up actions to be taken.

Genome-wide cfDNA analysis also enabled the detection of structural chromosomal abnormalities. Among the eight pregnancies with fetal segmental chromosomal imbalances identified, confirmed by invasive prenatal diagnosis, the smaller chromosome segment detected was 1.9 Mb in size (Supplementary Figure 2), demonstrating the power of this approach.

Genome-wide cfDNA screening has also aided in detection of a previously unknown familial translocation. In fact, two out of eight structural chromosomal abnormalities identified involved a fetus with unbalanced translocations, which was subsequently found to be inherited from the mother, who was unaware to be a balanced translocation carrier. Therefore, genome-wide cfDNA analysis may also represent a valuable option for families with a known translocation.

CONCLUSION

In conclusion, this study demonstrated that MPS-based NIPT protocols for common aneuploidies can also be used to detect all fetal chromosomal aneuploidies, segmental imbalances and even submicroscopic CNVs, by sequencing cfDNA from maternal plasma. Genome-wide cfDNA analysis represents an improved screening tool for prenatal detection of chromosomal abnormalities, allowing identification of clinically relevant imbalances that are not detectable by conventional cfDNA screening. Although genome-wide cfDNA analysis has shown the potential to improve overall pregnancy management, additional clinical data must be obtained before this approach can be evaluated for routine integration into NIPT programs. Further prospective studies in this area, with a large cohort of patients analyzed, will further enhance understanding of clinical effectiveness of genome-wide screening, elucidating the role that this technique will come to play in NIPT, including whether it may replace the use of standard cfDNA screening.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Conventional cfDNA-based NIPT focuses on detection of common aneuploidies, leaving a gap of ~17% of clinically relevant chromosomal abnormalities that would go undetected. Genome-wide NIPT would greatly expand the range of chromosomal rearrangements detectable, but it could lead to a decrease of the specificity and, consequentially, to an increase in unnecessary invasive testing.

WHAT DOES THIS STUDY ADD?

- This study demonstrates the clinical utility of expanding NIPT to cover the entire genome. Genome-wide cfDNA analysis provides a significant higher sensitivity compared to standard screening while maintaining a high specificity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

THE CLINICAL UTILITY OF GENOME-WIDE NON INVASIVE PRENATAL SCREENING

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SUPPLEMENTARY APPENDIX

SUPPLEMENTARY TABLES

Table S1. Demographic and pregnancy characteristics of the study patients

Characteristics	n
No. of eligible patients	12.114
Maternal age-yr	
- **Mean ±SD**	35.3±4.1
- **Min-max**	20-58
Gestational age at sample collection -wk	
- **Mean ±SD**	12.3±2.1
- **Min-max**	10-29
Indications for NIPT	
- **Parental Anxiety**	3804 (31.4%)
- **Advanced maternal age**[a]	4446 (36.7%)
- **Positive prenatal screen**	1199 (9.9%)
- **Fetal ultrasound abnormality**	472 (3.9%)
- **Prior pregnancy with fetal aneuploidy**	157 (1.3%)
- **More than one indication**	2035 (16.8%)

[a] age e35y

wk: weeks; **SD**: Standard deviation; **yr**: years

24

25 **Table S2: Details of genome-wide cfDNA testing positive cases for rare trisomies and segmental imbalances, with pregnancy outcome**

Case No.	Indication for cfDNA testing	Gastational Age	Genome-Wide cfDNA analysis results	Invasive testing results		CNV Size	Type of prenatal sample	cfDNA Results confirmed (Y/N)	Clinical outcome
				Array-CGH	Karyotype				
Segmental imbalances									
1	PA	13	del5p15.32p13.2	arr[GRCh37] 5p15.33p13.2(50,093-34,156,679)x1	-	34.1 Mb	AF	Y	TOP
2	FUA	20	del18p11.32p11.31 / del18q21.32q23	arr[GRCh37] 18p11.32p11.31(99,191-3,965,460)x1 - arr[GRCh37] 18q21.32q23(56,506,375-77,856,022)x1	46,XX,-18,r(18)(p11.32q23)	3.9 Mb; 21.3 Mb	AF	Y	TOP
3	PA	12	del13q33.1q34 / dup20q13.33	arr[GRCh37] 13q33.1q34(103,568,640-115,092,581)x1, 20q13.33(61,053,598-62,908,679)x3	46,XY,der(13)t(13;20)(q33q13.3)mat	11.4 Mb; 1.9 Mb	CVS	Y	TOP
4	PA	11	dup7p22.3p21.1 / dup9p24.1q31.3	arr[GRCh37] 7p22.3p21.2(14,916-14,993,340)x3; arr[GRCh37] 9p24.3q31.1(104,475-107,581,987)x3	46,XX,der(7)t(7;9)(p22;q31.3); der(9)t(7;9)(p22;p24.1)mat	15.0 Mb; 107.5 Mb	CVS	Y	TOP
5	AMA	11	dup18p11.32p11.21	arr[GRCh37] 18p11.32p11.21(99,191-14,076,542)x3		14.0 Mb	CVS	Y	TOP
6	AMA - FUA	18	del22q11.21	arr[GRCh37] 22q11.21(19,172,841-19,843,647)x1	-	4.3 Mb	AF	Y	TOP
7	AMA	12	delXp22.33p11.1	arr[GRCh37] Xp22.33p11.1(452,569-58,508,126)x1	46,X,i(X)(q10)[25]/45,X[25]	58.5Mb	AF	Y	Unknown
8	AMA	10	dup11p15.1p15.4	arr[GRCh37] 11p15.4p15.1(5,040,052-21,069,514)x3	46,XX,dup(11)(p15.4p15.1)	16.0 Mb	AF	Y	Unknown
9	PA	14	Dup9p24.3p13.1	arr(1-22,X)x2	46,XX	38.8 Mb	AF	N	Baby healthy at birth
10	PA	13	del5q14.3q32	arr(1-22,X)x2	46,XX	68.5 Mb	AF	N	Baby healthy at birth
11	PA	16	del7q21.11q31.1	arr(1-22)x2,(XY)x1	46,XY	36.8 Mb	AF	N	Baby healthy at birth
12	PA	15	dup8p23.3q13.3	arr(1-22,X)x2	46,XX	62.2 Mb	AF	N	Baby healthy at birth

13	AMA	11	del20q11.21q13.32	arr(1-22)x2,(XY)x1	46,XY	29.2 Mb	AF	N	Baby healthy at birth
Rare autosomal trisomies									
14	PA	12	T7	-	46,XX[48]/47,XX,+7[2]	-	AF	Y	Ongoing pregnancy
15	AMA	14	T9	-	46,XX[80]/47,XX,+9[20]	-	AF	Y	TOP
16	AMA	10	T22	-	46,XY[42]/47,XY,+22[8]	-	AF	Y	TOP
17	AMA	11	T22	-	47,XY,+22	-	POC	Y	Fetal loss
18	AMA	10	T15	-	47,XX,+15	-	POC	Y	Fetal loss
19	AMA	10	T22	-	47,XY,+22	-	POC	Y	Fetal loss
20	AMA - PPFA	10	T15	-	47,XY,+15	-	POC	Y	Fetal loss
21	AMA - PPFA	10	T22	-	47,XX,+22	-	POC	Y	Fetal loss
22	AMA	12	T22	-	47,XY,+22	-	POC	Y	Fetal loss
23	PA	11	T15	-	47,XY,+15	-	POC	Y	Fetal loss
24	AMA	11	T7	-	46,XY	-	AF	N	Baby healthy at birth
25	AMA - PPS	15	T7	-	46,XX	-	AF	N	Baby healthy at birth
26	PA	11	T7	-	46,XX	-	AF	N	Baby healthy at birth
27	PA	13	T14	-	46,XY	-	AF	N	Baby healthy at birth
28	AMA	11	T16	-	46,XY	-	AF	N	Baby healthy at birth
29	AMA	11	T12	-	46,XX	-	AF	N	Baby healthy at birth
30	AMA	10	T15	-	46,XX	-	AF	N	UPD 15

AF: Amniotic Fluid; CVS: Chorionic Villi Sampling; POC: Product Of Conception; UPD: Uniparental Disomy; AMA: Advanced Maternal Age; PPS: Positive Prenatal Screen; FUA: Fetal Ultrasound Abnormality; PPFA: Prior Pregnancy with Fetal Aneuploidy; PA: parental anxiety. T: Trisomy; Y: Yes; N: No; TOP: Termination of Pregnancy; CNV: Copy Number Variant.

