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META-ANALYSIS ***Andrology***

CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis

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BACKGROUND: Numerous studies have reported *CFTR* mutations in CBAVD (congenital bilateral absence of the vas deferens) patients, but their results are not completely consistent. Here, we present a systemic review and meta-analysis with emphasis on clarifying further the genetic association of *CFTR* mutations with CBAVD.

METHODS: We searched the MEDLINE database until March, 2011 for eligible articles reporting *CFTR* mutations in CBAVD. Relevant data from each included study were abstracted by two independent reviewers. The overall frequency of *CFTR* mutations in CBAVD and the odds ratio (OR) for common specific alleles were pooled under random-effect or fixed-effect model as appropriate. Subgroup analysis was performed by ethnicity, and potential heterogeneity and bias were both assessed.

RESULTS: Among CBAVD patients, 78% had at least one *CFTR* mutation, 46% having two and 28% only one. Moreover, the common heterozygous F508del/5T and F508del/R117H were observed in 17 and 4% of CBAVD cases respectively, and the allele frequency in CBAVD was 17% for F508del, 25% for 5T and 3% for R117H. Subgroup analysis indicated an increased frequency of cases with two mutations in Caucasian patients than in Non-Caucasian (68 versus 50%, $P = 0.012$), but no differences for cases with at least one mutation (88 versus 77%, $P = 0.163$) or with only one mutation (17 versus 25%, $P = 0.115$). Caucasian patients had higher F508del frequency, but lower 5T frequency, than Non-Caucasian (22 versus 8%, $P = 0.001$; 20 versus 31%, $P = 0.009$). Summary OR was 9.25 for 5T [95% confidence interval (CI) 7.07–12.11, $P = 0.000$], with moderate heterogeneity ($I^2 = 49.20\%$, $P = 0.019$) and evident bias (Egger's test, $P = 0.005$), and it was 19.43 for 5T/(TG)12_13 (95% CI 10.48–30.03, $P = 0.000$) without any evidence of heterogeneity ($I^2 = 0.1\%$, $P = 0.391$) and bias (Egger's test, $P = 0.160$). The OR for 5T/(TG)12_13 was significantly higher than that for 5T allele ($P = 0.000$).

CONCLUSIONS: In summary, our results demonstrate a high frequency of *CFTR* mutations in CBAVD patients, and these exhibit evident ethnic differences. In addition, 5T allele and 5T/(TG)12_13 may contribute to the increased risk for CBAVD, with the 5T penetrance probably being modulated by adjacent (TG)12_13.

Key words: CBAVD / CFTR / genetic association study / systemic review and meta-analysis

Introduction

Congenital bilateral absence of the vas deferens (CBAVD) accounts for ~1–2% of the population of infertile, but otherwise healthy, males and up to 25% of those with obstructive azoospermia (Jequier *et al.*, 1985; Patrizio *et al.*, 1993; Oates and Amos, 1994). It may occur either as an isolated reproductive disorder or an atypical symptom of cystic fibrosis (CF). Since almost all the males with CF also exhibit CBAVD and are infertile due to obstructive azoospermia, isolated CBAVD was postulated to have a common genetic origin with CF (Kaplan *et al.*, 1968; Holsclaw *et al.*, 1971; Stern *et al.*, 1982; Wilschanski *et al.*, 1996). This hypothesis was confirmed first by the observation that a substantial fraction of men with isolated CBAVD carried F508del (c.1521_1523delCTT), one of the most frequent mutations in the *CFTR* gene that are responsible for CF (Dumur *et al.*, 1990; Anguiano *et al.*, 1992). Subsequent studies have also confirmed this, and the isolated CBAVD has been proposed as a primarily genital form of CF (Chillon *et al.*, 1995; Stuhrmann and Dork, 2000; Timmreck *et al.*, 2003).

