Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Laboratory reporting on the clinical spectrum of CFTR p.Arg117His: Still room for improvement

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ARTICLE INFO

Article history:

Received 14 February 2020

Revised 14 May 2020

Accepted 19 May 2020

Available online xxx

Keywords:

Cystic fibrosis

CFTR-related disorders

External quality assessment

p.Arg117His

ABSTRACT

Background: The clinical spectrum associated with cystic fibrosis transmembrane conductance regulator (CFTR) variant p.Arg117His is highly variable, ranging from full-blown cystic fibrosis (CF) in a small number of cases to CFTR-related disorders (CFTR-RDs) or no symptoms at all. Therefore, taking into account phenotype variability is essential for interpretation. External quality assessment (EQA) schemes can help laboratories to objectively assess the quality of genotyping and reporting by the laboratory.

Methods: We performed a retrospective longitudinal data analysis on laboratory performance regarding the interpretation of p.Arg117His during CF EQA scheme participation. Completeness and accuracy of reporting on two mock clinical cases were each compared over time (case 1: 2005, 2007 and 2012; case 2: 2015 and 2018). These cases concerned subjects compound heterozygous for p.Phe508del and p.Arg117His *in cis* with 7T, but with different clinical backgrounds (family planning (case 1) versus diagnostic testing for a child (case 2)). Furthermore, we analyzed the influence of previous participations, annual test volume, accreditation status and laboratory setting on overall performance.

Results: Overall performance improved over time, except during the 2007 CF EQA scheme. In addition, previous participations had a beneficial effect on laboratory performance. Accreditation status, annual test volume and laboratory setting did not significantly influence total interpretation scores.

Conclusions: In general, laboratories performed well on both cases, although reporting on the variable clinical spectrum of p.Arg117His *in cis* with 7T and on the disease liability of individual CFTR variants can still improve. Moreover, this study underlined the educational role of CF EQA schemes.

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1. Introduction

Cystic fibrosis (CF) is a common autosomal recessive disorder in Caucasian populations, caused by pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Currently, more than 2000 CFTR variants have already been identified [1].

CFTR variant LRG_663t1: c.350G>A (p.Arg117His, traditional name: R117H) is a missense variant in exon 4, leading to conductance (class IV) as well as CFTR gating (class III) defects [2,3]. It has an estimated allele frequency of 0.3% in Caucasian populations [1].

Clinical presentation among individuals who are compound heterozygous for p.Arg117His and a CF-causing variant can vary from full-blown CF to CFTR-related disorders (CFTR-RDs) or no symptoms at all [4–8]. According to Bombieri *et al.*, a CFTR-RD is defined as “a clinical entity associated with CFTR dysfunction that does

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not fulfill the diagnostic criteria for CF", mainly congenital bilateral absence of the vas deference (CBAVD), acute recurrent or chronic pancreatitis and disseminated bronchiectasis [8]. The phenotype is highly dependent on the length of the polythymidine (poly-T) tract (LRG_663t1: c.1210-12T[5_9]) in intron 9 *in cis* [9,10]. A p.Arg117His allele on a 5T background is considered CF-causing if accompanied by a CF-causing variant *in trans* and p.Arg117His *in cis* with 7T is regarded as a variant of varying clinical consequence (VVCC) by CFTR2 [11]. In the CFTR-France database, p.Arg117His is reported as a CFTR-RD-causing variant [12]. The majority of individuals carrying the latter allele do not develop CF, although male infertility and severe pulmonary disease have been reported among these patients [4–8,13–15]. Thus, for patients and healthy individuals in whom p.Arg117His has been detected, reflex poly-T analysis should always be carried out.

