Carriers of cystic fibrosis among sperm donors: complete *CFTR* gene analysis versus *CFTR* genotyping

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Objective: To determine the frequency of cystic fibrosis (CF) carriers among sperm donors in Spain studied through a complete analysis of the *CFTR* gene and to compare the results with those that would have been obtained by the 4 genotyping panels of the *CFTR* gene most commonly used as a carrier test in the context of assisted reproduction in our country.
Design: Descriptive observational study.
Setting: Private center.
Patients: Nine hundred thirty-five sperm donors, from January 2014 to June 2019.
Intervention: None.
Main Outcome Measure: Presence of pathogenic variants in the *CFTR* gene.
Results: 17% of the donors were carriers of at least 1 pathogenic variant in *CFTR*, with 39 different pathogenic variants detected. Only 4 of these 39 variants (10.27%) would have been detected by the 4 genotyping tests considered, and 22 variants (56.41%) would not have been detected by any of the genotyping tests. The pathogenic variants of the *CFTR* gene included in the different genotyping tests analyzed vary widely, and <50% are common to all of them.
Conclusions: Although the was not based in the general population, these results show that the use of genotyping tests is associated with a high reproductive risk, because the rate of detection of CF carriers was lower when these panels were applied, in comparison with the complete study of the *CFTR* gene. We recommend that complete sequencing of the *CFTR* gene by next-generation sequencing be performed as a screening method for CF in sperm donors. (Fertil Steril® 2020;114:524–34. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Allelic heterogeneity, carrier screening, *CFTR* gene, cystic fibrosis, genotyping test, sperm donor

With an approximate incidence of 1 in 2500 live births, cystic fibrosis (CF) is one of the most common severe autosomal recessive diseases in the white population. It is caused by mutations in the cystic fibrosis transmembrane regulatory duct gene (CFTR, OMIM602421) (1).

Since the *CFTR* gene, located on chromosome 7q31 (2), was first cloned, >2,000 disease-causing sequence variants have been identified, thanks to major advances in molecular biology techniques (3). Most mutations are rare, with a population frequency <0.01%.

The large number of mutations detected and their genetic and phenotypic variability make the study of carriers in a healthy population a complex task. There is a wide variety of mutation panels of the *CFTR* gene, each one studying a limited number of variants and targeting particular populations and ethnic groups. These panels usually incorporate variants based on criteria of population frequency and phenotypic severity. Owing to the great diversity of mutations described in the *CFTR* gene, the effective detection of CF carriers by means of such targeted panels is limited. This is especially apparent with respect to subpopulations such as Hispanic individuals, among whom the mutations are not sufficiently well characterized despite the great efforts that have been made in this respect (4).

The genetic heterogeneity of the disease is further complicated by the existence of different classifications of

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the recognized clinical forms of CF, such as the classic form, disorders related to CF (lung disease without pancreatic disease, isolated pancreatitis, chronic sinusitis and/or lung disease in adults), and isolated congenital bilateral absence of the vas deferens (1, 5). Therefore, a given genetic test may present different detection rates, depending on whether its application is directed toward a specific clinical form (6).

Nevertheless, owing to the high prevalence of CF, many scientific societies recommend screening for sperm donors (7). However, none has specified which variants should be included in the study and which should not, or whether the screening should be directed toward a specific clinical form or should address the entire spectrum of clinical symptoms associated with CF, or whether the *CFTR* gene should be studied by complete sequencing and nondirected analysis of variants or by using a panel of targeted variants, i.e., a genotyping test.

The aim of this study was to determine, through a complete study of the *CFTR* gene by next-generation sequencing (NGS), the frequency of sperm donors carrying pathogenic variants of the *CFTR* gene and to analyze the detection rate of these carriers that would have been obtained by applying the genotyping tests for *CFTR* gene mutations that are most commonly used in Spain.

MATERIALS AND METHODS

Population

From January 2014 to June 2019, 5,872 potential sperm donors were interviewed. A semen analysis was performed on the 3,604 men who's personal and family medical histories met the criteria for continuing in the donation program. After the selection process established by the sperm bank was applied, 935 men were finally accepted as donors.

The study was based on these 935 men, ages 18 to 35 years, who were accepted as donors in the framework of a sperm donation program conducted by the Ceifer Biobanco sperm bank (Granada, Spain) from January 2014 to June 2019. All the sperm donors included in the study were white. Of the 935 individuals, 6 were North African, 923 were of Mediterranean origin (from Spain, Italy, or France), and 6 were Nordic.

