ORIGINAL ARTICLE

Karyotype versus Microarray Testing for Genetic Abnormalities after Stillbirth

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ABSTRACT

BACKGROUND

Genetic abnormalities have been associated with 6 to 13% of stillbirths, but the true prevalence may be higher. Unlike karyotype analysis, microarray analysis does not require live cells, and it detects small deletions and duplications called copy-number variants.

METHODS

The Stillbirth Collaborative Research Network conducted a population-based study of stillbirth in five geographic catchment areas. Standardized postmortem examinations and karyotype analyses were performed. A single-nucleotide polymorphism array was used to detect copy-number variants of at least 500 kb in placental or fetal tissue. Variants that were not identified in any of three databases of apparently unaffected persons were then classified into three groups: probably benign, clinical significance unknown, or pathogenic. We compared the results of karyotype and microarray analyses of samples obtained after delivery.

RESULTS

In our analysis of samples from 532 stillbirths, microarray analysis yielded results more often than did karyotype analysis (87.4% vs. 70.5%, P<0.001) and provided better detection of genetic abnormalities (aneuploidy or pathogenic copy-number variants, 8.3% vs. 5.8%; P=0.007). Microarray analysis also identified more genetic abnormalities among 443 antepartum stillbirths (8.8% vs. 6.5%, P=0.02) and 67 stillbirths with congenital anomalies (29.9% vs. 19.4%, P=0.008). As compared with karyotype analysis, microarray analysis provided a relative increase in the diagnosis of genetic abnormalities of 41.9% in all stillbirths, 34.5% in antepartum stillbirths, and 53.8% in stillbirths with anomalies.

CONCLUSIONS

Microarray analysis is more likely than karyotype analysis to provide a genetic diagnosis, primarily because of its success with nonviable tissue, and is especially valuable in analyses of stillbirths with congenital anomalies or in cases in which karyotype results cannot be obtained. (Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development.)

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Stillbirth, which is defined as fetal death at or after 20 weeks of gestation, occurs in 1 of every 160 births in the United States.[1] Despite extensive evaluation, 25 to 60% of stillbirths remain unexplained.[2]

Karyotypic abnormalities are detected in 6 to 13% of stillbirths with a successful karyotype analysis.[3,4] Some stillbirths may have chromosomal imbalances below the resolution of conventional cytogenetic analysis, which is typically 5 to 10 Mb. Single-nucleotide polymorphism (SNP) oligonucleotide microarray analysis detects almost all genomic imbalances recognized by karyotyping, as well as smaller deletions and duplications in the kilobase range, termed copy-number variants. Microarray analysis can be performed on DNA from nonviable, or even macerated, tissue. We tested the hypothesis that microarray analysis detects abnormalities in stillbirth samples more often than karyotype analysis.

Methods

Study Design

From March 2006 through September 2008, the Stillbirth Collaborative Research Network (SCRN) conducted a population-based study of stillbirth in a racially and ethnically diverse cohort in five geographic catchment areas.[5] Induced abortions of a live fetus were excluded.

The study was approved by the institutional review board at each clinical site, the 59 participating hospitals, and the data-coordinating center. An advisory board reviewed the progress and safety of the study. We obtained maternal written informed consent.[5] Full participation included a maternal interview, chart abstraction, standardized postmortem examination[6] and placental pathological examination,[7] karyotype analysis, and the collection and testing of maternal and fetal biospecimens. Women could decline any one of these components. Separate consent was obtained for future genetic testing. Biospecimens included cord blood, placental tissue, and fetal liver and muscle tissue. Karyotypes were analyzed in university-affiliated cytogenetic laboratories.

DNA was extracted with the use of established methods (Puregene, Qiagen Systems). DNA from placenta and cord blood was stored at −20°C for 2 to 5 years before microarray analysis, which was performed at a single laboratory (Columbia University Medical Center). DNA from stored frozen muscle and liver specimens was extracted immediately before microarray analysis.

