Fetal Diagnosis and Therapy

Identifying Robertsonian Translocation Carriers by Microarray-Based DNA Analysis

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Abstract

Objective: To develop a noninvasive prenatal testing improvement that allows identification of Robertsonian translocation carriers. *Methods:* Blood samples from 191 subjects, including 7 pregnant and 9 non-pregnant Robertsonian translocation carriers, were analyzed for fetal trisomy and Robertsonian translocation status. Digital Analysis of Selected Regions (DANSR™) assays targeting sequences common to the p arms of 5 acrocentric chromosomes were developed and added to existing DANSR assays. DANSR products were hybridized onto a custom DNA microarray for DNA analysis. The Fetal-Fraction Optimized Risk of Trisomy Evaluation (FORTE™) algorithm measures the fraction of fetal DNA and accounts for both the fetal and maternal fractions in the cell-free DNA sample to determine Robertsonian risk. The expectation in a Robertsonian translocation carrier is that DANSR assays on acrocentric p arms should have a concentration 20% less than that of controls. *Results:* The FORTE algorithm correctly classified the fetal trisomy status and maternal Robertsonian translocation status in all 191 samples. Sixteen samples had a Robertsonian risk score above 99%, while 175 samples had a Robertsonian risk score below 0.01%. *Conclusions:* Robertsonian translocations are the most common chromosomal translocations and can have significant reproductive consequences. A maternal screen for Robertsonian translocation carriers would provide women valuable information regarding the risk of fetal trisomy.

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Introduction

A Robertsonian translocation is a whole arm exchange between 2 acrocentric chromosomes (human chromosomes 13, 14, 15, 21, and 22), resulting in a fused chromosome with 2 long arms. Individuals with balanced Robertsonian translocations have a loss of 2 of the 10 p arms of the acrocentric chromosomes. The Robertsonian translocation is the most common chromosomal translocation, occurring at a frequency of ~1 in 1,000 [1]. Carri-

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ers of a balanced Robertsonian translocation are phenotypically normal, but have a higher risk of infertility, miscarriages, and aneuploid offspring [2, 3].

The most common Robertsonian translocation is between chromosomes 13 and 14, accounting for ~75% of Robertsonian cases [4]. The second most common Robertsonian translocation is between chromosomes 14 and 21, accounting for ~10% of all Robertsonian translocations. These maternal carriers have an estimated risk of fetal trisomy 21 of 15% in any given pregnancy [4].

The array platform of the Harmony test [5] allows for the easy expansion of the Harmony test. Because of the amount of available space on the custom DNA microarray, additional assays can be easily incorporated into the Harmony™ Prenatal test. As Robertsonian translocations are the most common chromosomal translocation and can have significant reproductive consequences, a screen for Robertsonian translocations would provide women valuable information regarding their risk of trisomy. By expanding the test to include Robertsonian translocation status, pregnant women can be provided the correct fetal trisomy risk information for both their current and subsequent pregnancies.

The purpose of this study was to demonstrate the development of a Harmony test improvement that can detect Robertsonian translocations carriers.

Materials and Methods

Subjects

Archived plasma from a total of 191 patients was used in this study. They consisted of 16 Robertsonian translocation carriers and 175 pregnant women 18 years and older. Seven of the Robertsonian translocation carriers were pregnant at the time of blood draw. The trisomy status of the 175 pregnant women was determined by the Harmony Prenatal Test from Ariosa Diagnostics Inc. (San Jose, Calif., USA). For 15 of the Robertsonian translocation carriers, karyotype information had been previously obtained. The blood from one Robertsonian translocation patient was provided as part of the Robertsonian translocation study; however, we are unable to locate the karyotype confirmation for this patient.

Subjects were prospectively enrolled into the Robertsonian study after informed consent with Institutional Review Board approval. Blood was collected from each subject into a cell-free™ BCT tube (Streck, Omaha, Nebr., USA) and sent directly to the lab for processing.