Sperm donors were selected in accordance with the legal regulations in force in Spain (Act 14/2006, of May 26, on assisted human reproduction techniques) and following the sperm bank's own criteria for minimum seminal quality (sperm concentration $>50 \times 10^6$ spz/mL, progressive motility >50%, sperm morphology [normal forms] >4%, and semen volume >2 mL), which are much stricter than the reference limits of the World Health Organization (8). An extended carrier test for recessive diseases was performed on all donors who were accepted, in accordance with applicable legal criteria and with the sperm parameters established. In addition, all donors underwent a pretest and a posttest genetic counseling session to discuss basic genetic concepts, technical aspects of mass sequencing, and the implications of possible outcomes from the study. Institutional Review Board approval for the study was not necessary because the donors underwent routine genetic screening by NGS in our center, and no additional intervention was applied. Written informed consent, which complies with the legal requirements in Spain (Act 14/2006, of May 26, on assisted human reproduction techniques) and with the recommendations of the Spanish Fertility Society, was provided by each subject before participation in the study, accepting the use of their genetic data for research purposes. Donor information and data were anonymized and deidentified before analysis.

Assessment of Sperm Quality

Donor candidates introduced the semen samples into sterile polypropylene containers by masturbation. After liquefaction, sperm volume, concentration, progressive motility, and morphology were determined by trained laboratory technicians following the guidelines of the World Health Organization (8). Total motility count was calculated by multiplying sperm volume by concentration and the percentage of progressive motility. All semen determinations were performed by professional technicians using the same apparatus, and all took part in internal and external quality control programs. The values obtained from the first semen samples provided by each donor candidate were used for this study.

CFTR gene study

The *CFTR* gene study is included in the qCarrier Plus carrier test (qGenomics, Barcelona, Spain), which uses NGS to detect the presence of genetic variants related to >300 recessive inheritance disorders. Only high-quality readings are used, with an average sequencing error of <1 base in 1,000. This technique is capable of detecting single nucleotide variants and small insertions and deletions (in/dels) of ≤9 nucleotides, with high analytic sensitivity (single nucleotide variant >99%; in/dels >85%) and specificity (>99%) The Carrier Plus Test sequences the *CFTR* gene completely (exons and introns), analyzes the variants found in the exonic and intronic regions (±5 nucleotides), and the variants of the deep intronic regions that are classified in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) as pathogenic.

To obtain this level of performance, the samples are sequenced at high average coverage values, generally >150×. This ensures that a large majority of the sequenced bases (>96%) are read at a minimum depth of 30×. At this depth, the probability of not identifying a heterozygous variant as such, i.e., of taking an alternative heterozygous variant as a reference, is very low (0.016%) in the detection of ≤5 alternative reads out of 30 within a heterozygous variant, corresponding to an allele balance of 20%. In other words, the sensitivity achieved is 99.984% at 30×. Moreover, with this coverage the probability of a sequencing error being observed in >20% of the reads is also very low (this value depends on the error rate of the sequencing platform, usually <1%), thus minimizing the possibility of false positives.

The reading depth of each variant is taken into account to assess its potential accuracy, without hard filters. In regions that have been sequenced with bases <15×, 10 times below the minimum average coverage, the probability of a

sequencing error being interpreted as a real variant (false positive, lack of specificity) or of a heterozygous position being seen as a homozygous reference (lack of sensitivity) increases substantially. At 15×, the probability of observing a real heterozygous variant with an allele balance of <20% (as if it were a reference) is very low, at 0.36%, i.e. the sensitivity is 99.64%. When we approach very low depth values (<10×), we try to maximize both sensitivity and specificity. To do so, the reading depth is taken into account so as to assess the potential accuracy of the variant, but only after the filtering and interpretation process has concluded. All the variants that are considered potentially pathogenic are inspected individually and manually. When a known pathogenic variant is identified at a very low depth (i.e., a doubtful case), it may be validated by an alternative technique if this is considered appropriate (Sanger).

Interpretation of Variants

All the variants found were classified as pathogenic (pathogenic and/or probably pathogenic), common variants (polymorphisms), or variants of uncertain significance (9), according to the following criteria.

Pathogenic variants (pathogenic and/or probably pathogenic variants): mutations classed as pathogenic or probably pathogenic in the ClinVar database, in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk), or so described in the literature associated with the disease. Also included in this group are the de novo truncating variants (nonsense, splicing, frameshift, and large copy number variations [CNVs]).

Common variants/polymorphisms: these variants are present in the databases of control individuals (1,000 genomes (http://1000genomes.org), Exome Variant Server (http://evs.gs.washington.edu/EVS/), and Exome Aggregation Consortium (http://exac.broadinstitute.org). These variants appear more frequently in the population than in the expected frequency of carriers, are present in homozygous controls, are reported as benign or probably benign in the ClinVar database, and are not reported to be associated with the disease.

Variants of uncertain significance: these variants have not previously been reported as pathogenic in the ClinVar or Human Gene Mutation Database databases and present frequencies in the population that are compatible with those of the disease.

