DIAGNOSTIC TESTING UPTAKE RATES IN FRAGILE X PREMUTATION CARRIERS AND COUPLES AT RISK FOR AN AUTOSOMAL RECESSIVE CONDITION IN THEIR OFFSPRING

By

KENDALL SNYDER

A thesis submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Microbiology and Molecular Genetics

Written under the direction of

Elena Ashkinadze

And approved by

_____

_____

_____

_____

_____

New Brunswick, New Jersey

May 2020

ABSTRACT OF THE THESIS

Diagnostic Testing Update Rates in Fragile X Premutation Carriers and Couples At Risk for an Autosomal Recessive Condition in their Offspring

By KENDALL SNYDER

Thesis Director:

Elena Ashkinadze

Expanded Carrier Screening (ECS) is a blood test designed to identify carrier status for hundreds of recessive some X-linked conditions. This allows identification of carrier couples and female X-linked recessive carriers at risk to have an affected child. Very little research has been done to assess the reproductive decision-making process in at-risk pregnancies. Our goal was to investigate whether patients, whose fetus is at risk, undergo diagnostic, invasive testing and when affected, if they opt for termination of pregnancy. This study was a retrospective chart review. A total of 116 at-risk pregnancies were reviewed, of which 73/116 (63%) were at risk for an autosomal recessive condition. Of these patients 41/73 (56%) chose to undergo invasive testing. Those that chose diagnostic testing, 7/41 (17%) had an affected fetus and the majority, 6/7 (86%), opted for termination of pregnancy. A total of 43 female Fragile X premutation carriers, of which 18/43 (42%) opted for invasive diagnostic testing. Of these, three were confirmed to have a fetus with >200 CGG repeats and all three opted for termination of pregnancy. Increasing CGG repeats in premutation carriers correlated with higher invasive testing uptake rates. Our study demonstrates that more than half of patients at-risk for a fetus

with a single gene disorder will undergo diagnostic fetal testing and when affected, the majority chose termination of pregnancy.

Acknowledgements

Thank you, Elena Ashkinadze, for all of your guidance through this process.

Thank you to the rest of my thesis committee, Shama Khan and Laurie Simone.

Thank you, Vania Lyons, for your help during data collection and tracking down patient records.

Thank you Jessica Rispoli Joines, Christy Seymour and Gary Heiman for always having my back.

Table of Contents

Abstract…… ii

Acknowledgements…….. iv

Introduction……. 1

Material and Methods……..4

Results……..7

Discussion……..12

Study Limitations and Research Recommendations……..15

References……..16

Introduction

Carrier screening is a genetic tool used to identify carrier couples of the same autosomal recessive condition or women who are carriers for an X-linked condition such as Fragile X Syndrome. By knowing their status, carrier couples and Fragile X premutation carriers have reproductive options to prevent or reduce the risk of having an affected child. These options include preimplantation genetic diagnosis, adoption, gamete donation, and prenatal testing with the option of termination of an affected pregnancy.

Traditionally, ethnicity-based carrier screening only focused on certain populations and included a limited number of conditions. The American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics and Genomics (ACMG), recommend that screening for Cystic Fibrosis (CF) and Spinal Muscular Atrophy (SMA) be offered to all women (pregnant or preconception), regardless of ethnicity.[1] Other practice guidelines outline available screening for Ashkenazi Jewish individuals,[2] hemoglobinopathy and thalassemia screening,[3] Fragile X screening[4] and implementation of expanded carrier screening.[5] Given the increased prevalence of conditions such as Tay-Sachs Disease (TSD) in the Ashkenazi Jewish population, screening guidelines gave rise to community wide screening programs whose main goal was to decrease the incidence of these ethnically relevant, autosomal recessive conditions in certain population.[6,7] CF is another autosomal recessive condition that has been widely screened for, originally offered only to Caucasian individuals or those with a family history of the disease, but is now standard of care to be offered to all pregnant or planning to be pregnant patients.[8]

While the use of ethnic-based screening has proven beneficial in the past, it has become increasingly hard to define ancestry and limit individuals to specific ethnic panels.[8] Newly developed pan-ethnic, or expanded carrier screening (ECS) panels, account for mixed or unknown ancestry and adoption history, and provide individuals with the opportunity to screen for hundreds of mutations associated with recessive and X-linked conditions. As ECS has become more widespread and recognized by ACOG, identified carrier couples and female carriers of X-linked conditions have been able to use this information for reproductive decision-making, specifically regarding invasive testing. An increasing number of single gene disorders have been identified prenatally through invasive testing and ECS results have become a growing indication for prenatal diagnosis.[9]