Over the years, numerous studies have investigated the genetic link between *CFTR* mutations and CBAVD risk. In CBAVD patients, a large number of different mutant *CFTR* alleles have been identified, and these mutations exhibited extreme heterogeneity in spectrum and frequency (Chillon *et al*., 1995; Costes *et al*., 1995; Dork *et al*., 1997; Claustres *et al*., 2000; Dayangac *et al*., 2004; Ratbi *et al*., 2007). In particular, several large studies that extensively screened *CFTR* mutations in CBAVD demonstrated that the most common mutations are F508del, 5T (c.1210-12T[5]) and R117H (c.350G > A) (Chillon *et al*., 1995; Dork *et al*., 1997; Casals *et al*., 2000; Claustres *et al*., 2000; Ratbi *et al*., 2007). These three alleles account for all but a small fraction of *CFTR* mutations observed in CBAVD, and the remaining mutations were detected in only a few cases with very low frequency or reported by one or two studies in specific populations (Claustres *et al*., 2000; Cuppens and Cassiman, 2004; Claustres, 2005).

CFTR mutations have been classified into five classes according to their functional effects on the protein (Welsh and Smith, 1993; Zielenski and Tsui, 1995). Mutations of classes 1, 2 and 3 that result in the complete functional loss of CFTR protein are known as severe mutations, while classes 4 and 5 are referred to as mild mutations given that they provide residual *CFTR* function to compensate for the functional defect of a severe mutant allele (Claustres *et al*., 2000; Claustres, 2005). Although the molecular mechanism underling CBAVD development remains largely unclear, evidence suggested that it might be associated with defects of the Wolffian ducts caused by *CFTR* mutations (Claustres, 2005). Clinical studies have demonstrated that most CBAVD patients were compound heterozygotes with two different mutant alleles, and that nearly all of the CBAVD males with heterozygous genotypes would have at least one mild mutation. Generally speaking, F508del/5T and F508del/R117H are the two most common kinds of compound heterozygote, in men with CBAVD, which clearly differs from those observed in typical CF (De Braekeleer and Ferec, 1996; Claustres *et al*., 2000; Claustres, 2005). Moreover, the penetrance of polymorphic variant 5T might be determined predominantly by the length of adjacent TG repeats (c.1210-34TG[9_13]) (Cuppens *et al*., 1998; Groman *et al*., 2004), and the allele combination of 5T with longer (TG)12 (c.1210-34TG[12]) or (TG)13 (c.1210-34TG[13]) would probably result in a higher disease risk than 5T itself.

Results from different studies are not completely consistent concerning the cause–effect link between *CFTR* mutations and CBAVD, and even controversial in some aspects. The discrepancies might be due, at least in part, to confusing factors such as ethnic differences, variation in scanning methods and/or case heterogeneity. Therefore, these factors should be taken into consideration when interpreting the implications of *CFTR* mutations in CBAVD. In CF, *CFTR* mutations exhibited striking ethnic or geographical differences, with a higher frequency in European Caucasian than in others, especially than in Asian or Oriental people (Estivill *et al*., 1997; Bobadilla *et al*., 2002). Similarly, evident geographical or ethnic differences have been demonstrated in *CFTR* mutations in CBAVD. For instance, some studies reported an exceptional high frequency of 5T in CBAVD patients of non-Caucasian origin, such as Egyptian (43.7%) (Lissens *et al*., 1999), Taiwanese (44.4%) (Wu *et al*., 2004), Japanese (30%) (Anzai *et al*., 2003) and Turkish (20%) (Dayangac *et al*., 2004), but a very high frequency of F508del in Caucasian cases. The screening methods employed by most studies can be classified into three categories according to their different coverage on *CFTR* sequence: (i) whole exon/flanking sequence; (ii) most frequent mutations; (iii) specific mutant alleles. Such coverage differences are likely to contribute to inconsistent results among different studies. Finally, case heterogeneity may be also a confusing factor, since some studies have included CBAVD patients with renal malformations, a condition that has been suggested to be associated with lower incidence of *CFTR* mutations and thought to be genetically different from that with normal kidney (Augarten *et al*., 1994; Schlegel *et al*., 1996; Dork *et al*., 1997; de la Taille *et al*., 1998).