For each of the pathogenic variants found, we reviewed its classification in the Cystic Fibrosis Mutation Database (http://www.cftr2.org/) (categories: CF-causing variant, Non–CF-causing variant, Variant of varying clinical consequence, Still under evaluation). In addition, we conducted a bibliographic review of all these variants and determined the expected population frequency according to the GenomAD database (https://gnomad.broadinstitute.org/), the population frequency observed in the sperm donors studied, and the clinical classification of the variants according to the categories of Castellani et al. (10): class A: mutations that cause CF; class B: mutations that are associated with clinically related CF; class C: clinical consequence of the mutations not known; class D: mutations with unlikely or unknown clinical significance).

Targeted Tests of Mutations of the *CFTR* Gene (Genotyping Test)

The study included an analysis of the 4 genotyping tests most commonly used in assisted reproduction centers in Spain to detect carriers of recessive diseases: panel H (HERES-GEN [Full Genomics]: 149 variants associated with CF), panel I (CGT 600 [iGenomix]: 146 variants associated with CF), panel SG (Preconception Focus [Sistemas Genómicos]: 327 variants associated with CF, and panel E (CFEU2v1 + CF Iberian + CF Poly-T [Elucigene]: 69 variants associated with CF, targeting European and Iberian populations).

The comparison with these 4 genotype tests was performed only at the theoretical level, checking whether or not the variants found in semen donors by NGS were included in the genotyping tests.

The variants addressed in all 4 targeted tests were termed common variants, and those studied by a single targeted test were termed exclusive.

Statistical Methods

The frequencies of CF carrier status observed in the sperm donors and those described in GenomAD were compared by the Fisher test, assuming statistical significance at $P<.05$. The 4 genotyping tests were compared by using a Venn diagram to assess their similarities and differences. In addition, we wished to determine the diagnostic sensitivity of each genotyping test, i.e., the number of pathogenic variants found in the sperm donors and included in a specific genotyping panel, and the clinical sensitivity, i.e., the number of sperm donors bearing a pathogenic variant in the *CFTR* gene that was detectable by any of the genotyping tests. The diagnostic and clinical sensitivities of the different tests were compared by the χ^2 test, with a level of statistical significance of $P<.05$.

Following the international guidelines for semen quality studies (11), the semen quality parameters were analyzed on a logarithmic scale to normalize the distribution. Student's *t*-test was used to compare the semen parameters obtained for carrier donors of at least 1 pathogenic variant in the *CFTR* gene with those of noncarrier donors Statistical significance was assumed at $P<.05$.

RESULTS

Frequency of Sperm Donor Carriers of Pathogenic Variants in the *CFTR* Gene

Of the 935 sperm donors, 159 (17%) were carriers of at least 1 pathogenic variant in the *CFTR* gene. The frequency, therefore, was approximately 1 in 6.

In total, 39 different variants were found, of which the most frequent were c.1210-34TG(11)T(5) (26.4% of the carriers), c.1210-34TG(12)T(5) (15.1% of the carriers), R75Q (11.3% of the carriers), L997F (10.1% of the carriers), G576A (6.9% of the carriers), V754M, and F508del (1 each, 3.8% of the carriers). Of those 39 variants, 1 was a deletion,

TABLE 1

Pathogenic variants of the *CFTR* gene found in sperm donors.

