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ORIGINAL ARTICLE

CFTR p.Arg117His associated with CBAVD and other CFTR-related disorders

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ABSTRACT

Background The high frequency of the cystic fibrosis (CF) transmembrane conductance regulator (*CFTR*) gene mutation p.Arg117His in patients with congenital bilateral absence of the vas deferens (CBAVD) and in newborns screened for CF has created a dilemma.

Methods Phenotypic and genotypic data were retrospectively collected in 179 non-newborn French individuals carrying p.Arg117His and a second *CFTR* mutation referred for symptoms or family history, by all French molecular genetics laboratories, referring physicians, CF care centres and infertility clinics.

Results 97% of the patients had the intronic T7 normal variant in *cis* with p.Arg117His. 89% patients were male, with CBAVD being the reason for referral in 76%. In 166/179 patients with available detailed clinical features, final diagnoses were: four late-onset marked pulmonary disease, 83 isolated CBAVD, 67 other CFTR-related phenotypes, including 44 CBAVD with pulmonary and/or pancreatic symptoms and 12 asymptomatic cases. Respiratory symptoms were observed in 30% of the patients, but the overall phenotype was mild. No correlation was observed between sweat chloride concentrations and disease severity. Five couples at risk of CF offspring were identified and four benefited from prenatal or preimplantation genetic diagnoses (PND or PGD). Eight children were born, including four who were compound heterozygous for p.Arg117His and one with a severe CF mutation.

Conclusions Patients with CBAVD carrying p.Arg117His and a severe CF mutation should benefit from a clinical evaluation and follow-up. Depending on the CBAVD patients' genotype, a *CFTR* analysis should be considered in their partners in order to identify CF carrier couples and offer PND or PGD.

INTRODUCTION

Male infertility with congenital bilateral absence of the vas deferens (CBAVD) is mainly a disorder related to cystic fibrosis (CF) transmembrane conductance regulator (*CFTR*) gene mutations. Around 1900 *CFTR* mutations, involved in a broad range of phenotypes including CF[1] and CFTR-related disorders (CFTR-RD),[2] have been reported so far. The diagnosis of CF is based on consensus clinical and laboratory criteria,[3] which include positive sweat tests (chloride value ≥60 mEq/l), and/or the presence of two CF-causing mutations. In CFTR-RD, there is evidence of CFTR dysfunction, but the diagnostic criteria for CF are not met. This is also the case for CBAVD patients,[2 4] most of whom carry one CF mutation combined with a so-called CFTR-RD associated mutation, which has a milder effect and is not observed in classical CF, or two CFTR-RD mutations. The wide phenotypic expression is mainly due to the *CFTR* mutation and genotype heterogeneity (http://www.genet.sickkids.on.ca/cftr), but disease variability, especially in the pulmonary disease, is also observed in patients carrying the same *CFTR* genotype. Genetic counselling may be particularly delicate in CBAVD patients who request assisted reproduction, as they may carry mutations with a wide range of effects.

The c.350G>A (p.Arg117His, R117H) mutation, though initially described in CF patients,[5] has been considered predominantly associated with CBAVD.[6–9] Phenotypic variability was mostly attributed to the presence of a polymorphic intronic polyT variant, c.1210-12T(5) or T(7) in *cis* with p.Arg117His (ie, T5 or T7 on the same chromosome as p.Arg117His). While the T7 variant is considered neutral, the T5 variant in *cis* with p.Arg117His has been associated with a more severe outcome.[10 11] The recent observation of a high frequency of p.Arg117His in newborns screened for CF has reinforced diagnostic and genetic counselling dilemmas,[12] even for CBAVD couples because of reproductive concerns. Epidemiological data from a large collaborative French survey on this mutation have already identified very low penetrance of classical CF in individuals carrying p.Arg117His in *cis* with T7 and the most frequent CF mutation, c.1521_1523del (p.Phe508del, F508del), with penetrance estimated at 0.03%.[13]

The aim of the present study was to focus on and detail phenotype and genotype data in CBAVD patients who carried two *CFTR* mutations with p.Arg117His on at least one allele, and to document the outcomes of assisted reproduction techniques (ART), and prenatal and preimplantation diagnoses (PND and PGD) for the couples, with a view to improving practices for follow-up and genetic counselling.

SUBJECTS AND METHODS

Clinical and genotype-phenotype correlation studies

A retrospective cohort study was initiated by the French CF laboratory network to collect phenotype and genotype data of all individuals with two *CFTR* mutations including p.Arg117His on at least one allele, identified from 1990 until 1 January 2008. *CFTR* mutations in *trans* of p.Arg117His (ie, on the other chromosome) were classified by their potential for causing disease (A: CF-causing; B: associated with CFTR-RD; C: no clinical consequences; D: unknown or uncertain clinical relevance).[2 4] Genotype data also included the polyT variant at c.1210–12T(5_9). The study was approved by the national ethics committee and the French Association for Newborn Screening.

