PGT-A INCORPORATING SENSITIVE AND SPECIFIC DETECTION OF RECURRENT MICRODELETION AND MICRODUPLICATION SYNDROMES USING HIGH-RESOLUTION TARGETED SEQUENCING

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OBJECTIVE:

The primary objective of this study was to evaluate the accuracy, sensitivity and specificity of a PGT-A platform incorporating detection of ten common microdeletion and microduplication disorders. A secondary objective was to survey syndromic prevalence in a large cohort of preimplantation embryos.

BACKGROUND:

Recurrent microdeletion and microduplication (del/dup) syndromes:

- Segmental (<5 mb) gains and losses associated with a clinical phenotype
  - Approximately 200 del/dup syndromes have been associated with intellectual disability, developmental delay and physical dysmorphia.
  - Phenotype and severity varies depending on expressivity, penetrance, and the individual genes impacted by copy number variation (CNV).
- Estimates of collective occurrence vary from 1/1000 to 1/200 live births
  - This may be an underestimation due to genotype/phenotype variability and inconsistent diagnosis (1,2).
- Embryonic occurrence rates may be higher
  - Del/Dup disorders are difficult to detect reliably using standard PGT-A.
  - Platform bias and poor resolution frequently lead to low sensitivity and high false positive rates.
  - ***There are currently no preimplantation del/dup screening tests, leaving patients at risk of transferring an impacted embryo.***

MICRODELETION AND MICRODUPLICATION TARGETS:

Disorders were selected based on prevalence and severity, and align closely with the those most frequently tested by NIPT providers. Genomic location and length are displayed, along with post-natal occurrence rates.

Microdeletion / Microduplication	Location	Length (mb)	Occurrence (%)
Langer-Giedion *Multiple osteochondromas, hypertrichosis, facial features*	8q23.2–q24.1	10.9	1 / 100,000 (0.001%)
Jacobsen *Craniofacial dysmorphia, cong. heart disease, intellectual disability*	11q23-qter	15.4	1 / 100,000 (0.001%)
2q33.1 Syndrome *Seizures, speech and development delay*	2q33.1	2.4	1 / 100,000 (0.001%)
Wolf-Hirschhorn *Craniofacial phenotype, growth restriction, intellectual disability*	4p16.3	1.7	1 / 50,000 (0.002%)
Potocki-Lupski / Smith-Magenis *Hypotonia, dysphagia, failure to thrive, heart abnormalities*	17p11.2	4.9	1 / 15,000 (0.007%)
Cri-du-chat *Low birth weight, severe intellectual disability, behavioral issues*	5p15	19	1 / 15,000 (0.007%)
1p36 Syndrome *Intellectual disability, heart defects, epilepsy*	1p36	5.5	1 / 5,000 (0.02%)
Angelman / Prader-Willi *Delayed milestones and intellectual disability, seizures*	15q11.2-q13.1	5.5	1 / 5,000 (0.02%)
DiGeorge / Velo-Cardio-Facial Syndrome *Heart defects, immune system, and intellectual disability*	22q11.2	2.6	1 / 2,000 (0.05%)

~ 1 / 1000 (0.1%) Live Births*

*Pre-natal occurrence presumed to be higher

ASSAY DEVELOPMENT:

Cell lines and clinical control samples with confirmed del/dups were used to develop and verify the method. Samples were processed using Primary Template-directed Amplification (PTA, 3), prepared for next-generation sequencing and target enriched for genome-wide and del/dup specific SNPs. A bioinformatics pipeline was applied to identify presence of del/dups through allelic balance analysis.

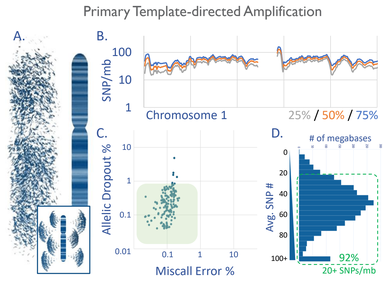


Figure 1: Primary Template-directed Amplification. *(A) Illustration comparing PTA with standard WGA (inset). (B) Smoothed SNP density across Chr. 1 from PTA sample. (C) Error rate vs ADO in PTA samples. (D) Genome-wide SNP distribution across 1 mb bins.*

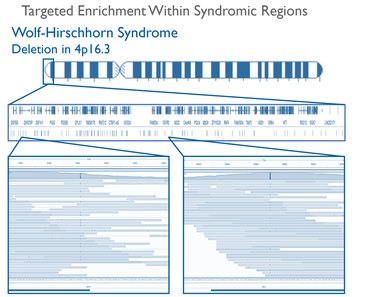


Figure 2: Target Enrichment for SNPs within syndromic regions. *Increasing resolution and representative read depth of SNP data generated within the Wolf-Hirschhorn region from target-enrichment NGS.*

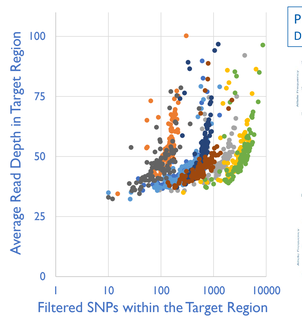


Figure 3: Quality SNPs by Del/Dup Region *Number and average read depth (>30x) for SNPs within indicated del/dup regions. Regions correspond to colors shown on table on left.*

