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Isochromosome 21q is overrepresented among false-negative cell-free DNA prenatal screening results involving Down syndrome

Huijsdens-van Amsterdam K, Page-Christiaens L, Flower N, Bonifacio M, Ellis KMB, Vogel I, Vestergaard EM, Miguelez J, Carvalho MHB, Sistermans EA, Pertile MD

Fleury Medicina & Saude, Sao Paulo, Brazil

Objective

False-negative cell-free DNA (cfDNA) screening results involving Down syndrome are rare but have high clinical impact on patients and their healthcare providers. Postzygotic mutations have been described as a cause of karyotype discordance between the fetus and the placenta and could potentially result in a false negative cfDNA result. About 2% of Down syndrome cases are due to 21q;21q rearrangements, most representing true isochromosomes, which arise de novo from a postfertilisation event. We aimed to investigate, if isochromosome 21q is overrepresented among false-negative cell-free DNA prenatal screening results.

Methods

Five clinical laboratories [Academic Medical Center Amsterdam, the Netherlands; Victorian Clinical Genetics Services (VCGS), Melbourne, Australia; Genea, Sydney, Australia; Aarhus University Hospital, Denmark and Fleury Medicina and Saude, Sao Paulo, Brazil], reviewed their cfDNA screening outcome records from 29 July 2013 to 29 September 2017. All trisomy 21 true-positive (TP) and false-negative (FN) cases were recorded and the false-negative rate was calculated. All prevailing cfDNA screening technologies were utilized: massive parallel shotgun sequencing (MPSS), targeted MPS (tMPS), targeted microarray (tMA) and targeted single nucleotide polymorphism (tSNP)-based methodologies. Laboratory databases were interrogated for cytogenetic summary reports for all trisomy 21 cases, for chromosome 21 z-scores or equivalent (when available) and for fetal fraction (FF) estimates. This data was combined with previously published FN cfDNA screening cases. Molecular studies of 21q rearrangements and placental karyotype were reviewed, when available.

Results

There were 9 false-negative results documented in 646 confirmed cases of trisomy 21; a false negative rate of 1. 4% (95% CI, 0. 7-2. 6). False-negative results included 4 cases of classical trisomy 21 and 5 cases with a de novo 21q;21q rearrangement. The average fetal fraction at first analysis in FN cases with 21q;21q rearrangements was 10. 1% (range 3-17. 2%). When combined with reports from the cfDNA screening literature, 8 out of 29 (28%) Down syndrome cases with a false-negative cfDNA test were associated with a 21q;21q rearrangement, a 14-fold increase over the 2% of cases reported in live born children with Down syndrome. There was evidence for placental or fetal mosaicism in all four 21q;21q rearrangement cases, in which placental tissue was available (one FN and 3 TP), and a true isochromosome was confirmed in both cases where molecular studies were performed.

Conclusion

Isochromosome 21q rearrangements are overrepresented among false-negative cfDNA screening results involving Down syndrome. Postzygotic isochromosome formation leading to placental mosaicism provides a biological cause for the increased prevalence of these rearrangements among false-negative cases. False-negative results can occur through mechanisms other than low fetal fraction, poor quality or technical error.