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ORIGINAL ARTICLE

Expanded carrier screening for preconception reproductive risk assessment: Prevalence of carrier status in a Mexican population

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Abstract

Objective: Genetic carrier screening has the potential to identify couples at risk of having a child affected with an autosomal recessive or X-linked disorder. However, the current prevalence of carrier status for these conditions in developing countries is not well defined. This study assesses the prevalence of carrier status of selected genetic conditions utilizing an expanded, pan-ethnic genetic carrier screening panel (ECS) in a large population of Mexican patients.

Methods: Retrospective chart review of all patients tested with a single ECS panel at an international infertility center from 2012 to 2018 were included, and the prevalence of positive carrier status in a Mexican population was evaluated.

Results: Eight hundred five individuals were analyzed with ECS testing for 283 genetic conditions. Three hundred fifty-two carriers (43.7%) were identified with 503 pathogenic variants in 145 different genes. Seventeen of the 391 participating couples (4.34%) were identified as being at-risk couples. The most prevalent alleles found were associated with alpha thalassemia, cystic fibrosis, *GJB2* nonsyndromic hearing loss, biotinidase deficiency, and familial Mediterranean fever.

Conclusion: Based on the prevalence and severity of Mendelian disorders, we recommend that couples who wish to conceive regardless of their ethnicity background explore carrier screening and genetic counseling prior to reproductive medical treatment.

1 | INTRODUCTION

With increased global awareness of infertility, reproductive specialists continue to refine and develop therapeutic treatments. Preconception expanded genetic carrier screening (ECS) is used to identify healthy individuals that carry a pathogenic variant, or mutation, in a gene associated with an X-linked or autosomal recessive disorder. Couples are at an elevated risk for conceiving an affected child when both partners are carriers of a pathogenic variant(s) of the same gene or when the female carries a pathogenic variant in a gene associated with an X-linked recessive disorder. Couples planning to utilize assisted reproductive technology (ART) have the option to undergo preconception ECS, which informs the patient of their reproductive potential/compatibility and engenders better decision making prior to pursuing treatment. In particular, preconception ECS helps patients and clinicians to navigate whether a couple could benefit from the use of preimplantation diagnosis testing for monogenic/single gene disorders (PGT-M), a non-carrier oocyte or sperm donor, or other alternatives like pursuing adoption. Additionally, for couples not utilizing ART, the use of ECS may help reduce the morbidity and mortality of

affected offspring who otherwise would not be detected or treated by enabling the couple to enlist timely care with appropriate medical providers at an earlier stage prior to birth.

Currently, it is estimated that worldwide for every 10 000 children, 30 will be affected by a genetic condition.[1] According to Online Mendelian Inheritance in Man (OMIM) database, there are approximately 2933 X-linked and autosomal recessive genetic conditions.[2] The relative high frequency of a number of these disorders has motivated the American Congress of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics and Genomics (ACMG) to create clinical guidelines that provide recommendations to physicians regarding risk assessment and how to screen patients appropriately.[3]

Originally, genetic carrier screening focused on the conditions thought to be most prevalent in particular ethnic groups, such as cystic fibrosis in Caucasians and sickle cell anemia in African Americans.[4] Today, expanded carrier screening through high-throughput genotyping and sequencing has advanced to be pan-ethnic and broadened the scope of detectable conditions.[5] Despite the multiple benefits observed by using ECS,[6,7] interpretation of the genomic data by clinicians remains a central challenge.[8] Variant interpretation, clinical relevance, standardized practice, economic sensibility, and social implications are ongoing challenges for the modern practitioner.[7,9] Due to variability in clinical preference arising from these concerns, ACOG or ACMG guidelines state that ECS is acceptable, but each clinician, health care provider, or practice should establish a standard approach for prenatal screening.[3,10] Furthermore, genetic carrier screening test implementation is nearly nonexistent in certain areas of the world due to a lack of exposure and standardization. The use of an ECS test in a diverse population that includes many races and ethnicities could increase the detection of carrier status for a variety of genetic conditions and prompt professional societies to support greater clinical implementation.[11,12]