Clinical details were provided by the referring physicians through standardised forms and included: gender; date of birth; age and reason for referral (classical CF presentation, respiratory symptoms, chronic sinus disease, pancreatitis, CBAVD, fetal bowel abnormalities, positive family history or CF newborn screening (CFNBS)); clinical features during the follow-up period with sputum cultures, pulmonary function tests (PFT), presence of disseminated bronchiectasis as assessed by CT scan and evaluation of pancreatic function. In adult male patients, clinical details about fertility and sterility were provided through specific forms and included data on clinical examinations, pelvic ultrasound (endorectal and testicular), hormone tests, standard chromosomal analysis and spermogram. Details about in vitro fertilisation were also collected and included intracytoplasmic sperm injection (ICSI), number of cycles, fresh or frozen embryos, pregnancy and liveborn children. Data on genetic counselling sessions, PND and PGD were also recorded.

Sweat testing was performed at least twice, mainly by sweat chloride determination with the Gibson and Cooke pad technique or conductivity.[14 15] Only the results of chloride concentrations were considered for frequency calculations (indicative of CF: ≥60 mEq/l; borderline: 40–59 mEq/l)[3] and the most elevated chloride values were recorded. Individuals with no detailed clinical information were excluded from the genotype-phenotype correlation studies.

Statistical analysis

Associations between *CFTR* genotypes and clinical features associated with a diagnosis of CF or CFTR-RD were studied. Since the occurrence of many features increases with age, we used the Kaplan–Meier method to estimate the cumulative probability of observing clinical features at 30 years of age.

Clinical differences between patients according to the classification of the in *trans* mutation were tested using the non-parametric log-rank test.

Stata software V.10 (StataCorp, College Station, Texas, USA) was used for all statistical analyses. Only p values <0.01 were considered significant.

Mutation nomenclature

Mutation names were given according to the recommendations of the Human Genome Variation Society, together with a reference to the traditional names at their first occurrence.

RESULTS

A total of 278 individuals with two *CFTR* mutations including at least one p.Arg117His were collected. These included 99 newborn screened (NBS) children (48 male (48%) and 51 female (52%); median age at last follow-up: 21 months; IQR (6–40)) and 179 individuals who were not identified through CFNBS (non-NBS individuals) (160 males (89%) and 19 (11%) females; median age at *CFTR* analysis: 31 years; IQR (27–36)). In order to accurately document genotype-phenotype correlations, especially in CBAVD patients, and because of limited follow-up in NBS children, we focused on the 179 non-NBS individuals.

The overall cohort

Genotype description

In the 278 individuals, 44 different mutations were associated with p.Arg117His. CF mutations (class A) were present in *trans* of p.Arg117His in nearly 90% of individuals, including c.1521_1523del (p.Phe508del, F508del) in 121 individuals (67%). The other mutations consisted of c.3846G>A (p.Trp1282*, W1282X) (2%), c.1624G>T (p.Gly542*, G542X) (2%), c.262_263del (394delTT) (2%), c.1585–1G>A (1717–1G>A) (2%) and other mutations (25%). One individual was p.Arg117His homozygous. No significant clinical difference was noticed between patients according to the mutation in *trans* of p.Arg117His.

Among the 172 patients with a documented polyT variant, only five carried T5 in *cis* with p.Arg117His, two males and three females, including one sibship (see online supplementary data table A). Two of them presented with severe disseminated bronchiectasis, one with isolated CBAVD, one with bronchial hyperreactivity, and one had no symptoms. All the other patients had the T7 normal variant.

Phenotype description

In the 179 non-NBS individuals, a *CFTR* gene analysis was performed because of CBAVD (136/179; 76%), respiratory symptoms (18/179; 10%), family history (15/179; 8%), pancreatic disease (5/179; 3%), or other reasons (digestive symptoms or antenatal abnormalities) (5/179; 3%). Clinical data were available in 166/179 patients (table 1). Median age at last follow-up was 31.5 years (IQR (27.1–35.7)). Only one individual died at 71 years of age because of pulmonary complications and chronic hepatitis. The final diagnoses at the time of analysis were: four cases of late-onset marked pulmonary disease, 83 isolated CBAVD, 67 other CFTR-RD with no or mild pulmonary disease and 12 asymptomatic cases.