Variant cDNA name (Variant legacy name)	No. of donor carriers	GT	Mutation	Frequency total GenomAD (10^{-4})	Frequency CFTR+ Ceifer (10^{-3})
c.1001G>A (R334Q)	1	0	Missense	1.06	1.07
c.1043T>A (M348K)	1	0	Missense	1.27	1.07
c.1052C>G (T351S)	1	0	Missense	1.70	1.07
c.1210-34TG(11)T(5)	42	E	intron variant	N/S	44.92
c.1210-34TG(12)T(5)	24	E	intron variant	N/S	25.67
c.1210-34TG(13)T(5)	1	E	intron variant	N/S	1.07
c.1310G>A (G437D)	1	0	Missense	0.12	1.07*
c.1727G>C/c.2002C>T/c.1327G>T (G576A/R668C/D443Y)	2	0	Missense	50.42/59.79/2.60	11.77†/ 9.63/2.14*
c.1521_1523delCTT (F508del)	7	H-E-I-SG	Deletion	71.72	7.49
c.1624G>T (G542X)	1	H-E-I-SG	Nonsense	3.22	1.07
c.1727G>C (G576A)	2	0	Missense	50.42	11.77
c.1727G>C /c.2002C>T (G576A/R668C)	7	0	Missense	50.42/59.79	11.77/9.63
c.202A>G (K68E)	1	0	Missense	1.49	1.07
c.3808G>A/c.220C>T (D1270N/R74W)	2	0/SG	Missense	15.12/17.18	1.07/2.14
c.221G>A (R74Q)	1	I	Missense	2.55	1.07
c.224G>A (R75Q)	18	0	Missense	156.5	19.25
c.2260G>A (V754M)	7	0	Missense	18.16	7.49†
c.2855T>C (M952T)	1	I	Missense	2.51	1.07
c.2856G>C (M952I)	1	0	Missense	0.80	1.07
c.2900T>C (L967S)	1	0	Missense	7.04	1.07
c.2991G>C (L997F)	16	0	Missense	22.22	17.11‡
c.3023T>A (V1008D)	1	0	Missense	0.04	1.07†
c.3041A>G (Y1014C)	2	0	Missense	2.58	2.14*
c.3154T>G (F1052V)	1	SG	Missense	6.28	1.07
c.3276C>A (Y1092X)	1	I-E-SG	Nonsense	0.18	1.07*
c.3454G>C (D1152H)	1	H-E-SG	Missense	3.76	1.07
c.3458T>A (V1153E)	1	0	Missense	0.32	1.07*
c.3705T>G (S1235R)	2	0	Missense	50.39	2.14
c.3718-2477C>T (3849+10kbC->T)	1	E	Intron variant	N/S	1.07
c.3909C>G (N1303K)	2	H-E-I-SG	Missense	1.39	2.14†
c.4097T>C (I1366T)	1	0	Missense	0.12	1.07*
c.4333G>A (D1445N)	1	I	Missense	3.76	1
c.580-1G>T (712-1G->T)	1	H-E-SG	splice acceptor variant	0.04	1.07†
c.601G>A (V201M)	1	0	Missense	2.16	1.07
c.617T>G (L206W)	1	H-E-I-SG	Missense	1.80	1.07
c.772A>G (R258G)	1	0	Missense	1.72	1.07
c.91C>T (R31C)	1	0	Missense	16.40	1.07
c.958T>G (L320V)	2	0	Missense	5.77	2.139037

Note: 0 = none; E = panel E; GT = genotyping test, including study of the variant; H = panel H; I = panel I; SG = panel SG.
* $P<.05$; † $P<.01$; ‡ $P<.0001$; frequency total GenomAD vs. frequency CFTR+ Ceifer.

Molina. Cystic fibrosis carriers on sperm donors. Fertil Steril 2020.

31 were missense variants, 2 were nonsense variants, and 5 were abnormal splicing (Table 1). All of the mutations found were heterozygous. The individuals presenting >1 variant were all healthy.

Of the spermiogram analyses performed on the 3,604 donor candidates, 43 (1.19%) presented azoospermia. Of the group of azoospermic candidates, 16 (37.20%) had a low seminal volume (<1.5 mL), and 27 (62.80%) had a normal volume (>1.5 mL). Of the 16 individuals with hypospermia, 4 presented pH<6, 1 had pH between 6.1 and 6.5, 2 had pH 6.5 to 7.1, and 9 had pH between 7.2 and 8. Among the donors there were no statistically significant differences in the semen parameters analyzed between carrier and noncarrier donors of *CFTR* variants (Supplemental Table 1, available online).

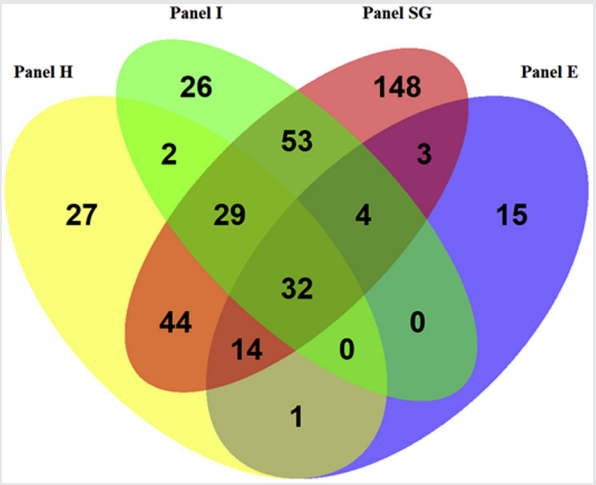
Comparison of the Variants of the Genotyping Tests for the *CFTR* Gene

Analysis of all the genotyping tests showed that only 32 variants were studied in all 4 tests. Panels E, I, H, and SG presented 15, 26, 27, and 148 variants exclusively, respectively (Fig. 1). The percentages corresponding to the common and exclusive variants in each panel are shown in Table 2.

Of the 39 variants found in the sperm donors, 22 variants (56.41%) would not have been detected by any of the 4 genotyping tests, and only 4 of the 39 variants (10.27%) would have been detected by all of the genotyping tests (Table 1).