Due to uncertainty on the clinical impact of p.Arg117His, its identification may lead to prognostic, diagnostic and therapeutic challenges for clinicians, as well as fear and uncertainty among patients and their family. In addition, p.Arg117His *in cis* with 7T is highly prevalent among cystic fibrosis screen positive, inconclusive diagnosis (CFSPID) children, also known as CFTR-related metabolic syndrome (CRMS) in the United States [16,17]. Moreover, CFTR-targeted therapies have become available for some CF patients depending on their genotype. For example, CF patients carrying at least one p.Arg117His allele are eligible for treatment with ivacaftor (Kalydeco®, Vertex Pharmaceuticals) [18]. Therefore, correct CFTR analysis and accurate interpretation are key features for molecular genetics laboratories.

External quality assessment (EQA) schemes, also known as proficiency testing, can help laboratories assess the quality of CFTR testing and interpretation within their laboratory over time, as well as compare their own performance to that of other laboratories. For accreditation according to International Organization for Standardization (ISO)/ International Electro-technical Commission (IEC) 17025 (testing and calibration laboratories) or ISO 15189 (medical laboratories), laboratories have to participate in EQA schemes on a regular basis [19,20]. Previous studies regarding molecular reporting for CF during EQA scheme participation indicated that there is still room for improvement [21–23].

2. Materials and methods

2.1. Study setting

For more than 20 years, the CF Network has organized CF EQA schemes annually, according to international standards, for which laboratories worldwide can enroll [24,25]. Therefore, the CF Network has access to a large database of molecular genetics laboratories. To respect laboratories' anonymity, each participant was assigned a unique laboratory- and year-specific identification number. The scheme course went as follows: after registration, participants received samples of extracted DNA, accompanied by mock clinical cases. Participants were requested to analyze and report on these samples as if it were real patients in routine practice. Laboratories had about eight weeks to submit reports and raw data. Reports were then scored on correctness and completeness regarding genotyping, interpretation and clerical accuracy. Interpretation was assessed based on well-defined interpretation criteria established by a group of international assessors, all experts in the field of molecular testing for CF, according to best practice guidelines and relevant literature [26–28]. Scores were given by two assessors independently. Afterwards, results were compared and discussed during an assessment meeting. At the end of the scheme course, participants received personalized feedback on their performance.

2.2. Study design

Similar mock clinical cases were reintroduced in the CF EQA schemes periodically in order to evaluate inter- and intra-laboratory performance over time. We conducted a retrospective longitudinal data analysis on the interpretation of p.Arg117His. We selected two similar mock clinical cases with the same genotype but with a different clinical background, i.e. a patient who is compound heterozygous for LRG_663t1: c.1521_1523del (p.Phe508del, traditional name: F508del), a CF-causing variant, and p.Arg117His *in cis* with 7T, which is now considered as a VVCC or CFTR-RD-causing variant with a very low risk of CF with a high potential for misinterpretation in analysis reports [11,12]. The first case involved an adult patient referred for CFTR testing in the context of family planning (2005, 2007 and 2012) (Table 1). The second case concerned a young child presenting with intermediate sweat test results, for which laboratories were requested to carry out diagnostic CFTR testing (2015 and 2018) (Table 1).

Reports were initially assessed by two assessors separately based on the presence and correctness of well-defined interpretation criteria. We compared individual scores on three interpretation criteria for case 1 and seven for case 2 (Table 2). These criteria were selected based on importance and comparability between the different scheme years. Since interpretation criteria "Variant p.Phe508del is a CF-causing variant" and "Variant p.Arg117His *in cis* with 7T is associated with CFTR-RD and highly unlikely to cause CF" were not assessed independently during the 2015 CF EQA scheme, we reevaluated reports of 2015 for these items. Afterwards, results were converted to specific points per interpretation criterion and added as total interpretation score, with a maximum total score of 2.00/2.00 for each mock clinical case. Points were allocated when the criterion was present and correctly mentioned, as well as when it was present but could not be clearly identified in the report. No points were assigned when the item was incorrectly reported or not present in the report.