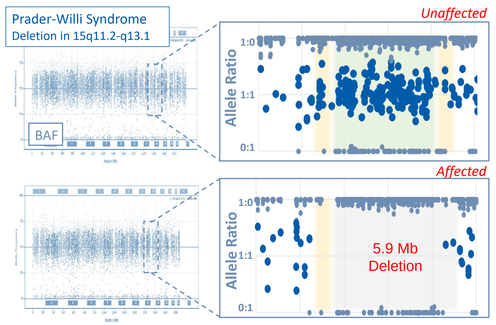


Figure 4: Region-Specific Allele Ratio dictates del/dup status in Control Samples *Bi-allelic SNP data expressed as a ratio of alleles indicates diploid regions with balanced allele contribution versus those regions of expected deletion where only one alle is present. Cultured cell lines from unaffected and confirmed affected individuals, processed as indicated.*

Dense SNP data within del/dup regions resulted in an average of 42-205 quality-filtered SNPs per megabase, resulting in a reportable rate of 96-99%. An initial estimate for overall accuracy was 97-99%, syndrome-specific sensitivity and specificity ranged from 95-99% and 92-99%, respectively.

References: 1. Wetzel et al., BMC Genomic Data, 2022, Vol. 23; Art.82. 2. Iordanescu et al., J.Pers Med. 2024 Mar; 14(3):290. 3.Gonzalez-Pena, V. et al., PNAS. 2021, Vol. 118: No. 24

		Control Type (detected/tested)		
Microdeletion / Microduplication		*Cell Line*	*Clinical*	*Overall*
Langer-Giedion		1/1	6/6	7/7
Jacobsen		2/2	5/5	7/7
2q33.1 Syndrome		1/1	6/6	7/7
Wolf-Hirschhorn		1/1	4/4	5/5
Potocki-Lupski / Smith-Magenis		1/2*	5/5	6/7
Cri-du-chat		3/3	5/5	8/8
1p36 Syndrome		2/3*	8/8	10/11
Angelman / Prader-Willi		2/2	3/3	5/5
DiGeorge / Velo-Cardio-Facial Syndrome		3/3	1/1	4/4
		16/18	*49/49*	*65/67*
Aneuploid Controls	0/11	*89%*	*100%*	*97%*
Euploid Controls	0/17			

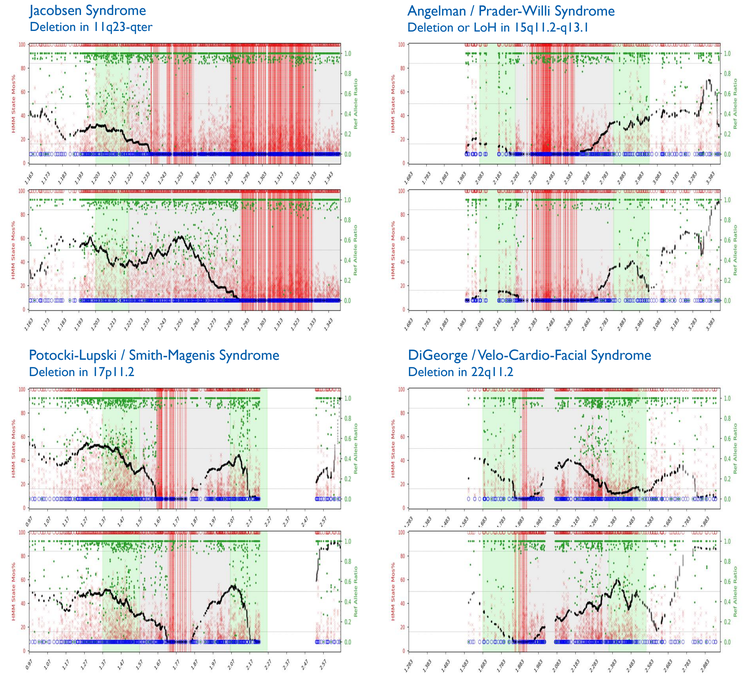
RETROSPECTIVE OCCURENCE IN PREIMPLANTATION COHORT:

Approximately 6,000 de-identified trophectoderm biopsies were retrospectively analyzed for this study. Samples with potential del/dup regions were identified and euploid embryos were visually checked to confirm the indicated gains or loss. Occurrence rates in this limited euploid dataset were above the post-natal rate.

Figure 5: Clinical Samples impacted by Del/Dup Disorders *Paired samples show a control cell line impacted by the indicated del/dup CNV at top and an embryo with analogous CNV at bottom. Green dots represent B-allele frequency, red Xs are read depth, red bars are megabase windows with likely loss, black dots are predicted ploidy over the region.*

Del/Dup Syndrome	*Positive Samples*
1P36 deletion	3 (0.04%)
2q33.1 deletion	9 (0.15%)
APW deletion	2 (0.03%)
CDC deletion	0 (0)
DG deletion	4 (0.06%)

Del/Dup Syndrome	*Positive Samples*
Jacobsen deletion	4 (0.06%)
LG deletion	3 (0.05%)
PLSM deletion	3 (0.05%)
WH deletion	6 (0.1%)
Total	***34 (0.55%)***



CONCLUSIONS:

This study confirms the utility of a high-resolution PGT-A platform to accurately detect causative microdeletions and microduplications underlying some syndromic disorders. This approach sensitively and specifically identifies regions of CNV that may otherwise go undetected with standard tests. The elevated incidence in this dataset could indicate a higher pre-natal vs post-natal occurrence rate. Overall, PGT-A with del/dup detection could deliver more complete transfer data.