Thirty per cent of patients presented with pulmonary symptoms, and we estimated the probability of developing respiratory symptoms at 30 years of age at 29% (table 1). PFT available in 21/46 pulmonary symptomatic individuals showed median Forced Expiratory Volume in 1 s (FEV_1) at 104.5% of the

Table 1 Probability of the occurrence of clinical features at a specific age in 166 compound heterozygous individuals carrying p.Arg117His

	Total cohort n=166			CBAVD patients n=125		
	No. of affected cases/no. of patients with available data	Frequency (%)	Probability of occurrence of clinical features (%)* at 30 years (99% CI)	No. of affected cases/no. of patients with available data	Frequency (%)	Probability of occurrence of clinical features (%)* at 30 years (99% CI)
Pulmonary symptoms	46/151	30	29 (20 to 40)	27/118	23	24 (11 to 31)
Asthma/bronchial hyper-reactivity	16/151	11	11 (6 to 20)	11/112	10	10 (5 to 20)
Disseminated bronchiectasis	11/150	7	6 (3 to 15)	1/112	1	0 Na
Nasopharyngeal symptoms	27/145	19	19 (12 to 30)	17/108	16	15 (8 to 27)
Chronic sinusitis	20/145	14	15 (9 to 25)	11/108	10	10 (5 to 21)
Nasal polyposis	10/145	7	7 (3 to 17)	8/108	7	6 (2 to 17)
Pancreatic symptoms	11/136	8	8 (3 to 19)	6/101	6	2 (1 to 16)
Pancreatic insufficiency	3/135	2	2 (0 to 10)	2/101	2	1 (0 to 14)
Chronic pancreatitis	0/135	0	0 na	0/101	0	0 Na
Acute pancreatitis	7/136	5	4 (1 to 13)	4/101	4	2 (0 to 11)
Staphylococcus aureus positive sputum cultures	16/59	27	24 (12 to 47)	4/30	13	14 (3 to 49)
Pseudomonas aeruginosa positive sputum cultures	4/59	7	2 (0 to 20)	1/30	3	0 Na

*Results are Kaplan–Meier estimates.
CBAVD, congenital bilateral absence of the vas deferens; na, not applicable.

predicted value (IQR (72–111)), and Forced Vital Capacity at 101% (IQR (79–110)) at a median age of 28.4 years (IQR (15–39)). Four of 11 individuals with disseminated bronchiectasis presented with a severe pulmonary disease in adulthood, as evaluated by PFT (FEV_1 <60%) at 30, 33, 65 and 80 years of age. Pulmonary transplantation was performed at 45 years of age in one case (see online supplementary data table B).

Nasopharyngeal or ear-nose-throat symptoms were present in 19% of patients with documented data and mainly consisted of chronic sinusitis and/or nasal polyposis (table 1).

Among the 11 individuals with pancreatic symptoms, three presented with pancreatic insufficiency (table 1; see online supplementary data table C).

Eighty-three of the 166 patients (50%) presented with isolated CBAVD at a median age at last follow-up of 31.6 years (IQR (28.2–34.7)). By contrast, four other men without CBAVD spontaneously fathered children: three carried a severe CF mutation and one had a mild, CFTR-RD mutation in *trans* of p.Arg117His. As evidence of their paternity, in each case, the child had inherited a paternal mutation. These four individuals were mostly diagnosed because of their family history.

Twelve of the 166 (7%) individuals remained totally asymptomatic, but their median age at last follow-up was low, and they were mainly identified through their family history (8.1 years; IQR (5.6–40.1)).

Sweat testing was available in 85/166 individuals (see online supplementary data table D). The median sweat chloride value was 73 mEq/l (IQR (52–90)): 76/85 (89%) individuals presented with their maximal value ≥40 mEq/l, of whom 53 (62%) were ≥60 mEq/l, and the other 23 (27%) were between 40 and 59 mEq/l.

The subpopulation referred for CBAVD

Among the 136 patients referred for CBAVD, complete clinical data were available for 100, and partial clinical data were available in 25 additional cases. No significant clinical difference was noticed between CBAVD patients according to the mutation in *trans* of p.Arg117His. No significant difference related to classification of the mutation in *trans* of p.Arg117His was noticed between patients with isolated CBAVD and other patients.

Among the 100 patients referred for CBAVD with complete clinical data, 66/100 presented with isolated CBAVD, and 34/100 presented with additional pancreatic, respiratory and/or nasopharyngeal symptoms, including two with pancreatic insufficiency (figure 1A). Only one CBAVD patient presented with moderate disseminated bronchiectasis (figure 1B). Sweat testing was available in 30 patients with isolated CBAVD and 18 patients with CBAVD and pulmonary symptoms. No significant difference was found in sweat test results between the two groups (see online supplementary data table D).

