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

Anne Girardet1,2,3, Victoria Viart1,3, Stéphanie Plaza1,3, Gemma Daina4, Martine De Rycke5, Marie Des Georges1,3, Francesco Fiorentino6, Gary Barton7, Aliya Ishmukhametova1,3, Joaquim Navarro4, Caroline Raynal1,3, Pamela Remwick8, Florielle Saguett1,3, Martin Schwarz9, Sioban SenGupta10, Maria Tzetis11, Anne-Françoise Roux1,3 and Mireille Claustres*1,2,3

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.

European Journal of Human Genetics (2016) 24, 469–478; doi:10.1038/ejhg.2015.99; published online 27 May 2015

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. 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.

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, including congenital bilateral absence of vas deferens (CBAVD), recurrent pancreatitis, disseminated bronchiectasis, or CFTR-related metabolic syndrome (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. 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 (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
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 ≥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)

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)

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 one only 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 cis with a severe variant 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.

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. 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.

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.

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.

Guidelines for CF-PGD

A Girardet et al

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. Blastomeres are then analyzed and embryos shown to be free of the disease are transferred to the maternal uterus. This procedure.

geneic screening tests, with worldwide probably more than 10 millions tests each year. Assessing disease causality for CFTR variants is extremely challenging owing to their number and variety 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.
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 trophectoderm biopsy at the blastocyst stage.23 However, blastocyst biopsy is more challenging than blastomere biopsy, and only some centers (especially those that test PGD embryos for aneuploidy26) 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.25

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.26,27 These guidelines describe general recommendations on genetic practices for PGD programs, in order to build a consensus 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,28,29 linkage analysis using informative microsatellites,30,31 or a combination of direct and indirect testing.32,33 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.34,35

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.36 The guidelines presented here are the result of a meeting held in December 2011 with the partnership of EuroGentest (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,27 for organization of a PGD center for PGD/PGS37 and for polar body and embryo biopsy38 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,39,40 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.41

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.32,43 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-
Table 1 (Continued)

| Legacy name | cDNA nucleotide name | Protein name |
|-------------|----------------------|--------------|
| R334W       | c.1000C>T            | p.Arg334Trp  |
| I336K       | c.1007T>A            | p.Ile336Lys  |
| T338I       | c.1013C>T            | p.Thr338Ile  |
| 1154insTC   | c.1021_1022dupTC     | p.Phe342His*28 |
| S341P       | c.1021T>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 |
| W401W(TAG)  | c.1202G>A            | p.Trp401*    |
| W401X(TQA)  | 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.1400T>C            | p.Leu467Pro  |
| S489X       | c.1466C>A            | p.Ser489*    |
| S492F       | c.1475C>T            | p.Ser492Phe  |
| 1677delTA   | c.1545_1546delTA     | p.Trp515*    |
| V520F       | c.1558G>T            | p.Val520Phe  |
| 1717-1G>A   | c.1585-1G>A          |             |
| 1717-8G>A   | c.1585-8G>A          |             |
| S549R       | c.1645A>C            | p.Ser549Arg  |
| S549N       | c.1646G>A            | p.Ser549Asn  |
| S549R       | c.1647T>G            | p.Ser549Arg  |
| Q552X       | c.1654C>T            | p.Gln552*    |
| A559T       | c.1675G>A            | p.Ala559Thr  |
| 1811+1.6kbA>G | c.1680-886A>G     | p.Ala559Thr  |
| 1812-1G>A   | c.1680-1G>A          |             |
| R560K       | c.1679A>G            | p.Arg560Lys  |
| E585X       | c.1753G>T            | p.Glu585*    |
| 1898+3G>A   | c.1766+3G>A          |             |
| 2184delA    | c.2125C>T            | p.Arg709*    |
| 2209delA    | c.2128A>T            | p.Leu710*    |
| 2184delA    | c.2125C>T            | p.Arg709*    |
| 2184delAT   | c.2128A>T            |             |
| 2184delA    | c.2125C>T            |             |
| 2209delA    | c.2128A>T            | p.Leu710*    |
| 2707insA    | c.2175dupA           | p.Ala559Thr  |
| 2195T>G     | c.2175T>G            | p.Leu732*    |
| 2347delG    | c.2215delG           | p.Val739Ilefs*16 |
| R764X       | c.2290C>T            | p.Arg764*    |
| 2585delT    | c.2453delT           | p.Leu818Trpfs*16 |
| 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*    |
| 2670TG+G>TGA | c.2551C>T          | p.Arg851*    |
| R851X       | c.2551C>T            | p.Arg851*    |
| 2711delT    | c.2583delT           | p.Phe861Leufs*3 |
| S945L       | c.2834C>T            | p.Ser945Lys  |
| 2789+2insA  | c.2657+2_2657+3insA  |             |
| Q890X       | c.2668C>T            | p.Gln890*    |
| L927P       | c.2780T>C            | p.Leu927Pro  |
| 3007delG    | c.2875delG           | p.Ala959His*9 |
| Q970R       | c.2908G>C            | p.Gly970Arg  |
| 3120G>A     | c.2988G>A            |             |