SUPPLEMENTARY FIGURES LEGEND

Supplementary Figure 1: Clinically relevant fetal copy number variants (CNVs) detected in maternal plasma by genome-wide cfDNA screening, with results confirmed by invasive testing. The right panel shows cfDNA sequencing results; Z-scores of 5Mb sliding windows are plotted across the chromosome, dotted lines represent ±2.5 z-scores and the areas above or below these cutoff values, colored in gray, highlight the aberrations. The left panel shows invasive testing results by microarray analysis. **A)** detection of a 34.1 Mb deletion at 5p15.32p13.2 (Case 1); **B)** detection of a 15.4 Mb duplication at 18p11.32p11.21 (Case 5). Microarray analysis result not shown. **C)** detection of a 58.5Mb deletion at Xp22.33p11.1 (Case 7). **D)** detection of a 16.0 Mb duplication at 11p15.1p15.4 (Case 8).

Supplementary Figure 2: Fetal unbalanced translocations detected in maternal plasma by genome-wide cfDNA screening, with results confirmed by invasive testing. The right panel shows cfDNA sequencing results; the left panel shows invasive testing results by microarray analysis. Detection unbalanced translocation derivatives consisting of a 11.4 Mb deletion at 13q33.1q34 and a 1.9 Mb duplication at 20q13.33 (Case 3). The arrow indicates the location of the latter CNV in the sequencing plot.

Supplementary Figure 3: Chromosome 22 ideogram showing (right panel) sequencing-based detection of a 4.3 Mb deletion at 22q11.21 (DiGeorge syndrome) (Case 6), that was confirmed by microarray analysis (left panel).

Supplementary Figure 4: Clinically relevant fetal copy number variants (CNVs) detected in maternal plasma by genome-wide cfDNA screening, with results not confirmed by invasive testing. **A)** detection of a 38.8 Mb duplication at 9p24.3p13.1 (Case 9); **B)** detection of a 68.5 Mb deletion at 5q14.3q32 (Case 10); **C)** detection of a 36.8 Mb deletion at 7q21.11q31.1 (Case 11); **D)** detection of a 62.2 Mb duplication at 8p23.3q13.3 (Case 12); **E)** detection of a 29.2 Mb deletion at 20q11.21q13.32 (Case 13)

Supplementary Figure 5: Rare autosomal trisomies detected in maternal plasma by genome-wide cfDNA screening, with results confirmed by invasive testing or product of conception (POC) karyotyping. **A)** Trisomy 22 (Case 17); **B)** Trisomy 15 (Case 18); **C)** Trisomy 22 (Case 19); **D)** Trisomy 15 (Case 20); **E)** Trisomy 22 (Case 21); **F)** Trisomy 22 (Case 22); **G)** Trisomy 15 (Case 23); **H)** Trisomy 9, confirmed as fetal mosaicim 46,XX[80]/47,XX,+9[20] by traditional karyotyping (Case 15); **I)** Trisomy 7, confirmed as fetal mosaicim 46,XX[48]/47,XX,+7[2] by traditional karyotyping (Case 14); **J)** Trisomy 22, confirmed as fetal mosaicim 46,XY[42]/47,XY,+22[8] by traditional karyotyping (Case 16).

Supplementary Figure 6: Rare trisomies detected in maternal plasma by genome-wide cfDNA screening, with results not confirmed by invasive testing. **A-C)** Trisomy 7 (Cases 24-26); **D)** Trisomy 14 (Case 27); **E)** Trisomy 16 (Case 28); **F)** Trisomy 12 (Case 29); **G)** Trisomy 15 (Case 30), in which a uniparental disomy of chromosome 15 occurred, because of a trisomy rescue event.

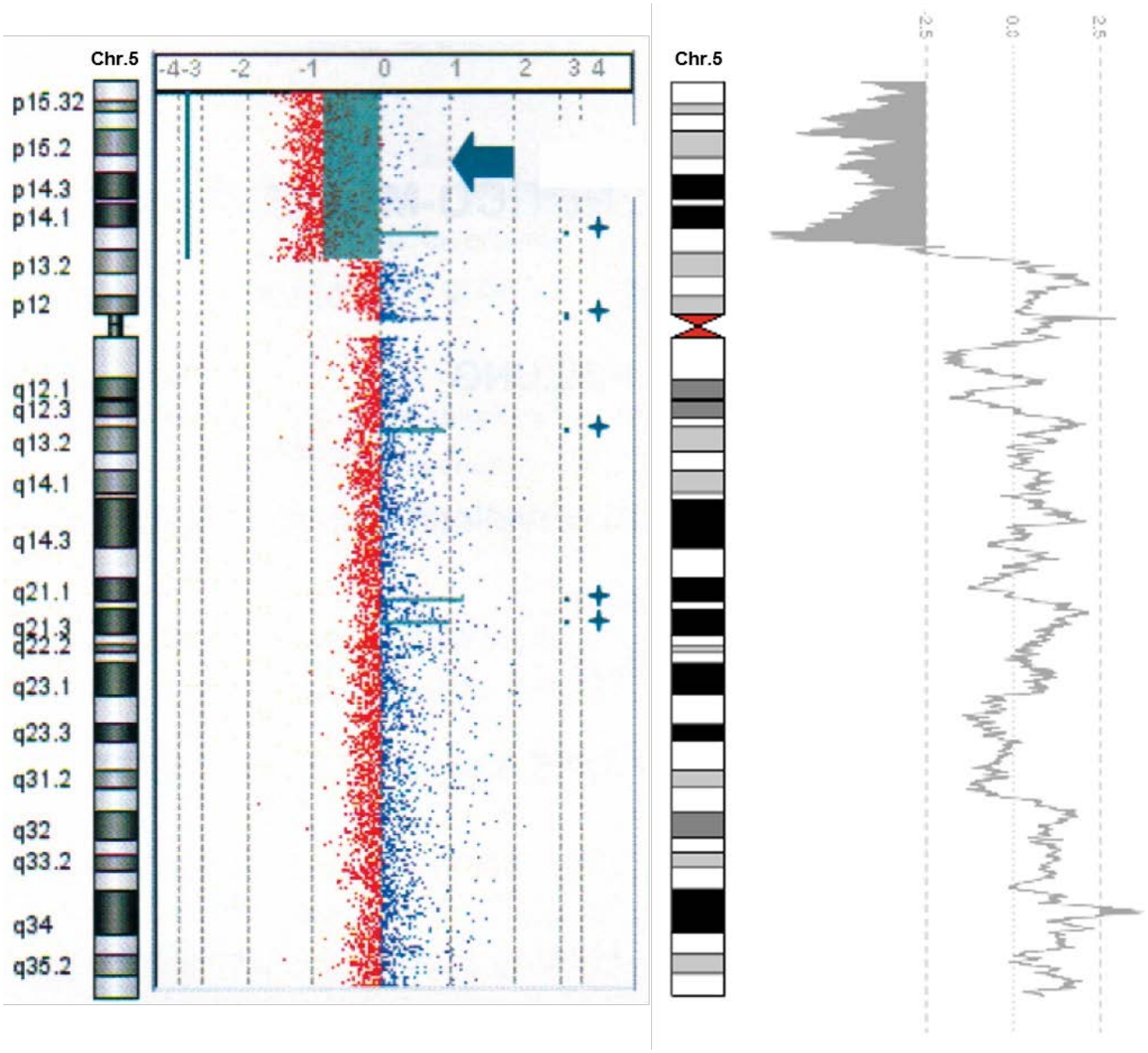
Supplementary Figure 7: Clinically relevant chromosomal abnormalities classes detected by genome-wide cfDNA analysis, with results confirmed by invasive testing or product of conception (POC) karyotyping. The absolute numbers of the aneuploidy classes are shown from left to right: the common trisomies (trisomy 21, 18, and 13), the sex-chromosome anomalies (monosomy X, triple X,

Jacobs and Klinefelter syndromes), the rare autosomal trisomies, and the deletion and duplication copy-number variations.