Fertility features

Specific forms about fertility and sterility were collected for 96 of the 136 CBAVD patients from urologists and assisted-reproduction clinics, and data could be analysed for 67 of them.

Upon clinical examination in 50 patients, the testes were mostly normal (47/50, 94%) or hypotrophic (3/50, 6%). Endorectal ultrasound documented in 28 CBAVD patients revealed absent (12/28, 43%), hypotrophic (10/28, 36%) or normal (6/28, 21%) seminal vesicles. Testicular ultrasound documented in 34 patients showed partial absence of the epididymis (14/34, 41%), presence of an epididymal cyst or nodules (4/34, 12%), and appeared normal in about half the patients (16/34, 47%). Hormone tests, performed in 54 patients and including measurements of FSH, Follicle-Stimulating Hormone (FSH), LH, Luteinizing Hormone (LH) testosterone and inhibin B, were all within the normal range except for one patient who had low FSH, LH and testosterone levels. Standard chromosomal analysis performed in 47/58 patients was normal. Spermograms, documented in 58 patients, showed azoospermia in all cases. All investigations, also including seminal markers, revealed mostly an obstructive origin (41/42 documented, 98%) and only one secretory origin, the same patient as mentioned above, with low FSH, LH and testosterone levels (1/42, 2%). Results of the testicular biopsy revealed sperm cells usable for intracytoplasmic injections in 46/47 patients.

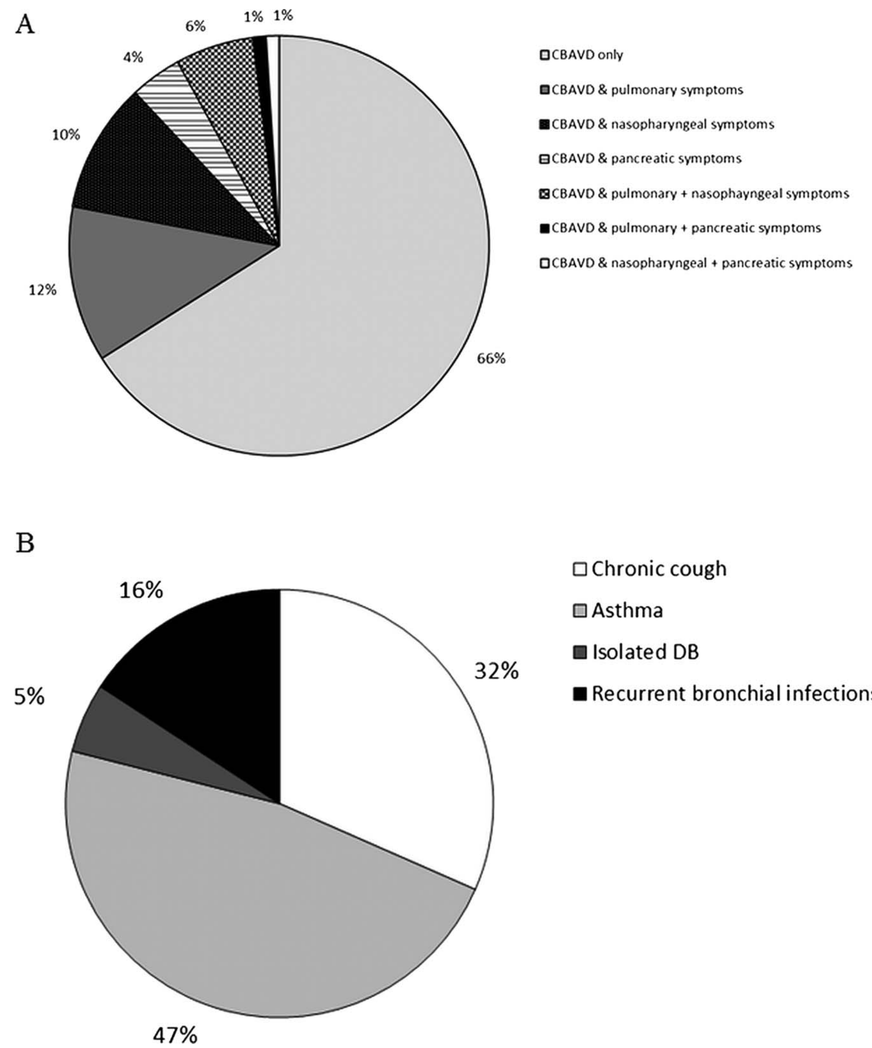

Figure 1 (A) Clinical presentations according to organ diseases in 100 patients referred for congenital bilateral absence of the vas deferens (CBAVD), and for whom complete clinical data were recorded. (B) Detailed pulmonary symptoms in the 19/100 patients referred for CBAVD and having pulmonary symptoms, with complete available clinical data. DB, disseminated bronchiectasis.