Numerous studies have assessed the reproductive outcomes of CF carrier couples,[10,11] and even TSD couples[12], but there is limited data regarding decision-making of carrier couples identified through these larger pan-ethnic screening panels. One recent survey study by Ghiossi et al. (2018), analyzed the outcomes of at-risk carrier couples identified by ECS. A total of 537 carrier couples were identified and sent a survey asking how they will proceed with reproductive decision-making. Only 64/537 (12%) completed the survey and met inclusion criteria. The data demonstrated that at-risk carrier couples of a severe or profound autosomal recessive condition were significantly more likely to pursue diagnostic testing, as compared to those who were at risk for a moderate condition. However, there were a range of study limitations including response bias, participant memory and level of medical literacy, disproportionate demographics and that planned behaviors may not correlate to future actions.[13]

The purpose of our study was to evaluate whether carrier couples of recessive conditions or Fragile X premutation carriers opted for diagnostic testing. For those that opted for diagnostic testing, we assessed how many decided to pursue termination of pregnancy, when the fetus was affected.

Given the increasing utilization of ECS, we hypothesize that the majority of couples, who have an at-risk pregnancy for an autosomal recessive or Fragile X Syndrome, will pursue invasive diagnostic testing to determine the status of the fetus. We also evaluated if testing uptake rates were influenced by the severity of the disease for autosomal recessive conditions. In premutation Fragile X carriers, we wanted to assess whether increasing CGG repeats and thus higher likelihood of a full mutation fetus would correlate with increasing invasive testing uptake rates.

Materials and Methods

We conducted a retrospective chart review from January 2010 to August 2019 at the Division of Maternal Fetal Medicine at Rutgers-Robert Wood Johnson Medical School in New Brunswick, New Jersey. Female patients who were seen for genetic counseling because she and her partner were both carriers for the same autosomal recessive condition and females who were Fragile X premutation carriers, were included in the study. Females less than 18 years old were excluded. Carrier couples who had undergone in-vitro fertilization with preimplantation genetic diagnosis for a single gene disorder (IVF with PGT-M) and were using prenatal diagnosis as confirmation, were excluded. We also excluded female patients who were carriers of Glucose-6-phosphate-dehydrogenase deficiency (G6PD) and intermediate Fragile X carriers, as the potential phenotype in their offspring is typically not serious enough to warrant prenatal diagnosis. Carrier couples who were only each a silent carrier for alpha thalassemia, were also excluded because their offspring were not at risk for a disease phenotype.

The number of patients who met eligibility criteria were 116 pregnancies. Of these, 43 were Fragile X premutation carriers and 73 were carrier couples for an autosomal recessive condition. All extracted data was de-identified and the study was approved by the Rutgers University New Brunswick Health Sciences Institutional Review Board (IRB).

Patients were selected from the genetic counseling patient logs, which track all patients seen and their reason for referral. Once the patients were selected, their individual records were reviewed. Data collected included their carrier screen results, type of inheritance pattern (autosomal recessive versus X-Linked), ethnicity and age of

patient, level of education for patient, gestational age at the time of genetic counseling appointment, whether they opted for and underwent invasive testing such as chorionic villus sampling (CVS) or amniocentesis, and whether they chose to terminate an affected pregnancy. All the information was collected and tabulated in Microsoft Excel.

We further assessed whether the decision to pursue diagnostic testing was influenced by severity of the disease with autosomal recessive conditions. Specifically, we categorized autosomal recessive conditions as "moderate" or "severe." Severe conditions were defined as a condition that shortened lifespan or caused cognitive impairment. This category included SMA, CF, Familial Dysautonomia, all hemoglobinopathies, Congenital Disorder of Glycosylation Type 1a (PMM2 Type), and Smith Lemli Opitz Syndrome (SLOS). Moderate conditions were defined as a condition with normal lifespan or no impact on cognition, if treated. This category included Phenylketonuria (PKU), Factor XI Deficiency, Familial Mediterranean Fever (FMF), Non-Syndromic Hearing Loss GJB2 Related, Medium-Chain Acyl CoA Dehydrogenase Deficiency (MCAD), Non-Classical CAH, Usher Syndrome Type 1b, and Gaucher Disease Type 1. For autosomal recessive conditions, the moderate and severe disease categories were compared using Fisher's Exact Test with statistical significance set at $P < 0.05$.