Table 1 Examples of common CF-causing, indetermined, and non-CF-causing variants (modified from5,8,17)
## Table 1 (Continued)

### HGVS nomenclature

| Legacy name | cDNA nucleotide name | Protein name |
|-------------|---------------------|--------------|
| 3121-G>A    | c.2989-1G>A         | p.Leu1065Pro |
| 3199del6 (3195del6) | c.3067_3072delATAAGT | p.Ile1023_Val1024del |
| 3272-26 A>G  | c.3140-26A>G        |              |
| L1065P      | c.3194T>C           | p.Leu1065Pro |
| R1066C      | c.3196C>T           | p.Arg1066Cys|
| R1066H      | c.3197G>A           | p.Arg1066His |
| L1077P      | c.3230T>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(3747G>A) | c.3611G>T        | p.Trp1204*  |
| W1204X(3747G>A) | c.3612G>A          | p.Trp1204*  |
| 3791delC    | c.3659delC          | p.Leu1220LysfsX8 |
| 3849+10kbC>T | c.3718-2477C>T     | p.(?)       |
| G1244E      | c.3731G>A           | p.Gly1244Glu|
| 3876delA    | c.3744delA          | p.Lys1250ArgfsX9 |
| S1251N      | c.3752G>A           | p.Ser1251Asn|
| 3905insT    | c.3773dupT          | p.Leu1258PhefsX7 |
| 4005+1G>A   | c.3873+1G>A         | p.Ser1257PhefsX5 |
| Q1313X      | c.3913C>T           | p.Gln1313*  |
| CFTRdel22,23| c.3964-78_4242+577del| p.(Gly1323_Val1415del) |
| 4209GTT>AA  | c.4077_4080delTGT| |
| 4382delA    | c.4251delA          | p.Glu1418ArgfsX14 |

### Examples of common variants with varying or indelimited clinical consequences

- **R117H**: c.350G>A, p.Arg117His
- **L227R**: c.680T>G, p.Leu227Arg
- **Q359K/T360K**: (c.1075C>A,1079C>A), p.(Gln359Lys, Thr360Gly)
- **L558S**: c.1673T>C, p.Leu558Ser
- **Y569D**: c.1705T>G, p.Thr569Asp
- **D579G**: c.1736A>G, p.Asp579Gly
- **D614G**: c.1841A>G, p.Asp614Glu
- **S977F**: c.2930C>T, p.Ser977Phe
- **F1052V**: c.3154T>G, p.Phe1052Val
- **G1069R**: c.3205G>A, p.Gly1069Arg
- **R1070Q**: c.3209G>A, p.Arg1070Gln
- **D1152H**: c.3454G>C, p.Asp1152His
- **I1234V**: c.3700G>A, p.Ile1234Val
- **5T**: c.1210-12[5]

### Examples of common non CF-causing variants

- **R31C**: c.91C>T, p.Arg31Cys
- **R747W**: c.2240T>C, p.Arg747Trp
- **R75Q**: c.2244G>A, p.Arg75Gln
- **I148T**: c.443T>C, p.Ile148Thr
- **M470W**: c.1408A>G, p.Met470Val
- **G576A**: c.1727G>C, p.Gly576Ala
- **R668C**: c.2002T>C, p.Arg668Cys
- **V754M**: c.2260G>A, p.Val754Met
- **L997F**: c.2991G>C, p.Leu997Pro
- **I1027T**: c.3080T>C, p.Ile1027Thr
- **R1070W**: c.3208C>T, p.Arg1070Trp
- **R1162L**: c.3485T>G, p.Arg1162Leu

---

### Table 1 (Continued)

### HGVS nomenclature

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

**Abbreviation**: HGVS, Human Genome Variation Society.

(CF-causing variants: when paired together, they cause disease. Non CF-causing variants: when paired with a severe variant in cis, they cause 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, 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. 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.)
Heterozygote carrier F508del/normal c.[1521_1523delCTT];
Compound heterozygote (two alleles in trans)
Two variants, phase unknown example 1b
Two variants, phase unknown example 2b
Two heterozygote variants on one allele (in cis)
Compound heterozygote for polyT variants T5/T7
Compound heterozygote for the S T allele and a CF variant
Compound heterozygote for polyTG-T variants and a CF variant