Assisted reproductive techniques were performed in 44/67 couples, and data were available in 40/44 couples (table 2). Female partners had a median age of 31 years (IQR: (28–33)) at the first ICSI. A total of 92 ICSI cycles were performed with 738 injected oocytes (median 8.4 injected oocytes/cycle, 426 fertilised oocytes (median 4.5 fertilised oocytes/cycle), and 143 transferred fresh embryos (median 1.6 transferred fresh embryos/cycle), 82 frozen embryos. The 22 cycles performed with frozen embryos led to four pregnancies. Finally, 18/40 couples gave birth to at least one liveborn child (29 from fresh transfer and three from frozen transfer).

Genetic counselling

A genetic counselling session was given to 60 patients and their couples, and the results of *CFTR* gene analysis were available in 53 partners. Six carriers of a severe CF mutation and one of a mild, CFTR-RD mutation were identified and PND or PGD was offered (table 3). Two couples chose to turn to a sperm donor. Eight children were born, including four who were compound heterozygous for p.Arg117His and one severe CF mutation. They are healthy so far.

DISCUSSION

We have recently shown that the penetrance of classical CF in individuals carrying p.Arg117His on a T7 background and p.Phe508del is very low, at 0.03%, and that only 3.1% of individuals expected to carry this genotype have come to clinical attention and been tested for *CFTR* mutations.[13] The objective of the present study was to describe the overall phenotype

Table 2 Fertility data in the 40 couples followed in assisted medical reproduction centres with available data

		Per puncture		Per fresh transfer	
	n	**Mean**	**Std**	**Mean**	**Std**
Injected ovocytes	738	8.4	4.7		
Fertilised ovocytes	426	4.5	4.5		
Embryos	361	4.2	4.2		
Fertilisation rate*	–	51%	29%		
Fresh cycle outcomes					
Punctures with fresh transfers	78	85%	36%		
Transferred embryos	143	1.6	1.0		
Clinical pregnancy rate	26	29%	45%	34%	48%
Miscarriage rate†	5	6%	25%		
Delivery rate	19	22%	42%	26%	44%
Frozen embryos	82	0.9	1.9		

*Fertilisation rate=number of embryos/number of ovocytes injected.
†Data were not available for 13 patients.
n, number.

Table 3 Details of fertility outcome in CBAVD couples at risk of CF or CFTR-RD in offspring

CBAVD patient's genotype	Partner's *CFTR* mutation	Year of molecular diagnosis	Reproductive options	Liveborn children	Risk for offspring
p.(Phe508del);(Arg117His)	p.Phe508del	1998	Offer of sperm donor	na	CF or CFTR-RD
p.(Phe508del);(Arg117His)	p.Phe508del	2000	2001: PND leading to twin pregnancy 2005: PGD leading to twin pregnancy	2 2*	CF or CFTR-RD
p.(Phe508del);(Arg117His)	p.Phe508del	2004	2005: PGD, no pregnancy	–	CF or CFTR-RD
c.(3705T>G;1210–12T5);(350G>A)	c.262_263del	1996	2 ICSI cycles, 2 transferred embryos, 1 twin pregnancy, no PND	2†	CFTR-RD
p.(Ala455Glu);(Arg117His)	p.Gly551Asp	1995	No spontaneous pregnancy and no in vitro fertilisation	–	CF or CFTR-RD
p.(Leu206Trp);(Arg117His)	p.Ser1235Arg	2000	2 ICSI cycles, no pregnancy Offer of donor sperm	–	CFTR-RD
p.(Phe508del);(Arg117His)	c.3528del	2001	2001–2006: 6 preimplantation cycles, 7 transferred embryos, 3 pregnancies (including 1 triple)	2	CF or CFTR-RD

Genotypes are written according to HGVS Human Genome Variation Society (HGVS) recommendations. Notably, c.350G>A corresponds to p.Arg117His and c.3705T>G to p.Ser1235Arg. All CBAVD patients carried the T7 variant in *cis* with p.Arg117His.
*Both sibs present the p.(Phe508del);(Arg117His) genotype.
†Both sibs present the c.(262_263del);(350G>A) genotype.
CBAVD, congenital bilateral absence of the vas deferens; CF, Cystic Fibrosis; CFTR-RD, CFTR-related disorders; ICSI, intra-cytoplasmic sperm injection; na, not applicable; PGD, preimplantation genetic diagnosis; PND, prenatal diagnosis.

associated with p.Arg117His plus a second *CFTR* mutation, in order to improve practices for the diagnosis and follow-up of patients and improve genetic counselling, in particular, for couples requiring assisted reproduction. Indeed, p.Arg117His was shown to be the third most frequent mutation found in French CBAVD patients.[8 9]

The frequency of the T5 variant in *cis* with p.Arg117His was much lower in France than that reported in Australian and UK populations,[10 11] thus preventing any correlation between phenotype and polyT variation in the present study. Our conclusions can thus be drawn only for populations in whom p.Arg117His on a T7 background is predominant.

