

## POLICY

# The improvement of the best practice guidelines for preimplantation genetic diagnosis of cystic fibrosis: toward an international consensus

Anne Girardet<sup>1,2,3</sup>, Victoria Viart<sup>1,3</sup>, Stéphanie Plaza<sup>1,3</sup>, Gemma Daina<sup>4</sup>, Martine De Rycke<sup>5</sup>, Marie Des Georges<sup>1,3</sup>, Francesco Fiorentino<sup>6</sup>, Gary Harton<sup>7</sup>, Aliya Ishmukhametova<sup>1,3</sup>, Joaquina Navarro<sup>4</sup>, Caroline Raynal<sup>1,3</sup>, Pamela Renwick<sup>8</sup>, Florielle Saguet<sup>1,3</sup>, Martin Schwarz<sup>9</sup>, Sioban SenGupta<sup>10</sup>, Maria Tzetis<sup>11</sup>, Anne-Françoise Roux<sup>1,3</sup> and Mireille Claustres<sup>\*,1,2,3</sup>

Cystic fibrosis (CF) is one of the most common indications for preimplantation genetic diagnosis (PGD) for single gene disorders, giving couples the opportunity to conceive unaffected children without having to consider termination of pregnancy. However, there are no available standardized protocols, so that each center has to develop its own diagnostic strategies and procedures. Furthermore, reproductive decisions are complicated by the diversity of disease-causing variants in the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene and the complexity of correlations between genotypes and associated phenotypes, so that attitudes and practices toward the risks for future offspring can vary greatly between countries. On behalf of the EuroGentest Network, eighteen experts in PGD and/or molecular diagnosis of CF from seven countries attended a workshop held in Montpellier, France, on 14 December 2011. Building on the best practice guidelines for amplification-based PGD established by ESHRE (European Society of Human Reproduction and Embryology), the goal of this meeting was to formulate specific guidelines for CF-PGD in order to contribute to a better harmonization of practices across Europe. Different topics were covered including variant nomenclature, inclusion criteria, genetic counseling, PGD strategy and reporting of results. The recommendations are summarized here, and updated information on the clinical significance of *CFTR* variants and associated phenotypes is presented.

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## INTRODUCTION

Cystic fibrosis (CF, OMIM #219700) is one of the most common life-shortening autosomal recessive disorders in populations of European descent, affecting about 1 in 4000 individuals.<sup>1</sup> It is also present in most other ethnicities, but the disease frequency varies greatly. CF is caused by sequence variations in the Cystic fibrosis transmembrane conductance regulator (*CFTR*, OMIM #602421) gene on 7q31.2, which lead to absent or decreased chloride and bicarbonate transports across the apical membranes of secretory epithelial cells, elevated intracellular sodium and decreased extracellular water. This results in thickened secretions in affected structures (airways, pancreatic and biliary ducts, intestines and vas deferens). Although the severity of symptoms is variable, most patients with CF typically present progressive obstructive lung disease with persistent airway colonization and bronchiectasis, pancreatic insufficiency, gastrointestinal and nutritional anomalies, abnormally high sweat chloride levels and male infertility due to absent or occluded vasa deferentia. Early detection of CF through newborn screening programs is of major importance for the improvement of both survival and quality of life of patients.<sup>2–4</sup>

Close to 2000 unique variations have been identified so far in the *CFTR* gene (<http://www.genet.sickkids.on.ca>), either in CF, in *CFTR*-related disorders (CFTR-RD), or in asymptomatic individuals. The term CFTR-RD refers to a distinct spectrum of nonlethal diseases that do not fulfill diagnostic criteria for CF but show evidence of *CFTR* dysfunction,<sup>5</sup> including congenital bilateral absence of vas deferens (CBAVD),<sup>6–12</sup> recurrent pancreatitis,<sup>13,14</sup> disseminated bronchiectasis,<sup>15</sup> or *CFTR*-related metabolic syndrome<sup>16</sup> (Box 1, Table 1). Besides the most common CF disease-causing variant, a 3-bp deletion c.1521\_1523delCTT known as p.Phe508del, that accounts for approximately 67% of *CFTR* mutant alleles in Europe, another 130 variants have been clinically, genetically and experimentally classified as CF-causing.<sup>17</sup> The clinical consequences of many other variants, particularly those that change an amino acid in the protein (missense variants) are largely uncharacterized. In addition, some pathogenic variants are not necessarily fully penetrant<sup>18</sup> (eg their deleterious effect depends on other factors). The number of variants of unknown (clinical) significance (VUCS or VUS) is predicted to increase with the expansion of next generation sequencing methods for CF carrier screening, which is among the first general population

<sup>1</sup>Laboratoire de Génétique Moléculaire, CHU Arnaud de Villeneuve, Montpellier, France; <sup>2</sup>Faculté de Médecine, Université Montpellier I, Montpellier, France; <sup>3</sup>Unité INSERM U827, Montpellier, France; <sup>4</sup>Unitat de Biologia Cel·lular i Genètica Mèdica, Universitat Autònoma de Barcelona, Bellaterra, Spain; <sup>5</sup>Center for Medical Genetics UZ Brussel, Brussels, Belgium; <sup>6</sup>Genoma Laboratories, Via di Castel Giubileo, Roma, Italy; <sup>7</sup>Reprogenetics, Livingston, NJ, USA; <sup>8</sup>Center for Preimplantation Genetic Diagnosis, Guy's and St Thomas' NHS Foundation Trust, Great Maze Pond, London, UK; <sup>9</sup>Manchester Centre for Genomic Medicine, Manchester, UK; <sup>10</sup>UCL Center for PGD, Institute for Women's Health, University College London, London, UK; <sup>11</sup>Department of Medical Genetics, University of Athens, Greece

\*Correspondence: Professor M Claustres, Genetic Diagnosis Laboratory, IURC, 641 avenue du Doyen Gaston Giraud, 34095 Montpellier Cedex 3, 34095, France. Tel: +33 4 11 75 98 47; Fax: +33 4 11 75 98 82; E-mail: Mireille.Claustres@inserm.fr

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**Box 1 The spectrum of CFTR-associated diseases**

## ✓ Classical or typical cystic fibrosis (CF) includes the following features:

- Chronic sinopulmonary disease with persistent colonization with typical CF pathogens,
- Specific gastrointestinal or nutritional abnormalities,
- Salt-loss syndromes,
- Obstructive azoospermia,
- Abnormal sweat test (chloride concentration  $\geq 60$  mmol/l),
- Two CF-causing variants in *trans*.