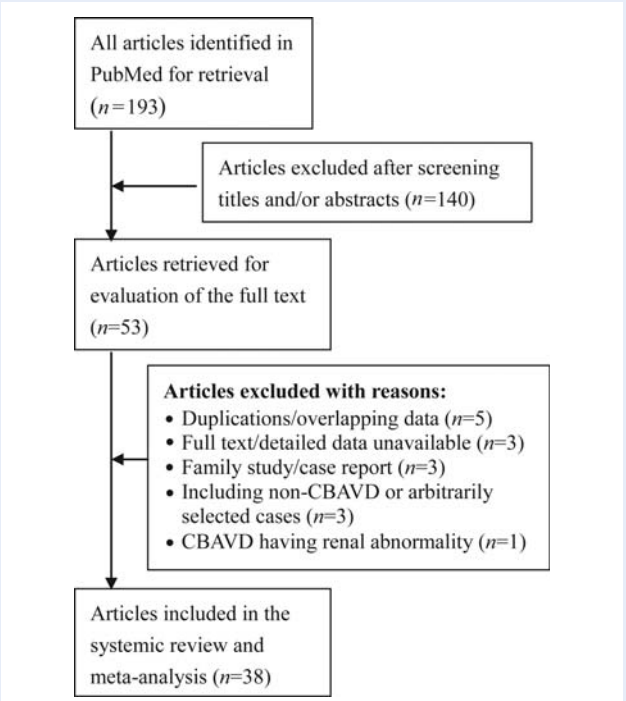

Figure 1 The flow chart for study selection.

Several excellent reviews have documented the link between *CFTR* mutations and CBAVD (De Braekeleer and Ferec, 1996; Stuhrmann and Dork, 2000; Cuppens and Cassiman, 2004; Claustres, 2005; Radpour *et al*., 2008). Here, we have performed a systemic review and meta-analysis with particular emphasis on clarifying the factors that might confuse our understanding of the association of *CFTR* mutations with CBAVD. Therefore, the results from this study should help elucidate better the critical roles of *CFTR* mutations in CBAVD development.

Materials and Methods

Literature search and study selection

We searched the PubMed database for eligible articles. The search terms covered Medical Subject Headings and/or text words relating to the *CFTR* gene and CBAVD disease. Search conditions were limited to publications in English until March 2011. The abstracts and/or titles of all the retrieved papers were screened and subsequently the full text was evaluated in