Since for this study we only focused on interpretation, we excluded laboratories that did not submit reports or made genotyping errors, i.e. p.Arg117His or p.Phe508del was not correctly identified. Results from laboratories that did not test or wrongly reported the length of the poly-T tract were also excluded. To determine if the poly-T tract was indeed analyzed, reports and raw data uploaded by participants were checked on applied testing method(s) and correctness of reporting. We consulted manuals of commercial assays online to assess the extent of the test panel. In addition, we evaluated the influence of accreditation status of the laboratory, laboratory setting, average test volume and number of previous participations on overall performance. Previous participations were defined as enrollments in the CF Network EQA scheme years during which p.Arg117His *in cis* with 7T was introduced (2005, 2007, 2012 or 2015) [24]. During each EQA course, participants were requested to provide additional information regarding accreditation status, laboratory setting and average amount of CFTR tests performed in their laboratories (test volume) in an online questionnaire. Since there is often confusion on the term "accreditation", we validated this feature by consulting the websites of national accreditation bodies in November 2018. Examples of accepted accreditation standards included ISO/IEC 17025 and ISO 15189 [19,20]. The laboratory setting was confirmed by visiting the website of each laboratory. Industrial laboratories were considered laboratories involved in the development of diagnostic commercial assays. Independent non-industrial laboratories consisted of laboratories that are not located within a hospital's infrastructure. Meanwhile hospital laboratories encompassed laboratories associated with private or public hospitals, and lastly, university and research laboratories referred to laboratories of university hospitals or research centers. Annual test volumes, i.e. the average amount

Table 1

Overview of mock clinical cases per CF EQA scheme year.

Year	Patient	Case description
Case 1		
2005	Harriet Carter 02/07/1979 Leicester, United Kingdom	Harriet Carter is planning a pregnancy and would like to know her carrier status, because her brother, Mark Carter, is infertile because of CBAVD. He is thought to have been tested for <i>CFTR</i> variants, although results are not available. Harriet and Mark are from the United Kingdom.
2007	Lasse Lindgren 18/02/1972 Bullerbyn, Sweden	Lasse Lindgren and his wife Lotta Langstrop want to undergo assisted reproduction, because of male infertility. Andrological examinations of Lasse Lindgren had revealed azoospermia and CBAVD. There are no known cases of CF in Lasse's family. Lotta Langstrop is healthy but her sister is affected with CF. From previous molecular testing in another laboratory, it is known that Lotta is heterozygous for the <i>CFTR</i> variant LRG_663t1: c.262_263del (p.Leu88Ilefs*22, traditional name: 394delTT). Your laboratory receives a DNA sample from Lasse Lindgren for molecular testing of the <i>CFTR</i> gene, prior to ICSI/assisted reproduction. The referring clinician asks you to comment on possible health implications for Lasse and his offspring.
2012	Elisa Pennec 10/03/1986 Brittany, France	CF carrier testing is requested for Elisa Pennec who is a healthy woman and whose partner, Erwan Pennec, has a nephew diagnosed with CF at birth. Erwan Pennec was found to be heterozygous for LRG_663t1: c.1521_1523del (p.Phe508del, traditional name: F508del) in your laboratory. The couple is planning a pregnancy and would like to know their risk of having a child with CF. There is no history of CF in Elisa's family, but she knows that her brother is followed for infertility. Elisa and Erwan are both from Brittany.
Case 2		
2015	Jolene Belgard 20/07/2010 Poitiers, France	Jolene is a five-year-old girl suspected of having CF based on two episodes of bronchiolitis and an intermediate sweat test value. There is no family history of CF and molecular testing is requested to confirm the diagnosis of CF. Her parents are from France.
2018	Isabelle Girac 02/01/2018 Clermont Ferrand, France	Isabelle Girac is a one-month-old child screened positive to CF newborn screening. She was found to be heterozygous for the variant for LRG_663t1: c.1521_1523del (p.Phe508del, traditional name: F508del) (analysis performed with the CF30v2 kit, Elucigene). The sweat test gave intermediate results. The child is growing well and has no signs of CF disease. The parents are of French origin and there is no history of CF in the family. Additional information sent by the physician on 06/02/2018: Isabelle Girac has had a bronchitis the last two weeks; please can you perform an additional molecular test to the screening test.