We further assessed whether an increasing number of CGG repeats in premutation Fragile X carriers correlated with diagnostic testing uptake rates. Fragile X premutation carriers were divided into three categories. These categories were based on the guidelines established by ACOG: low risk (55-69 CGG repeats with a 4-5% risk of expansion), medium risk (70-89 CGG repeats with a 31-58% risk of expansion), and high risk (>90

CGG repeats with a >80% of expansion).[1] The three groups of Fragile X premutation carriers were compared using the Cochran-Armitage Chi-Square Test for trend to analyze this data, with a statistical significance set at $P < 0.05$.

Results

A total of 116 patients were evaluated. Forty-three out of 116 were Fragile X premutation carriers, and 73/116 were autosomal recessive carrier couples.

The demographic data for all 116 of our female patients is summarized in Table 1. The majority were between the ages of 30 and 34 years old (46/116 = 40%), of European decent (35/116 = 30%), and of those that provided highest level of education level, had obtained a graduate or professional degree (24/49 = 49%). There was no statistical significance on how patients made reproductive decisions, when sorted by age, level of education, or ethnicity.

Table 1 – Patient demographic information.

Age (of female patient)	**Reported (n)**
18-24	7
25-29	34
30-34	46
35-39	23
40-44	6
Ethnicity (of female patient)	**Reported (n)**
African American	23
Ashkenazi Jewish	19
Asian	8
European	34
Hispanic	15
Middle Eastern	10
Unspecified	7
Highest Level of Education (of female patient)	**Reported (n)**
High School/Vocational	8
College	17
Graduate/Professional	24
Not Provided	67

Couples At Risk for a Fetal Autosomal Recessive Condition

For couples specifically at risk of having a child with an autosomal recessive condition (n = 73), CF (12/73 = 16%) and Hemoglobinopathies/Thalassemia (32/73 = 44%) were the two most common groups. Table 2 summarizes which autosomal recessive conditions couples were at risk for, how we classified each disease, and how many couples opted or declined invasive testing. Of the 73 at-risk couples, 41 (56%) chose to undergo invasive testing; 17/41 (42%) had a CVS, and 24/41 (59%) had an amniocentesis.

Table 2 – Autosomal recessive disease classifications and invasive testing outcomes.

Disease	N	Disease Classification	Opted for Invasive Testing	Declined Invasive Testing
Factor XI Deficiency	1	Moderate	0/1	1/1
Familial Mediterranean Fever (FMF)	1	Moderate	0/1	1/1
Gaucher Disease Type 1	4	Moderate	2/4	2/4
Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)	1	Moderate	1/1	0/1
Non-Classical Congenital Adrenal Hyperplasia (CAH)	2	Moderate	0/2	2/2
Non-Syndromic Hearing Loss – GJB2 Associated	6	Moderate	4/6	2/6
Phenylketonuria (PKU)	2	Moderate	2/2	0/2
Usher Syndrome Type 1b	1	Moderate	1/1	0/1
Hemoglobinopathies/Thalassemia*	32	Severe	14/32	18/32
Cystic Fibrosis	12	Severe	8/12	4/12
Congenital Disorder of Glycosylation Type 1a (PMM2)	1	Severe	1/1	0/1
Familial Dysautonomia	3	Severe	2/3	1/3
Smith Lemli Opitz Syndrome (SLOS)	1	Severe	1/1	0/1
Spinal Muscular Atrophy (SMA)	6	Severe	5/6	1/6
Totals	**73**		**N = 41/73 (56%)**	**N = 32/73 (44%)**

*Hemoglobinopathies/Thalassemia include; Sickle Cell Disease (SS or SC), Hemoglobin C Disease, Beta Thalassemia, Sickle-Beta Thalassemia, Beta Thalassemia-Hemoglobin E Disease, and Hemoglobin H Disease.