Analysis of Copy-Number Variants

We analyzed samples using the Affymetrix GenomeWide Human SNP Array 6.0. Array data were analyzed with the use of Chromosome Analysis Suite, version 1.0.1, and the NetAffx annotation database, version 28 (Affymetrix), with data aligned to the Human Genome release 18 (hg18).

Array data were analyzed to identify aneuploidy, potential maternal–fetal contamination, and sex discordance. We included all copy-number variants of 500 kb or larger in our analysis. Categorization of variants was based on the American College of Medical Genetics standards and guidelines for interpretation and reporting,[8] with modifications. A copy-number variant was categorized as benign if its full length was listed in any of three databases of apparently unaffected persons: the Database of Genomic Variants,[9] the benign database[10] of the International Standards for Cytogenomic Arrays Consortium, or the Children's Hospital of Philadelphia database[11] converted from hg17 to hg18.[9-13] The remaining copy-number variants were classified as pathogenic, probably benign, or of unknown significance. Pathogenic variants had evidence of pathogenicity according to the published literature, contained a gene listed in the Online Mendelian Inheritance in Man (OMIM) database that is known to cause disease relevant to stillbirth or development,[14] or were included in the pathogenic database[15] of the International Standards for Cytogenomic Arrays Consortium. For variants that were classified as probably benign, the variant contained no genes at all or evidence in the literature suggested that the variants were benign. Variants that did not meet the criteria for classification as pathogenic, probably benign, or benign were classified as having unknown significance. We considered a variant to be confirmed on observing a variant of the same type and approximately the same size in an independent DNA sample from the same stillbirth.

Statistical Analysis

Individual stillbirths were the units of analysis. Statistical analysis was performed with the use of SAS software, version 9.2 (SAS Institute), or R software, version 2.13.1 (www.r-project.org). Fisher's exact test was used to compare detection rates

across subgroups. We used McNemar's test for paired data to evaluate differences between karyotype analysis and microarray analysis in the detection of variants. We combined two Wilson score intervals to estimate confidence intervals for detection rate ratios.[16]

RESULTS

STUDY POPULATION

The study series (953 women with a stillbirth) is described in Figure S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. The 290 women who did not enroll did not differ significantly from those who enrolled with respect to age, race or ethnic group, insurance or method of payment, or gestational age at delivery (Table S1 in the Supplementary Appendix).

COMPARISON OF KARYOTYPE AND MICROARRAY ANALYSES

We compared the performances of karyotype and microarray analyses using samples obtained after delivery from 532 stillbirths in which both karyotype and microarray testing were attempted. These samples included tissue from 492 singleton stillbirths, 19 twin gestations with 1 stillbirth, 4 twin gestations with 2 stillbirths (but only 1 assessed by karyotype and microarray testing), 8 twin gestations with 2 stillbirths and both assessed, and 1 triplet gestation with 1 stillbirth, for a total of 524 pregnancies. A comparison of the characteristics of the 524 pregnancies that were included in the analysis and the 139 pregnancies that were not included, among all women enrolled in the study, is provided in Table S1 in the Supplementary Appendix.

We karyotyped both fetal and placental tissue in 158 of 532 stillbirths (29.7%), fetal tissue only in 309 stillbirths (58.1%), placental tissue only in 64 stillbirths (12.0%), and tissue of unknown type in 1 stillbirth. If placental DNA was unavailable, we used cord blood, fetal muscle, or fetal liver for microarray analysis (106 cases, 19.9%).

Of the karyotype analyses we attempted, 375 of 532 (70.5%) yielded a result; 29.5% did not yield a result in any tissues tested. Of karyotypes yielding results, 31 of 375 (8.3%) were classified as abnormal (Fig. 1). Abnormalities included trisomy 21 in 9 stillbirths, trisomy 18 in 8 stillbirths, trisomy 13 in 2 stillbirths, monosomy X in 5 stillbirths, other sex-chromosome abnormalities in 2 stillbirths, 46,XY,dup(2)(q37) in 1 stillbirth, 46,XY,del(18)(q22) in 1 stillbirth, and 3 stillbirths with mosaic cell lines in the placenta (Table S3 in the Supplementary Appendix).