No statistically significant differences were observed between the diagnostic sensitivities of the different genotyping tests evaluated. However, there were differences in the

FIGURE 1



Number of variants of the *CFTR* gene included in 4 different genotyping panels (panel H: HERES-GEN; panel I: CGT 600; panel SG: Preconception Focus; panel E: CFEU2v1 + CF Iberian + CF Poly-T).

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clinical sensitivities, with panel E being clearly more sensitive in this respect than the other 3 tests (Table 2).

The pathogenic variants most frequently detected were c.1210-34TG(11,12,13)T(5), R75Q, L997F, R668C, G756A, and V754M. These variants were not detected by any of the 4 genotyping tests, with the exception of Poly 5T (c.1210-34TG(11,12,13)T(5)), which was detected only by panel E. Twelve of the 39 variants presented a statistically significantly higher frequency ($P<.05$) in the study population than those reported in GenomAD (Table 1).

Regarding the clinical classification of the variants found, 7 of the 39 variants detected would be classified by CFTR2 as CF-causing variant (F508del, G542X, Y1092X, 3849+10kbC->T, N1303K, 712-1G->T, and L206W), and 12 as variants of varying clinical consequence or still under classification. Of those 19 variants associated with CF, 6 would not have been detected by any of the 4 genotyping tests. Of the 7 variants classified as causing CF by CFTR2, only 4 would have been detected by all 4 genotyping tests. Moreover, 12 of the 39 variants found in our study would be classified by Castellani et al. (10) as class A or class A–B (c.1210-34TG(12)T(5), c.1210-34TG(13)T(5), F508del, G542X, G576A, Y1092X, D1152H, S1235R, 3849+10kbC->T, N1303K, 712-1G->T, and L206W), whereas 6 would be classified as class B or class B–C. Of those 18 variants, 7 would not have been detected by any of the 4 genotyping tests (Table 3). Of the 12 variants classified by Castellani et al. (10) as class A or A–B, only 4 would have been detected by the 4 genotyping tests considered.

DISCUSSION

The carrier frequency of CF pathogenic variants (1 in 6) (17% carriers) found in sperm donors is well above that described in previous research focused on a white European population (1 in 29) (12). This may be because our study was conducted by the complete sequencing of the *CFTR* gene and by nontargeted analysis of variants, whereas the population frequency described in the literature was based on the use of panels that included a limited number of variants of the *CFTR* gene.

Some publications have reported rates of CF carriers in sperm donors, but those papers refer to studies conducted with genotype tests analyzing a limited number of variants. In consequence, the carrier rate obtained is lower than if the analysis had been based on NGS (13). According to Landaburu et al. (14), the rate of CF carriers in sperm donors is 1.3%. However, another study of sperm donors reported a carrier rate of 4.6% when the Poly 5T variant was excluded from the analysis, and 7.8% when it was included (15).

The high CF carrier frequency observed in our study means that if all donor carriers are rejected, problems of donor gamete supply will arise. One means of reducing the risk of having offspring affected by CF after assisted reproduction treatment with sperm donation is genetic matching, i.e., selecting a donor who does not carry pathogenic variants in the *CFTR* gene when the prospective recipient is a carrier of a pathogenic variant in the *CFTR* gene (16–18).

Between the 2 extremes that could be adopted to reduce the reproductive risk of CF—excluding all donor carriers of pathogenic variants in the *CFTR* gene, or including donors carrying CF only when security is assured by genetic matching—we propose an alternative intermediate measure, by which reproductive risk may be reduced. An "exclusion

TABLE 2

Percentage of common-exclusive variants and clinical-diagnostic sensitivity in each of the genotyping tests.

Variable	Panel E (n = 69)	Panel I (n = 146)	Panel H (n = 149)	Panel SG (n = 327)
% Variants				
Common	46.38% (32/69)	21.92% (32/146)	21.48% (32/149)	9.79% (32/327)
Specific	21.74% (15/69)	17.81 (26/146)	18.12% (27/149)	45.26% (148/327)
Sensitivity				
Diagnostic	28.2% (11/39)	20.5% (8/39)	15.4% (6/39)	23.1% (9/39)
Clinical	51.6% (82/159)	9.4% (15/159)[a]	8.2% (13/159)[a]	10.7% (17/159)[a]

Note: Clinical sensitivity = number of carrier donors detected by the test / total number of carrier donors; Diagnostic sensitivity = number of variants detected by the test / total number of variants found in the study population; n = number of variants included in each genotyping panel.

[a] Statistical differences in clinical sensitivity ($P<.05$) of the different panels vs. panel E.

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TABLE 3

Classification of the variants found in the sperm donors according to CFTR2, Castellani et al. (10), and their clinical expressiveness.