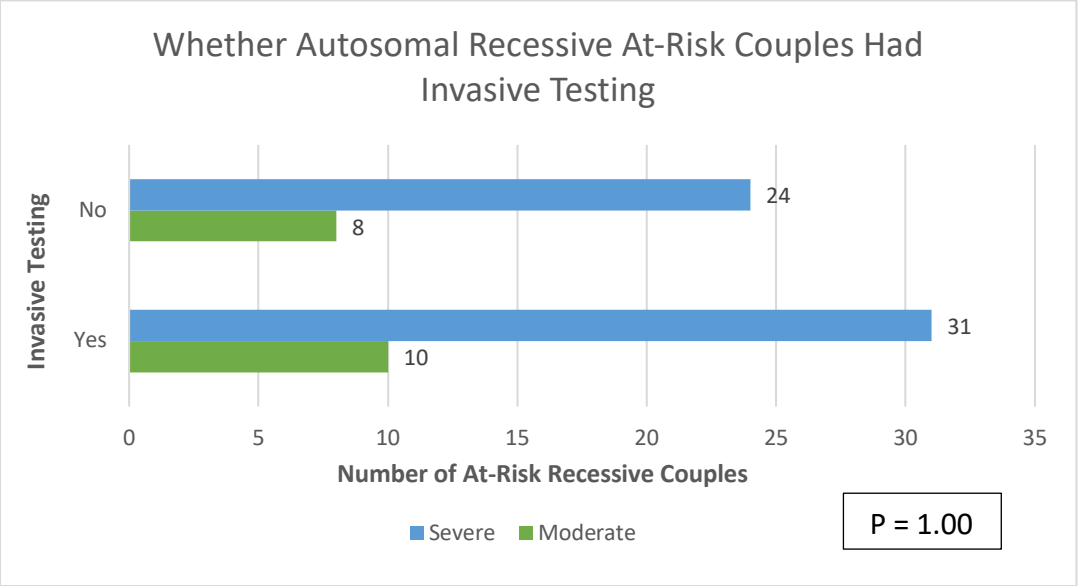
Out of these 41 couples who had invasive testing, 7/41 (17%) pregnancies were found to be affected. Table 3 displays the results for these seven pregnancies.

Table 3 – Rate of affected pregnancies and rate of terminations.

Fetal Risk for Disease	Disease Classification	Affected Pregnancies (n = 7)	Number of Patients Who Pursued Termination
Familial Dysautonomia	**Severe**	**1**/7 (14%)	100% (1/1)
Beta Thalassemia	**Severe**	**1**/7 (14%)	100% (1/1)
Cystic Fibrosis	**Severe**	**3**/7 (43%)	67% (2/3)
Spinal Muscular Atrophy	**Severe**	**1**/7 (14%)	100% (1/1)
Sickle Cell Disease	**Severe**	**1**/7 (14%)	100% (1/1)

When comparing whether there was a difference in uptake rates for invasive testing between the autosomal recessive moderate and severe disease groups, we did not find a statistical difference ($p = 1.00$). Disease severity, by our classifications, was not found to be a significant factor in reproductive decision-making. This data is demonstrated in Figure 1.

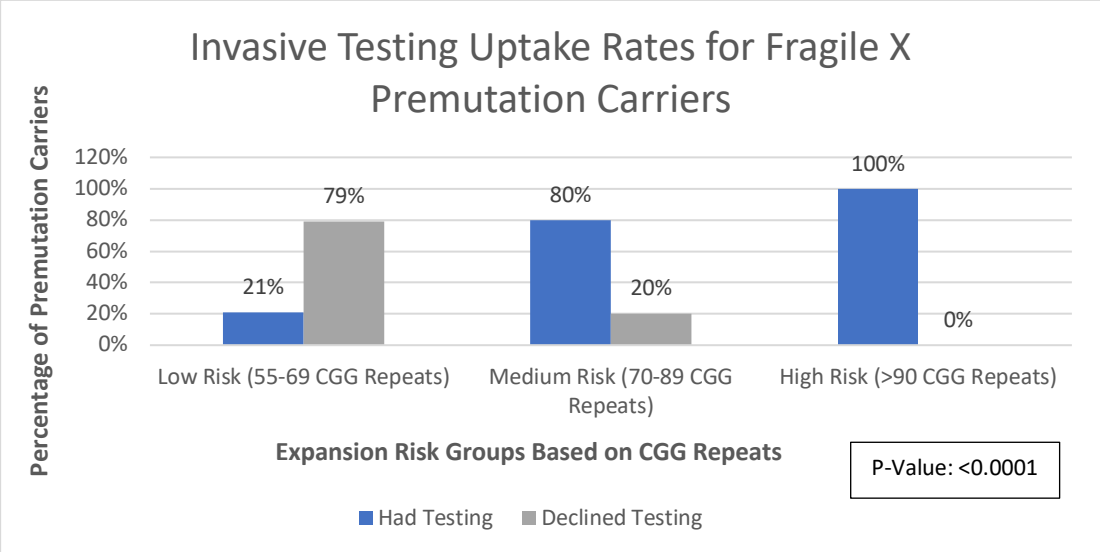
Figure 1 – Invasive testing uptake rates for fetal risk of autosomal recessive condition, based on our disease classifications.