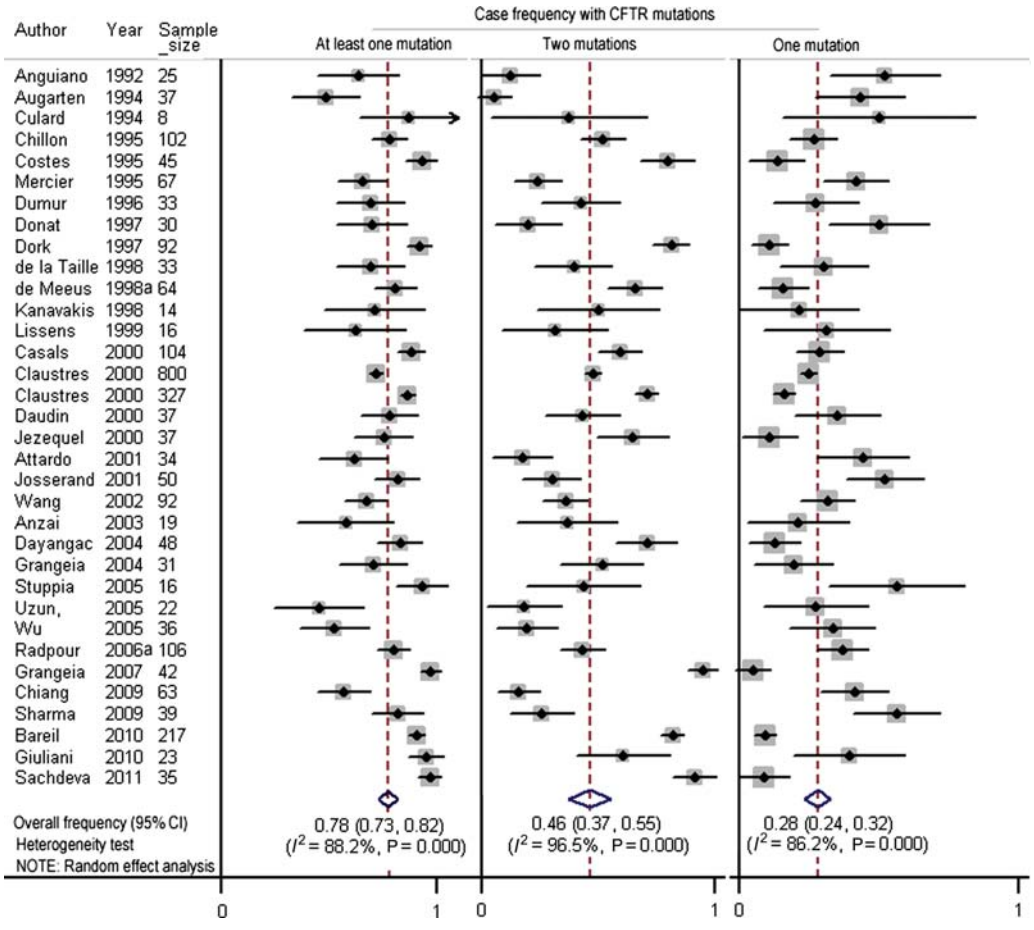

Figure 2 Forest plot for meta-analysis of *CFTR* mutation frequency in CBAVD patients. Summary frequency of CBAVD cases with at least one mutation, two mutations and one mutation and their 95% CI were calculated by random-effect model. Summary frequency and 95% CI are indicated by diamond. Solid grey square marks the frequency from each study with square size directly proportional to the weight, and the horizontal lines represent 95% CI.

detail to confirm fulfillment of the inclusion criteria. Moreover, reference lists of the retrieved papers and reviews were hand-searched for additional relevant articles.

Inclusion criteria were: reporting *CFTR* mutations in CBAVD cases and describing the genotyping protocols. CBAVD diagnosis should be conducted through a comprehensive strategy including physical examination, semen analysis and transrectal ultrasonography. In detail, the otherwise healthy, infertile males were diagnosed as CBAVD if they had azoospermia concurrently with low semen plasma volume, normal plasma FSH, LH and testosterone levels and bilateral non-palpable/or rudimentary vas deferens. CBAVD patients with typical CF symptoms or renal ultrasound abnormalities were excluded, since such cases may represent a distinct clinical entity with different genetic etiology from isolated CBAVD (Stuhrmann and Dork, 2000). In case of duplicate publications or overlapping data, only those published most recently or with the largest samples were included (Little *et al*., 2002).

Data abstraction and synthesis

According to the MOOSE guidelines, the variables were abstracted from each eligible study directly, and some data were calculated using the original results provided in the text if the direct data were unavailable (Stroup *et al*., 2000). Data analyses were conducted mainly concerning two aspects: the overall frequency of *CFTR* mutations in CBAVD cases, and the summary odds ratios (ORs) for common specific mutant alleles. For the purpose of stratification analysis, the studies were classified into Caucasian and non-Caucasian groups according to the subject ancestry. When ethnicity information was lacking, the determination was made based on the geographical location of the study. The overall frequency of *CFTR* mutations in CBAVD cases and 95% confidence interval (CI) values were pooled by a random-effects model as commonly done since such frequency outcomes are usually heterogeneous between studies. The summary ORs and 95% CI values for specific mutant alleles were calculated using a random-effect model or fixed-effect model as appropriate depending on the heterogeneity among studies. The significance of the summary ORs was determined by the *Z*-test.

Heterogeneity and bias

For each outcome pooled, the heterogeneity was assessed by Cochran's Q-statistic and I^2 statistic among included studies (Higgins and Thompson, 2002; Higgins *et al*., 2003). To assess publication bias, we first used a funnel plot that depicts the effect size (on a logarithmic scale) against its standard error for each study, and visual confirmation of any plot