Over 85% of these patients have two severe CF-causing variants and are pancreatic insufficient (CF-PI). A minority of CF patients carry at least one 'moderate' variant, which confers some residual CFTR channel function maintaining sufficient exocrine pancreatic function to allow normal digestion of nutrients (pancreatic sufficiency, CF-PS).

In many countries, CF is now diagnosed through newborn screening detecting abnormal values of immunoreactive trypsinogen in the blood, on the basis of abnormal sweat test and two CF-causing variants.

## ✓ Nonclassical or atypical CF

A small proportion of patients have symptoms characteristic of CF in at least one organ but a negative (<30 mmol/l) or borderline (30–60 mmol/l) sweat test. They are CF-PS and exhibit a milder and late lung disease. The diagnosis is confirmed by the identification of variants (either one mild in *trans* with a severe one, or two mild variants), and by abnormal nasal potential difference measurement or intestinal current measurement using rectal biopsy. Individuals in this group have an increased risk of pancreatitis.

✓ CFTR-related disorders (CFTR-RD)<sup>5</sup>

A CFTR-RD is defined as a clinical entity with evident CFTR dysfunction that does not fulfill the CF diagnostic criteria. Three distinct subgroups must be distinguished: congenital bilateral absence of the vas deferens, acute recurrent or chronic pancreatitis, and disseminated bronchiectasis.

*Congenital bilateral absence of the vas deferens (CBAVD)*<sup>6–12</sup>

CBAVD is the most common cause of infertility in otherwise healthy males, with an incidence of 1:1000 men, and is present in all ethnicities. The male reproductive tract is the most sensitive tissue to CFTR dysfunction, so that *CFTR* variations not severe enough to cause CF can be responsible for vas deferens aplasia. Up to 80% of CBAVD men have two *CFTR* variants in compound heterozygosity, whereas 20% have either only one or no variant. None of CBAVD men carry two severe *CFTR* variants: the vast majority carry a mild in *trans* with a severe variant, and a minor proportion has two mild variants. In CBAVD men from European descent, the two most common genotypes are p.Phe508del in *trans* either with the '5T allele' in intron 8 (now designated as c.1210-12T[5] in intron 9) (30%) or with the variants R117H-7T (p. Arg117His associated with a 7T allele (c.1210-12T[7]) (6%). In humans, the variant 5T, also present in the general population at a frequency of 0.05, alters the splicing signals and leads to the skipping of exon 9 (10 in the HGVS nomenclature) in a proportion of transcripts. The skipping varies among tissues and individuals, which generates a proportion of incomplete and nonfunctional CFTR proteins; the result is a reduced quantity of normal CFTR protein. The disease penetrance of 5T is incomplete (some men carrying a 5T in *trans* with a severe variant are fertile), and is influenced by another polymorphic sequence immediately upstream, the 'TG tract': the longer the TGn and the shorter the Tn repeats are, the greater is the reduction in full-length *CFTR* transcripts. CBAVD is more likely to occur in males carrying a 5T associated in *cis* with 12TG (c.[1210-34TG[12];1210-12T[5]] or 13TG (c.[1210-34TG[13];1210-12T[5]]) than 11TG (c.[1210-34TG[11];1210-12T[5]]) repeats. Thus the determination of TG repeat number is predictive of the pathogenicity of the 5T alleles.

Spermatogenesis in CBAVD males is normal, thus patients can be treated by sperm extraction followed by ICSI. If the female partner also carries a CF-causing variant, the offspring has an increased risk (25%) of CF (the CF variants of both partners), and male offspring have, depending on the penetrance of mild variants, up to 25% additional chance of having CBAVD (the mild variant of the father and the CF variant of the mother). Genetic counselling may be particularly difficult in couples with a CBAVD partner as they often carry variants with a wide range of effects from normal to CFTR-RD.<sup>11,12</sup>

*Idiopathic chronic pancreatitis and recurrent acute pancreatitis*: approximately 30% of individuals in this group who do not fulfill the diagnostic criteria for CF are found with at least one *CFTR* variant and sweat chloride values in the intermediate range.<sup>13</sup> They include a small proportion with compound heterozygosity for a CF-causing and a mild *CFTR* variant (including the 5T allele) and a majority of carriers of a single CF or CFTR-RD variant with increased susceptibility to pancreas dysfunction. Recent studies suggest that certain missense variants could selectively alter the bicarbonate transport while chloride function is preserved, thus affecting only tissues that necessitate CFTR for bicarbonate secretion such as pancreas.<sup>14</sup>

*Disseminated bronchiectasis*: 15–25% of individuals with abnormal and irreversible dilatation of thick-walled bronchi are found to carry *CFTR* variants, mostly with residual CFTR function (including a high proportion of allele 5T) and mostly in the heterozygous state. *CFTR* variants may act as risk alleles for multigenic disorders in the general population.<sup>15</sup>

*CFTR-related metabolic syndrome (CRMS)*: Asymptomatic newborns with an inconclusive diagnosis following a positive NBS test (abnormal immunoreactive trypsinogen levels but nondiagnostic sweat chloride or genetic analysis). They may either remain symptom free or develop mild CF or CFTR-RD later in life, and therefore need regular follow-up.<sup>16</sup>

genetic screening tests, with worldwide probably more than 10 millions tests each year. Assessing disease causality for *CFTR* variants is extremely challenging<sup>18–20</sup> owing to their number and variety<sup>21</sup> and the complexity of genotype-phenotype relationship in CF or CFTR-RDs (some identical genotypes may be associated with different phenotypes). Interpreting *CFTR* variations as 'pathogenic' or 'neutral' and, beyond, as 'CF-causing' or 'non CF-causing', has evident impact on diagnosis, genetic counseling and reproductive choices of patients and families.

Evidence from practice indicates that whilst natural conception followed by prenatal diagnostic (PND) was in the past the most common reproductive choice for many couples at risk of passing on CF, preimplantation genetic diagnosis (PGD) is increasingly being seen as a preferred choice. So far, the most used approach for PGD for CF involves the biopsy of one blastomere from 3 days-old embryos following ICSI (intracytoplasmic sperm injection) at the 6–10 cell stage.<sup>22</sup> Blastomeres are then analyzed and embryos shown to be free of the disease are transferred to the maternal uterus. This procedure

avoids the dilemma of therapeutic abortion following PND by chorionic villus sampling or amniocentesis in the first or second trimester of pregnancy. As the blastomere biopsy procedure on day 3 can be traumatic to the embryo, reducing its developmental potential, there is an increasing tendency to move to the day 5 or 6 trophoctoderm biopsy at the blastocyst stage.<sup>23</sup> However, blastocyst biopsy is more challenging than blastomere biopsy, and only some centers (especially those that test PGD embryos for aneuploidy<sup>24</sup>) have changed their protocols so far. CF is one of the most common indications of PGD worldwide, accounting for almost 10% of all indications for monogenic disorders, as recorded in the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium data collection XII.<sup>25</sup>