Microarray analysis that was performed on the same samples yielded a result in 465 of 532 stillbirths (87.4%), significantly more than were successfully karyotyped (P<0.001) (Fig. 1). In 396 of these 465 stillbirths (85.2%), we observed no variants larger than 500 kb, benign variants, or probably benign variants; 32 stillbirths (6.9%) were aneuploid, 12 (2.6%) harbored a pathogenic variant, and 25 (5.4%) harbored a variant of unknown significance. Among the aneuploid stillbirths, we observed trisomy 21 in 10 stillbirths, trisomy 18 in 10 stillbirths, trisomy 13 in 2 stillbirths, monosomy X in 8 stillbirths, and other sex-chromosome abnormalities in 3 stillbirths (with 1 stillbirth having both trisomy 21 and sex-chromosome aneuploidy). Table S2 in the Supplementary Appendix provides information that was used to classify each of the 41 stillbirths with variants meeting the criteria of 500 kb or more that were not found in the three databases of apparently unaffected persons. In samples from 37 stillbirths, there were 38 pathogenic variants or variants of unknown significance. These genomic events included 10 deletions (584 kb to 25.3 Mb) and 28 duplications (500 kb to 2.8 Mb). One stillbirth had a pathogenic deletion and a duplication that were consistent with an unbalanced translocation (Fig. S2 in the Supplementary Appendix).

On microarray analysis (but not on karyotype analysis), we observed three copy-number variants in three stillbirths (one in each stillbirth) at chromosome 22q11.2, a region disrupted in the DiGeorge (also called velocardiofacial) syndrome. Two of these variants were deletions typical of those causing the DiGeorge syndrome, and one was a duplication. One of the stillbirths carrying a deletion had multiple cardiopulmonary anomalies, abnormal facies, skeletal anomalies, a urogenital anomaly, and a hypoplastic thymus (Fig. S3 in the Supplementary Appendix).

Eight stillbirths with variants of unknown significance had overlapping duplications of the 19p13.3 region, which is known to contain five OMIM loci and many benign copy-number variants (Table 1). In these eight stillbirths, no congenital anomalies were noted on postmortem