This study aims to assess the results of ECS in infertile couples from Mexico in order to better understand the prevalence of carrier status for autosomal recessive and X-linked conditions. Additionally, the study will assess the prevalence of at-risk couples within our population. The study results are anticipated to expand clinical knowledge of genetic risks that are specific to the Mexican population. Additionally, we expect that increased uptake of carrier screening in the Mexican population could increase knowledge in reproductive genetics and help optimize early detection methods to prevent inheritance of pathogenic genetic conditions. Thus, we anticipate the results of this study might better facilitate reproductive counseling and decision making for couples planning to pursue reproductive medical care.

What's already known about this topic?

- The implementation of genetic carrier screening has the potential to identify couples at risk of having a child affected with an autosomal recessive or X-linked disorder. However, the current prevalence of carrier status for screened genetic conditions in developing countries is not well defined.

What does this study add?

- This study assesses the prevalence of carrier status for a selection of genetic conditions utilizing an expanded, pan-ethnic genetic carrier screening panel in a large population of Mexican patients. The aim of the study is to increase the current knowledgebase about specific polymorphisms and inheritable genetic conditions in the Mexican population to enhance preconception genetic screening awareness and advise proper genetic counseling.

2 | MATERIAL AND METHODS

2.1 | Participants

A retrospective analysis of patients from Mexico that underwent ART treatment was performed. All study participants reported being born in Mexico and received primary care at our practice's Mexico City office. The Mexico City office receives infertile patient referrals from other different entities or states within the Mexican country. However, for high complexity ART treatments such as IVF and preimplantation genetic testing, some patients are required to travel to our office's headquarters in the United States. In our fertility clinics, upon initiation of care during the first consultation, expanded genetic carrier screening is offered to all couples or patients interested in fertility care, regardless of whether genetic screening had been performed in prior treatments. Patients reported demographic data, including country of birth, state, and self-reported familiar ancestry or ethnicity. Informed consent was obtained from all patients, and testing was conducted simultaneously within couples when applicable. Only patients who underwent ECS from January 2015 to January 2019 were included in the analysis.

Included participants were representative of different geographic regions of Mexico (25 of the 32 states within the country). The self-reported ethnicities were categorized as Hispanic (patients with Latin-American or Ibero-American ancestry) and non-Hispanic (any other ancestry); afterwards, groups were subdivided by representative groups based on the race origin and patients self-reported familiar ancestry (Latino [n = 640], European [n = 72], Jewish [n = 68], Middle Eastern [n = 22], and others [n = 3]).

2.1.1 | ECS panel description

A single genetic testing laboratory was used throughout the duration of the study. The ECS panel tests for 283 clinically impactful diseases (Sema4-Expanded Carrier Screen, Sema4 Genomics, CT, USA) (Table S1); 5 to 10 mL of blood serum was collected from participating

patients and was used to test for clinically significant pathogenic variants using the following methodologies (depending on the targeted genes and/or variants): next generation sequencing, genotyping with multiplex PCR amplification, multiple ligation-dependent probe amplification (MLPA), array CGH, and long-range PCR. Quantitative PCR, Exon oligonucleotide microarray, and Sanger sequencing were used as confirmation methods when appropriate. For each patient, status (carrier or non-carrier) was determined by the genetic testing laboratory, and patients found to be a carrier were offered genetic counseling. Detected at-risk couples discussed the option to use prenatal diagnostic testing and/or preimplantation genetic testing for monogenic (PGT-M) to avert disease inheritance.