Best practice guidelines for amplification-based PGD dealing with infrastructures, equipment and materials as well as molecular procedures (pre-examination, examination and post-examinations processes) have been established and updated by the ESHRE consortium.<sup>26,27</sup> These guidelines describe general recommendations on genetic practices for PGD programs, in order to build a consensus opinion on best practices within the PGD community and to achieve a minimum standard across all centers. However, due to the absence of a universal, standardized PGD assay, each center develops its own in-house methods. Such methods are based on direct screening of specific variants,<sup>28,29</sup> linkage analysis using informative microsatellites,<sup>30,31</sup> or a combination of direct and indirect testing.<sup>32,33</sup> Some centers use fluorescent multiplex PCR protocols, others perform nested PCRs, and a few centers use Whole Genome Amplification techniques (mainly Multiple Displacement Amplification, MDA) to generate larger quantities of DNA template, followed by haplotyping of several polymorphic markers.<sup>34,35</sup>

Many health practitioners, even among highly trained specialists in academic medical institutions and including those in reproductive medicine, wish more information about genetic testing and counseling.<sup>36</sup> The guidelines presented here are the result of a meeting held in December 2011 with the partnership of EuroGentest ([www.eurogentest.org](http://www.eurogentest.org)). The main purpose of the meeting was to formulate more specific PGD guidelines for CF-PGD, in line with the general guidelines for amplification-based PGD,<sup>27</sup> for organization of a PGD center for PGD/PGS<sup>37</sup> and for polar body and embryo biopsy<sup>38</sup> previously published by ESHRE. The specific guidelines are based on experience with *CFTR* genetic testing, and will contribute to the harmonization and quality of procedures, methods and protocols and to the standardization of reporting results for cystic fibrosis. We also present updated information on *CFTR* variation including nomenclature, classification of variants, and genotype-phenotype correlations.

## MATERIALS AND METHODS

In order to discuss relevant technical and genetic issues in PGD for cystic fibrosis, a 'Best practice guidelines for CF-PGD meeting' was held in Montpellier, France, on 14 December 2011. The meeting brought together eighteen experts in the fields of PGD and molecular diagnosis of CF and *CFTR*-RDs from seven countries (Spain, Belgium, Italy, USA, UK, Greece and France). Presentations and subsequent discussions formed the basis for establishing specific guidelines.

## NOMENCLATURE

Since the discovery of the *CFTR* gene in 1989, the CF scientific community developed its own nomenclature system (Cystic Fibrosis Mutation Database, (<http://www.genet.sickkids.on.ca/>)). Despite the introduction of a worldwide standardized Human Genome Variation

Society (HGVS) nomenclature,<sup>39,40</sup> the original ('legacy') names are still commonly used for *CFTR* variants and polymorphisms in clinical services, whereas diagnostic laboratories use both nomenclatures simultaneously to ensure compatibility with previous clinical reports and understanding by clinicians and couples. The HGVS DNA variant numbering system is based on cDNA, defining position '+1' as the A of the ATG translation initiation codon in the *CFTR* reference sequence (NM\_000492.3), with initiation codon as codon 1 and exons numbered 1–27. In contrast, in the legacy (traditional) annotation, '+1' was the first base of the mRNA major transcript, and the A of the ATG was consequently nucleotide 133. These two different nomenclatures can lead to confusion and serious erroneous interpretation and reporting of the location of variants. For example, the well-known 1717-1G>A and 3120+1G>A variants (legacy names) are designated as c.1585-1G>A and c.2988+1G>A, respectively, according to the HGVS nomenclature. Not being aware of nomenclature changes and testing a variant at a wrong position in the gene will ultimately lead to erroneous PND or PGD. Variants nomenclature is a complex and evolving process, therefore constant vigilance is needed for accurate reporting.<sup>41</sup>

It is strongly recommended to obtain the original molecular genetic reports including the description of identified variants in both the HGVS and legacy nomenclatures. Then, it is recommended to verify the reported variants on new DNA samples from the couples, using standard molecular techniques (Box 2).

Traditional and HGVS nomenclatures for the most common CF-causing, non CF-causing and variants with varying clinical consequences, and examples of common *CFTR* genotypes nomenclatures, are presented in Tables 1 and 2, respectively. Useful Internet resources are compiled in Box 3.

## INCLUSION CRITERIA

Multiple occasions arise to detect individuals carrying *CFTR* variants (Table 3), who may become future candidates for PGD. Some variants are not causing CF but have been included in commercially available population-based carrier screening panels. These kits may be used by accredited and non-accredited laboratories, thus accreditation does not guarantee the non-detection of clinically non-significant variants. Couples may not have been correctly counseled about the risks.<sup>42,43</sup> Before enrolling a couple for a PGD procedure for CF, it is recommended to evaluate within a multidisciplinary team whether the variants identified in both partners should be considered for PND or PGD. Variants may be grouped in four categories: (i) loss-of-

### Box 2 Importance of verifying the presence of variants initially reported in each member of the couple requesting a CF-PGD

Analysing the DNA of each member of the couple will confirm the initial result and will confirm that both parents carry at least one CF-causing variant.

For example, inability to confirm the variants in both parents may be found in cases with apparent homozygosity for a common or a rare variant:

- Nonpaternity
- Presence on the opposite allele of a large deletion, nonrevealed by routine tests
- Presence of a rare SNP in primer-binding sites or a rare DNA variant that causes failure of amplification and/or hybridization of a probe: for example, false homozygosity for p.Phe508del due to the presence of the variant F508C (c.1523T>G); false homozygosity for p.Ile507del due to the presence of p.Ile507Val (I597V) (c.1519A>G).
- Uniparental disomy of chromosome 7, more rarely.