Sample Preparation

cfDNA was purified from each plasma sample as described previously [6]. Samples were analyzed for fetal fraction, trisomy status and Robertsonian status using the array hybridization system as previously described [5].

In addition to the previously described 2592 DANSR assays targeting chromosomes 13, 18, and 21, 63 DANSR™ assays targeting sequences that are common to the p arms of the 5 acrocentric chromosomes were developed to assess Robertsonian translocation status. To estimate fetal fraction, DANSR products from 576 polymorphic assays were analyzed.

Custom DNA microarrays from Affymetrix Inc. (Santa Clara, Calif., USA) with >100,000, 6-µm features were manufactured to specifically quantify products of the DANSR assays. Microarrays were imaged on an Affymetrix GeneTitan® Multi-Channel Instrument. Each patient sample was assayed on a single custom microarray.

Data Analysis

A previously published algorithm, Fetal-Fraction Optimized Risk of Trisomy Evaluation (FORTE™), was used to calculate risk scores [6]. The FORTE algorithm accounts for both the fetal and maternal fractions in the cell-free DNA sample to determine Robertsonian risk. The expectation in a Robertsonian sample is that, in the maternal fraction of the cfDNA, the DANSR assays on the 5 acrocentric p arms should have a concentration that is 20% less than that of the controls.

Results

A total of 191 patients were included in this study. Samples had the following Robertsonian translocation karyotypes: 10 samples were der(13;14)(q10;q10), 4 were pregnant, 2 pregnant samples were der(14;21)(q10;q10), 1 der(13;15)(q10;q10), 1 der(14;15)(q10;q10), 1 der (21;22)(q10;q10), and 1 pregnant Robertsonian translocation with an unknown karyotype. Demographic characteristics for samples in this study are described in table 1.

Harmony results correctly classified all 191 patients as non-trisomy. The FORTE™ algorithm correctly classified the Robertsonian translocation status in all 191 samples regardless of pregnancy status. All of the samples with translocation karyotypes had a Robertsonian risk score of greater than 99%, while the 175 non-Robertsonian samples had a Robertsonian risk score of less than 0.01% (fig. 1).

Table 1. Demographic characteristics of the analysis population

Karyotype	Number of subjects	Number pregnant
45,XY,der(13;14)(q10;q10)	8	4
45,XX,der(13;14)(q10;q10)	2	–
45,XX,der(14;21)(q10;q10)	2	2
45,XY,der(13;15)(q10;q10)	1	–
45,XX,der(14;15)(q10;q10)	1	–
45,XY,der(21;22)(q10;q10)	1	–
Robertsonian translocation, XX	1	1
Euploid	175	175

Fig. 1. Microarray analysis correctly classified the Robertsonian carrier status for all 191 samples. Fetal fraction is plotted on the x-axis and the Robertsonian $\log_{10}$ odds are plotted on the y-axis. There is a separation of 6 LODs between the control samples and the Robertsonian carrier samples. Seven samples from the non-pregnant balanced Robertsonian carrier samples appear in the upper left with zero fetal fraction.
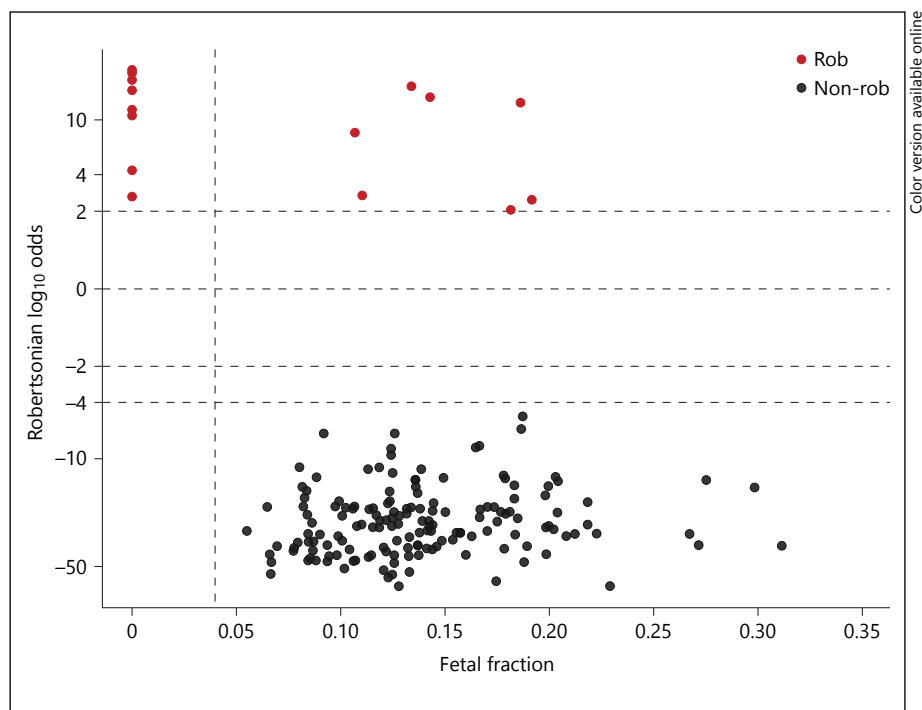
Discussion

