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1Title: **False Positive Cell Free DNA Screening for Microdeletions Due to Non-Pathogenic**

2Copy Number Variants

3Short Title: False positive cfDNA due to alternate CNVs

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19What's Already Known About This Topic?: Aneuploidy screening using cell free DNA (cfDNA)
20has recently been expanded to include selected microdeletions. However, validation has been
21limited and the real-world positive-predictive value is unknown.

22

23What Does This Study Add?: Here we describe three cases of false positive cfDNA
24microdeletions due to small, non-pathogenic copy number variants near the region of interest.
25These findings elucidate one mechanism of false-positive results with this screening test and
26suggest that additional validation is necessary before wider use in clinical practice.

27

28

29Abstract

30*Objective:* Fetal aneuploidy screening using cell free DNA (cfDNA) has recently been expanded
31by some laboratories to cover selected microduplications/microdeletions. While validation
32testing has reported high sensitivity and specificity for these disorders, the real-world positive
33predictive value of cfDNA is largely unknown.

34*Methods:* Confirmatory cytogenomic microarray analysis was performed on three consecutive
35amniotic fluid samples with positive cfDNA screening results for microdeletion syndromes.

36*Results:* All three tests were false positives, likely due to small copy number variants near the
37critical regions for the disorders reported by cfDNA.

38*Conclusion:* Copy number variants other than those related to microduplication/microdeletion
39syndromes may cause false positive cfDNA results, reaffirms the necessity of follow-up testing
40to confirm cfDNA screening, and underscores the need for additional evaluation before
41implementing these tests in practice.

42

43Main Text

44The use of aneuploidy screening using fetal cell-free DNA has recently been introduced as a
45method to identify chromosomal aneuploidies in fetuses from maternal plasma samples^{1,2}. The
46high sensitivity and specificity of cfDNA screening for common aneuploidies has made this a
47very attractive screening test for pregnant women. Within the last year, commercially-
48available cfDNA testing as performed in some laboratories has been extended to cover a
49number of microduplication/microdeletion syndromes, most of which are very rare³. The
50sensitivity and specificity of the assays for these regions is high in laboratory validation
51studies^{3,4}, but the positive predictive value, which has been reported only for the detection of the
52relatively common 22q11.22 microdeletion (DiGeorge syndrome), is low in clinical testing⁵. In
53this report, we describe the first three cases of positive cfDNA microduplication/microdeletion
54results subsequently sent to our laboratory for confirmatory microarray following amniocentesis.

In all three cases, the cfDNA result was false positive due to likely benign copy number variants near the critical genomic region for the disorder in question. Our findings may disclose a common mechanism for the low positive predictive value of such tests. These results also underscore the need for additional validation before routine use in practice as well as the necessity for confirmatory diagnostic testing after a positive cfDNA result.

60

61Case 1:

Amniotic fluid from a 42 year old female at 17 weeks, 6 days gestation was sent for single nucleotide polymorphism (SNP) array testing due to a high likelihood of DiGeorge syndrome on cfDNA testing (quoted a priori risk 1/2000, post-test risk 1/19). The cfDNA test had been performed at 9 weeks, 2 days gestation and revealed a male fetus with a fetal cell-free DNA fraction of 8.4%. On array, using an Illumina CytoSNP 850k platform with hg19 genome build, a 270 Mb interstitial deletion was found at chromosomal positions 22q11.22(22,307,381-22,573,637), which is downstream of the DiGeorge critical region and is not associated with any known phenotypic consequences (Figure 1A). No deletions or duplications were identified in the DiGeorge critical region (Figure 1A).

71

72Case 2:

Amniotic fluid from a 31 year old female at 16 weeks, 4 days gestation was sent for SNP array testing due to a high likelihood of DiGeorge syndrome on cfDNA testing (quoted a priori risk 1/2000, post-test risk 1/19). The cfDNA test had been performed at 12 weeks, 4 days gestation and revealed a male fetus with a fetal cell-free DNA fraction of 7.3%. On array, a 240 Mb interstitial duplication was identified at chromosomal positions 22q11.22(22,314,463-22,555,078), which is similar to the region deleted in case 1 and is likewise not associated with known phenotypic consequences (Figure 1A). No deletions or duplications were identified in the DiGeorge critical region (Figure 1A).