**Table 1 Examples of common CF-causing, indetermined, and non CF-causing variants (modified from<sup>5,8,17</sup>)**

HGVS nomenclature		
Legacy name	cDNA nucleotide name	Protein name
<i>CF-causing variants<sup>a</sup></i>		
F508del	c.1521_1523delCTT	p.Phe508del
G542X	c.1624G>T	p.Gly542*
G551D	c.1652G>A	p.Gly551Asp
N1303K	c.3909C>G	p.Asn1303Lys
W1282X	c.3846G>A	p.Trp1282*
621+1G>T	c.489+1G>T	
CFTRdele2,3	c.54-5940_273 +10250del21080	p.Ser18Argfs*16
E60X	c.178G>T	p.Glu60*
G85E	c.254G>A	p.Gly85Glu
394delTT	c.262_263delTT	p.Leu88Ilefs*22
711+1G>T	c.579+1G>T	
R347P	c.1040G>C	p.Arg347Pro
A455E	c.1364C>A	p.Ala455Glu
Q493X	c.1477C>T	p.Gln493*
I507del	c.1519_1521delATC	p.Ile507del
R553X	c.1657C>T	p.Arg553*
R560T	c.1679G>C	p.Arg560Thr
1898+1G>A	c.1766+1G>A	
2183AA>G	c.2051_2052delAAinsG	p.Lys684Serfs*38
2789+5G>A	c.2657+5G>A	
3120+1G>A	c.2988+1G>A	
M1101K	c.3302 T>A	p.Met1101Lys
R1162X	c.3484C>T	p.Arg1162*
3659delC	c.3528delC	p.Lys1177Serfs*15
M1V	c.1 A>G	p.? (unknown)
Q39X	c.115C>T	p.Gln39*
P67L	c.200C>T	p.Pro67Leu
R75X	c.223C>T	p.Arg75*
405+1G>A	c.273+1G>A	
406-1G>A	c.274-1G>A	
E92X	c.274G>T	p.Glu92*
E92K	c.274G>A	p.Glu92Lys
Q98X	c.292C>T	p.Gln98*
457TAT>G	c.325_327delTATinsG	p.Tyr109Glyfs*4
D110H	c.328G>C	p.Asp110His
R117C	c.349C>T	p.Arg117Cys
Y122X	c.366 T>A	p.Tyr122*
574delA	c.442delA	p.Ile148Leufs*5
444delA	c.313delA	p.Ile105Serfs*2
663delT	c.531delT	p.Ile177Metfs*12
G178R	c.532G>A	p.Gly178Arg
711+3 A>G	c.579+3 A>G	
711+5G>A	c.579+5G>A	
712-1G>T	c.580-1G>T	
H199Y	c.595C>T	p.His199Tyr
P205S	c.613C>T	p.Pro205Ser
L206W	c.617 T>G	p.Leu206Trp
Q220X	c.658C>T	p.Gln220*
852del22	c.720_741delAGGGAGAAT GATGATGAAGTAC	p.Gly241Glufs*13
1078delT	c.948delT	p.Phe316Leufs*12
G330X	c.988G>T	p.Gly330*

**Table 1 (Continued)**

HGVS nomenclature		
Legacy name	cDNA nucleotide name	Protein name
R334W	c.1000C>T	p.Arg334Trp
I336K	c.1007 T>A	p.Ile336Lys
T338I	c.1013C>T	p.Thr338Ile
1154insTC	c.1021_1022dupTC	p.Phe342Hisfs*28
S341P	c.1021 T>C	p.Ser341Pro
R347H	c.1040G>A	p.Arg347His
1213delT	c.1081delT	p.Trp361Glyfs*8
1248+1G>A	c.1116+1G>A	
1259insA	c.1130dupA	p.Gln378Alafs*4
W401X(TAG)	c.1202G>A	p.Trp401*
W401X(TGA)	c.1203G>A	p.Trp401*
1341+1G>A	c.1209+1G>A	
1461ins4	c.1329_1330insAGAT	p.Ile444Argfs*3
1525-1G>A	c.1393-1G>A	
S466X	c.1397C>A or c.1397C>G	p.Ser466*
L467P	c.1400 T>C	p.Leu467Pro
S489X	c.1466C>A	p.Ser489*
S492F	c.1475C>T	p.Ser492Phe
1677delTA	c.1545_1546delTA	p.Tyr515*
V520F	c.1558G>T	p.Val520Phe
1717-1G>A	c.1585-1G>A	
1717-8G>A	c.1585-8G>A	
S549R	c.1645 A>C	p.Ser549Arg
S549N	c.1646G>A	p.Ser549Asn
S549R	c.1647 T>G	p.Ser549Arg
Q552X	c.1654C>T	p.Gln552*
A559T	c.1675G>A	p.Ala559Thr
1811+1.6kba>G	c.1680-886 A>G	
1812-1G>A	c.1680-1G>A	
R560K	c.1679G>A	p.Arg560Lys
E585X	c.1753G>T	p.Glu585*
1898+3 A>G	c.1766+3 A>G	
2143delT	c.2012delT	p.Leu671*
2184insA	c.2052_2053insA	p.Gln685Thrfs*4
2184delA	c.2052delA	p.Lys684Asnfs*38
R709X	c.2125C>T	p.Arg709*
K710X	c.2128 A>T	p.Lys710*
2307insA	c.2175dupA	p.Glu726Argfs*4
L732X	c.2195 T>G	p.Leu732*
2347delG	c.2215delG	p.Val739Tyrfs*16
R764X	c.2290C>T	p.Arg764*
2585delT	c.2453delT	p.Leu818Trpfs*3
E822X	c.2464G>T	p.Glu822*
2622+1G>A	c.2490+1G>A	
E831X	c.2491G>T	p.Glu831*
W846X	c.2537G>A	p.Trp846*
W846X	c.2538G>A	p.Trp846*
(2670TGG> TGA)		
R851X	c.2551C>T	p.Arg851*
2711delT	c.2583delT	p.Phe861Leufs*3
S945L	c.2834C>T	p.Ser945Leu
2799+2insA	c.2657+2_2657+3insA	
Q890X	c.2668C>T	p.Gln890*
L927P	c.2780 T>C	p.Leu927Pro
3007delG	c.2875delG	p.Ala959Hisfs*9
G970R	c.2908G>C	p.Gly970Arg
3120G>A	c.2988G>A	

Table 1 (Continued)