No correlation was found between the phenotype and the class of the second in *trans* mutation. As nearly 90% of patients carried a CF mutation in *trans* of p.Arg117His, it is hypothesized that compound heterozygosity for p.Arg117His on a T7 background and another mild mutation would predominantly be associated with no obvious CFTR disease.[13]

An overall mild phenotype with predominant CBAVD in males and a low penetrance in females

We present the genotypes and phenotypes of 166/179 individuals carrying two *CFTR* mutations with p.Arg117His on at least one allele, which is the largest cohort described to date. The highly skewed sex ratio (males/females=12.4) is consistent with CBAVD being the principal reason for referral in these patients (76%), as compared with a sex ratio of 0.94 in NBS children. This observation supports a very low disease penetrance in women.

Association with mild pulmonary disease overall, but rare pancreatic disease in CBAVD

Despite the fact that 30% of symptomatic individuals had pulmonary symptoms (23% considering only the patients referred for CBAVD), these were of moderate intensity overall. Among the 11 patients with disseminated bronchiectasis, only five were men, including three with CBAVD, one father referred for a family history and one young male (17 years of age at last follow-up) with unknown fertility status (see online supplementary data table B). In the three CBAVD patients, pulmonary symptoms were found in one case at 34 years of age, at the same time as the CBAVD diagnosis, and at 54 years of age in the other two cases, long after the CBAVD diagnosis. Late presentation of CF has previously been reported in the literature, in two males at 61 and 64 years of age, carrying p.Arg117His in *cis* with T7 and presenting with infertility and severe bronchiectasis.[16] Thus, a complete initial evaluation and regular follow-up should be proposed in CBAVD patients, who might develop pulmonary symptoms a long time after the genetic analysis requested for assisted reproduction purposes.

Our data support the observation that *Staphylococcus aureus* is also a frequent respiratory pathogen in patients with CFTR-RD.[17 18] Regarding *Pseudomonas aeruginosa*, which is associated with a greater risk of shortened survival,[19] the probability of finding positive cultures was lower in our series than in French patients with a documented diagnosis of CF (data from the French national patient registry Vaincre La Mucoviscidose—INED 2008).

Pancreatic disease remained rare with only 2% of individuals presenting with pancreatic insufficiency, while the majority of the few patients with pancreatic symptoms presented with an isolated episode of acute pancreatitis (see online supplementary data table C). All the patients with pancreatic symptoms had the T7 allele in *cis* with p.Arg117His. This is in contrast with the few previously reported cases, which were associated with the T5 variant.[11]

Absence of a correlation between the disease phenotype and sweat chloride values

Sweat chloride values were ≥60 mEq/l in 62% of patients, which is consistent with a diagnosis of CF. However, no difference was found in sweat chloride values whether patients had isolated CBAVD or a multiorgan disease with pulmonary symptoms (see online supplementary data table D). These observations question the positive diagnostic value of sweat testing in individuals carrying p.Arg117His in *cis* with T7, as previously reported.[20]

Males and fertility

CFTR-RD is not the rule in individuals carrying p.Arg117His plus a CF mutation, as previously demonstrated[13] and exemplified by the four healthy men of the study who fathered spontaneously. Moreover, we previously estimated that only 3% of individuals carrying such a genotype present clinical