Variant cDNA name (variant legacy name)	CFTR2	Castellani et al. (10)	Clinical expressiveness (reference)
c.1001G>A (R334Q)	VC	N/S	46, 47, 48
c.1043T>A (M348K)	N/S	N/S	49, 50, 51
c.1052C>G (T351S)	N/S	N/S	52, 53
c.1210-34TG(11)T(5)	VC	B–C	12
c.1210-34TG(12)T(5)	VC	A–B	12, 54
c.1210-34TG(13)T(5)	VC	A–B	12
c.1310G>A (G437D)	N/S	N/S	32, 55
c.1727G>C/ c.2002C>T/ c.1327G>T (G576A/ R668C/D443Y)	NCC/NCC/VC	B	36, 37, 56, 57
c.1521_1523delCTT (F508del)	CC	A	58, 59, 60
c.1624G>T (G542X)	CC	A	61, 62
c.1727G>C (G576A)	NCC	A-B	37
c.1727G>C/c.2002C>T (G576A/R668C)	NCC	B	36, 56
c.202A>G (K68E)	N/S	N/S	58, 63, 64
c.3808G>A/c.220C>T (D1270N/R74W)	VC	B	40, 65, 66
c.221G>A (R74Q)	N/S	N/S	46
c.224G>A (R75Q)	NCC	NCC	33, 34, 35, 67
c.2260G>A (V754M)	NCC	N/S	32, 41, 39
c.2855T>C (M952T)	N/S	N/S	68, 69
c.2856G>C (M952I)	N/S	B	70
c.2900T>C (L967S)	VC	N/S	49, 67
c.2991G>C (L997F)	NCC	B	5, 54, 62, 67, 71, 72
c.3023T>A (V1008D)	N/S	N/S	73
c.3041A>G (Y1014C)	SUE	N/S	54, 73, 74
c.3154T>G (F1052V)	VC	N/S	35, 46, 71, 75, 76
c.3276C>A (Y1092X)	CC	A–B	77, 78
c.3454G>C (D1152H)	VC	A–B	79, 80
c.3458T>A (V1153E)	VC	N/S	52, 59
c.3705T>G (S1235R)	NCC	A–B	39, 76, 81
c.3718-2477C>T (3849+10kbC->T)	CC	A	66, 82, 83
c.3909C>G (N1303K)	CC	A	58, 66, 84, 85, 86
c.4097T>C (I1366T)	N/S	N/S	87
c.4333G>A (D1445N)	N/S	N/S	53, 88
c.580-1G>T (712-1G->T)	CC	A–B	60, 74, 89
c.601G>A (V201M)	SUE	N/S	90, 91, 92
c.617T>G (L206W)	CC	A–B	62, 75, 93, 94
c.772A>G (R258G)	N/S	N/S	39, 95, 96
c.91C>T (R31C)	NCC	N/S	25, 97, 98
c.958T>G (L320V)	NCC	N/S	53, 72, 99

A = mutations that cause CF; C = clinical consequences of the mutations are unknown; CC = CF-causing variant; class B = mutations that are associated with CF-related clinical presentation; NCC = non CF-causing variant; NCF = not cause CF; N/S = not found in CFTR2 database; SUE = still under evaluation; VC = variant of varying clinical consequences;

Molina. Cystic fibrosis carriers on sperm donors. Fertil Steril 2020.

panel" of variants, considered to be at high risk for the classic form of CF or of a severe phenotype, is defined. Donors carrying any of these variants are excluded from the donation program. The sperm bank should inform the collaborating centers that donors may be carriers of other variants in the *CFTR* gene, not included in the "exclusion panel," and that if they wish to reduce the risk of CF associated with a nonclassic or less severe phenotype, they should perform genetic matching. If the recipient is a carrier of CF, a semen donor who has been studied by complete sequencing and is not a carrier of any variant of the *CFTR* gene will be selected.

The American Society for Reproductive Medicine emphasizes the importance of providing patients with adequate genetic counseling and informing them about the residual risks of the genetic test, about the population risks of being a carrier of a recessive disease, and about the possibility of reducing the reproductive risk by selecting a donor on the basis of genetic matching (19). The reproductive risk for a prospective recipient of sperm donors of having a child with CF depends on the ethnic origin of the recipient, the tests performed on the recipient and on the sperm donor, the results obtained in those tests, and the exclusion criteria applied to the donor. Accordingly, the reproductive risk should be assessed individually, case by case (20).