Legacy name	HGVS nomenclature	
	cDNA nucleotide name	Protein name
3121-1G>A	c.2989-1G>A	
3199del6 (3195del6)	c.3067_3072delATAGTG	p.Ile1023_Val1024del
3272-26 A>G	c.3140-26 A>G	
L1065P	c.3194 T>C	p.Leu1065Pro
R1066C	c.3196C>T	p.Arg1066Cys
R1066H	c.3197G>A	p.Arg1066His
L1077P	c.3230 T>C	p.Leu1077Pro
W1089X	c.3266G>A	p.Trp1089*
Y1092X	c.3276C>A	p.Tyr1092*
E1104X	c.3310G>T	p.Glu1104*
R1158X	c.3472C>T	p.Arg1158*
S1196X	c.3587C>G	p.Ser1196*
W1204X(3743G>A)	c.3611G>A	p.Trp1204*
W1204X(3744G>A)	c.3612G>A	p.Trp1204*
3791delC	c.3659delC	p.Thr1220Lysfs*8
3849+10kbC>T	c.3718-2477C>T	p.(?)
G1244E	c.3731G>A	p.Gly1244Glu
3876delA	c.3744delA	p.Lys1250Argfs*9
S1251N	c.3752G>A	p.Ser1251Asn
3905insT	c.3773dupT	p.Leu1258Phefs*7
4005+1G>A	c.3873+1G>A	
4016insT	c.3889dupT	p.Ser1297Phefs*5
Q1313X	c.3937C>T	p.Gln1313*
CFTRdele22,23	c.3964-78_4242+577del	p.(Gly1323_Val1415del)
4209TGTT>AA	c.4077_4080delTGTTinsAA	
4382delA	c.4251delA	p.Glu1418Argfs*14
<i>Examples of common variants with varying or indetermined clinical consequences<sup>b</sup></i>		
R117H	c.350G>A	p.Arg117His
L227R	c.680 T>G	p.Leu227Arg
Q359K/T360K	c.[1075C>A;1079C>A]	p.[Gln359Lys; Thr360Lys]
L558S	c.1673 T>C	p.Leu558Ser
Y569D	c.1705 T>G	p.Tyr569Asp
D579G	c.1736 A>G	p.Asp579Gly
D614G	c.1841 A>G	p.Asp614Gly
S977F	c.2930C>T	p.Ser977Phe
F1052V	c.3154 T>G	p.Phe1052Val
G1069R	c.3205G>A	p.Gly1069Arg
R1070Q	c.3209G>A	p.Arg1070Gln
D1152H	c.3454G>C	p.Asp1152His
I1234V	c.3700 A>G	p.Ile1234Val
5T	c.1210-12[5]	
<i>Examples of common not CF-causing variants<sup>c</sup></i>		
R31C	c.91C>T	p.Arg31Cys
R74W	c.220C>T	p.Arg74Trp
R75Q	c.224G>A	p.Arg75Gln
I148T	c.443 T>C	p.Ile148Thr
M470V	c.1408 A>G	p.Met470Val
G576A	c.1727G>C	p.Gly576Ala
R668C	c.2002C>T	p.Arg668Cys
V754M	c.2260G>A	p.Val754Met
L997F	c.2991G>C	p.Leu997Phe
I1027T	c.3080 T>C	p.Ile1027Thr
R1070W	c.3208C>T	p.Arg1070Trp
R1162L	c.3485G>T	p.Arg1162Leu

Table 1 (Continued)

Legacy name	HGVS nomenclature	
	cDNA nucleotide name	Protein name
S1235R	c.3705 T>G	p.Ser1235Arg
D1270N	c.3808G>A	p.Asp1270Asn
7T	c.1210-12[7]	

Abbreviation: HGVS, Human Genome Variation Society.

<sup>a</sup>CF-causing variants: when paired together in *trans*, they cause cystic fibrosis in most patients\*. The six most frequent non-p.Phe508del variants are listed first followed by 18 other common variants, then 110 other variants are listed by cDNA nucleotide numbering (middle column). CF-causing variants include: (i) variants that are predicted to introduce a premature termination codon (PTC) into *CFTR* mRNA and therefore result in absence of protein such as nonsense variants (sequence changes that introduce a stop codon), variants that affect canonical nucleotides of the donor or acceptor splice sites (gt or ag) or insertions and deletions that cause a frameshift of the ORF (Open Reading Frame); (ii) variants occurring within or near splice sites that lead to severe reduction (>90%) in the level of full-length *CFTR* mRNA and *CFTR* protein; (iii) missense variants that drastically alter folding, processing, trafficking and/or function of *CFTR* protein. \*However, several variants in the list above may retain residual function and can be found either in CF or in *CFTR*-RDs. Well known examples include missense variants D110H, R117C, L206W, R347P, R347H, R1066H, or splice variants that produce both aberrant and full-length transcript such as 3849+10kbC>T, 2789+5G>A, 3272-26 A>G, 711+3 A>G. <sup>b</sup>Variants with varying or indetermined clinical consequences: when paired with a severe variant in *trans*, they cause either no disease phenotype, or *CFTR*-RD or mild CF-PS. The 5T allele is the most common allele associated with *CFTR*-RDs worldwide. The disease penetrance of some of these variants is dependent on other factors, such as other sequence changes associated in *cis*. The most studied examples are R117H and the 5T allele. When paired in *trans* with a severe CF variant, R117H is considered as causing *CFTR*-RD (or rarely mild CF-PS) if associated in *cis* with a 5T allele (rare occurrence), whereas it is considered as a neutral or *CFTR*-RD variant when associated in *cis* with the common 7T allele.<sup>11</sup> Owing to the extremely low penetrance of R117H for CF,<sup>18</sup> in some countries this variant has been removed from the newborn CF screening panels, as its presence created both management and counseling dilemmas (many healthy carriers of R117H-7T could be wrongly considered as CF carriers and prenatal diagnosis inappropriately proposed). <sup>c</sup>Non CF-causing variants: when paired with a severe variant in *trans*, they cause usually no disease phenotype, or a *CFTR*-RD in some individuals. Missense variants R74W, R1070W, D1270N are classified as 'indeterminate' by Sosnay *et al.*,<sup>17</sup> however, as they are frequently found in *trans* with a severe CF variant in asymptomatic individuals (including fertile fathers), they may not be sufficient to cause disease.<sup>19</sup> Moreover, they are often associated within the same allele (eg in *cis*), forming various combinations ('complex alleles') depending on individuals, so that their disease liability is questionable.

Examples:

[R74W;D1270N] c.[220C>T;3808G>A]

[R74W;V201M;D1270N] c.[220C>T;601G>A;3808G>A]

[R74W;R1070W;D1270N] c.[220C>T;c.3208C>T;3808G>A]

I148T is a neutral variant, but can be associated in *cis* with a severe CF variant

c.3067\_3072del (legacy 3199del6 or 3195del6) that, in isolation causes CF, whereas I148T in isolation does not.<sup>19,20</sup> G576A is found in *cis* with R668C and R668C can be found alone or in *cis* with G576A. I1027T is usually found in *cis* with F508del.