2.1.2 | Statistics

Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc, Cary, North Carolina). Summary statistics including median and average age were calculated for the entire population. Descriptive data were compared by Chi-squared test and Student *t* test when appropriate. The results were expressed as percentages, means, and standard deviations (SDs) with Clopper-Pearson binomial 95% confidence intervals (95% CI). Prevalence of carrier status was calculated for the group as a whole, and then by sex and self-reported ethnicity. We defined "at-risk couples" as couples found to be carriers of deleterious pathogenic variants in the same gene.

3 | RESULTS

3.1 | Demographics

A total of 805 patients (391 couples) underwent ECS testing prior to entering ART treatment. Overall, the MEAN age of the tested population was 38.5 SD± 6.5. Positive carrier status was found in 43.7% (n = 352) of the individuals tested and consisted of individuals who were heterozygous carriers for at least one pathogenic variant associated with an autosomal recessive or X-linked condition. Within this group, 62.5% (n = 220) were found to be carriers for one condition, 26.45% (n = 93) for two conditions, 6.5% (n = 23) for three conditions, and 1.9% (n = 7) were carriers for four conditions. No patient carried more than four conditions on our study. A total of 503 pathogenic variants in 145 genes were identified in the study population.

The average age of the female group was 35.2 SD ± 4.9. Of these females (n = 391), 188 were carriers for at least one condition (48.09%), and 203 (51.91%) were negative for all conditions tested. In the male population group, the average age was found to be 41.7 SD ± 6.31. Of the 414 males who were tested, 164 (39.61%) were found to be carriers of at least one condition, and 250 (60.39%) were negative for all conditions tested (Table 1). The percentage of carrier females was 48.08% (n = 188/391) compared with 39.61% in males (n = 164/414) (*P* = .02; OR = 0.7; 95% CI, 0.5%-0.96%). These differences can be explained by X-linked disorders, which were only detectable in women within our study. After excluding X-linked conditions (n = 10/391), there was no statistical difference in the prevalence of abnormal ECS tests when comparing females 45.55% (n = 178/391) vs males 39.61% (n = 164/414) (OR 0.7; 95% CI, 0.5-1.02; *P* = .07) (Table S2).

3.2 | Recessive/X-linked single gene disorders

Of the 283 different genes analyzed on the ECS panel used, the study cohort was found to have 503 pathogenic variants in 145 different genes associated with autosomal recessive or X-linked genetic conditions. The full list of most common conditions is depicted in Table 2. Alpha thalassemia (*HBA1/HBA2* genes) 4.10% (n = 33/805) was the most common variant found in our population, followed by cystic fibrosis (*CFTR*) 3.85% (n = 31/805), nonsyndromic hearing loss (*GJB2* related) 3.35% (n = 27/805), biotinidase deficiency (*BTD*) 2.98% (n = 24/805), and familial Mediterranean fever (*MEFV*) 2.36% (n = 19/805) (Table 2).

TABLE 1 Demographic and carrier prevalence information in a Mexican population based by self-reported ethnicity and ancestry

Self-Reported Ethnicity	Hispanic-Mexican		Non-Hispanic–Mexican							
Subclassification by self-reported ancestry	Latino		European		Jewish		Middle Eastern		Others	
	N	%	N	%	N	%	N	%	N	%
Total patients (n = 805)	640	79.5	72	9	68	8.5	22	2.7	3	0.3
Females (n = 391)	306	78.3	34	8.7	38	9.7	11	2.8	2	0.5
Positive carrier	145	47.4	7	20.5	26	68.4	6	54.5	1	50
Polymorphisms	209		9		41		7		1	
Males (n = 414)	334	80.7	38	9.2	30	7.3	11	2.7	1	0.01
Positive carrier	123	36.8	18	47.3	17	56.6	5	45.4	1	100
Polymorphisms	179		28		26		5		1	

TABLE 2 Top 10 diagnosed pathogenic variants in the Mexican-Hispanic population analyzed (N = 805 individuals)