Our study shows there is great heterogeneity in the pathogenic variants of the *CFTR* gene that are included in the genotyping tests most commonly used in the context of assisted reproduction. Fewer than 50% of the variants

included in the panels are common to all these tests. The clinical and diagnostic sensitivity of the test results obtained highlights the limitations of the tests currently used to detect CF carriers, corroborating previous research findings (4, 21). Except for panel E, which is aimed specifically at the European and Hispanic population, panels I, H, and SG have been validated in accordance with the standards and guidelines of the American College of Medical Genetics and Genomics. Perhaps this is why these 3 tests indicate a lower rate of CF carriers in our population than is reported by the Elucigene test, although the latter includes the smallest number of variants. Our study findings in this respect are in line with those obtained previously in southern European populations, which present high molecular heterogeneity and in which commercial panels cover 50% to 75% of the alleles (22).

Only 4 of the 7 variants classified as CF-causing variants by CFTR2 (57.14% of CF-causing variants by CFTR2) would have been detected by all 4 genotyping tests. On the other hand, of the 12 variants classified by Castellani et al. (10) as class A or A–B, only 4 would have been detected by the 4 genotyping tests (33.33% of the A–B variants by Castellani et al. [10]). These results highlight the low detection rate of genotyping tests in comparison with the results obtained by complete sequencing of the *CFTR* gene by NGS. In addition, they show that detection rates vary depending on whether the test is focused on a specific clinical form (4, 6, 21). Thus, the application of genotyping tests is associated with a higher reproductive risk, although this can be reduced by performing a complete study of the *CFTR* gene by NGS.

The lower detection rate of CF carriers with panels I, H, and SG is mainly due to the fact that none of them studies the 5T-TG variants as a possible cause of CF.

The pathogenic variants most frequently detected in our study were c.1210-34TG (11)T(5), c.1210-34TG (12)T(5), c.1210-34TG(13)T(5), R75Q, L997F, R668C, G756A, and V754M. None of them were detected by any of the 4 genotyping tests analyzed, with the exception of the Poly 5T variants, which only the Elucigene test detected as a cause of CF. It should be noted that the Preconception Focus test includes a study of 5T-TG and of the L997F variant of the *CFTR* gene; however, it does not report them as causative variants of CF, but as a different clinical entity that only provokes the congenital bilateral absence of the vas deferens (CBAVD). Both variants have been associated with other CF-related clinical characteristics (23–26); thus, limiting the presence of these variants to CBAVD, as panel SG does, would not be correct.

Although the L997F variant has been known since 1992, its functional role and impact on pathogenicity remain unclear. Initially it was described as a polymorphism, owing to its high population frequency and because, according to CFTR2, it does not cause CF (27). This variant has been described in a patient with genotype p.Leu997Phe/c.2909-92G>A, who showed a typical clinical presentation of CF (23). An in vitro functional study has shown that the presence of this variant reduces the conductivity of the Cl^- ion with respect to the reference protein. In that same study, conducted in newborns with a positive sweat test result, the variant was identified in 4% of patients. However, it is not known how many subsequently experienced other respiratory or gastrointestinal disorders or manifestations of infertility associated with CF (24). In addition, the presence of this variant has been associated with pancreatic insufficiency (25, 27), lung disease (5, 27), disseminated bronchiectasis, normal sweat test result with neonatal hypertripsinemia, and congenital absence of the vas deferens (27).

The 5T allele in intron 8 (IVS8) causes abnormal splicing in the *CFTR* gene and is considered a mutation of incomplete penetrance (26). The genotype-phenotype correlation is not clear; thus, it has been reported that when the 5T variant is found in trans with a severe *CFTR* mutation it can lead to male infertility, nonclassic CF, or a normal phenotype (26, 28); that the number of TG repeats adjacent to 5T is correlated with the phenotype and disease penetrance; that the number of 11TG repeats is associated with the absence of the vas deferens or with a normal phenotype; and that 12TG and 13TG are more frequent in affected individuals, whether producing the absence of the vas deferens or a nonclassic CF phenotype (26).

Although the 5T variant is found in 10% of the general population (26), in our study its frequency was approximately 7%. This discrepancy may be due to the fact that the variant has been associated with obstructive azoospermia and oligozoospermia (28–30), and donor candidates with these sperm characteristics are not included in the donation program. Moreover, donor candidates who reported a family history of CF were excluded from the donation program. In consequence, our population is biased in this respect.