CFTR: LRG_663. Abbreviations: CBAVD, Congenital Bilateral Absence of the Vas Deferens; CF, Cystic Fibrosis; *CFTR*, Cystic Fibrosis Transmembrane conductance Regulator; ICSI, Intracytoplasmic Sperm Injection; LRG, Locus Reference Genomic.

Table 2

Scoring of specific interpretation criteria.

Interpretation criteria	Score
Case 1	
a) Comment on variable phenotype associated with p.Phe508del/p.Arg117His <i>in cis</i> with 7T	0.67
b) Suggesting/Offering cascade screening for p.Phe508del in relatives	0.67
c) Suggesting/Offering genetic counselling	0.67
Case 2	
a) Variant p.Phe508del is a CF-causing variant	0.25
b) Variant p.Arg117His <i>in cis</i> with 7T is associated with <i>CFTR</i> -RD and highly unlikely to cause CF	0.50
c) No confirmation of CF, <i>CFTR</i> -RD/CF cannot be ruled out	0.50
d) Suggesting/Offering confirmation analysis in the parents	0.25
e) Suggesting/Offering cascade screening for p.Phe508del in relatives	0.25
f) Suggesting/Offering genetic counselling	0.25
g) Inappropriately mentioning or offering PGD/PND in case only one CF-causing variant was found (without mentioning any further specifications: e.g. it can be done in case a 2 nd CF-causing variant is found):	-0.50
- Consider p.Arg117His as a CF risk allele	
- Mentioning or offering PGD/PND for p.Arg117His <i>in cis</i> with 7T	

CFTR: LRG_663. Abbreviations: CF, Cystic fibrosis; *CFTR*-RD, *CFTR*-Related Disorder; PGD, Preimplantation Genetic Diagnosis; PND, Prenatal Diagnosis.

of *CFTR* tests conducted by the laboratory a year, were not validated further.

2.3. Statistical analysis

Generalized linear models were applied for data analysis with estimation based on generalized estimating equations (GEEs) to account for clustering in the data. Logistic regression was used for the analysis of the specific criteria as binary outcomes, and proportional odds models for total interpretation scores as ordinal outcome. Results are presented by odds ratios (ORs) with 95% confi-

dence intervals [95% CI]. A *p*-value of <0.05 was considered statistically significant. Analyses have been performed using SAS software (version 9.4 of the SAS System for Windows).

3. Results

3.1. Study population

We included data of 320 laboratories from 41 different countries. Of these laboratories, 98% (314/320) offered *CFTR* analysis to referring clinicians and patients. More details on laboratory char-

Table 3

Statistical results on laboratory performance over time for case 1 and 2.

Interpretation criteria	OR [95% CI]	p-value
Case 1:		
a) Comment on variable phenotype associated with p.Phe508del/p.Arg117His <i>in cis</i> with 7T	NA	<0.0001
b) Suggesting/Offering cascade screening for p.Phe508del in relatives	NA	<0.0001
c) Suggesting/Offering genetic counselling	NA	0.0015
Total interpretation score	NA	<0.0001
Case 2:		
a) Variant p.Phe508del is a CF-causing variant	1.07 [0.68; 1.68]	0.7800
b) Variant p.Arg117His <i>in cis</i> with 7T is associated with CFTR-RD and highly unlikely to cause CF	1.56 [0.87; 2.79]	0.1326
c) No confirmation of CF, CFTR-RD/CF cannot be ruled out	1.50 [0.91; 2.48]	0.1116
d) Suggesting/Offering confirmation analysis in the parents	1.29 [0.57; 2.94]	0.5415
e) Suggesting/Offering cascade screening for p.Phe508del in relatives	0.72 [0.42; 1.24]	0.2383
f) Suggesting/Offering genetic counselling	1.98 [0.36; 10.87]	0.4331
g) Inappropriately mentioning or offering PGD/PND in case only one CF-causing variant was found	0.78 [0.45; 1.36]	0.3853
Total interpretation score	1.20 [0.82; 1.76]	0.3416