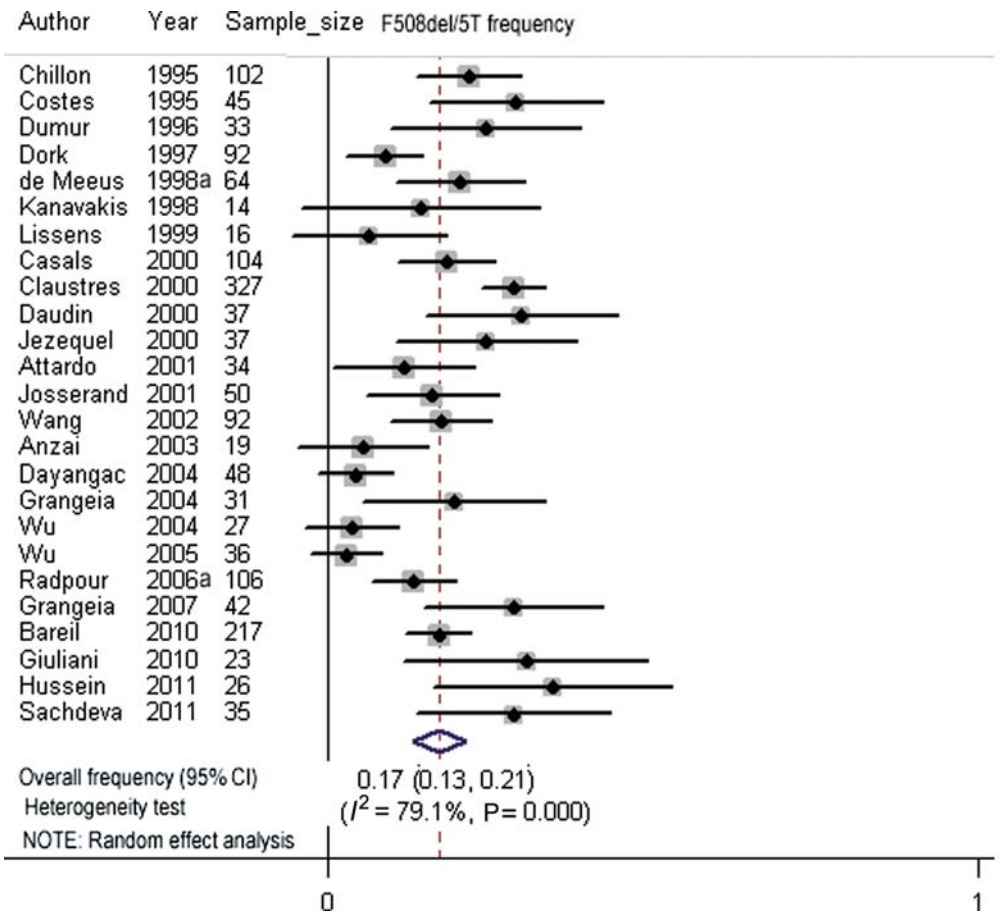

Figure 3 Forest plot for meta-analysis of F508del/5T frequency in CBAVD patients. For details see Fig. 2.

asymmetry indicated bias presentation. Further, Egger's test was also performed to statistically assess the presence of publication bias (Egger *et al.*, 1997).

Analysis software

All statistical analyses were carried out in Stata software11.0 (Stata Corporation, College Station, TX, USA).

Results

Study selection

A total of 193 articles were identified through a comprehensive literature search in the PubMed database, of which 38 studies met the inclusion criteria after careful comprehensive evaluation. Overall, 53 of all the identified articles were reviewed in full text for the eligible data of which 15 were ultimately excluded. The main reasons for exclusion were as follows: five studies had duplicate or overlapping data (Casals *et al.*, 1995; Jezequel *et al.*, 1995; Cuppens *et al.*, 1998; Ratbi *et al.*, 2007; Radpour *et al.*, 2008); three studies were unavailable or lacked sufficient data for analysis (Bienvenu *et al.*, 1997; Ravnik-Glavac *et al.*, 2000; Radpour *et al.*, 2006b); three were family or case studies (Dumur *et al.*, 1990; Rave-Harel *et al.*, 1995; Zielenski *et al.*, 1995); three studies included non-CBAVD or arbitrarily selected cases (Groman *et al.*, 2004; Tamburino *et al.*, 2008; Gallati *et al.*, 2009); one study involved 18.3% CBAVD cases with renal abnormalities (Samli *et al.*, 2006). One article provided data on two cohorts of subjects that were evaluated for *CFTR* mutations by different methods, and it was counted as two separate studies when pooling the results (Claustres *et al.*, 2000). Figure 1 provides an overview of the process of literature search and review.