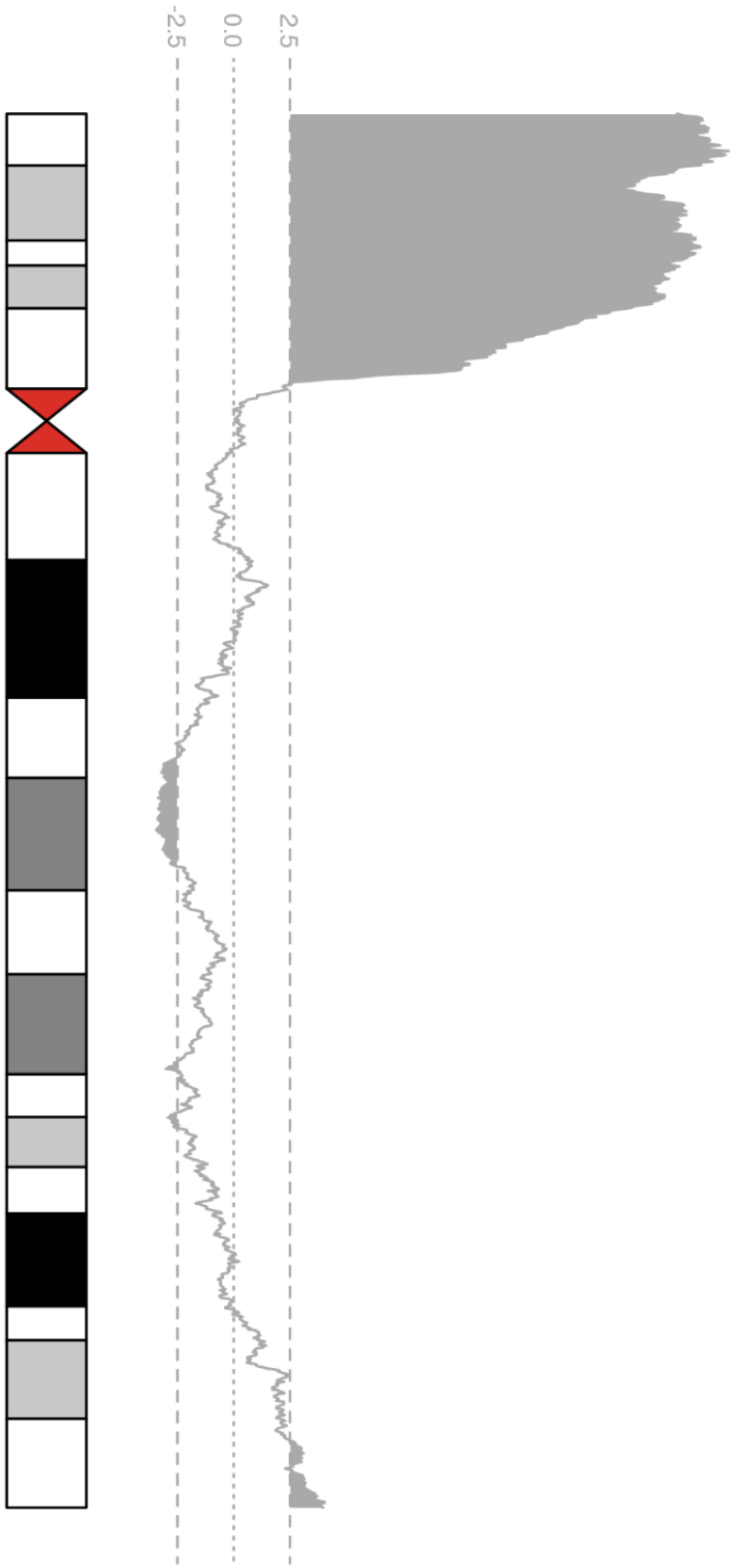
Supplementary Figure 8: The pie chart depicts the types of clinically relevant chromosome anomalies detected by genome-wide cell-free fetal DNA (cfDNA) testing and conventional cfDNA-based non-invasive prenatal testing (NIPT) for common fetal aneuploidy. The size of each pie segment represents the relative contribution of the specific category of abnormality to the overall chromosome abnormalities identified in the samples analyzed. The category "common aneuploidies" includes common autosomal trisomies (chromosomes 21, 18, 13) and sex chromosome abnormalities (monosomy X, triple X, Jacobs and Klinefelter syndromes); the category "Chr. Abnormalities not detected by standard cfDNA testing" includes rare autosomal trisomies and structural chromosomal aberrations.