GENE	Disease or Condition Associated	Positive Cases, N	Prevalence %
HBA	Alpha-Thalassemia	33	4.10
CFTR	Cystic Fibrosis	31	3.85
GJB2	Non-Syndromic Hearing Loss (*GJB2*-Related)	27	3.35
BTD	Biotinidase Deficiency	24	2.98
MEFV	Familial Mediterranean Fever	19	2.36
PAH	Phenylalanine Hydroxylase Deficiency	15	1.86
SMN1	Spinal Muscular Atrophy	15	1.86
PMM2	Congenital Disorder of Glycosylation, Type Ia	12	1.49
FMR1	Fragile X Syndrome	10	1.24
GAA	Glycogen Storage Disease, Type II	9	1.12

Note: Full list of variants can be found on the Supporting Information.

When analyzing carrier status by different ethnicities within our Mexican population, patients who were identified as Jewish were most frequently found to be carrier of at least one condition 64.7% (n = 44/68), followed by the Middle eastern 50% (n = 11/22), Latino 42.03% (n = 269/640), and Europeans 34.7% (n = 25/72). Also, two of the three tested patients from the "Others" ethnicities group, such as Asian, were found to be carriers of at least one polymorphism. The full list of positive carrier status for different conditions by self-reported ethnicity is reported in Table 3.

3.3 | Alpha thalassemia

HBA1/HBA2 gene variants were found in 4.10% (n = 33/805) of the population. The ECS panel used in our study tested all patients for deletions/duplications of the four functional alpha-globin genes, two copies of *HBA1* and two copies of *HBA2*. Nineteen patients (57.3%) were found to be silent carriers with a deletion of one copy of the *HBA2* gene (aa/a−). Thirteen patients (39.3%) were found to have a duplication in the *HBA2* gene (aaa/aa). One patient (3.3%) was found to be a homozygous trait carrier (a−/a−). An at-risk couple was observed, yet, consisted of two silent carriers (aa/a−). Thus, there was minimal risk of having symptomatic offspring. This couple after counselling decided to not pursue PGT-M.

3.4 | Cystic fibrosis

A cystic fibrosis (CF) transmembrane conductance regulator (*CFTR*) gene pathogenic variant was found in 3.85% of the study patients (n = 31/805). CF carrier frequency was found to be higher in infertile males. We observed a screen-positive rate of 4.35% (n = 18/414) in males compared with 3.32% (n = 13/391) within tested females, albeit this difference was not statistically significant (P = .46).

The genetic panel used in our study tested for 576 variants and sequenced 26 of the 27 exons of the *CFTR* gene. The most common pathogenic *CFTR* variant found in our Mexican population analysis was the delta F508 variant, which was observed in 15 patients (51.6%), followed by F1052V (9.6%) in three patients, and D1152H (9.6%) in three patients. Other single variants, found at a prevalence of 3.2%, were as follows: W1282X, W1089X, c.3367+2T>C, M952I, Y1014C, R117H, 1624G>T (G542*), 711+1G>T and p.Y1014C.

3.5 | Non-syndromic hearing loss (*GJB2* related)

Next generation sequencing and targeted genotyping were performed for 21 pathogenic variants and two out of two exons on the *GJB2* gene as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del (*GJB6*-D13S1830) and del (*GJB6*-D13S1854). Twenty-seven cases (3.35%) of the Mexican population carried at least one pathogenic variant in *GJB2*. The most common variant found in the study was c.35delIG (10 cases [37%]), followed by c.101T>C (five cases [18.5%]), c.617A>G (four cases [14.8%]), c.109G>A (two cases [7.4%]), and other variants (deletion GJB6-D13S1830, c.416G>A, p.Leu90Pro, c.365A>T, c.169C>T, c.269T>C) (one case [3.7%] each).