Study characters

In total, 38 eligible studies provided the frequency of *CFTR* mutations in CBAVD cases, but only 14 of them, which determined the CBAVD risk for the important alleles 5T and 5T/(TG)12_13, selected the fertile males as controls. Therefore, we first calculated the frequency of overall *CFTR* mutations as well as some specific genotype/allele in CBAVD patients, and then pooled the summary OR to evaluate the CBAVD risk for individuals carrying 5T or 5T/(TG)12_13. Most studies stated clearly the diagnostic strategies, while three lacked detailed information (de Meeus *et al.*, 1998b; Ravnik-Glavac *et al.*, 2001; Stuppia *et al.*, 2005; Bareil *et al.*, 2010). Some studies involved the CBAVD cases with minor CF-related symptoms such as respiratory tract symptoms or pancreatitis episodes. As demonstrated in Supplementary data, Table S1, 12 studies included CBAVD cases with renal abnormalities among study subjects and these cases have

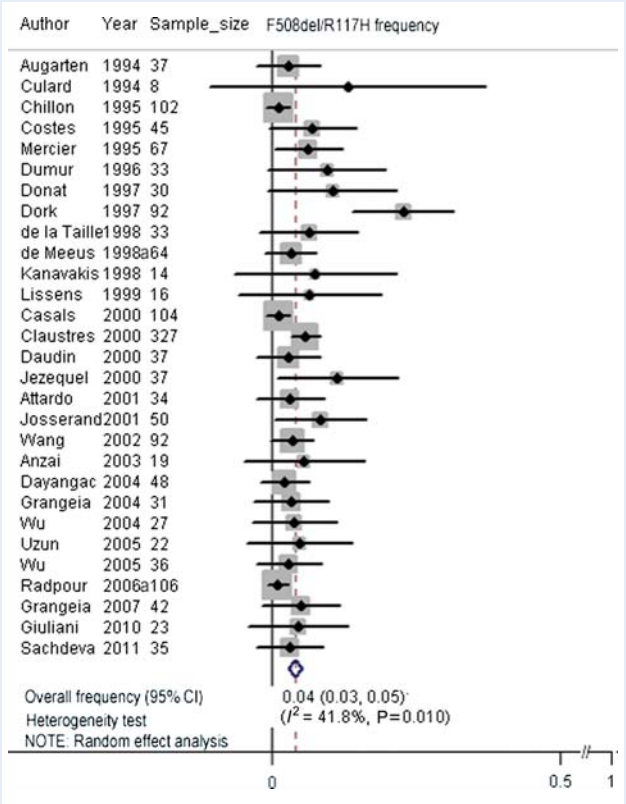

Figure 4 Forest plot for meta-analysis of F508del/R117H frequency in CBAVD patients. For details see Fig. 2.

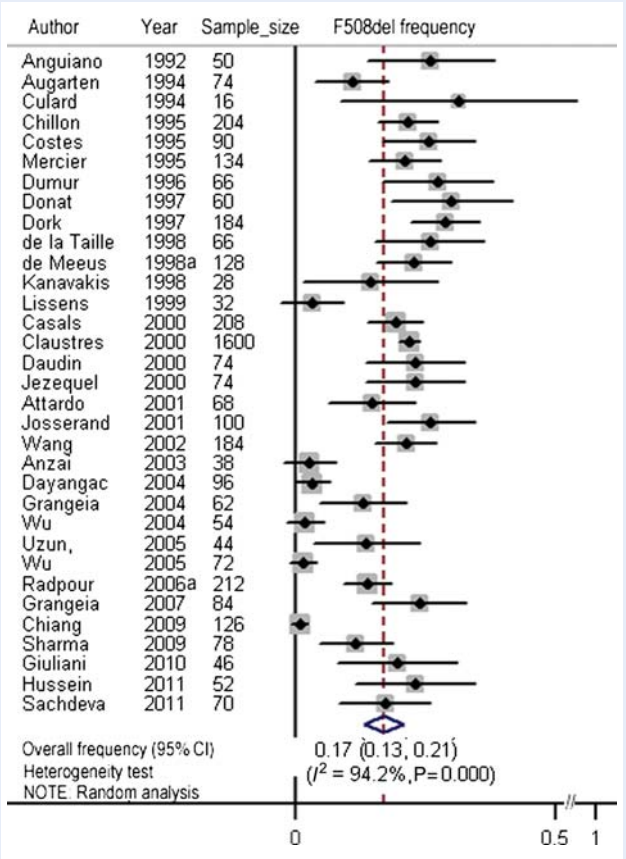

Figure 5 Forest plot for meta-analysis of F508del frequency in CBAVD patients. For details see Fig. 2.