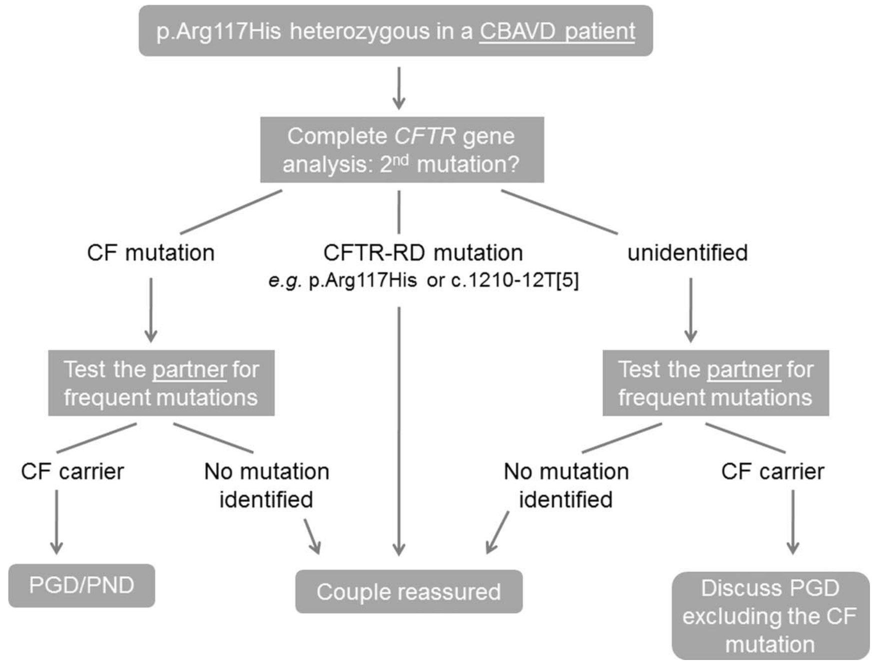

Figure 2 Strategy for genetic counselling in congenital bilateral absence of the vas deferens couples. PGD, preimplantation genetic diagnosis; PND, prenatal diagnosis.

manifestations and had a genetic analysis,[13] thus illustrating the low penetrance for CF and CFTR-RD. Nevertheless, given that CBAVD is the most frequent symptom observed in our cohort, we suggest that male individuals carrying p.Arg117His in combination with another *CFTR* mutation and referred for a family study, or respiratory or pancreatic symptoms be offered fertility investigations in an ART clinic, according to the clinical context and the patients' wishes.

Implications for genetic counselling for CBAVD couples

An important issue for CBAVD couples is the accurate evaluation of their risk of having a CF child. Such couples require appropriate genetic counselling and should be offered reproductive options. Based on the recent reclassification of p.Arg117His in *cis* with T7 as a CFTR-RD mutation, it should not be considered for either cascade testing in relatives, or for a risk of classical CF in offspring. As a consequence, depending on the second mutation carried by the CBAVD patient, different reproductive options, summarised in figure 2, may be discussed. These options should be explained to the couple in a genetic counselling session. CBAVD patients carrying two mild CFTR-RD mutations are not considered at risk of having a CF child, as was the case for 10% of all patients. On the other hand, once CBAVD patients are found to carry a CF-causing mutation in *trans* of p.Arg117His (90% of cases in our series), their partner should be offered testing for frequent CF mutations. If the partner is found to be a CF carrier, PGD could be considered primarily because of the need for assisted reproductive techniques. The partner should also be tested if the patient has no other identified mutation, because of the remaining risk that he carries an undetected CF mutation in an unexplored region. In our series, surprisingly, six of the 53 (11.3%) female partners were carriers of a frequent CF mutation, while the frequency of such carriers in the general population in France is about 2.9%.[13] There could be a bias for such a high frequency, as positive results were probably more easily tracked than negative ones. Because of the increased risk of obtaining embryos carrying two CF-causing mutations, it could be discussed to implant embryos with a CFTR-RD genotype in a PGD process, as was the case for two couples in our series who associated p.Arg117His and a CF mutation. Indeed, the parent's desire for PGD was to rule out the CF-associated genotype only, and some embryos with CFTR-RD-associated compound heterozygous genotypes could be transferred, depending on the embryos available (table 3).

In conclusion, our results indicate the need for a complete initial clinical evaluation in patients with at least one p.Arg117His mutation, especially in CBAVD patients who may be free of respiratory symptoms at the time of the genetic analysis requested for assisted reproduction purposes, and for appropriate genetic counselling.