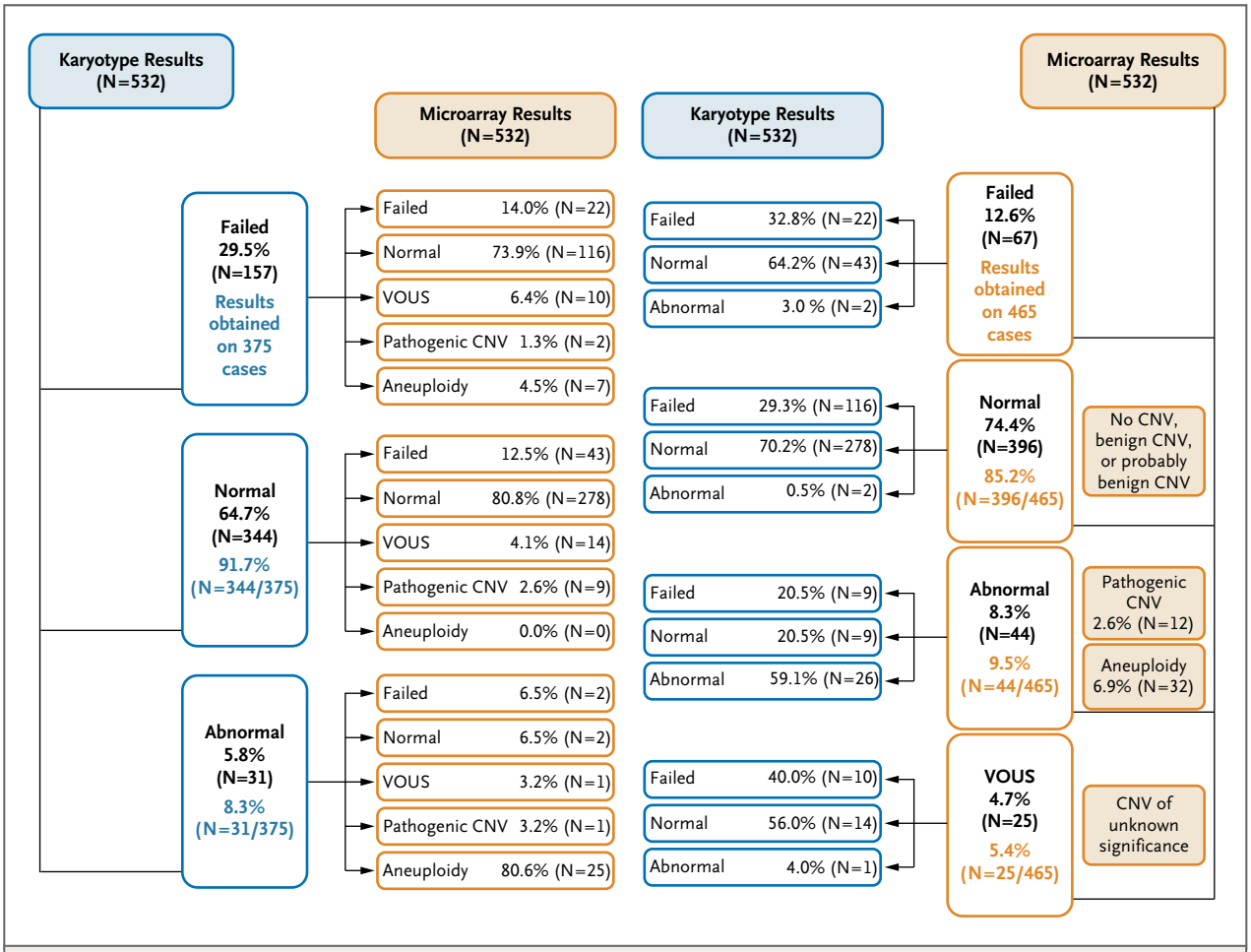

Figure 1. Performance of Karyotype and Microarray Analyses in Samples Obtained after Delivery in 532 Stillbirths.

On the left, karyotype results (blue) are categorized as failed, normal, or abnormal, as compared with findings from the same cases on microarray analysis (orange). On the right, the microarray results are categorized as failed, normal, abnormal, or variants of unknown significance (VOUS), with the corresponding karyotype results in the same cases. CNV denotes copy-number variant.

examination. However, these stillbirths had substantially abnormal placental findings, including chronic deciduitis and villous infarction (in three), chronic cytomegalovirus villitis (in one), villous infarction (in two), and abruption (in two).

Microarray analysis provided improved detection of genomic abnormalities (aneuploidy plus pathogenic variants), as compared with karyotype analysis (8.3% vs. 5.8%, P=0.007), a 41.9% increase (detection rate ratio, 1.42; 95% confidence interval [CI], 1.07 to 1.89) (Fig. 1). When we included variants of unknown significance in this comparison, we observed an even greater detection of abnormalities with the use of microarray analysis, as compared with karyotype analysis (13.0% vs. 5.8%, P<0.001), a 122.6% increase (detection rate ratio, 2.23; 95% CI, 1.63 to 3.04). Of the 157 stillbirths for which karyotype analysis failed to provide a definitive result, 79.6% yielded a definitive microarray result: 73.9% were normal or probably benign and 5.7% were abnormal (with aneuploidy or a pathogenic variant). Table 1 shows microarray results for stillbirths with aneuploidy, variants of unknown significance, or pathogenic variants in which the karyotype was normal or the test failed. Of the 44 stillbirths with aneuploidy or a pathogenic variant detected on microarray analysis, 41% had a normal karyotype or the test failed (Fig. 1).