We have shown the development of a sensitive blood-based test to identify Robertsonian translocation carriers using cfDNA. An array platform allows for the easy expansion of the Harmony test because of the very large number of features available on the array. A total of 63 DANSR™ assays targeting sequences that are common to the p arms of the 5 acrocentric chromosomes were developed and added to the existing Harmony assays. All 16 of the Robertsonian translocation carriers were successfully identified.

Humans have 5 pairs of acrocentric chromosomes – chromosomes 13, 14, 15, 21, and 22. A balanced Robertsonian translocation occurs between 2 pairs of acrocentric chromosomes. Balanced Robertsonian translocation carriers have only 45 chromosomes and a loss of 2 of the 10 p arms of the acrocentric chromosomes. Because the short arms of the acrocentric chromosome contain mostly satellite DNA sequences and ribosomal RNA genes and share extensive sequence homology, a loss of 2 out of 10 copies of the p arms has no phenotypic consequence [7].

A Robertsonian translocation is found in ~1 out of 1,000 individuals, making it the most common chromosomal translocation [1]. Since carriers of a balanced Robertsonian translocation are phenotypically normal, most Robertsonian carriers are not aware of their Robertsonian translocation status. They have, however, higher risk for infertility, repeated miscarriages, and offspring with unbalanced karyotypes [8].

The most common Robertsonian translocation is between chromosomes 13 and 14, making up ~75% of all Robertsonian translocations [4]. Individuals carrying the der(13;14) translocation confer a 0.4% estimated risk of Patau syndrome to their fetus. Female carriers of der(14;21), the second most common type of Robertsonian translocation, have a 15% estimated risk of fetal trisomy 21 in any given pregnancy. Other Robertsonian translocations involving chromosome 21 also have a similar estimated risk of fetal trisomy 21 of 15% in female carriers [4].

To identify balanced Robertsonian translocation carriers, assays targeting the homologous regions of the p arms of the acrocentric chromosomes were designed in order to evaluate the number of maternal p arms that are present. Since a balanced Robertsonian translocation results in a loss of 2 out of 10 copies of the p arm of the acrocentric chromosomes, balanced Robertsonian translocation carriers have a 20% loss of the total signal of the Robertsonian assays in the maternal fraction of the cfDNA. Nine of the 16 Robertsonian translocation carriers in the study were not pregnant. Since the FORTE algorithm accounts the fetal and maternal fractions in the cell-free DNA sample,

the presence of a Robertsonian translocation carrier was correctly identified, regardless of the pregnancy status.

A screen for women with a balanced Robertsonian translocation has been successfully developed. This was done by adding just a few DANSR assays to the existing Harmony test. Pregnant women with this translocation can now be identified and have an appropriate prior risk used to evaluate the appropriate fetal trisomy risk. In addition, these women can be aware of the increased risk in subsequent pregnancies.

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