3.6 | Familial Mediterranean fever

Within the total of 805 patients tested using the ECS panel, 19 patients (2.36%) were found to carry a pathogenic variant in the *MEFV* gene, with higher prevalence in patients of Mexican-Middle Eastern and Mexican-Jewish ancestry (9.09% and 11.76%, respectively). One at-risk couple who both partners carried the p.V726A variant was found, and the couple waived pursuing PGT-M after specialist counseling. Lastly, one male patient was found on the screening to have two pathogenic variants in MEFV (c.2080A>G,

TABLE 3 Prevalence of positive carrier status for different conditions by self-reported ethnicity

Gene	Condition	Cases	Prevalence %
Hispanic Latino		N = 640	Carriers = 269
HBA	Alpha thalassemia	24	3.75
CFTR	Cystic fibrosis	22	3.44
GJB2	Non-syndromic hearing loss (GJB2-related)	20	3.13
BTD	Biotinidase deficiency	16	2.50
SMN1	Spinal muscular atrophy	14	2.19
PAH	Phenylalanine hydroxylase deficiency	11	1.72
PMM2	Congenital disorder of glycosylation, type Ia	9	1.41
MEFV	Familial Mediterranean fever	9	1.41
FMR1	Fragile X syndrome	8	1.25
GAA	Glycogen storage disease, type II	7	1.09
European		N = 72	Carriers = 25
CFTR	Cystic fibrosis	3	4.17
GJB2	Nonsyndromic hearing loss (*GJB2*-Related)	3	4.17
HBA	Alpha thalassemia	2	2.78
CYP11B2	Corticosterone methyloxidase deficiency	2	2.78
ALDOB	Hereditary fructose intolerance	2	2.78
CYP21A2	Congenital adrenal hyperplasia (CAH) due to 21-alpha-hydroxylase deficiency	2	2.78
HHB	Beta-globin-related hemoglobinopathies	1	1.39
BTD	Biotinidase deficiency	1	1.39
CPT2	Carnitine palmitoyltransferase II deficiency	1	1.39
RMRP	Cartilage-hair hypoplasia	1	1.39
Jewish		N = 68	Carriers = 44
MEFV	Familial Mediterranean fever	8	11.76
BTD	Biotinidase deficiency	6	8.82
CFTR	Cystic fibrosis	5	7.35
HBA	Alpha thalassemia	5	7.35
GJB2	Nonsyndromic hearing loss (*GJB2* related)	4	5.88
GBA	Gaucher disease	3	4.41
PMM2	Congenital disorder of glycosylation, type Ia	2	2.94
F11	Factor XI deficiency	2	2.94
DLD	Lipoamide dehydrogenase deficiency	2	2.94
AQP2	Nephrogenic diabetes insipidus, type II	2	2.94
Middle Eastern		N = 22	Carriers = 11
MEFV	Familial Mediterranean fever	2	9.09
HBA	Alpha thalassemia	2	9.09
MCCC2	3-methylcrotonyl-CoA carboxylase deficiency	1	4.55
MTTP	Abetalipoproteinemia	1	4.55
BTD	Biotinidase deficiency	1	4.55
CFTR	Cystic fibrosis	1	4.55
FMR1	Fragile X syndrome	1	4.55
SLC12A3	Gitelman syndrome	1	4.55
IVD	Isovaleric acidemia	1	4.55
PAH	Phenylalanine hydroxylase deficiency	1	4.55

(Continues)

TABLE 3 (Continued)

Gene	Condition	Cases	Prevalence %
Others		N = 3	Carriers = 2
ALPL	Hypophosphatasia	1	33.3
DHCR7	Smith-Lemli-Opitz syndrome	1	33.3

p.M694V (one copy) and c.2177T>C, p.V726A (one copy)), when correlating clinical symptoms patient had features of mild familial Mediterranean fever, therefore patient was referred to a specialist for treatment.

3.7 | Fragile X

The study observed a total of 10 patients who were carriers for an increased number of CGG repeats in the fragile X mental retardation 1 (*FMR1*) gene (1.24% carrier rate). Only females were detected to carry an *FMR1* gene polymorphism; 10 of our 391 patients (2.56%) were diagnosed to be carriers of fragile X disease. No other X-linked related disorders were found in both groups.