CFTR: LRG_663. A p-value of <0.05 was considered statistically significant. Abbreviations: CF, Cystic Fibrosis; CFTR-RD, CFTR-Related Disorder; [95% CI], [95% Confidence Interval]; NA, Not Applicable; PGD, Preimplantation Genetic Diagnosis; PND, Prenatal Diagnosis; OR, Odds Ratio.

acteristics per scheme year are described in Supplemental table S1. We included reports of respectively 261 and 223 laboratories for case 1 and 2, some of which participated in multiple scheme years. After exclusion of laboratories that did not submit reports, made genotyping errors or reported incomplete genotyping results, 151 (of 205 registered participants in 2005), 193 (of 220 registered participants in 2007) and 182 (of 219 registered participants in 2012) reports were retained for further analysis for the first case. Of these laboratories, 34% (88/261) provided one, 31% (81/261) two and 35% (92/261) three reports for the CF EQA schemes of 2005, 2007 and 2012. For the second case, we selected data of respectively 178 and 174 from the original 197 and 192 registered participants in 2015 and 2018. A total of 129 laboratories provided two reports, and only one report was taken into account for 94 laboratories.

3.2. Case 1

Overall performance (average total score in 2005=1.38/2.00, 2007=1.32/2.00 and 2012=1.85/2.00) improved significantly over time for **Case 1** ($p<0.0001$, Table 3). By pairwise comparing results between different scheme years, we observed an improvement in total score for 2012 in comparison to 2005 (OR=8.13 [5.18; 12.66]; $p<0.0001$) and 2007 (OR=9.90 [6.25; 15.63]; $p<0.0001$). During the 2007 CF EQA scheme, average total scoring slightly declined compared to 2005, but nonetheless not statistically significant (OR=0.82 [0.59; 1.13]; $p=0.2278$). Results regarding the presence and correctness of specific interpretation criteria in the reports over time are shown in Fig. 1(A). For case 1, more laboratories reported correctly on the variable clinical spectrum in 2012 compared to 2005 (OR=2.21 [1.19; 4.10]; $p=0.0117$) and 2007 (OR=8.85 [5.00; 15.63]; $p<0.0001$), although results on this interpretation criterion were significantly worse in 2007 than in 2005 (OR=0.25 [0.16; 0.40]; $p<0.0001$). Interpretation criterion "Suggesting/offering cascade screening for p.Phe508del in relatives" was more often reported in 2012 than in 2005 (OR=13.51 [7.69; 23.81]; $p<0.0001$) and 2007 (OR=6.58 [3.77; 11.49]; $p<0.0001$), as well as "Suggesting/offering genetic counselling" in 2012 compared to 2005 (OR=4.72 [2.02; 10.99]; $p=0.0003$) and 2007 (OR=4.07 [1.72; 9.62]; $p=0.0015$). Furthermore, overall performance of laboratories that already participated in two previous scheme years scored better for case 1, compared to laboratories that never participated before (OR=3.02 [1.35; 6.76]; $p=0.0070$).

3.3. Case 2

In general, participants of the 2018 CF EQA scheme (average total score=1.65/2.00) scored better than those in 2015 (average to-

Fig. 1. Performance on reporting specific interpretation criteria per scheme year. (A) Case 1. Interpretation criterion a, Comment on variable phenotype associated with p.Phe508del/p.Arg117His *in cis* with 7T; b, Suggesting/Offering cascade screening for p.Phe508del in relatives; c, Suggesting/Offering genetic counselling. (B) Case 2. Interpretation criterion a, Variant p.Phe508del is a CF-causing variant; b, Variant p.Arg117His *in cis* with 7T is associated with CFTR-RD and highly unlikely to cause CF; c, No confirmation of CF, CFTR-RD/CF cannot be ruled out; d, Suggesting/Offering confirmation analysis in the parents; e, Suggesting/Offering cascade screening for p.Phe508del in relatives; f, Suggesting/Offering genetic counselling; g, Inappropriately mentioning or offering PGD/PND in case only one CF-causing variant was found. Abbreviations: CF, cystic fibrosis; CFTR-RD, CFTR-Related Disorder; PGD, Preimplantation Genetic Diagnosis; PND, Prenatal Diagnosis.