We also assessed the ability of microarray analysis to detect abnormalities identified by karyotype analysis (Table S3 in the Supplemen-

tary Appendix). Of the 31 stillbirths with abnormal karyotypes, 29 had results when analyzed on microarray analysis. A total of 25 stillbirths had microarray results that were consistent with the results obtained on karyotyping. Two low-level mosaics with 10% or less abnormal cells on karyotyping were normal on microarray analysis (cases 1 and 2). Two stillbirths with abnormal karyotypes had a different abnormality on microarray analysis (cases 3 and 4), and another two with abnormal karyotypes (trisomy 21 and a duplication) did not yield microarray results because of DNA degradation (cases 5 and 6). One of the stillbirths with discordant results was a phenotypic male but was 45,X according to karyotype. On the basis of microarray results, much of the Y chromosome was missing — 46, XY del(Y)(q11.122 qter) — but the pseudoautosomal region down to and including the sex-determining region was present.

SUBGROUP ANALYSES

We performed subgroup analyses for antepartum stillbirths and stillbirths with structural anomalies (Table 2). Karyotype analysis yielded a result in 298 of 443 antepartum stillbirths (67.3%). Of these 298 stillbirths, 29 (9.7%) were abnormal. Microarray analysis yielded a result in 385 of the 443 antepartum stillbirths (86.9%). Of these 385 stillbirths, 31 (8.1%) were aneuploid, 8 (2.1%) had pathogenic variants, and 24 (6.2%) had variants of unknown significance. Microarray analysis detected more abnormalities in the antepartum subgroup than did karyotype analysis (8.8% vs. 6.5%, $P=0.02$), a 34.5% increase (detection rate ratio, 1.34; 95% CI, 1.01 to 1.78).

Of the 472 stillbirths with postmortem examinations, 67 (14.2%) had structural anomalies. Karyotype analysis yielded results in 45 of the 67 stillbirths (67.2%), of which 13 (28.9%) were abnormal. Microarray analysis was successful in 60 of the 67 stillbirths (89.6%), of which 17 (28.3%) had aneuploidy, 3 (5.0%) had pathogenic variants, and 3 (5.0%) had variants of unknown significance. Microarray analysis detected more abnormalities in this group (in 20 of 67 stillbirths, or 29.9%) than did karyotype analysis (in 13 of 67 stillbirths, or 19.4%; $P=0.008$), a 53.8% increase (detection rate ratio, 1.54; 95% CI, 1.03 to 2.26). Anomalous stillbirths were significantly more likely than nonanomalous stillbirths to have abnormal results on microarray analysis and karyotype analysis ($P<0.001$ for both comparisons).

DISCUSSION

Genomic techniques allow for the identification of chromosomal abnormalities at high resolution. The usefulness of these techniques has been shown in children with unexplained developmental delay or intellectual disability.[17-19] In addition, in this issue of the *Journal*, Wapner and colleagues report that microarray analysis improves the prenatal detection of clinically relevant genetic abnormalities.[20] Microarray analysis, as compared with conventional karyotype analysis, has also increased the detection of genetic abnormalities in pregnancy loss at a gestation of less than 20 weeks.[21-23] Two small studies have assessed the usefulness of microarray analysis for the evaluation of stillbirths. A study of 15 stillbirths with abnormalities in two organs and either normal results on karyotype analysis or failed karyotyping identified an instance of trisomy 21 and another instance of an unbalanced translocation.[24] The other study examined 29 unexplained stillbirths and identified copy-number variants in 24 cases, although only one variant was considered to be causative of stillbirth.[25]

The primary benefit of using microarray analysis over karyotype analysis is the greater likelihood of obtaining a result because of the ability to analyze nonviable tissue. We thus were able to obtain a result in 90 more cases (24.0% more) than we would have done using karyotype analysis alone. Of the stillbirths for which definitive results were obtained on either karyotype analysis or microarray analysis, the percentage of aneuploid stillbirths was 7%, which is consistent with the results of a large cytogenetic study.[4] Because of the improved yield of results obtained on microarray analysis, the actual number of aneuploid stillbirths detected on microarray analysis was greater than that detected on karyotype analysis. Moreover, as compared with karyotype analysis, microarray analysis was more sensitive to the presence of pathogenic variants. Some of the variants that were detected on microarray analysis may represent unbalanced translocations, which can be missed by karyotyping (Fig. S2 in the Supplementary Appendix). Detection of inherited translocations in stillbirths is important because of future reproductive risks for the carrier parent.