SUPPLEMENTARY FIGURES

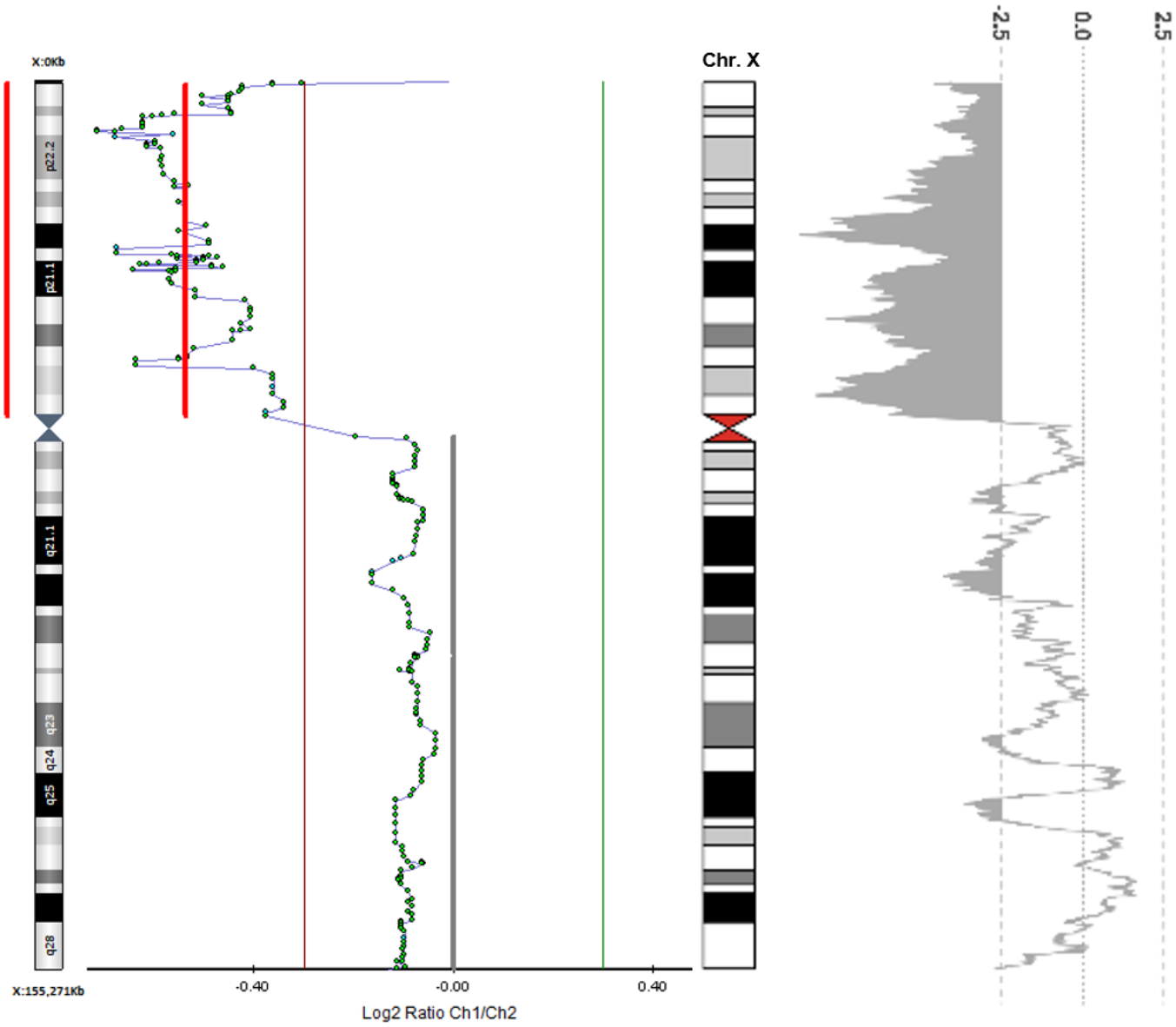
Supplementary Figure 1A



Supplementary Figure 1B



Supplementary Figure 1C



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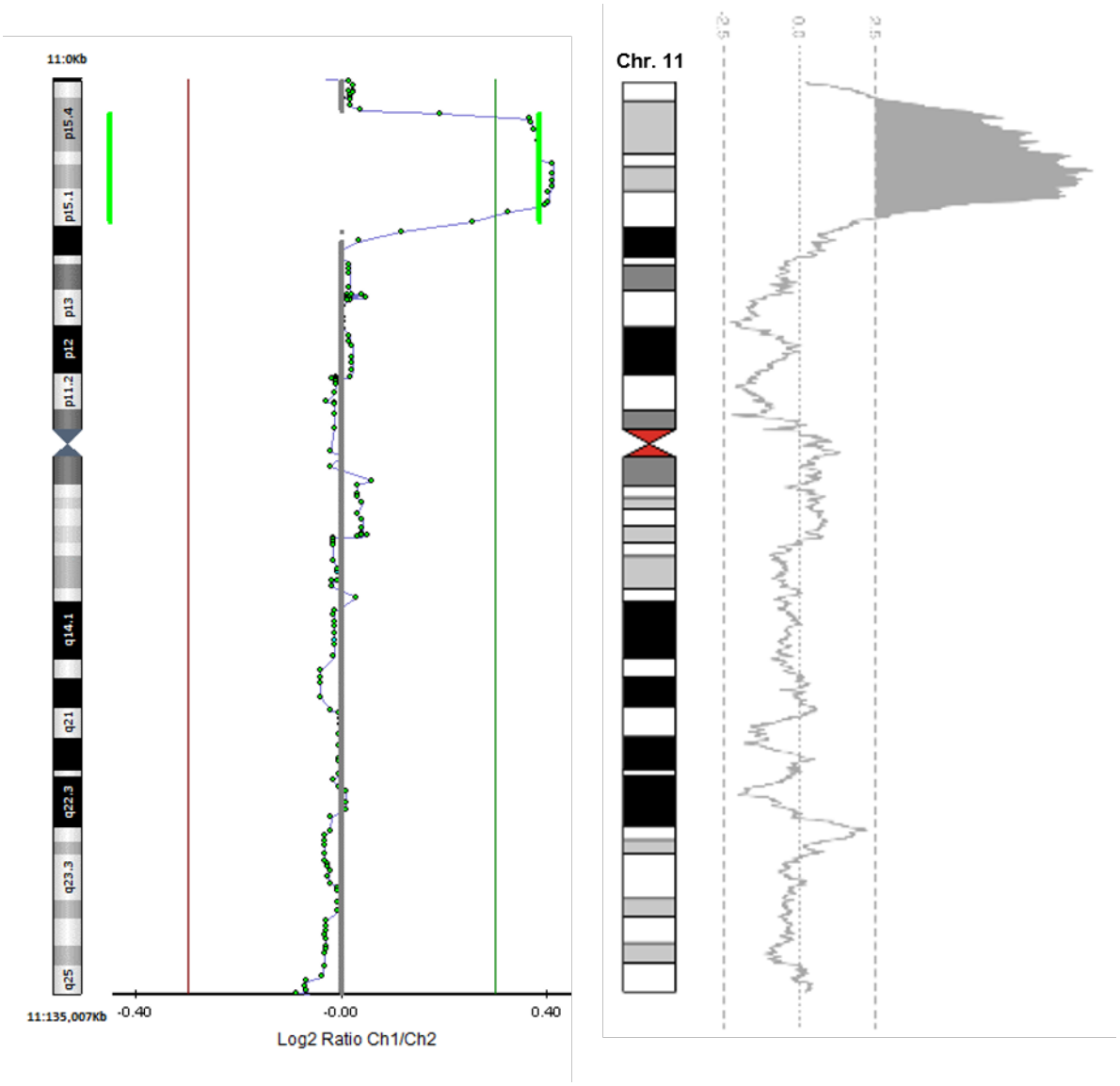

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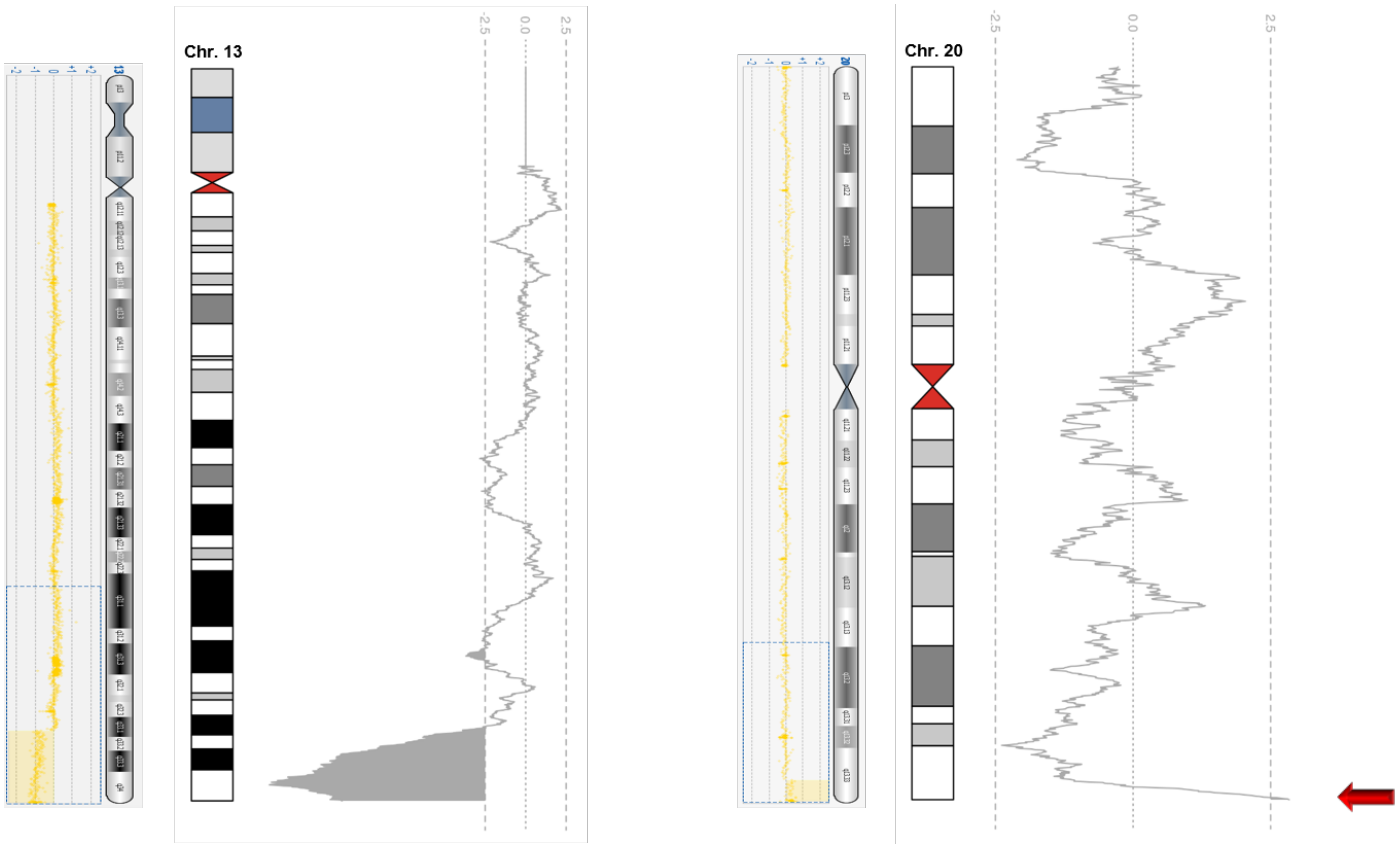