Fragile X Premutation Carriers at Risk for Fetal Fragile X Syndrome

A total of 43/116 (37%) of our sample size was represented by Fragile X premutation carriers. Eighteen out of 43 (42%) premutation carriers chose to undergo invasive testing; 17/18 (94%) had a CVS and 1/18 (6%) had an amniocentesis. When looking at risks associated with allele expansion specifically in our Fragile X cohort (n = 43), we found that there was a significant difference between CGG repeat groups and diagnostic testing uptake rates (p = <0.0001). Figure 2 illustrates diagnostic testing uptake rates by the three CGG repeat categories. For patients in the low risk category, 21% had diagnostic testing. For patients in the medium risk category, 80% had diagnostic testing. For patients in the high risk category, 100% had diagnostic testing. The higher the number of CGG repeats, the greater the likelihood that the patient would undergo prenatal testing.

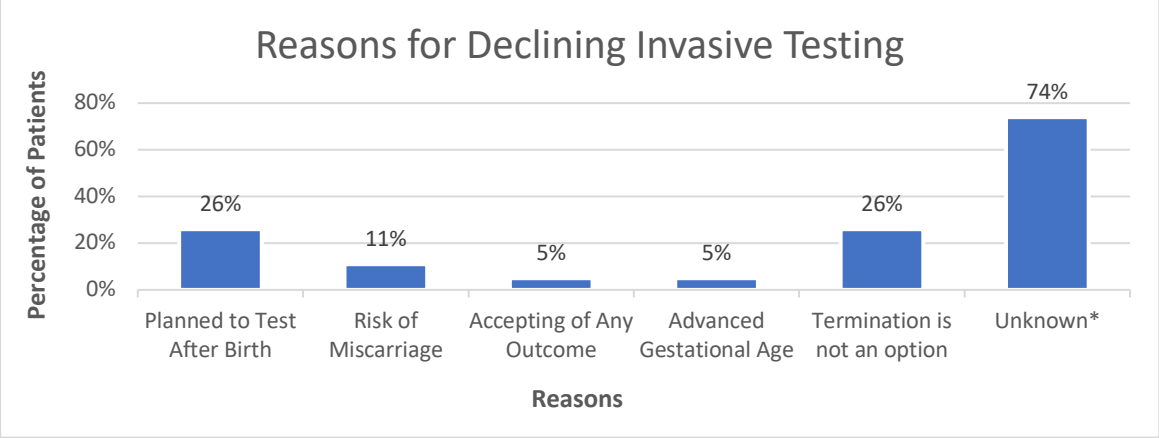
Figure 2 – Invasive testing uptake rates in Fragile X premutation carriers sorted by CGG repeats.



Reasons For Declining Invasive Testing

Fifty-seven of our 116 (49%) at-risk couples or female Fragile X premutation carriers declined prenatal testing. Twenty-five out of 57 (44%) were Fragile X premutation carriers, and 32/57 (56%) were couples at-risk of having a child with an autosomal recessive condition. Thirty-eight out of 57 (67%) provided a reason as to why they declined, and the most common responses are summarized in Figure 3.

Figure 3 – Reported reasons for declining invasive testing.



*Reason was not documented in the patient chart.

Discussion

The objective of this study was to investigate how patients navigate their reproductive options when their fetus is at risk for an autosomal recessive condition or Fragile X Syndrome. Based on our data, couples who were at risk for a fetal autosomal recessive condition in their fetus were not influenced by disease severity when deciding about undergoing prenatal testing. Of these patients, those that had an affected fetus, they mostly opted for termination of pregnancy. However, 44% of our patients declined fetal testing, with the most common reasons being that they planned to test the fetus after birth or termination of pregnancy was not an option, if the fetus was affected. Thus, if seems that patients who pursue fetal testing are the ones that are most likely to intervene, if a fetal diagnosis is established.