81

82Case 3:

83Amniotic fluid from a 34 year old female at 16 weeks, 0 days gestation was sent for SNP array
84testing due to a high likelihood of Angelman syndrome on cfDNA testing (quoted a priori risk
851/12,000, post-test risk 1/26). The cfDNA test had been performed at 11 weeks 4
86days gestation and revealed a female fetus with a fetal cell-free DNA fraction of 7.5%. On array,
87a small 0.026 Mb interstitial deletion was identified at chromosomal
88position 15q11.2(25,089,832-25,116,221) within intron 1 of the *SNRPN* gene, a deletion not
89associated with aberrant imprinting in Prader-Willi syndrome and with no change on the *UBE3A*
90gene related to Angelman syndrome (Figure 1B). Follow up parental testing revealed that the
91deletion was maternally inherited (Figure 1B). Testing of the amniotic fluid sample by Southern
92blot revealed normal imprinting for both alleles, confirming the absence of Angelman syndrome
93in the fetus (Figure 1C). We note that for all three cases our results are limited in that we have
94not yet obtained clinical follow-up postnatally. However, we sought to report our results
95immediately, as physicians are currently encountering such cfDNA testing with minimal
96guidance for incorporation into practice.

97

98In laboratory validation testing, largely involving mixed samples of normal and abnormal
99genomic DNA, cfDNA for the identification of microduplications/microdeletions has a high
100sensitivity and specificity⁴. The number of false positive cases in the recently published
101laboratory validation series for the cfDNA assay used in our three patients ranged from
1020 of 422 (Angelman syndrome) to 3 out of 397 (DiGeorge syndrome). With additional
103sequencing of positive samples, a false positive rate as low as 1 out of 396 (0.25%) has been
104reported for DiGeorge syndrome⁴. However, a recent report from real-world clinical testing of
105more than 21,000 women found that the false positive rate for 22q11.22 deletion is much higher,
106as the positive predictive value in that study was only 18%⁵. No real-world clinical data has

107been published to describe false positive rates for testing of the 15q11.2 region, nor other
108regions analyzed on commercially available testing such as 1q36 and 5p deletions.

109

110The difference between the reported specificity of the assay from laboratory-only validation
111studies and that seen in clinical testing may be that none or very few of the control samples
112used in the laboratory-only validation studies harbored non-pathogenic microduplications or
113microdeletions near or in the critical regions for these disorders. The non-pathogenic 22q11.22
114copy loss seen in case 1, for example, is seen in <0.05% of the population (DGV database,
115<http://dgv.tcag.ca>), and microduplications in that region as identified in case 2 are seen in <0.2%
116of the population per the DGV. The 26 kb microdeletion in *SNRPN* is likewise rare; less than
1170.01% of the population has been identified with similar or larger deletions per the DGV.

118

119Our results suggest that such likely benign microduplications/microdeletions may cause false
120positives for clinical syndromes using cfDNA. As cfDNA is a screening test, when reporting a
121positive result laboratories performing this test note that risk for a given clinical syndrome is
122greatly increased but the syndrome is not definitively present. Our results suggest that the
123methodology used by the testing laboratory is sufficient to detect small CNVs near a clinically
124relevant region, thus avoiding the more clinically concerning situation of a false negative test,
125but are perhaps not accurate enough to determine the precise genomic coordinates of the CNV
126and thus pathogenicity. We would encourage laboratory providers to report the precise genomic
127coordinates examined by their testing for each microduplication/microdeletion syndrome. Based
128on population studies, the likely benign CNVs near these regions we identify here, as well as
129additional likely benign variants also found near these regions, may be even more common than
130the pathogenic variants tested for on the cfDNA assay (for example, ~1 in 3000 for pathogenic
13122q11.2 microdeletion versus >1/1000 for other variants in the region based on the DGV
132database). If these alternate CNVs routinely lead to positive testing, this mechanism would

133strongly limit even the theoretical positive predictive value of cfDNA for
134microduplication/microdeletions.