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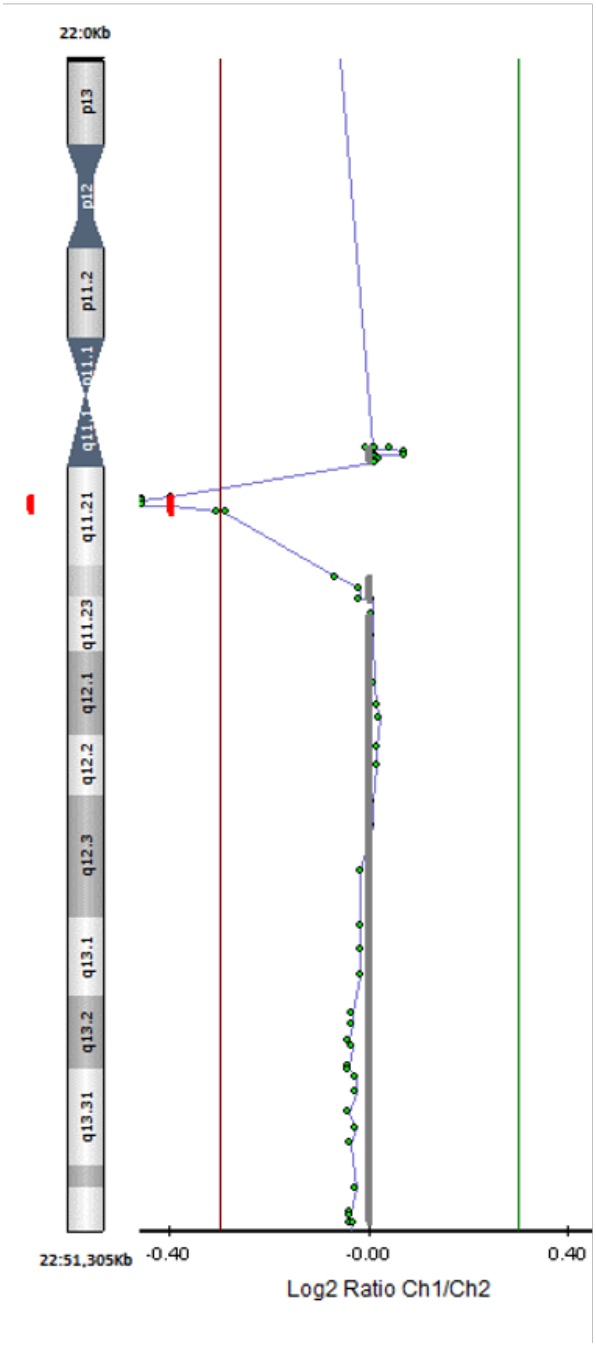