been excluded when abstracting data, while additional 8 studies lacked information about renal abnormalities in cases. The eligible studies were heterogeneous in terms of subject ethnicity, since these studies were conducted in a wide range of populations and a few studies even included individuals with different geographical locations and/or ethnic origins (Anguiano *et al.*, 1992; Chillon *et al.*, 1995; Mercier *et al.*, 1995; Dork *et al.*, 1997). Most studies employed a comprehensive strategy for mutation detection, of which 22 screened full 27 exons and flanking regions, 12 detected all or majority of the most common *CFTR* mutations and a few other studies screened a limited number of exons/introns or only several specific mutations. Along with these comprehensive scanning strategies, specific testing for 5T was also performed in most studies. The study characters were described in details in Supplementary data, Table S1.

Data analysis

Summary frequencies of CFTR *mutations in CBAVD patients*

In all, 38 eligible studies provided sufficient data for summary analysis of the overall frequency of *CFTR* mutations. We first calculated the CBAVD frequency with at least one mutation, two mutations and only one mutation respectively. Summary analysis showed that 78% cases had at least one mutation, 46% cases had two mutations and 28% had only one mutation, which was illustrated in Fig. 2. Second, the summary frequency for each of the several common genotypes and alleles was also calculated. As usual, allele frequency was defined as the percent of chromosomes with one allele, and genotype frequency as the percent of individuals having one genotype. Summary analysis demonstrated that two common heterozygous genotypes, F508del/5T and F508del/R117H, were observed in CBAVD cases with frequency of 17 and 4%, respectively (Figs 3 and 4). As for the frequent mutant alleles, the frequency was 17% for F508del, 25% for 5T and 3% for R117H, respectively (Figs 5–7). Heterogeneity was significant for the summary analysis of both overall mutations (Fig. 2) and common genotype/allele (Figs 2, 3, 5 and 6). Moreover, the bias was evident for all but two that pooled the case frequency of CBAVD with at least one mutation and with only one mutation as demonstrated by Egger's test ($P = 0.328$ and $P = 0.874$, respectively) and the symmetric funnel plot (Supplementary data, Figs S1 and 2).

Since *CFTR* mutations exhibit a significant ethnic difference, we performed a subgroup analysis by racial or regional origin. Data from each study were classified into Caucasian group or non-Caucasian group according to the racial information and/or the residential country of the study subjects. Subgroup analysis of the frequency of overall *CFTR* mutations included only studies that conducted comprehensive scanning of whole exons/flanking sequences and 5T testing, so as to reduce as possible confusing influence produced by method variations. The frequency of cases with at least one mutation was 88 versus 77% ($P = 0.163$) and of those with only one mutation was 17 versus 25% ($P = 0.115$) for Caucasian and non-Caucasian CBAVD, respectively. However, the frequency of cases with two mutations was higher in