Notes: (i) Some missense variants classified as either indeterminate or non CF-causing (R74Q, R75Q, R117H, R170H, L967S, L997F, D1152H, S1235R and D1270N) can selectively alter the bicarbonate permeation of the *CFTR* channel (but not the chloride channel), thus affecting primarily the organs that utilize *CFTR* for bicarbonate secretion (pancreas, nasal sinus, or vas deferens) and, consequently, they could be involved in the pathogenic mechanisms of *CFTR*-RDs.<sup>14</sup> (ii) In Table 1, the traditional name of common *CFTR* variants is referenced alongside the HGVS version in order to ensure compatibility with clinical reports and understanding by clinicians and couples. In the associated text, for reasons of space, some *CFTR* variants may be referred using traditional names.

function variants that cause CF disease when paired together; (ii) variants that retain residual *CFTR* function and are compatible with milder phenotypes such as *CFTR*-RD; (iii) variants with no clinical consequences; and (iv) variants of unproven or uncertain clinical relevance. A recent survey of genotype and phenotype data from almost 40 000 individuals recorded as 'affected with CF' in the clinical registries in Europe and North America, in conjunction with functional assessment, allowed to redefine the disease liability of 159 common variants in the *CFTR* gene.<sup>17</sup> However, the disease penetrance of the vast majority of hundreds of rare missense variants identified so far remains unknown. It is therefore recommended to work in close collaboration with specialized clinical geneticists and reference laboratories that have gained considerable knowledge in the interpretation of *CFTR* variants.

Otherwise, with respect to variations in local or national regulations and specific laboratory practices, the Montpellier meeting revealed some discrepancies in the way that couples are included or not in CF-PGD programs (Table 4). In several countries, when at least one

**Table 2** Examples of *CFTR* genotypes in traditional (legacy) and HGVS nomenclatures (modified and updated from Berwouts *et al.*, 2011)<sup>41</sup>

<i>CFTR</i> testing result	Traditional	HGVS DNA nucleotide name
No variant detected	normal/normal	c.[=];[=]
Heterozygote carrier	F508del/normal	c.[1521_1523delCTT];[=]
One variant found in a CF patient	F508del/unknown	c.[1521_1523delCTT];[?]
Compound heterozygote (two alleles in <i>trans</i> ) <sup>a</sup>	F508del/G21+1G>T	c.[1521_1523delCTT];[489+1G>T]
Two variants, phase unknown example 1 <sup>b</sup>	F508del and G551D	c.[1521_1523delCTT(;)1652G>A]
Two variants, phase unknown example 2 <sup>b</sup>	3849+10kbC>T and N1303K	c.[3718-2477C>T(;)3909C>G]
Two heterozygote variants on one allele (in <i>cis</i> )	R117H-T5/normal	c.[350G>A;1210-12 T[5]];[=]
Compound heterozygote for polyT variants	T5/T7	c.[1210-12 T[5]];[1210-12 T[7]]
Compound heterozygote for the 5 T allele and a CF variant	T5/F508del	c.[1210-12 T[5]];[1521_1523delCTT]
Compound heterozygote for polyTG-T variants and a CF variant	TG12-T5/F508del	c.[1210-34TG[12];1210-12 T[5]];[1521_1523delCTT]

'c.' indicates a coding DNA sequence. In the HGVS nomenclature, variations are described at the nucleotide level because the use of a protein-based nomenclature may be equivocal. Example: p.Phe508del can be caused by different sequence changes at the DNA level, including c.1521\_1523delCTT and c.1522\_1524delTTT. See the HGVS nomenclature website (<http://www.hgvs.org/mutnomen/recs.html>) for detailed descriptions of sequence variants and significance of punctuation symbols.

<sup>a</sup>The term 'compound heterozygote' means that the phase of variants has been checked by testing both parents, which confirmed that each of them is a carrier of one of the two variants, which consequently are in *trans* (on opposite chromosomes) in the affected individual.

<sup>b</sup>The phase of the two variants has not been checked by parental testing: compound heterozygosity cannot be confirmed.

### Box 3 Internet resources

CF-Network EQA—External Quality Assessment process	<a href="http://www.cfnetwork.be">http://www.cfnetwork.be</a>
United Kingdom National External Quality Assessment Service for Molecular Genetics (UKNEQAS)	<a href="http://www.ukneqas-molgen.org.uk">http://www.ukneqas-molgen.org.uk</a>
EuroGentest	<a href="http://www.eurogentest.org/web/qa/basic/xhtml">http://www.eurogentest.org/web/qa/basic/xhtml</a>
HGVS nomenclature	<a href="http://www.hgvs.org/mutnomen/">http://www.hgvs.org/mutnomen/</a>
European Molecular Genetics Quality Network (EMQN)	<a href="http://www.emqn.org/emqn/Best+Practice">http://www.emqn.org/emqn/Best+Practice</a>
Cystic Fibrosis Mutation Database	<a href="http://www.genet.sickkids.on.ca/">http://www.genet.sickkids.on.ca/</a>
CFTR2	<a href="http://www.cftr2.org">http://www.cftr2.org</a>
Testing guidelines for molecular diagnosis of cystic fibrosis (2008)	<a href="http://www.acgs.uk.com/media/774379/cfbp26may.pdf">www.acgs.uk.com/media/774379/cfbp26may.pdf</a>
Orphanet	<a href="http://www.orpha.net">www.orpha.net</a>
OCDE guidelines for quality assurance in molecular genetic testing	<a href="http://www.oecd.org/sti/biotech/38839788.pdf">http://www.oecd.org/sti/biotech/38839788.pdf</a>
University of California Santa Cruz (UCSC) genome browser	<a href="http://genome.ucsc.edu">http://genome.ucsc.edu</a>

**Table 3** Main indications for *CFTR* testing

<i>Newborn screening for CF</i>
<i>Diagnostic testing</i>
Typical CF clinical presentations (including newborns with meconium ileus)
Atypical clinical presentations and/or borderline or negative sweat test
Male infertility with CBAVD
CFTR-Related Disorder in adults
Fetuses with bowel hyperechogenicity and/or loop dilatation in the second trimester of pregnancy (risk of CF estimated as 3%)
<i>Carrier testing</i>
Individuals with a family history of CF ('cascade testing')
Individuals without a family history (essentially for preconception carrier screening)
Partner of a CF patient
Partner of a CBAVD patient
Infertile couples seeking for IVF (not usually recommended among European genetic societies, as the risk of carrying a CF variant is not significantly higher than that of the general population).
<i>Prenatal diagnosis, Preimplantation genetic diagnosis, offered to</i>
Parents of a CF patient
Carrier couples identified through carrier testing
Carrier couples identified through investigations for fetal bowel anomalies
Couples with one individual affected with CF and a carrier partner
Couples with one individual affected with CBAVD and a carrier partner

**Table 4** Inclusion and exclusion criteria for CF-PGD according to countries

	France	Italy	Spain	Belgium	Greece	UK	USA
<i>Parents</i>							
1 affected+1 carrier S/LS	A	A	A	A	A	A	A
1 affected+1 carrier M	R	A	A	A	A	A	A
1 affected+1 carrier U	A	A	A	A	R	A★	A
2 carriers S/LS	A	A	A	A	A	A	A
1 carrier S/LS+1 carrier M	R	A	A	A	A	A	A
1 carrier S/LS+1 carrier U	A	A	A	A	R	A★	A

Abbreviations: S, severe CF-causing variant (p.Phe508del, p.Gly542\*...); LS, large spectrum variant (p.Leu206Trp, c.2657+5G>A ...); M, mild variant with variable disease penetrance (IVS8-5 T allele, p.Arg117His-7 T ...); U, variants of unproven or uncertain CF clinical relevance (p.Pro1013His, p.Arg1162Leu ...); A, accepted; R, refused.