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REFERENCES

1 Welsh MJ, Ramsey BW, Accurso F, Cutting GR. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D.eds. *Metabolic and molecular bases of inherited disease*. 8th edn. New York: McGraw-Hill, 2001: 5121–88.
2 Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, Sermet I, Schwarz M, Tzetis M, Wilschanski M, Bareil C, Bilton D, Castellani C, Cuppens H, Cutting GR, Drevínek P, Farrell P, Elborn JS, Jarvi K, Kerem B, Kerem E, Knowles M, Macek M Jr, Munck A, Radojkovic D, Seia M, Sheppard DN, Southern KW, Stuhrmann M, Tullis E, Zielenski J, Pignatti PF, Ferec C. Recommendations for the classification of diseases as CFTR-related disorders. *J Cyst Fibros* 2011;10:S86–102.
3 Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, Durie PR, Legrys VA, Massie J, Parad RB, Rock MJ, Campbell PW 3rd. Cystic Fibrosis Foundation. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008;153:S4–14.
4 Dequeker E, Stuhrmann M, Morris MA, Casals T, Castellani C, Claustres M, Cuppens H, des Georges M, Ferec C, Macek M, Pignatti PF, Scheffer H, Schwartz M, Witt M, Schwarz M, Girodon E. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders–updated European recommendations. *Eur J Hum Genet* 2009;17:51–65.
5 Dean M, White MB, Amos J, Gerrard B, Stewart C, Khaw KT, Leppert M. Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* 1990;61:863–70.
6 Gervais R, Dumur V, Rigot JM, Lafitte JJ, Roussel P, Claustres M, Demaille J. High frequency of the p.Arg117His cystic fibrosis mutation in patients with congenital absence of the vas deferens. *N Engl J Med* 1993;328:447–57.
7 Chillon M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey MC, Ruiz-Romero J, Verlingue C, Claustres M, Nunes V, Férec C, Estivill X. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N Engl J Med* 1995;332:1475–80.
8 Claustres M, Guittard C, Bozon D, Chevalier F, Verlingue C, Ferec C, Girodon E, Cazeneuve C, Bienvenu T, Lalau G, Dumur V, Feldmann D, Bieth E, Blayau M, Clavel C, Creveaux I, Malinge MC, Monnier N, Malzac P, Mittre H, Chomel JC, Bonnefont JP, Iron A, Chery M, Georges MD. Spectrum of CFTR mutations in cystic fibrosis and in congenital absence of the vas deferens in France. *Hum Mutat* 2000;16:143–56.
9 Ratbi I, Legendre M, Niel F, Martin J, Soufir JC, Izard V, Costes B, Costa C, Goossens M, Girodon E. Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. *Hum Reprod* 2007;22:1285–91.
10 Kiesewetter S, Macek M Jr, Davis C, Curristin SM, Chu CS, Graham C, Shrimpton AE, Cashman SM, Tsui LC, Mickle J, Amos J, Highsmith WE, Shuber A, Witt DR, Crystal RG, Cutting GR. A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nature Genet* 1993;5:274–8.
11 Massie RJ, Poplawski N, Wilcken B, Goldblatt J, Byrnes C, Robertson C. Intron-8 polythymidine sequence in Australasian individuals with CF mutations p.Arg117His and R117C. *Eur Respir J* 2001;17:1195–200.
12 Munck A, Dhondt JL, Sahler C, Roussey M. Implementation of the French nationwide cystic fibrosis newborn screening program. *J Pediatr* 2008;153:228–33.
13 Thauvin-Robinet C, Munck A, Huet F, Génin E, Bellis G, Gautier E, Audrézet MP, Férec C, Lalau G, Georges MD, Claustres M, Bienvenu T, Gérard B, Boisseau P, Cabet-Bey F, Feldmann D, Clavel C, Bieth E, Iron A, Simon-Bouy B, Costa C, Medina R, Leclerc J, Hubert D, Nové-Josserand R, Sermet-Gaudelus I, Rault G, Flori J, Leroy S, Wizla N, Bellon G, Haloun A, Perez-Martin S, d'Acremont G, Corvol H, Clément A, Houssin E, Binquet C, Bonithon-Kopp C, Alberti-Boulmé C, Morris MA, Faivre L, Goossens M, Roussey M, Girodon E, Collaborating Working Group on R117H. The very low penetrance of cystic fibrosis for the p.Arg117His

mutation: a reappraisal for genetic counselling and newborn screening. *J Med Genet* 2009;46:752–68.
14 Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 1959;23:545–9.
15 Hammond KB, Turcios NL, Gibson LE. Clinical evaluation of the macroduct sweat collection system and conductivity analyzer in the diagnosis of cystic fibrosis. *J Pediatr* 1994;124:255–60.
16 Peckham D, Conway SP, Morton A, Jones A, Webb K. Delayed diagnosis of cystic fibrosis associated with p.Arg117His on a background of 7T polythymidine tract at intron 8. *J Cyst Fibros* 2006;5:63–5.
17 Armstrong DS, Grimwood K, Carlin JB, Carzino R, Olinsky A, Phelan PD. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. *Pediatr Pulmonol* 1996;21:267–75.
18 Lording A, McGaw J, Dalton A, Beal G, Everard M, Taylor CJ. Pulmonary infection in mild variant cystic fibrosis: implications for care. *J Cyst Fibros* 2006;5:101–4.
19 Lai HJ, Cheng Y, Cho H, Kosorok MR, Farrell PM. Association between initial disease presentation, lung disease outcomes, and survival in patients with cystic fibrosis. *Am J Epidemiol* 2004;159:537–46.
20 Davis PB, Schluchter MD, Konstan MW. Relation of sweat chloride concentration to severity of lung disease in cystic fibrosis. *Pediatr Pulmonol* 2004;38:204–9.

CFTR p.Arg117His associated with CBAVD and other CFTR-related disorders

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