135

136Our finding is similar to that seen by Yatsenko *et al.*⁶ in their recent report of a single patient with
137a high risk of DiGeorge syndrome identified using cfDNA. Similar to Case 1 here, their patient
138had a deletion downstream of the DiGeorge critical region that is present in <0.1% of the
139phenotypically normal population. A more recent report of a variety of non-trisomic
140chromosomal anomalies reported by cfDNA has also described limited concordance with
141cytogenomic microarray⁷. Here we report additional variants both in the 22q11 and 15q11
142regions that suggest a general mechanism for false positive cfDNA testing. A recent study
143found that false positive results for trisomy 18 on cfDNA testing may be caused by maternal
144copy number duplications on this chromosome⁸. While we were unable to obtain maternal
145follow-up samples for Cases 1 and 2, our findings in Case 3 suggest these false positive
146screens for microdeletion syndromes may be more likely to occur in the case of inherited
147maternal copy number variants. If maternal copy number variants are frequently causative of
148cfDNA false positives in microdeletion testing, this result would further emphasize the
149importance of parental testing to alleviate anxiety and inform testing decisions in future
150pregnancies. Our experience also further reaffirms the necessity of confirming all positive
151findings from cfDNA by cytogenetic analysis, using cytogenomic microarray for
152microduplication/microdeletion calls in particular. Our results also further emphasize the
153necessity of additional clinical and analytical validation of these testing methodologies before
154routine use in clinical practice.

155

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162**References:**

1631. Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A et al.
164 Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and
165 low-risk cohort. *Obstet Gynecol.* 2014;124(2 Pt 1):210-8.

166

1672. Porreco RP, Garite TJ, Maurel K, Marusiak B, Obstetrix Collaborative Research
168 Network, Ehrich M et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13
169 and the common sex chromosome aneuploidies from maternal blood using massively
170 parallel genomic sequencing of DNA. *Am J Obstet Gynecol.* 2014;211(4):365.e1-12.

171

1723. Wapner RJ, Babiarz JE, Levy B, Stosic M, Zimmermann B, Sigurjonsson S et al.
173 Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion
174 syndromes. *Am J Obstet Gynecol.* 2015;212(3):332.e1-9.

175

1764. Gross SJ, Ryan A, Benn P. Noninvasive prenatal testing for 22q11.2 deletion syndrome:
177 deeper sequencing increases the positive predictive value. *Am J Obstet Gynecol.*
178 2015;213(2):254-5.

179

1805. Gross SJ, Stosic M, McDonald-McGinn DM, Bassett AS, Norvez A, Dhamankar R et al.
181 Clinical Experience with Single-Nucleotide Polymorphism-Based Noninvasive Prenatal
182 Screening for 22q11.2 Deletion Syndrome. *Ultrasound Obstet Gynecol.*
183 2015; epub ahead of print September 23, 2015

184

1856. Yatsenko SA, Peters DG, Saller DN, Chu T, Clemens M, Rajkovic A. Maternal cell-free
186 DNA-based screening for fetal microdeletion and the importance of careful diagnostic
187 follow-up. *Genet Med*. 2015;17(10):836-8.
188
1897. Sahoo T, Hovanes K, Strecker MN, Dzidic, N, Commander, S, Travis MK. Expanding
190 noninvasive prenatal testing to include microduplications and segmental aneuploidy:
191 cause for concern? *Genet Med*. 2016;18(3):275-6.
192
1938. Snyder MW, Simmons LE, Kitzman JO, Coe BP, Henson JM, Daza RM, Eichler EE,
194 Shendure J, Gammill HS. Copy-number variation and false-positive aneuploidy
195 screening results. *N Engl J Med* 2015;372(17):1639-45.
196

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Figure 1. A. Positive cfDNA testing resulting from microdeletion (top panel) and microduplication (middle panel) downstream of the typical region deleted in DiGeorge syndrome (bottom panel: unrelated case including the critical gene *TBX1*). **B.** Maternally inherited small 0.026 Mb Microdeletion in intron 1 of the *SNRPN* gene (zoom in, right), which is much smaller than a typical pathogenic deletion within the 15q11.2 region (zoom out, left; unrelated case), not associated with any imprinting defects, and seen at low frequency in the normal population. **C.** Southern blot for Prader-Willi and Angelman syndrome reveals normal methylation of both alleles. PW+ and AS+ = positive controls.