During *FMR*1 gene analysis, CGG repeats were confirmed by Southern blot analysis and assessed the size and methylation status. A total of six cases (60%) were diagnosed as premutation carriers with CGG repeats ranging between 55 and 200, and four cases (40%) diagnosed as intermediate carriers with a CGG repeat range of 45 to 54 repeats. No full mutations were found within the study population. After specialized and thorough genetic counseling, all patients in the *FMR1* premutation group who were at risk of passing on a full mutation to offspring and all intermediate cases pursued PGT-M before embryo transfer selection.

3.8 | At-risk couples

When analyzing the data by couple tested, 391 couples underwent ECS, two of these couples had family history of disease (one couple was a carrier for cystic fibrosis and another couple for Ellis-van Creveld syndrome). Of the 391 couples, 17 (4.34%) were identified as at-risk couples for being carriers of pathogenic variants in the same gene or carrying fragile X disease. Ten couples of 391 (2.55%) carried an *FMR1* gene intermediate mutation (n = 6) or premutation (n = 4), and seven of 391 couples had pathogenic variant associated with an autosomal recessive condition (1.79%). Of these couples, three were found to be carriers for cystic fibrosis (*CFTR*) (42.8%), one couple for non-syndromic hearing loss (*GJB2*) (14.2%), one for familial Mediterranean fever (*MEFV*) (14.2%), one for Ellis van Creveld syndrome (*EVC*) (14.2%), and lastly, one for alpha thalassemia (both partners had silent carrier status, aa/a–) (14.2%). After specialized counseling with a genetic counselor and a reproductive endocrinologist, 15 of the 17 at-risk couples (88.2%) elected to screen embryos using PGT-M with the goal of transferring an unaffected embryo.

4 | DISCUSSION

The implementation of high-throughput sequencing technology facilitates the screening of multiple genes simultaneously in a manner that is efficient both in terms of cost and labor. Along with advances in ART treatment, especially PGT-M, fertility centers are rapidly adopting expanded carrier screening as a routine standard of practice for patients who aim to achieve a successful, healthy pregnancy.

Mendelian disorders have been reported to account for almost 20% of infant mortality and up to 18% hospitalizations in developed countries.[13] Lamentably, there is no published data to describe the prevalence and incidences of carrier status for many recessive genetic diseases in Mexican-born and Mexican-based populations. To date, reproductive specialized medical centers in Mexico and other developing countries do not have standardized guidelines approved by a multidisciplinary organization that recommend genetic carrier screening to patients undergoing ART treatment.

Our study includes a population of Mexican patients who were treated at US and Mexico City offices. Our current standard of care offers genetic carrier screening to all patients, abided by the American College of Obstetricians and Gynecologist and the American College of Medical Genetics current practice guidelines.[14,15] This study is the first to review the prevalence of carrier status for multiple recessive disorders simultaneously in a Mexican population, and our results can be used by clinicians to better ascertain reproductive decision making throughout ART treatment and could reduce the incidence of disease inheritance.

In this analysis, we demonstrate that 43.7% (n = 352) of the Mexican population screened (n = 805) carried at least one pathogenic variant associated with an autosomal recessive or X-linked condition. This number contrasts with prior published works demonstrating positive carrier status ranging from a 25.1% incidence within a US population utilizing different commercially available panels that aimed to detect between 97 and 117 conditions,[16] to a reported 78% in a European population utilizing a broad ECS panel aimed to detect pathogenic variants for 728 different genes/conditions.[17] The variation in carrier frequencies among the published studies and our analysis may be dependent on the population analyzed, different genetic carrier panels used, and screening platforms used.[18]

Our study population included 391 couples. The number of males tested (n = 414) was greater than females (n = 391); this difference can be attributed to patients that were utilizing donor sperm or egg recipients (n = 23). Carrier status was higher in females compared with males (48.08% vs 39.6%); but after adjusting for the presence of X-linked disorders, the prevalence of abnormal tests was comparable among cohorts.