Our results indicate that microarray analysis identifies more abnormalities of unknown significance than does karyotype analysis. A major

challenge with microarray testing in stillbirths is determining the clinical implications, since the clinical relevance of many variants is unknown (hence the classification of variant of unknown significance). These unknown factors pose problems for genetic counseling. However, the challenge of counseling couples about unpredictable outcomes is not new.[26]

Table 1. Microarray Results for Stillbirths with Aneuploidy, Variants of Unknown Significance, or Pathogenic Variants in Which Karyotyping Failed or Was Normal.

Microarray Result	Case No.	Observed Change on Microarray*	Size
Failed karyotyping			
Aneuploidy			
Trisomy 18	1	18p11.32q23(1,543–76,116,030)×3	Chromosome
Trisomy 18	2	18p11.32q23(1,542–76,116,029)×3	Chromosome
Trisomy 21	3	21q11.q22.3(13,286,390–46,921,374)×3	Chromosome
Trisomy 21 and XXY	4	21p11.1q22.3(9,758,730–46,921,374)×3, Xp22.33q28(2,401,346–154,843,252)×2, 19q13.12(41,553,395–42,220,583)×1	Chromosome Chromosome 667 kb
Monosomy X	5	Xp22.33q28(108,464–154,849,094)×1	Chromosome
Monosomy X	6	Xp22.33q28(2,401,346–154,843,252)×1	Chromosome
Monosomy X	7	Xp22.33q28(108,464–154,849,094)×1	Chromosome
Pathogenic variant			
Deletion	8	1q21.1(143,845,772–146,838,707)×1	4.0 Mb
Deletion	9	22q11.21q11.23(17,256,416–22,140,054)×1	4.9 Mb
Variant of unknown significance			
Duplication	10	19p13.3(363,729–965,377)×3	602 kb
Duplication	11	19p13.3(441,414–965,377)×3	524 kb
Duplication	12	19p13.3(339,937–1,270,320)×3	931 kb
Duplication	13	19p13.3(441,414–1,261,136)×3	820 kb
Duplication	14	19p13.3(388,808 - 1,270,320)×3	882 kb
Duplication	15	19q13.12(57,198,183–57,722,222)×3	524 kb
Duplication	16	21q21.3(27,162,033–28,340,061)×3	1.2 Mb
Duplication	17	21q22.13(36,685,848–37,185,921)×3	500 kb
Duplication	18	Xq27.1(138,676,821–139,311,901)×3	635 kb
Duplication	19	5p15.2(10,908,334 - 11,459,739)×3	551 kb
Normal karyotyping			
Pathogenic variant			
Deletion	20	22q11.21(17,256,416–19,795,836)×1	2.5 Mb
Deletion	21	Xp22.31(6,903,881–7,774,557)×0	869 kb
Deletion	22	7q11.23(73,247,250–73,753,322)×1	506 kb
Unbalanced translocation: deletion and duplication	23	4q32.3q35.2(165,903,367–191,254,120)×1 17p13.3(514–2,811,647)×3	25.3 Mb 2.8 Mb
Duplication	24	22q11.21(17,128,427–18,647,705)×3	1.5 Mb
Duplication	25	18p11.21(13,574,399–14,760,946)×3	1.2 Mb
Duplication	26	16p13.11p12.3(15,224,214–18,286,344)×3	3.0 Mb
Duplication	27	16p13.11p12.3(15,389,423–18,464,701)×3	3.1 Mb
Duplication	28	17q21.31(31,890,369–33,552,890)×3	1.7 Mb

Table 1. (Continued.)