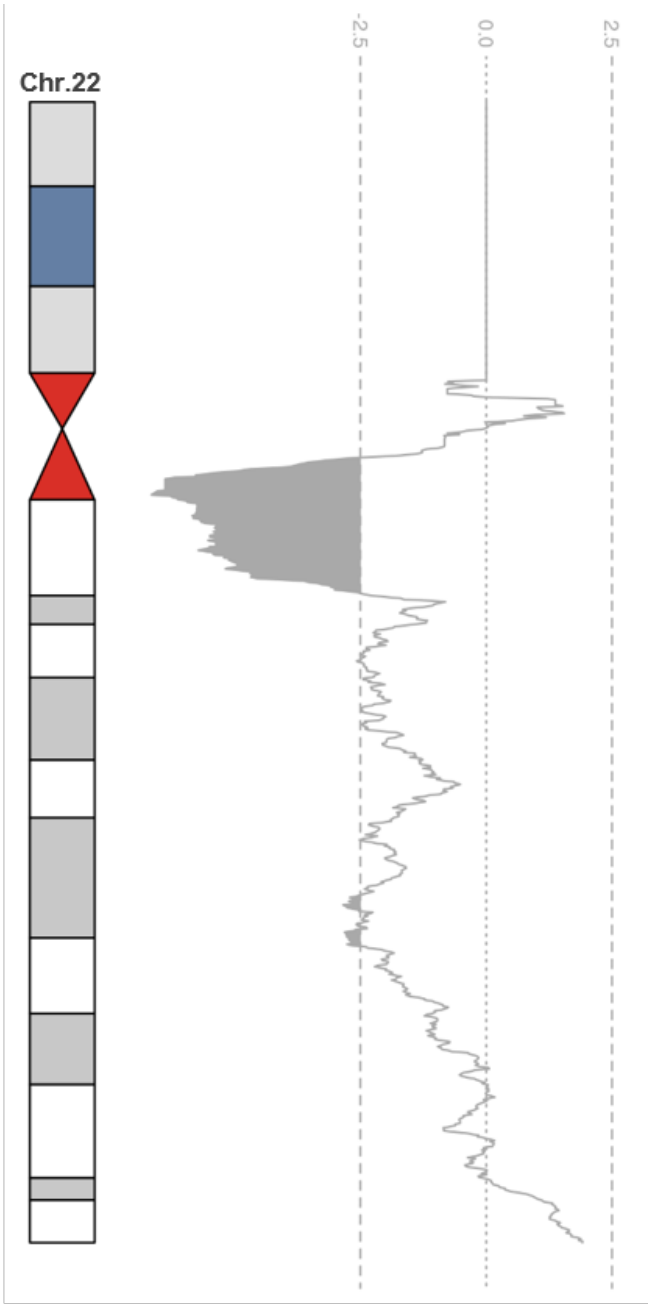

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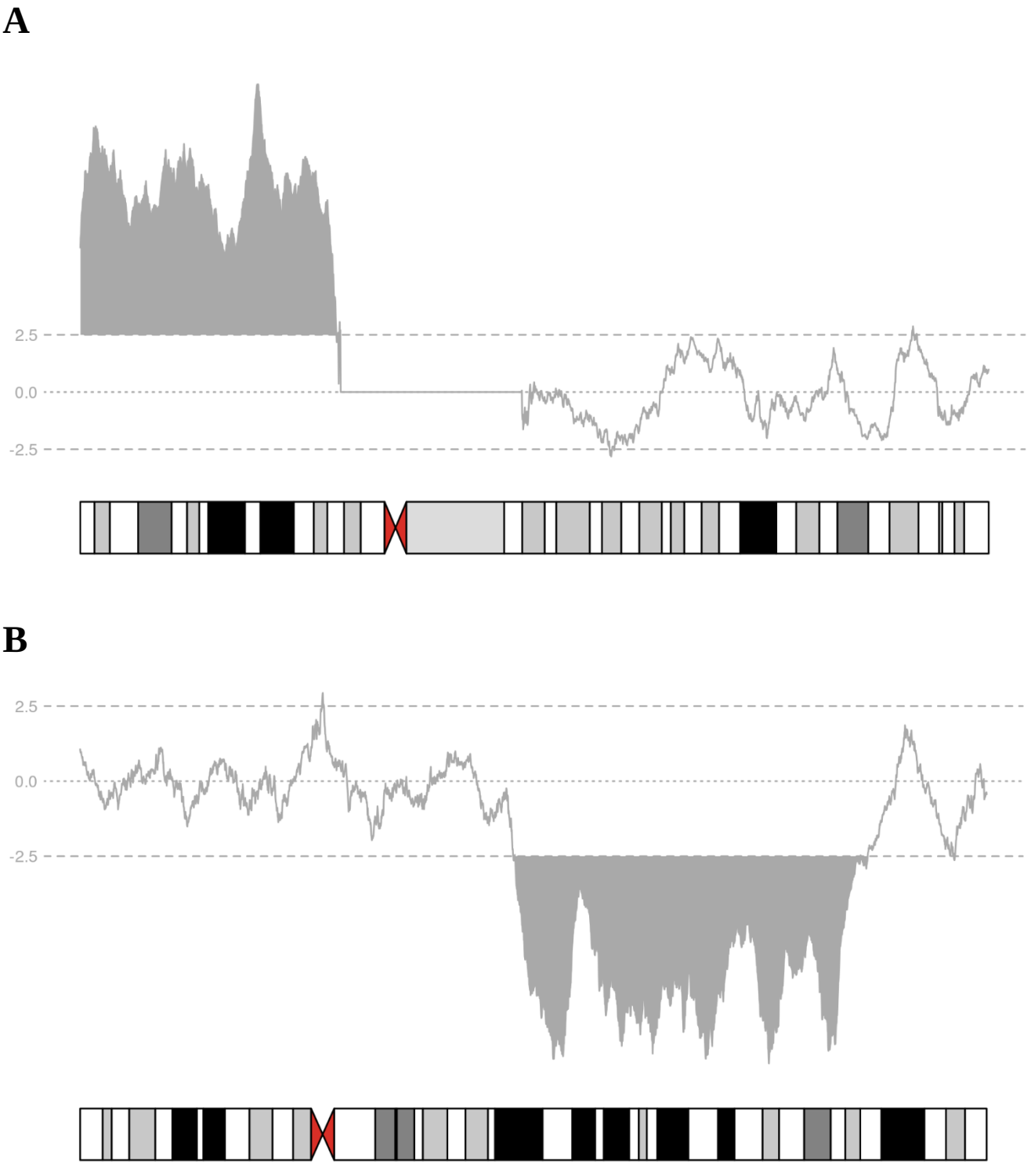

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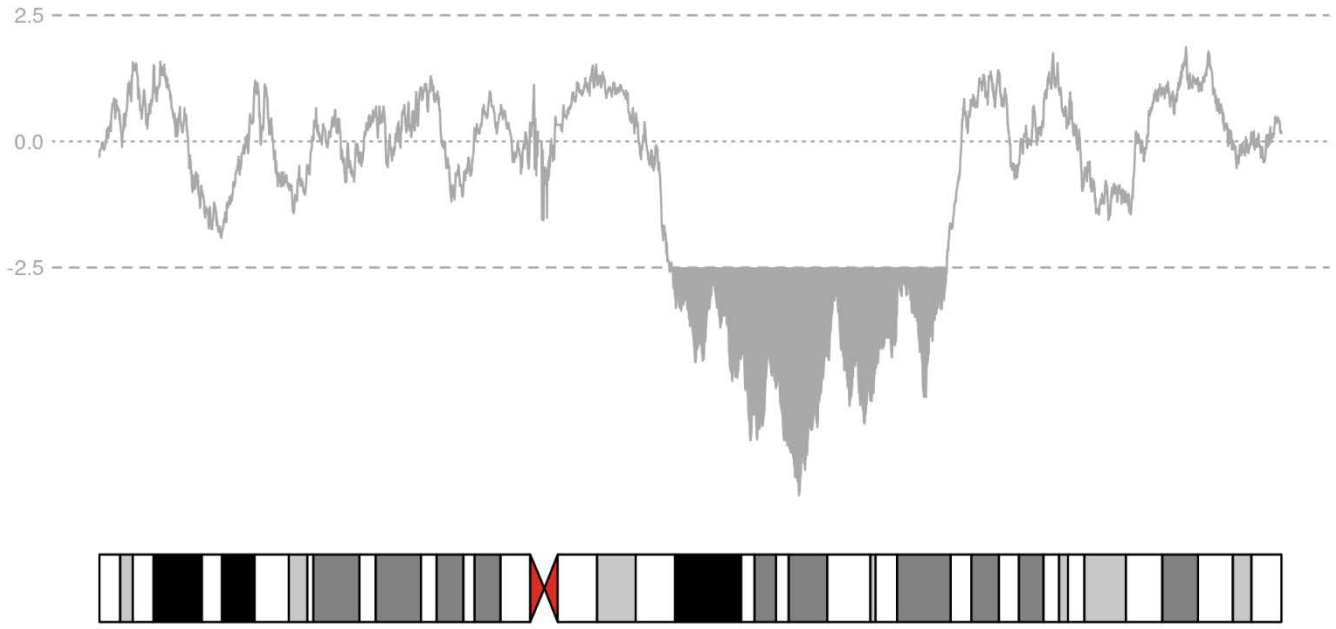

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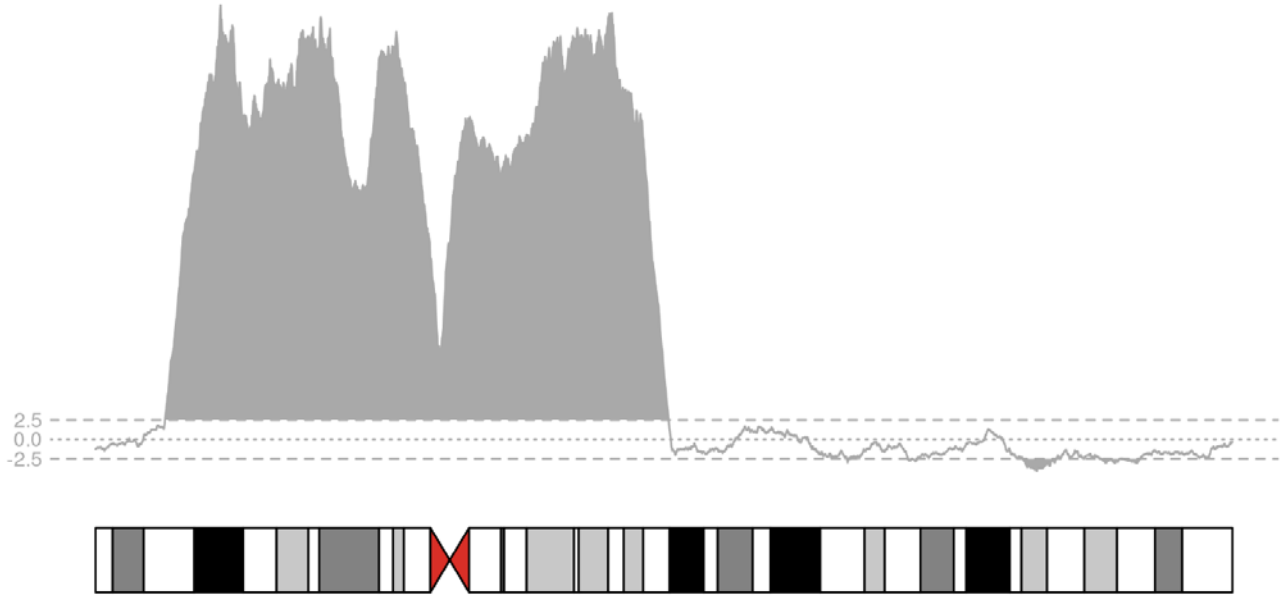