About the prevalence of common recessive conditions, in our study, the most common polymorphism found in the population analyzed was alpha thalassemia (*HBA*) gene with a variant prevalence of 4.10% (n = 33). Our finding is consistent with the reported 7% prevalence of any thalassemia-related genetic variants in Mexican patients by De la Cruz Salcedo et al[19] In our study both affected members of the couple were silent carriers for alpha thalassemia (aa/a-), this particular combination of variants is not causative of disease (HbH disease or Barts hydrops), and therefore did not warrant prenatal/preconception intervention.

Also, we observed *CFTR* gene polymorphisms to be the second most common variants found in our dataset, the prevalence was found to be 3.58% (n = 31). Furthermore, *CFTR* variants were more commonly detected in males compared with females, even though the difference did not reach statistical significance ($P = .46$). The CF carrier status within our study was consistent with prior published reports of CF carrier prevalence on infertile male and females.[20-22] The overall CF prevalence in our population is higher than the prevalence showed by Sugarman et al. who found a 2.3% *CFTR* carrier frequency in Hispanic populations, although the population analyzed in that study consisted a broad U.S. based Hispanic residents and did not assess native Mexican Hispanics.[23] Moreover, Haque et al. published a large population study that found a high prevalence of *CFTR* variants in a Hispanic population (12%), albeit the screened population consisted of 517 Hispanic couples from different countries and included patients that were not born in Mexico.[24]

The *CFTR* delta F508 was the most common variant found in our population (51.6%). This finding is consistent with the 40.7% prevalence reported in a publication by Flores et al. in which the authors characterized different *CFTR* variants in a diverse Mexican-Hispanic population.[25]

Another common and relevant variant found in our study was the *GJB2* polymorphism, which is causative of non-syndromic hearing loss. This gene had a 3.35% carrier frequency in our population, with c.35delG being the most common found variant (37%). Our finding is similar to the reported 2.14% carrier frequency in northeastern populations of Mexico, and that study also found the most common variant to be c.35delG.[26,27]

A 2.36% prevalence for variants in the *MEFV* gene was found in our population. Published data about the prevalence of these familiar Mediterranean fever causing variants in Mexican population is lacking, although our study's finding is comparable with those reported in Middle Eastern populations.[28,29]

Finally, 1.24% (n = 10/805) of the participants in our study were found to carry a *FMR1* variant. Our cohorts prevalence of fragile X is consistent with a reported 1.5% frequency in Mexican and Amerindian populations.[30] While fragile X syndrome is expected to occur more commonly among women in an infertility setting,[31] no data has been published about the general prevalence of *FMR1* polymorphisms in a broader noninfertile Mexican population.

An objective of our study was to describe the prevalence of couples at increased risk to conceive a child with an autosomal or X-linked disorder based on the ECS results. Interestingly, within our population, couples were found to be carriers of the same condition at a rate of 1.79%. This exceeds the frequency of infertile at-risk couples reported by Franasiak et al, who described 0.2% prevalence. However, possible explanations for this difference in at-risk couple's rates could be explained by the use of alternative commercial ECS offerings and/or the evaluation of only an American-based population.[32] Other potential explanation is that within our population, there are two couples who underwent ECS due to history of an affected sibling or family history of a specific disorder, as it is one couple carrying Ellis van Creveld syndrome and another couple with history of CF affected offspring. This may raise the rate of at-risk couples found within our study; moreover, if we analyze only de novo findings, five of 391 couples would be catalogued as at-risk couples, meaning a prevalence of 1.27%. Thus, our study's finding is similar to the 1.2% reported in another large study by Peyser et al, which focused exclusively within a noninfertile Eastern American population.[32] Lastly, by including fragile X disease in the at-risk couples group, a total 4.34% (n = 17/391) prevalence of at-risk couples were found in our population, being this overall prevalence similar as the reported in a largest study published by Hogan et al,[33] who showed a frequency of at-risk couples of 4.5% (n = 335/7498) of tested couples utilizing a next generation based screening platform. Although that study evaluated a broad and mixed US-based noninfertile population, ours was more centralized on patients born in Mexico.