★In the context of a family history of Cystic Fibrosis (see text). Population carrier screening is not performed in the UK.

severe CF-causing or large spectrum variant is identified in one of the members of the couple, PGD is accepted even though a mild or benign variant is found in the other partner. This is an exclusion criterion in France where PGD is restricted to couples 'having a high probability to give birth to a child affected by a particularly severe genetic disease and recognized as being incurable at the time of diagnosis'. Consequently, only the risk of transmitting CF (and not milder phenotypes such as *CFTR*-RD) is considered for PGD. In the UK, when one couple member is heterozygous for a variant of unproven or uncertain clinical relevance, PGD is accepted only in the context of a

**Table 5** List of markers commonly used in PGD for Cystic Fibrosis

Marker	Type	UniSTS <sup>a</sup>	Location on chromosome 7 <sup>b</sup>	Position according to the CFTR gene <sup>c</sup>	Heterozygosity rate (%)
IVS1CA	(ca)n	NA	117,490,314_117,490,356	Intron 1	75
D7S677	(ca)n	18390	117,499,431_117,499,463	Intron 1	85
IVS8CA	(ca)n	NA	117,548,335_117,548,367	Intron 9	75
IVS10CA	(ca)n	NA	117,566,250_117,566,288	Intron 11	89
IVS17BCA	(ca)n	NA	117,612,236_117,612,262	Intron 20	40
IVS17BTA	(ta)n	NA	117,612,009_117,612,069	Intron 20	80
D7S2502	(ca)n	30517	115,818,136_115,818,163	Centromeric, 1.65 Mb	78
D7S486	(ca)n	1556	116,254,761_116,254,799	Centromeric, 1.21 Mb	80
D7S2460	(ca)n	24290	116,767,976_116,768,008	Centromeric, 0.7 Mb	71
D7S23	(ca)n	31363	117,322,538_117,322,572	Centromeric, 0.18 Mb	85
D7S633	(ca)n	70412	117,370,804_117,370,850	Centromeric, 0.1 Mb	45
D7S2847	(tacc)n	64614	119,179,488_119,179,552	Telomeric, 1.46 Mb	84
AFM320vb5	(ca)n	31676	118,351,454_118,351,480	Telomeric, 0.64 Mb	51

Abbreviation: NA, not applicable.

<sup>a</sup>UniSTS has migrated to UCSC.

<sup>b</sup>According to UCSC GRCh38/hg38, December 2013.

<sup>c</sup>Exon numbering according to the HGVS nomenclature. Physical distances are taken from location along chromosome 7 according to the UCSC genome browser.

family history of CF. As a precaution, in all participating centres, novel rare variants of unproven or uncertain clinical significance may be considered as potentially CF-causing upon advice of specialists of *CFTR* alterations.

### GENETIC COUNSELING AND EMBRYO TRANSFER

Similarly, discrepancies in the regulations about embryo transfer may exist from one country to another. It is recommended that all couples are individually counseled before the clinical cycle and the expected embryo genotypes and corresponding phenotypes are clearly explained.

(i) Rare variants or variants with incomplete penetrance in disease

Couples should be aware that in case of a genotype that includes rare variants with incomplete penetrance, individual outcomes cannot be reliably predicted. For instance, an embryo with p.Phe508del in *trans* with the 5T allele (genotype c.[1210-12 T[5]]; [1521\_1523delCCT]) may be considered as suitable for transfer in some countries, while in others the parents' wish to preferentially transfer carrier embryos without the IVS8-5 T allele is taken into account. In countries in which embryos compound heterozygous for a severe and a mild/benign variant may be transferred, couples should be informed of possible risks for their children, ranging from no clinical signs to moderate and/or late-onset phenotypes. Some common or rare *CFTR* variants are associated with a wide phenotypic spectrum and updated information should be provided in light of current knowledge (Table 1).

(ii) Selection of embryos for transfer

Couples should be informed that following the identification of genetically transferable embryos a subsequent selection is made based upon the morphology of the embryo and its potential to implant. Thus morphology rather than distinction between carrier embryos and embryos free of both parental germ-line variants, forms the basis of the final selection of the embryo for transfer. Some centres may also include aneuploidy screening in the PGD protocol in order to achieve higher implantation and live birth rates. In these cases, couples need to be appropriately counseled as the identification of aneuploidy may exclude embryos that had been considered transferable on the basis of *CFTR* analysis alone. Couples should also be made aware that diagnosis may not be achieved for some embryos for various reasons including a failure of amplification or due to cumulus cell

contamination. It is useful for the IVF unit to know the live birth rate per cycle started for CF-PGD at their center as well as the outcome following transfer after cryopreservation of surplus transferable embryos. Multiplex PCR protocols have proven to be robust, sensitive and highly accurate,<sup>12,31</sup> however it is recommended in most countries that the results should be confirmed with a prenatal diagnostic test.

### PGD STRATEGY

Recommended strategies and decision procedures for PGD are presented in the following section. In particularly difficult situations, it is essential that the diagnostic strategies be shared with the referring clinical geneticist.

As mentioned in the amplification-based PGD guidelines,<sup>26,27</sup> it is recommended to perform both indirect (marker based) and direct (variant based) genetic diagnosis.

### PGD diagnostic strategies based on polymorphic markers

Several microsatellite markers within or close to the *CFTR* gene can be used in PGD for CF (Table 5). Laboratories may have to search databases for previously unpublished microsatellite markers in order to obtain sufficient informative markers for a given couple.

It is recommended to offer PGD using at least two fully informative flanking markers within a 1-megabase (Mb, equivalent to one million base pairs) region on both sides of the gene and to include intragenic markers when available.

As recombination events within the *CFTR* gene have been very rarely described, it is acceptable to perform indirect genetic diagnosis using intragenic microsatellites located on the same side of the variant if markers flanking the *CFTR* gene are not informative or gave inconclusive results.

When MDA is performed, it is recommended to study at least four fully informative microsatellite markers (two each side of the gene) because of the higher Allele Drop Out (ADO) rates frequently reported. Furthermore, semi-informative or noninformative markers can still be used in an informative haplotype to detect the presence of contamination and/or chromosomal abnormality (presence of one or three haplotypes).