Our findings contrasted those of the study conducted by Ghiossi et al., who demonstrated couples at risk for a profound/severe condition in their fetus, were significantly more inclined to undergo invasive testing, compared to those at risk of a moderate disease.[13] Their method of disease severity classification mirrored those established in the Lazarin et al. study, which organized disease characteristics based on clinical impact to the affected individual, and assigned severity based on the combination and ranking of said characteristics.[13,14] Availability of treatment was not included as a characteristic, but rather a disease severity modifier, and study respondents were asked to consider the untreated course of each disease during severity ranking.[14] While their classification approach has proven useful in the past, we chose a different classification for the conditions in our cohort. We classified conditions such as PKU as moderate, rather than severe, because the disease may result in normal outcomes, if appropriate

treatment is implemented. Thus, we suspect that we did not observe a difference in invasive testing rates in our two categories, because we used a different classification system. It could be that when couples are facing a risk in their fetus for disease, having a moderate phenotype is enough of a risk threshold that invasive testing is undertaken, when those couples are seeking information prenatally. The couples that would not consider termination or simply can forgo having the information during pregnancy, feel the same regardless of the fetal phenotype.

Future studies, with larger number of subjects and broader range of phenotypes, may provide further information on how couples make reproductive decisions. One of the limitations of our study is that in 74% of individuals who declined testing, they were not able to elucidate the reason. The insight into why patients decline testing should be investigated in future studies.

In regards to our Fragile X premutation group (n = 43), risk for expansion to a full mutation in the offspring did significantly impact invasive testing uptake rates. In total, 18/43 (42%) of our premutation carriers underwent prenatal testing. The risk for allele expansion exclusively occurs through maternal meiosis. Hence, the higher the number of CGG repeats, the greater the risk that a fetus would have a full mutation. Consequently, it is not surprising that the higher the CGG repeats in our premutation female carriers, the higher the chance they underwent prenatal testing. Researchers at the Danek-Gertner Institute of Human Genetics in Israel also looked at invasive testing rates of identified Fragile X premutation and full mutation carriers, with and without family histories of the condition.[15] A total of 260 carriers were detected, and 214/260 (82%) underwent invasive testing. This was a higher rate than observed in our study. However, 67% of our

premutation carriers had a CGG repeat of 55-69, which is associated with a lower risk for expansion to a full mutation. Thus, our patient population may have overall had a lower risk than the study reported by Berkenstadt et al.

Currently, no professional organization advocates for Fragile X carrier screening in the general population. However, unlike CF and SMA, Fragile X syndrome is not included on newborn screening panels. In addition, phenotypic features of Fragile X are not apparent until later in development, which can lead to a longer diagnostic odyssey.[16] In a parent survey study conducted by Bailey et al., the average age of a Fragile X diagnosis was at 40 months of life (male or female), and about 25-39% of families had another child with a full mutation (>200 CGG repeats), before their first child was also diagnosed. Their data also showed that 76% of their survey respondents (n = 250) said the Fragile X diagnosis affected their future reproductive decisions, whether they would have opted to not have any more children, utilize IVF and PGT technologies, or terminate an affected pregnancy.[17]

Study Limitations and Research Recommendations

Since this is a retrospective chart review, our samples were selected based on certain parameters, specifically female patients who were referred to genetic counseling because she and her partner were both carriers of the same recessive condition or females who were Fragile X premutation carriers, at a single institution, and thus not representative of an entire population of at-risk couples and Fragile X premutation carriers. We also had a small sample size. A prospective multi-center analysis evaluating patient decision-making immediately after carrier status identification and over a five-year period would be of interest. This would allow us to study couples across different pregnancies and assess if reproductive decision-making changes over time and with increasing family size. We also did not collect information on whether these patients already had an affected child and whether first-hand knowledge of the condition would impact reproductive decision-making. A potential future study would compare carrier couples found through carrier screening with carrier couples ascertained after having an affected child. The same analysis can apply to Fragile X carriers found through routine screening as compared to Fragile X carriers identified after an affected child or a family history of the condition.