Microarray Result	Case No.	Observed Change on Microarray*	Size
Variant of unknown significance			
Deletion	29	Yq11.221(18,148,539–18,999,761)×0	850 kb
Deletion	30	1p35.3(28,444,904–28,952,754)×1	508 kb
Deletion	31	16p11.2(29,333,900 – 30,038,055)×1	704 kb
Duplication	32	19p13.3(373,237–1,261,136)×3	887 kb
Duplication	33	19p13.3(441,414–965,377)×3	524 kb
Duplication	34	19p13.3(392,194–972,725)×3	580 kb
Duplication	35	15q12q13.1(25,366,691–26,087,702)×3	721 kb
Duplication	36	19p12(23,613,361–24,388,578)×3	775 kb
Duplication	37	6p25.1p24.3(6,844,061–7,457,081)×3	613 kb
Duplication	38	10q23.31(90,658,193–91,207,964)×3	549 kb
Duplication	39	19q13.12(41,961,955–42,487,630)×3	526 kb
Duplication	40	8q24.23(137,042,624–139,247,552)×3	2.2 Mb
Duplication	41	3p21.31(45,806,446–46,455,963)×3	649 kb
Duplication	42	11p13(33,005,102–33,592,112)×3	587 kb

* For each observed change on microarray analysis, the times sign specifies the number of copies of the genomic region indicated in the parentheses.

We observed a recurrent variant of unknown significance in a telomeric region of chromosome 19p13.3 (ranging in size from 632 to 930 kb) in eight stillbirths. This region is known to contain multiple benign variants as well as five loci in the OMIM database that have been associated with disease but not with stillbirth or developmental disorders. The variant of unknown significance that we observed in this region may be benign or may confer a risk of stillbirth. Similarly, we observed two deletions categorized as variants of unknown significance in two stillbirths (one per stillbirth) with major anomalies on postmortem examination. Whether these variants are pathogenic remains to be determined. Girirajan and colleagues[18] have recently reported that children who carry two large variants of unknown clinical significance are eight times as likely to have developmental delay as are controls from the general population.

We detected genomic imbalances in the 22q11.2 region in three cases on microarray analysis but not on karyotype analysis. Microdeletions in the 22q11.2 region are associated with the DiGeorge syndrome, and microduplications give rise to the 22q11.2 microduplication syndrome. The phenotype of both syndromes is variable, with shared clinical anomalies that include heart defects, urogenital abnormalities, and velopharyngeal insufficiency.[27-29] The incidence of the DiGeorge syndrome is estimated to be 1 case in 4000 births.[30] The 22q11.2 microduplication syndrome appears to be less prevalent. We detected three variants of 500 kb or more in the typical 22q11.2 region and a typical DiGeorge deletion (2.8 Mb) in a stillbirth that was not included in the primary analysis because karyotyping was not attempted. The three stillbirths with a pathogenic variant in the 22q11.2 region represent an increase in the prevalence of this abnormality by a factor of 22.6 ($P=3.5\times10^{-4}$), as compared with the frequency in the general population (1 in 4000 births). If we count all four variants in the 22q11.2 region and the 41 stillbirths that underwent microarray analysis but not karyotype analysis, the prevalence is increased by a factor of 27.3 ($P=1.5\times10^{-5}$). These results suggest that genomic imbalances in this region may be associated with stillbirth. Identifying the 22q11.2 variant in the stillbirth is important because the DiGeorge syndrome is a haploinsufficiency disorder in which parental studies are recommended.[30] The risk of recurrence in subsequent pregnancies increases from less than 0.1% for genotypically normal parents to 50% if a parent has the deletion.[30] In some cases, affected offspring may serve as the index case

Table 2. Comparison of Karyotype Analysis and Microarray Analysis in the Diagnosis of Genetic Abnormalities in 532 Stillbirths, According to Subgroup.*