tal score=1.57/2.00), but there was no significant effect measured (OR=1.20 [0.82; 1.76]; $p=0.3416$). Laboratories with a higher number of previous participations also performed better for this case (per extra previous participation: OR=1.24 [1.07; 1.44]; $p=0.0043$).

We did not observe a significant difference in scoring results for specific interpretation criteria between the two scheme years (Table 3, Fig. 1(B)).

3.4. Influence of laboratory characteristics on overall performance

Total scores of accredited laboratories did not differ significantly compared to those of non-accredited laboratories, neither for case 1 (OR=1.26 [0.88; 1.82]; $p=0.2102$), nor for case 2 (OR=1.18 [0.78; 1.79]; $p=0.4227$). Overall performance of laboratories that conduct more *CFTR* tests a year was not significantly better than laboratories that analyze on average less samples a year (case 1: OR=1.06 [0.96; 1.18]; $p=0.2621$; case 2: OR=1.00 [0.88; 1.14]; $p=0.9964$). The laboratory setting did not influence laboratory performance (case 1: $p=0.3146$; case 2: $p=0.2031$). See Supplemental Table S2 for more details.

4. Discussion

Data of a broad range of molecular genetics laboratories were included in this study. Since the CF Network is a European organization, the majority of the participating laboratories (89% (286/320)) are located in Europe [24]. In the first case, we mainly focused on p.Arg117His *in cis* with 7T in the context of male infertility. In the second case, the role of p.Arg117His on a 7T background in children with intermediate sweat test results was covered. Since the scheme years regarding case 1 already date from some years ago, it could be possible that laboratory interpretation on this case would differ today [5]. For case 2, more recent data were included. Nevertheless, it should be taken into account that laboratories may adapt their reporting during EQA participation in order to meet EQA recommendations on laboratory reporting. Therefore, reporting during EQA scheme participation could differ from reporting in routine practice. Although striving for maximum harmonization, fluctuation in rigorosity of evaluating between assessors as well as within the same assessors over time could be possible. In addition, with evolving knowledge on the genotype, EQA recommendations for p.Arg117His *in cis* with 7T slightly changed over time [5]. Since this variant is relatively frequent among asymptomatic individuals, it is now recommended not to suggest preimplantation genetic diagnosis (PGD) and prenatal diagnosis (PND), nor cascade screening for this variant.

4.1. Laboratory performance over time

With the exception of the 2007 CF EQA scheme, average total interpretation scores improved over time. This can be due to enhanced knowledge of this genotype through publications and best practice guidelines, as well as experience gained during previous EQA scheme participation or in routine practice. For case 2, we did not find a statistically significant improvement in overall scoring. This could be due to shorter time span (three years), compared to case 1 (seven years). In general, laboratories performed better on case 2 than case 1. However, we could not determine if this was due to time (improved knowledge or adaptation to EQA scheme requirements) or case difficulty. The contextual differences between the original mock clinical cases in 2007 in contrast to those in 2005 and 2012 could explain the decline in scoring in 2007. During the CF EQA scheme of 2007, the tested individual himself presented with male infertility due to CBAVD. The mock clinical cases in 2005 and 2012 on the other hand involved an asymptomatic female individual with a family history of male infertility. Participants in 2007 mainly focused on the association of genotype p.Phe508del/p.Arg117His on a 7T background with CBAVD and on the offspring's risk for having CF. Nonetheless, many participants

did not further discuss additional health implications for the tested individual.

