PGT-A INCORPORATING SENSITIVE AND SPECIFIC DETECTION OF NINE RECURRENT DELETION AND DUPLICATION SYNDROMES USING HIGH RESOLUTION TARGETED SEQUENCING

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Background: Recurrent deletion and duplication (del/dup) syndromes are a group of genetic disorders arising from chromosomal segmental loss or gain which results in distinct clinical phenotypes. Approximately 200 such del/dup syndromes have been characterized, many of which involve intellectual disability, developmental delay and physical dysmorphia, though individual severity can vary significantly depending on expressivity, penetrance, and the specific genes impacted by copy number variation (CNV). Estimates of collective occurrence vary from 1/1000 to 1/200 live births, but this is likely an underestimation due to genotype/phenotype variability and inconsistent diagnosis (1,2). Data suggests that embryonic occurrence rates may be higher but given that del/dup CNV is generally less than 10 megabases in length, and frequently much shorter, these disorders are difficult to detect reliably using standard PGT-A methods. Platform bias and poor resolution frequently lead to low sensitivity and specificity with elevated false positive rates. This has translated to a lack of accurate del/dup screening options in preimplantation embryos prior to transfer.

Objective: The primary objective of this study was to evaluate the accuracy, sensitivity and specificity of a PGT-A platform incorporating detection of nine del/dup disorders (based on occurrence and severity). A secondary objective was to survey the frequency of syndromic occurrence in a large cohort of preimplantation embryos.

Materials and Methods: De-identified trophectoderm biopsies submitted for standard PGT-A were retrospectively analyzed for this study. Approximately 25K samples were processed using primary template-directed amplification (PTA, 3), prepared for next-generation sequencing and enriched for selected SNP data. A bioinformatics pipeline was applied to identify genome-wide and del/dup region-specific CNV through allelic balance analysis. Cell lines and clinical samples with confirmed deletions and duplications were used to develop and verify the method.

Results: Genome-wide PGT-A results displayed 99.5% concordance with standard PGT-A outcomes. Sequencing metrics showed that the Del/Dup-enabled PGT-A pipeline resulted in consistent, deep, and dense collection of SNP data within syndromic regions. The enrichment pipeline generated, on average, 42-205 quality-filtered SNPs *per megabase* across the defined del/dup regions, resulting in a reportable rate of 96-99%. An initial estimate for overall accuracy was 97-99% and on first pass, syndrome-specific sensitivity and specificity ranged from 82-96% and 92-99%, respectively. Occurrence rates in this limited dataset varied between 1-2x the reported live birth occurrence.

Conclusions: This study confirms the overall strength and utility of a high-resolution PGT-A method to accurately detect short segmental deletions and duplications that underly often severe syndromic disorders. By leveraging PTA which has been shown to generate greater genomic coverage while preserving allelic balance, and targeted sequencing to achieve critical SNP-specific depth and density, this approach sensitively and specifically identifies regions of CNV that may otherwise go undetected with standard tests. A small number of samples displayed novel subregion and complex CNV that may explain some of the variability observed in del/dup phenotypes. The slightly elevated occurrence rate suggests that these disorders may occur more frequently than observed in live birth. Overall, PGT-A with del/dup detection could deliver more complete, actionable test results to further inform embryo transfer decisions.

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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