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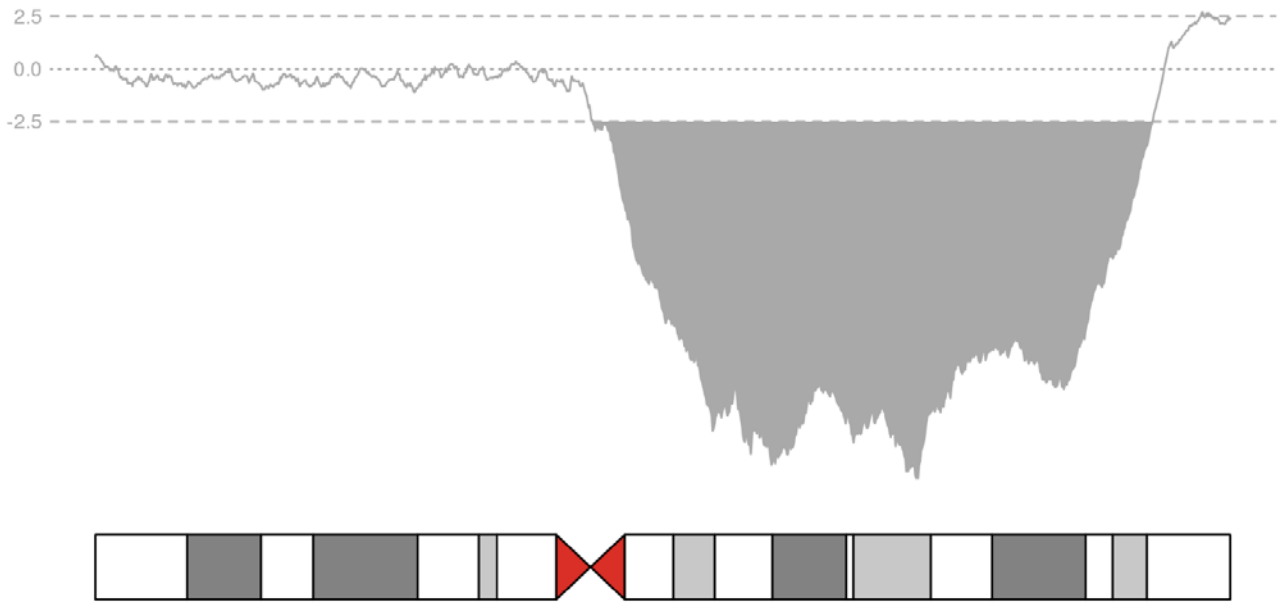
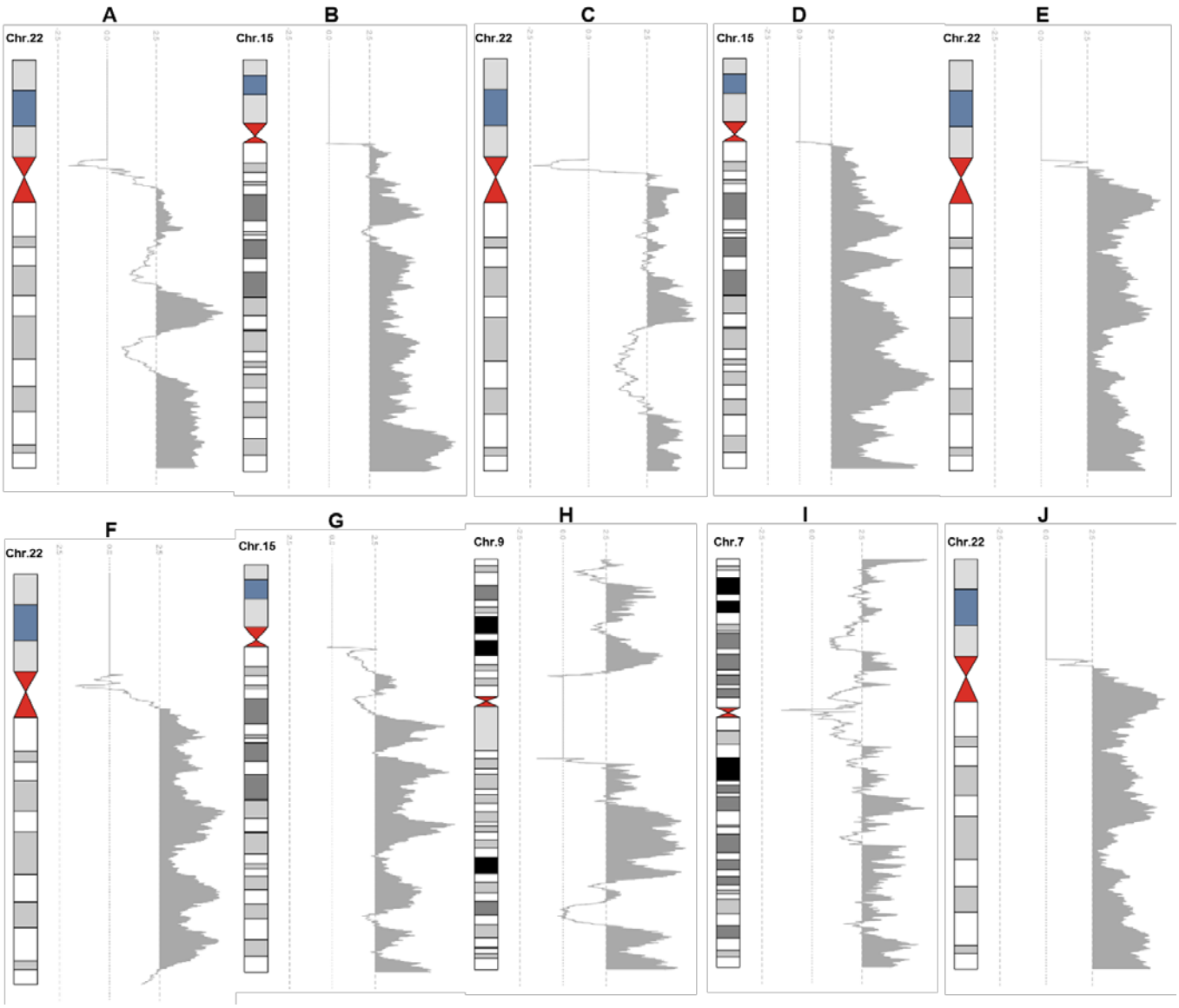