4.2. Reporting of specific interpretation criteria

The elected interpretation criteria in this study represent important elements in laboratory reporting for the particular mock clinical cases. In 2012, laboratories reported better on all interpretation criteria compared to 2005 and 2007. Especially discussing the variable clinical spectrum of p.Phe508del/p.Arg117His on a 7T background was worse in 2007 compared to 2005 and 2012. Besides the difference in original mock clinical cases, another contributing factor for this could be the slight difference in phrasing of the original interpretation criteria in the specific scheme years. As such, the focus in 2007 was mainly on discussing health implications and further clinical evaluation of the patient, compared to only reporting on the variable clinical spectrum in 2005 and 2012. For case 2, there was a trend of improved scoring on all interpretation criteria, except for "Suggesting/Offering cascade screening for p.Phe508del in relatives", although this improvement was not statistically significant. In addition, still 23% (40/174) of the laboratories did not clearly mention the disease liability of p.Phe508del in their report and 11% (20/174) did not comment on p.Arg117His *in cis* with 7T being a *CFTR*-RD-causing and highly unlikely to cause CF during the 2018 CF EQA scheme. The disease liability of individual *CFTR* variants should be clearly mentioned in the report since this has implications for follow-up of the tested individual as well as for relatives. In addition, molecular genetics laboratories may not rely on good knowledge of *CFTR* variants among referring clinicians. The use of online databases like *CFTR2* and *CFTR-France*, as well as the published literature, are indicated for this matter [11,12]. Furthermore, 18% (32/174) of the laboratories participating in the 2018 CF EQA scheme (case 2) did not clearly state that the tested child was unlikely to have CF and that this particular genotype can be associated with *CFTR*-RD. In case of p.Arg117His *in cis* with 7T, laboratories should indicate that most individuals will not develop CF and that some will have *CFTR*-RDs [4–8,13–15]. Due to uncertainty and very low risk of having CF, PGD and PND are not indicated for p.Arg117His *in cis* with 7T.

4.3. Educational role of EQA schemes

Similar to Berwouts *et al.*, we did not observe any effect of accreditation status, test volume and laboratory setting on laboratory performance [21]. Although we found no significant effect of accreditation on laboratory performance, previous longitudinal studies in biomarker analysis for colon and lung cancer showed that accredited laboratories performed better in EQA schemes in the first years after biomarker implementation, but this effect stopped over time [29]. Since most laboratories have a long experience in *CFTR* analysis, our results seem to be in concordance with these data. Furthermore, laboratories that previously participated in CF EQA scheme years in which p.Arg117His was introduced, performed better than laboratories that enrolled less often in these schemes. This highlights the role of EQA schemes in continuous learning.

5. Conclusions

In general, laboratories reported well on mock clinical cases containing p.Arg117His *in cis* with 7T, although the knowledge on the clinical significance of this *CFTR* variant can still be improved. Laboratories should clearly indicate that p.Arg117His *in cis* with 7T can be associated with *CFTR*-RD, but is highly unlikely to cause CF. In addition, laboratories should clearly address the disease liability of each *CFTR* variant identified during testing separately, since this

could have implications for further investigations for the tested individual, as well as for relatives. Furthermore, this study highlights the educational role of EQA schemes for all types of molecular genetics laboratories performing *CFTR* analyses.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

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Acknowledgements

We would like to thank Dr. Sarah Berwouts, Dr. Marie des Georges, Valentina Giannone, Dr. Christof Meyer-Kleine, Dr. Martin Schwarz, Prof. Manfred Stuhmann and Dr. Véronique Tack for thoroughly assessing reports and Dr. Annouschka Laenen, from the Leuven Biostatistics and Statistical Bioinformatics Centre, for the statistical support. We also like to pay gratitude to the laboratories that participated in the CF EQA schemes, for their dedication to aim at providing the highest quality of testing for patients. Finally, we thank Romy Gentens and Dr. Inne Nauwelaers, from the Biomedical Quality Assurance Research Unit, for the administrative assistance and for carefully reading this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.jcf.2020.05.005](https://doi.org/10.1016/j.jcf.2020.05.005).

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