References

1. Committee Opinion No. 691: Carrier Screening for Genetic Conditions. (2017). *Obstet Gynecol, 129*(3), e41-e55. doi:10.1097/aog.0000000000001952
2. Gross, S. J., Pletcher, B. A., Monaghan, K. G., & Professional Practice and Guidelines Committee (2008). Carrier screening in individuals of Ashkenazi Jewish descent. *Genetics in medicine : official journal of the American College of Medical Genetics*, *10*(1), 54–56. https://doi.org/10.1097/GIM.0b013e31815f247c
3. ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy. (2007). Obstet Gynecol, 109(1), 229-237. doi:10.1097/00006250-200701000-00055
4. ACOG Committee Opinion No. 469: Carrier screening for fragile X syndrome. (2010). Obstet Gynecol, 116(4), 1008-1010. doi:10.1097/AOG.0b013e3181fae884
5. Committee Opinion No. 690 Summary: Carrier Screening in the Age of Genomic Medicine. (2017). Obstet Gynecol, 129(3), 595-596. doi:10.1097/aog.0000000000001947
6. Arjunan, A., Litwack, K., Collins, N., & Charrow, J. (2016). Carrier Screening in the era of expanding genetic technology. *Genet Med, 18*(12), 1214-1217. doi:10.1038/gim.2016.30
7. Kaback, M. M. (2000). Population-based genetic screening for reproductive counseling: the Tay-Sachs disease model. *Eur J Pediatr, 159 Suppl 3*, S192-195. doi:10.1007/pl00014401
8. Nazareth, S. B., Lazarin, G. A., & Goldberg, J. D. (2015). Changing trends in carrier screening for genetic disease in the United States. *Prenat Diagn, 35*(10), 931-935. doi:10.1002/pd.4647
9. Awomolo, A., Palomares, K., Garcia, G. H., Rosen, T., Duzyj, C., & Ashkinadze, E. (2018). Trends in invasive prenatal diagnostic testing at a single institution. *Prenat Diagn, 38*(10), 735-739. doi:10.1002/pd.5290
10. Ioannou, L., Delatycki, M. B., Massie, J., Hodgson, J., & Lewis, S. (2015). "Suddenly Having two Positive People who are Carriers is a Whole New Thing" - Experiences of Couples Both Identified as Carriers of Cystic Fibrosis Through a Population-Based Carrier Screening Program in Australia. *J Genet Couns, 24*(6), 987-1000. doi:10.1007/s10897-015-9833-9
11. Scotet, V., Dugueperoux, I., Audrezet, M. P., Blayau, M., Boisseau, P., Journel, H., . . . Ferec, C. (2008). Prenatal diagnosis of cystic fibrosis: the 18-year experience of Brittany (western France). *Prenat Diagn, 28*(3), 197-202. doi:10.1002/pd.1910
12. Kaback, M., Lim-Steele, J., Dabholkar, D., Brown, D., Levy, N., & Zeiger, K. (1993). Tay-Sachs disease--carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. *Jama, 270*(19), 2307-2315.
13. Ghiossi, C. E., Goldberg, J. D., Haque, I. S., Lazarin, G. A., & Wong, K. K. (2018). Clinical Utility of Expanded Carrier Screening: Reproductive Behaviors of At-Risk Couples. *J Genet Couns, 27*(3), 616-625. doi:10.1007/s10897-017-0160-1

14. Lazarin, G. A., Hawthorne, F., Collins, N. S., Platt, E. A., Evans, E. A., & Haque, I. S. (2014). Systematic Classification of Disease Severity for Evaluation of Expanded Carrier Screening Panels. PLOS ONE, 9(12), e114391. doi:10.1371/journal.pone.0114391
15. Berkenstadt, M., Ries-Levavi, L., Cuckle, H., Peleg, L., & Barkai, G. (2007). Preconceptional and prenatal screening for fragile X syndrome: experience with 40,000 tests. *Prenat Diagn, 27*(11), 991-994. doi:10.1002/pd.1815
16. Gutierrez, J. F., Bajaj, K., & Klugman, S. D. (2013). Prenatal screening for fragile x: carriers, controversies, and counseling. *Rev Obstet Gynecol, 6*(1), e1-7.
17. Bailey, D. B., Jr., Raspa, M., Bishop, E., & Holiday, D. (2009). No change in the age of diagnosis for fragile x syndrome: findings from a national parent survey. *Pediatrics, 124*(2), 527-533. doi:10.1542/peds.2008-2992