Supplementary Figure 5



142 **Supplementary Figure 6**

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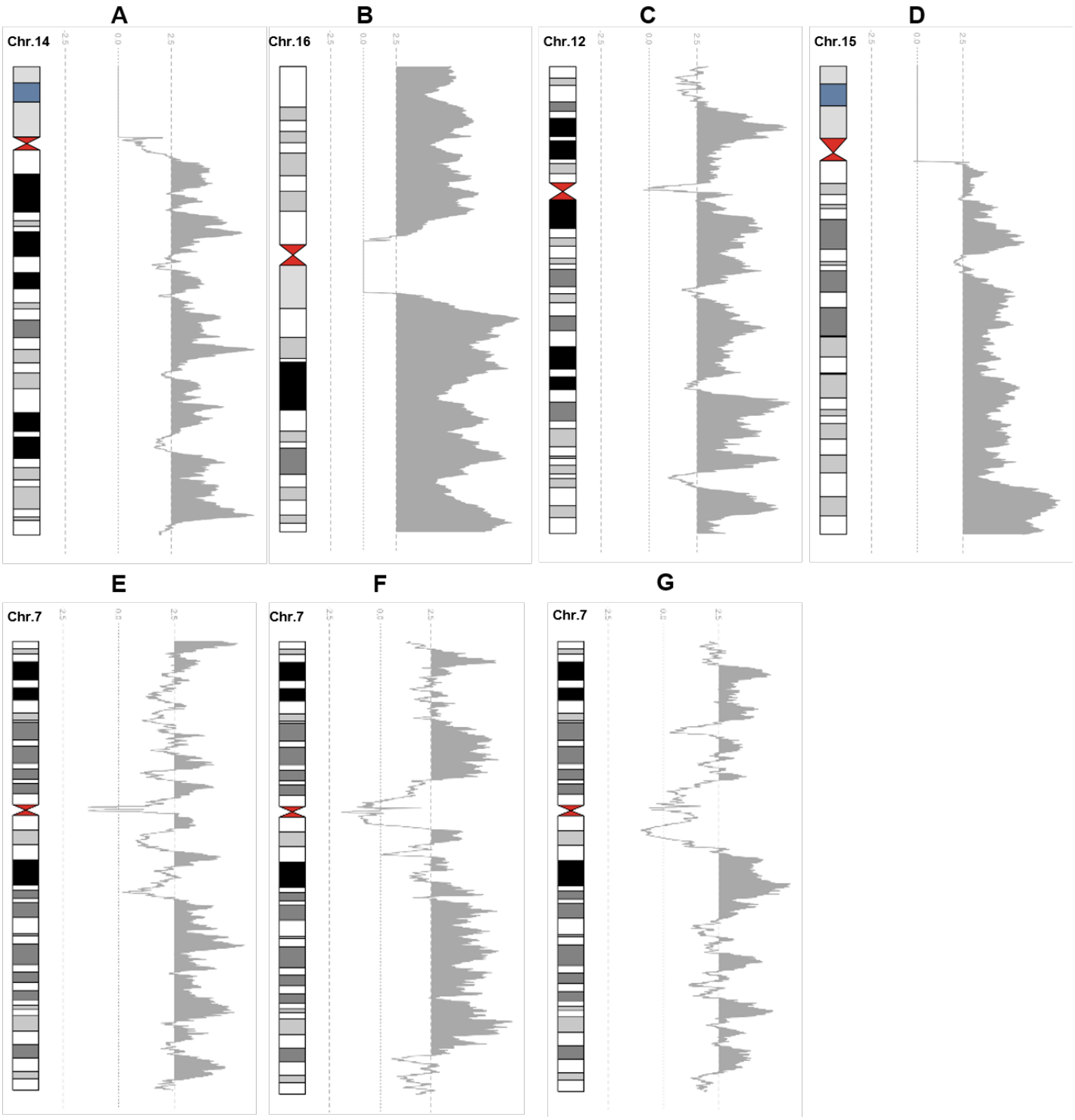
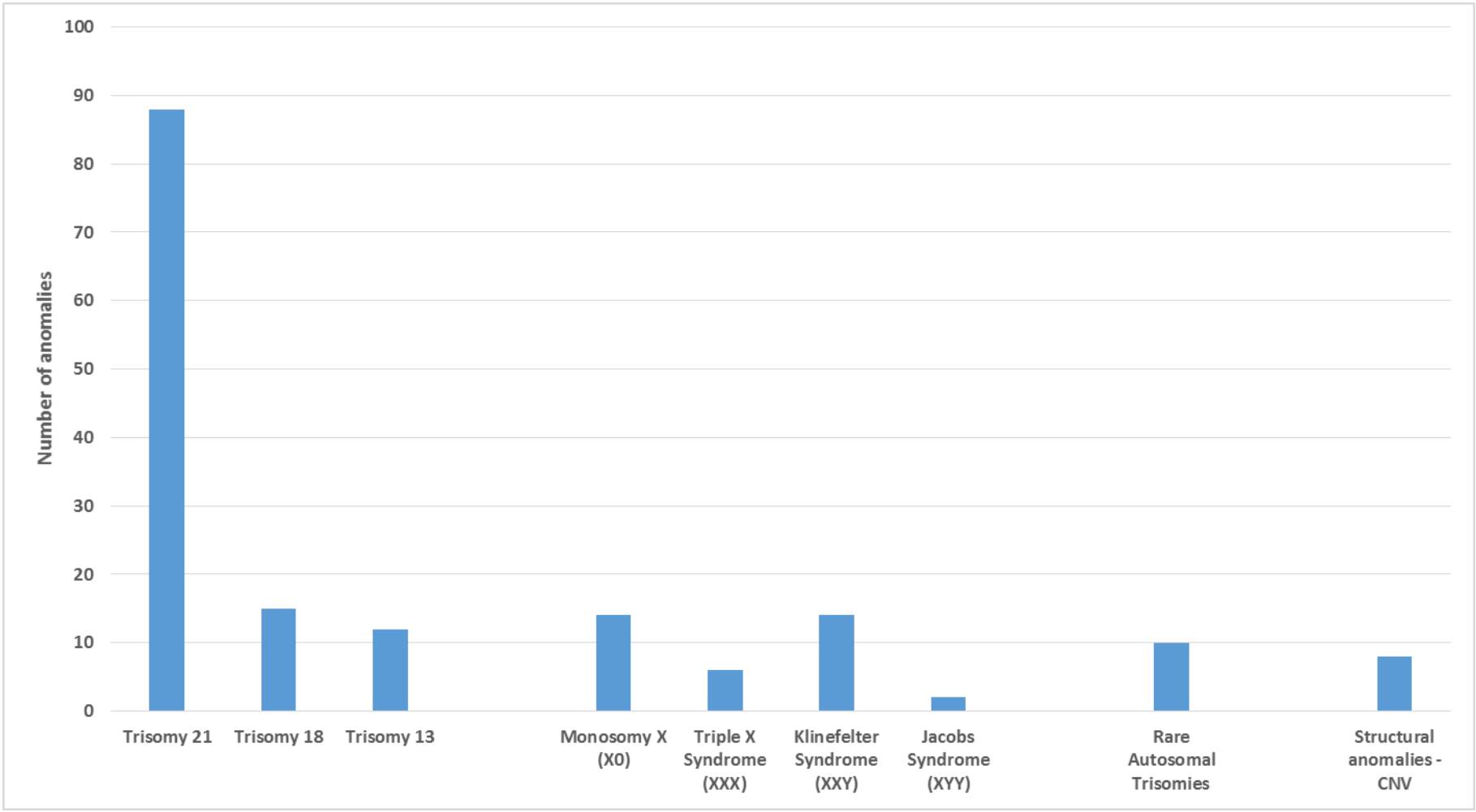


Supplementary Figure 7



Supplementary Figure 8