Some of the most common mutated alleles found within the study population placed carriers at risk for offspring with highly disabling diseases. Within our dataset, 16 of the 17 at-risk couples were found to be carriers of conditions with the potential for significant clinical impact: fragile X, cystic fibrosis, *GJB2*-related nonsyndromic hearing loss, familial Mediterranean fever, and a case of Ellis van Creveld syndrome. All of these at-risk couples required clinical oversight and counseling regarding reproductive options. Clinical practitioners are urged to inform all potential patients of the wide spectrum of autosomal recessive/X-linked diseases they may carry and include ECS as part of their infertility screening and preconception work up. Invariably, this effort will steer clinicians towards a more personalized treatment approach that includes genetic counseling, in order for patients to engender informed clinical-management decisions consistent with their values.[34,35]

Our study represents a diverse population of patients who were born in Mexico, which is inherently different from other geographic areas and countries.[36,37] Although the study was designed to be offered to a multiethnic population, the data from this study is biased toward the fact that the majority of our tested population is captured from an international private infertility practice, and the patients who need to pursue ART for building a family have to cover their expenses to travel internationally to receive specialized attention. In addition, the majority of Mexican citizens do not have access or coverage to pursue assisted reproductive technologies (ART) and/or PGT-M being that these treatments are not included at any insurance company or state owned hospitals.[38] Thus, our findings do not fully describe the entire Mexican population. However, the use of ECS can still benefit patients, as they can be counseled about other reproductive options

such as the use of gamete donors, adoption, or conceiving without further testing and future counseling.

Furthermore, the carrier screening panel used within this study utilized next generation sequencing that includes one the most up to date and efficient testing platforms currently available.[11,18,39] The panel is designed to target multiple ethnic populations by using broad disease panels and adding full gene sequencing, instead of being targeted to a specific ethnic group. In fact, some researchers have demonstrated that the use ethnic/racial labels provides little or no value when utilizing expanding carrier screening as opposed to panels targeted to specific ethnicities[40,41]; thus, carrier screening of an expanded list of genetic conditions should be offered to all individuals regardless of their specific ethnic background.[42,43]

To our knowledge, this is the first study to use a large expanded carrier screening panel in a Mexican population that investigates carrier status for a broad number of recessive and X-linked conditions. Genomic screening is rapidly evolving and making testing more accurate, efficient, and accessible to patients. In the coming years, we anticipate that the adoption of ECS by professional societies (ACOG, Society for Maternal Fetal Medicine, ACMG, and National Society of Genetic Counselors) will encourage its use within routine clinical care. Presently, we urge all health care providers, including primary care, family physicians, and gynecologists, to inform patients of the benefits of ECS. We suggest greater outreach and in-depth counseling for every patient, while managing the patient's clinical and emotional expectations as they pursue building a family.

Based on the prevalence and severity of Mendelian disorders, we recommend that couples who wish to conceive regardless of their ethnicity background explore carrier screening and genetic counseling prior to reproductive medical treatment.

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CONFLICTS OF INTEREST

None declared

ETHICAL STATEMENT AND TRIAL REGISTRATION NUMBER

Upon initiation care at these centers, patients were consented for the use of deidentified existing information for retrospective scientific study. This retrospective analysis was approved by the Western Institutional Review (WIRB PRO NUM: 20161791; Study Number: 1167398). The study complies with all ethical standards for medical research and all patient information was anonymized and de-identified prior to analysis.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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