Stillbirth Subgroup and Karyotype	Karyotype Analysis	Microarray Analysis					
		Failed	Normal or Benign	Probably Benign	Variant of Unknown Significance	Pathogenic Variant	Aneuploidy
		number of stillbirths (percent)					
Antepartum (N=443)							
Failed	145 (32.7)	22 (15.2)	104 (71.7)	0	10 (6.9)	2 (1.4)	7 (4.8)
Normal	269 (60.7)	35 (13.0)	214 (79.6)	2 (0.7)	13 (4.8)	5 (1.9)	0
Abnormal	29 (6.5)	1 (3.4)	2 (6.9)	0	1 (3.4)	1 (3.4)	24 (82.8)
Intrapartum (N=89)							
Failed	12 (13.5)	0	12 (100.0)	0	0	0	0
Normal	75 (84.3)	8 (10.7)	60 (80.0)	2 (2.7)	1 (1.3)	4 (5.3)	0
Abnormal	2 (2.2)	1 (50.0)	0	0	0	0	1 (50.0)
Anomalous (N=67)							
Failed	22 (32.8)	3 (13.6)	12 (54.5)	0	1 (4.5)	2 (9.1)	4 (18.2)
Normal	32 (47.8)	4 (12.5)	25 (78.1)	0	2 (6.2)	1 (3.1)	0
Abnormal	13 (19.4)	0	0	0	0	0	13 (100.0)
Nonanomalous (N=405)							
Failed	122 (30.1)	16 (13.1)	95 (77.9)	0	9 (7.4)	0	2 (1.6)
Normal	271 (66.9)	32 (11.8)	218 (80.4)	3 (1.1)	11 (4.1)	7 (2.6)	0
Abnormal	12 (3.0)	1 (8.3)	2 (16.7)	0	1 (8.3)	1 (8.3)	7 (58.3)

* Percentages for the karyotype analysis were calculated with the number of cases in the stillbirth subgroup (antepartum, intrapartum, anomalous, or nonanomalous) as the denominator. Percentages for the microarray analysis were calculated with the number of cases in the karyotype subgroup (failed, normal, or abnormal) as the denominator. A total of 472 stillbirths underwent complete postmortem examination and were classified as either anomalous or nonanomalous. The 60 stillbirths that did not undergo complete postmortem examination were not categorized.

leading to diagnosis in a parent with the 22q11.2 deletion who has a mild clinical phenotype.[30]

There are limitations of microarray-based technology. Truly balanced rearrangements cannot be detected on microarray analysis; however, they are unlikely to cause stillbirth. In addition, low-level mosaicism detected by means of karyotyping went undetected on microarray analysis in our study, although the clinical implications of this low-level mosaicism are unclear.

Concurrent karyotype and microarray testing on the same tissues would have been ideal. However, this was not possible because the study design necessitated karyotyping in real time. Another limitation was our inability to distinguish de novo from inherited variants owing to the unavailability of parental DNA. De novo variants in clinically significant gene regions are more likely to be causative. However, inherited pathogenic variants should not be discounted as a cause of stillbirth because of their variable expressivity and incomplete penetrance.[8,26] Our ability to assess confined placental mosaicism, in which the fetus is genetically normal but the placenta is genetically abnormal, was limited.

A major strength of our study was the large, geographically and racially diverse, population-based series of women with a stillbirth.[5] All the stillbirths that were included in the analysis were carefully phenotyped because the women provided consent for a complete evaluation, including fetal postmortem examination, placental pathological analysis, karyotyping, and maternal–fetal testing.[31] Microarray analysis was performed at an institution that was not part of the study, and the researchers who performed the analysis were unaware of the karyotyping results and the clinical history.

In conclusion, we found that microarray analysis could be useful in cases of stillbirth when

karyotyping results cannot be obtained or in cases in which there are congenital anomalies. Microarray analysis is more expensive than standard karyotype analysis, although its cost is expected to decrease[26] and may be offset by the higher yield of genomic abnormalities.

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