Among the donors predefined as individuals with good sperm quality, there were no statistically significant differences in sperm quality between carriers and noncarriers of *CFTR* variants. Studies in which a relationship has been found between CF carrier status and sperm quality have been conducted among the general population, but not in a study limited to individuals with high sperm quality (31). However, we would expect to find a higher rate of CF carriers among oocyte donors, inasmuch as heterozygous carrier status has not been associated with any marker analyzed in the screening of oocyte donors. At first, R75Q and R668C/G756A (isolated or in allelic complex in cis) variants were classified as benign polymorphisms. However, their frequent occurrence in individuals with a CF-related phenotype suggests that a pathogenic role may also be played. In addition, their presence is associated with a decrease of 30% to 50% in mRNA levels of CFTR and a decrease of 17% to 26% in mature CFTR protein (32). When the R75Q variant is in trans with another pathogenic variant, it is frequently detected in patients with asthma, CBAVD, disseminated bronchiectasis, obstructive pulmonary disease, or pancreatitis (33–35). Variants G576A and R668C are associated with abnormal splicing (32). Both variants are associated with a reduction in normal transcript quantity, of 57% and 37% respectively, with respect to the wild-type gene. The G576A/R668C haplotype in cis has been found in patients with disseminated and idiopathic bronchiectasis (33, 34) and with CF-related phenotypes such as cholestasis, nasal polyps, and idiopathic pancreatitis (36).

The R668C variant has been associated with CBAVD and azoospermia (33, 36). On the other hand, studies have associated the presence of the G576A variant with individuals presenting a classic CF phenotype, and it has been detected in patients with CF-related symptoms such as idiopathic pancreatitis, CBAVD, and lung disease (37).

The individuals who presented >1 variant were healthy. The variants detected in these individuals (p.[D443Y; G576A; R668C] and p.[R74W; V201M; D1270N]) have been reported as variants in cis on the same allele (38–40), which could explain the fact that these individuals did not present symptoms related to CF.

The presence of the V754M variant is also associated with a 30% to 50% decrease in mRNA levels of the *CFTR* gene and with a 39% decrease in mature CFTR protein levels (32). Moreover, it has been described in a patient with atypical CF, in whom a second mutation was not detected, and in patients with bronchiectasis and CBAVD (32, 39, 41).

The great clinical variability associated with the variants found in the *CFTR* gene is in accordance with the previously described genetic and clinical heterogeneity of the disease (10). Our findings suggest that CF could be influenced by multiple additive effects such as mutations in other alternative or modifier genes, epigenetic factors, or environmental influence (42–44).

In conclusion, in view of the evident clinical heterogeneity associated with the variants of the *CFTR* gene, population studies are needed of patients with CF to clarify the genotype-phenotype relationship in CF, thus facilitating genetic counseling for the individuals concerned and improving decision making in assisted reproduction treatment based on gamete donation. Despite the higher cost of studying the *CFTR* gene by NGS with nontargeted analysis of the variants compared with genotyping, the higher detection rate of healthy individuals who are carriers of CF helps reduce the number of children born with CF, which ultimately produces a cost-effectiveness benefit (45).

Although our study was not based on the general population, we recommend that complete sequencing of the *CFTR* gene be carried out using NGS to screen for CF in sperm donors, because genotype testing alone may result in many mutations being missed. The low rate of detection of CF carriers in the targeted panels and the differences between the results obtained highlight the need to seek a consensus on the basic variants that should be included in a study of CF carriers. In this regard, we emphasize the importance of identifying the risks involved and of informing patients of the existence of a statistically significantly high reproductive risk when targeted panels are used.

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Portadores de fibrosis quística entre donantes de esperma: análisis completo del gen CFTR versus genotipado CFTR.

Objetivo: Determinar la frecuencia de portadores de fibrosis quística (FQ) entre los donantes de esperma en España estudiados mediante un análisis completo del gen CFTR, y comparar los resultados con los que se habrían obtenido mediante los 4 paneles de genotipado del gen CFTR más utilizados como test de portadores en el contexto de la reproducción asistida en nuestro país.

Diseño: Estudio observacional descriptivo.

Entorno: Centro privado.

Pacientes: Novecientos treinta y cinco donantes de esperma, desde enero de 2014 a junio de 2019.

Intervención: Ninguna.

Principales medidas de resultado: Presencia de variantes patogénicas en el gen CFTR.

Resultados: El 17% de los donantes eran portadores de al menos 1 variante patogénica en CFTR, con detección de 39 diferentes variantes patogénicas detectadas. Solamente 4 de estas 39 variantes (10.27%) habrían sido detectadas por las 4 pruebas de genotipado consideradas, y 22 variantes (56.41%) no habrían sido detectadas por ninguna de las pruebas de genotipado. Las variantes patogénicas del gen CFTR incluidas en las diferentes pruebas de genotipado analizadas varían ampliamente, y $<$50% son comunes a todos ellos.

Conclusiones: Aunque no se basaron en la población general, estos resultados muestran que el uso de pruebas de genotipado está asociado con un alto riesgo reproductivo, debido a que la tasa de detección de portadores de FQ fue menor cuando se aplicaron estos paneles, en comparación con el estudio completo del gen CFTR. Recomendamos que la secuenciación completa del gen CFTR mediante secuenciación de última generación (NGS) sea realizada como método de detección de la FQ en donantes de esperma.