CFTR testing result

No variant detected normal/normal
c.[=];[=]
Heterozygote carrier F508del/normal c.[1521_1523delCTT];[=]
Compound heterozygote (two alleles in trans)
Two variants, phase unknown example 1b
Two variants, phase unknown example 2b
Two heterozygote variants on one allele (in cis)
Compound heterozygote for polyT variants T5/T7
Compound heterozygote for the S T allele and a CF variant
Compound heterozygote for polyTG-T variants and a CF variant

CF-Network EQA—External Quality Assessment process
United Kingdom National External Quality Assessment Service for Molecular Genetics (UKNEQAS)
EuroGentest
HGVS nomenclature
European Molecular Genetics Quality Network (EMQN)
Cystic Fibrosis Mutation Database
CFTR2
Testing guidelines for molecular diagnosis of cystic fibrosis (2008)
Orphanet
OCDE guidelines for quality assurance in molecular genetic testing
University of California Santa Cruz (UCSC) genome browser

Table 3 Main indications for CFTR testing

Newborn screening for CF

Diagnostic testing
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%)

Carrier testing
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).

Prenatal diagnosis, Preimplantation genetic diagnosis, offered to
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

| Parents | France | Italy | Spain | Belgium | Greece | UK | USA |
|---------|--------|-------|-------|---------|--------|----|-----|
| 1 affected+1 carrier S/LS | A | A | A | A | A | A |
| 1 affected+1 carrier M | R | A | A | A | A |
| 1 affected+1 carrier U | A | A | A | R | A | ★ |
| 2 carriers S/LS | A | A | A | A | A |
| 1 carrier S/LS+1 carrier M | R | A | A | A | A | |
| 1 carrier S/LS+1 carrier U | A | A | A | R | ★ | 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 unknown 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 unknown or uncertain clinical relevance, PGD is accepted only in the context of a
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 5'T allele (genotype c.[1210-12T]; [1521_1523delCTTT]) 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, 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 particular difficult situations, it is essential that the diagnostic strategies be shared with the referring clinical geneticist.

As mentioned in the amplification-based PGD guidelines, 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.
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,44 guidelines for accreditation of the PGD laboratory,45 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.

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 (i.e. Italy).

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.
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. 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.

ACKNOWLEDGEMENTS
We acknowledge EuroGentest for funding and all participants of this meeting for fruitful discussions. EuroGentest was funded by the European Commission’s Seventh Framework Program (contract HEALTH-F4-2010-261469 for the EuroGentest2 project). We express our sincere gratitude to Professor Gert Matthys for providing corrections, suggestions and encouragement.
Guidelines for CF-PGD

A Girardet et al

Hariton GL, Magli MC, Lundin K, Montag M, Lemmen J, Harper JC, ESHRE PGD: Consortium/Embryology Special Interest Group - best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Hum Reprod 2011; 26: 41–46.

Beutler E, McKusick VA, Motulsky AG, Scriver CR, Hutchinson F: Mutation nomenclature: nicknames, systematic names, and unique identifiers. Hum Mutat 1996; 8: 203–206.

Den Dunnen JT, Antonarakis SE: Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat 2000; 15: 7–12.

Berwouts S, Morris MA, Giroud E, Schwarz M, Stuhlmann M, Dequeker E: Mutation nomenclature in practice: findings and recommendations from the cystic fibrosis external quality assessment scheme. Hum Mutat 2011; 32: 1197–1203.

Strom CM, Redman JB, Peng M: The dangers of including nonclassical cystic fibrosis variants in population-based screening panels: p.I997T, further genotype/phenotype correlation data. Genet Med 2011; 13: 1042–1044.

Thomas M, Lemonnier L, Guilmans V et al. Is there evidence of correct diagnosis in cystic fibrosis registries? J Cyst Fibros 2014; 13: 275–280.

Claustres M, Kidlich V, Dequeker E et al. Recommendations for reporting results of diagnostic genetic testing (biochemical, cytogenetic and molecular genetic). Eur J Hum Genet 2014; 22: 160–170.

Harper JC, SenGupta S, Vesela K et al. Accreditation of the PGD laboratory. Hum Reprod 2010; 25: 1051–1065.

Deans Z, Fiorentino F, Bircik A et al. The experience of 3 years of external quality assessment of preimplantation genetic diagnosis for cystic fibrosis. Eur J Hum Genet 2013; 21: 800–806.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.