All customized protocols must be thoroughly validated before clinical use.<sup>25,26</sup>

### PGD strategies based on variants identified in couples

**Case 1: *p.Phe508del* in one or both couple members.** As the most frequent CF variant is the *p.Phe508del* in exon 11 of the *CFTR* gene (exon 10 in the traditional nomenclature), it is very common to identify this variant in at least one of the couple members (Box 4). Therefore, it is highly recommended to set up single-cell PCR conditions for the *p.Phe508del* variant in combination with indirect genetic diagnosis to monitor contamination and preclude misdiagnosis owing to ADO.

For couples in whom one of the partners is heterozygous for a variant other than *p.Phe508del*, it is acceptable to perform a direct diagnosis for the *p.Phe508del* and an indirect diagnosis for the other variant, provided sufficient informative markers are available to trace inheritance of both chromosomes from the non-*p.Phe508del* carrier.

When there is a lack of informative markers in the parent carrier of the other variant, it is recommended to specifically study this causative variant (in addition to the *p.Phe508del*) and also to biopsy and independently analyze two cells instead of one, preferably at the blastocyst stage.

**Case 2: Identical variant (other than *p.Phe508del*) in both couple members.** It is recommended to develop PCR conditions for the unique CF-causing variant in addition to indirect genetic diagnosis. It is acceptable to perform only indirect genetic diagnosis for fully informative couples (at least two informative markers <1 Mb flanking the gene or one intragenic marker and one flanking marker).

**Case 3: Different variants (other than *p.Phe508del*) in both couple members.** It is acceptable to perform only indirect genetic diagnosis for fully informative couples (at least two informative markers <1 Mb flanking the gene or one intragenic marker and one flanking marker). In cases that lack informativity for markers within or near the gene, it is recommended to specifically study both causative variants and to search and add informative markers located further up- or downstream. The biopsy and analysis of trophectoderm cells at the blastocyst stage, should also help in reducing the misdiagnosis risk due to ADO.

### REPORTING

There is currently no formal consensus regarding PGD reports. It also depends on legislation and ethical laws of each country. However, according to recommendations for reporting results of diagnostic genetic testing,<sup>44</sup> guidelines for accreditation of the PGD laboratory,<sup>45</sup> and recommendations from OECD (Organization for Economic Co-operation and Development), some important items should be included in a PGD report. They are listed below.

#### Box 4 Genotype coverage in CF

With a frequency of *p.Phe508del* reaching ~70% of CF alleles in populations from Northern European descent, almost half of individuals with CF have two copies of the *p.Phe508del* allele ( $p^2=(0.7)^2=0.49$ ), 42% have one *p.Phe508del* in *trans* with another *CFTR* variant ( $2pq=2\times0.7\times0.3=0.42$ ) and 9% have two non-*p.Phe508del* variants ( $q^2=(0.3)^2=0.09$ ). Commercially available testing panels including the 30 most common CF variants detect about 87% of CF alleles, so that it can be predicted that 24% of patients with CF carry at least one rare variant not included in these panels ( $2\times0.87\times0.13+(0.13)^2=0.24$ ). However, these features can be very different in Southern-European and non-European populations, who exhibit a much larger proportion of rare variants and a decreased frequency of *p.Phe508del*.

### Feasibility report

- Laboratory issuing results (affiliation, address, secretarial phone and fax numbers)
- Date of report
- Name of the clinician referring the couple
- Unequivocal identification of the couple (2 identifiers per individual, e.g. name and date of birth)
- Specimen information – type of sample and date of sampling (also time if appropriate)
- Disease (OMIM number, inheritance mode) and gene (OMIM number) being tested for
- Methods performed and markers used
- Clearly presented results with appropriate interpretative comments
- Appropriate reference intervals
- Error rates clearly stated
- Variant nomenclature using Human Genome Variation Society (HGVS) recommendations
- Accession number of reference sequence (including version number)
- Identification of individual providing results and authorizer

### PGD case report

- Laboratory issuing results (affiliation, address, secretarial phone and fax numbers)
- Referring clinician: name and affiliation
- Date of report
- Title, that is ‘PGD for cystic fibrosis’
- Disease (OMIM number and inheritance mode) and gene (OMIM number) being tested for
- Unequivocal identification of both couple members (2 identifiers per individual, e.g. name and date of birth)
- Specimen information: type of sample, date/time of sampling, date of arrival of samples in the lab
- Methods performed
  - List of DNA sequences studied
  - Names of the variants analyzed (using HGVS nomenclature)
  - Accession number of reference sequence (including version number)
  - Limits of the test (risk of recombination, ADO rate if indicated)
- Results obtained (genotype may be given or not depending on local rules). Clearly presented results with appropriate interpretative comments with clear indication of whether embryos should or should not be transferred
- Error rates clearly stated
- Identification of individual providing results and authorizer
- The report must be signed (some labs sign all steps: experiments, reading, validation)
- Pagination to include the actual and total number of pages

The reports must be sent (faxed or emailed, depending on legislation on confidentiality of medical information) to the referring physician and/or IVF team as described in the official test request form, in time for embryo transfer. Results should be reported only to persons authorized to receive and use this kind of medical information. In some jurisdictions, the lab report must also be sent to the patient (ie Italy).



## EXTERNAL QUALITY ASSESSMENT

To meet the ISO 15189 standards, accredited laboratories are required to participate in EQA schemes, which provide an independent evaluation of the quality of their service and inter-laboratory comparison, with an educational purpose. The United Kingdom National External Quality Assessment Service has developed a scheme specifically for molecular PGD, based on experience gained with a three-years pilot scheme for CF-PGD.<sup>46</sup> The scheme examines (i) the ability of each participating lab to genotype genomic DNA and single cells (ii) the interpretation of results (including the determination of the limitations of the protocol that was applied for the testing of single cell samples supplied in the EQA), and (iii) the reporting of PGD results. The scheme recommendations were followed by the participating laboratories and led to the improvement in the content of the reports.

## CONCLUDING REMARKS

This meeting organized on behalf of EuroGentest has highlighted important variation among the participating countries both in technical protocols and in the counseling of couples. It emphasized the need to pursue harmonization and quality assurance, preferably through some form of internationally or nationally recognized accreditation. Given the complexity of phenotype and genotype relationships, the extremely high number of variants found in the *CFTR* gene, and the reclassification of some variants as (non)-pathogenic as more data become available, genetic counseling is mandatory, and less common variants should be reviewed with *CFTR* experts before acceptance of a PGD CF request.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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