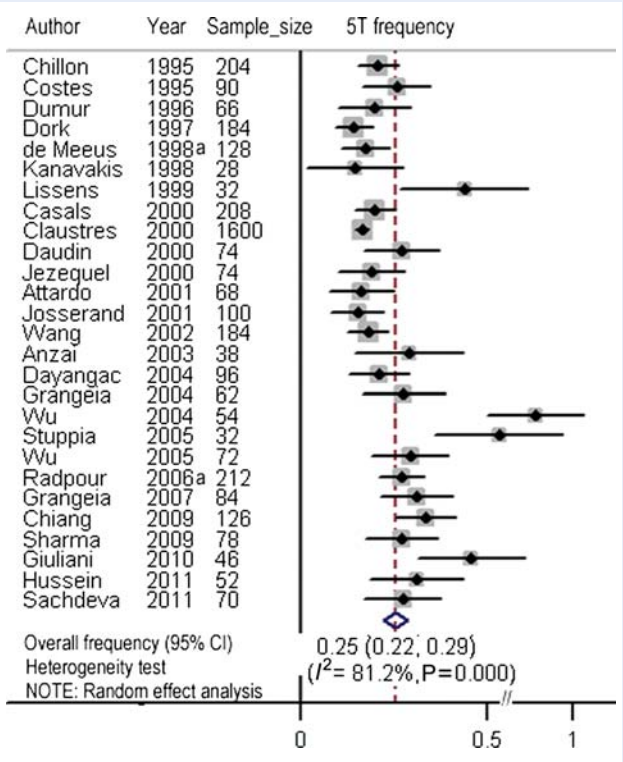

Figure 6 Forest plot for meta-analysis of 5T frequency in CBAVD patients. For details see Fig. 2.

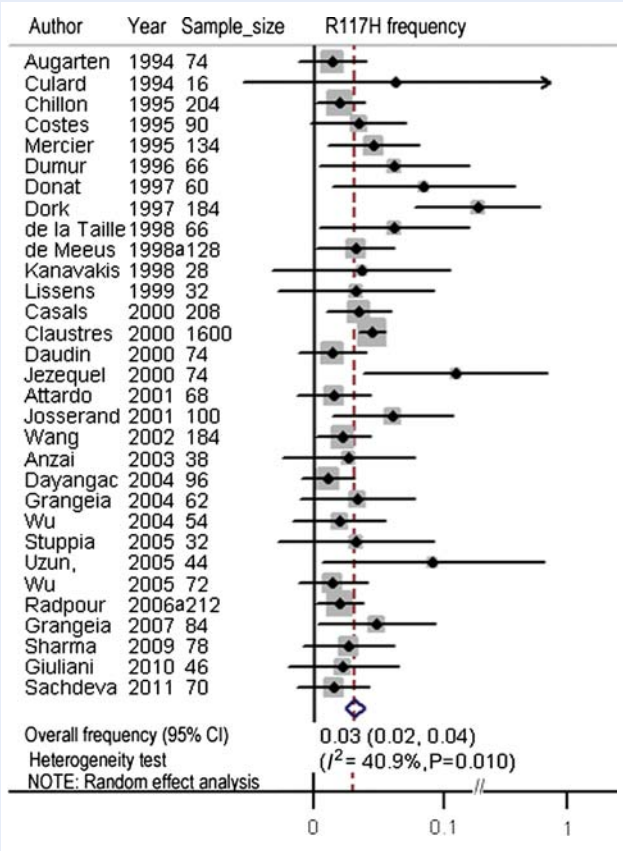

Figure 7 Forest plot for meta-analysis of R117H frequency in CBAVD patients. For details see Fig. 2.

the Caucasian population than that in the non-Caucasian (68 versus 50%, $P = 0.012$) (Supplementary data, Fig. S3). There were also significant racial differences in common alleles (Supplementary data, Figs S4–6). The F508del allele frequency was significantly higher in Caucasian, than non-Caucasian patients (22 versus 8%, $P = 0.001$), while the reverse was true for the 5T allele that presented in 20% of chromosomes in Caucasian cases versus 31% in other populations ($P = 0.009$). On the contrary, F508del/5T incidence was not significantly different between Caucasian patients and non-Caucasians (20 versus 12%, $P = 0.683$).

Pooling OR for CBAVD risk

Overall, 14 studies, which comprised 1624 CBAVD chromosomes and 2237 normal chromosomes, were eligible for the meta-analysis of ORs concerning the risk alleles 5T and 5T/(TG)12_13. Under a fixed-effect model, the pooled OR for 5T allele was 9.25 (95% CI 7.07–12.11, $P = 0.000$) compared with the other two alleles 7T and 9T (Fig. 8), and the heterogeneity among these studies was moderate ($I^2 = 49.20\%$; $\chi^2 = 25.61$, $P = 0.019$). The bias for 5T studies was evident as indicated by both the asymmetric funnel plot (Supplementary data, Fig. S7) and the Egger's test ($P = 0.005$). For the 5T/TG12_13, the pooled OR was 19.43 (95% CI 10.48–30.03, $P = 0.000$) (Fig. 8), and it was significantly higher than the OR for 5T allele (19.43 versus 9.25%, $P = 0.000$). There was no evidence of heterogeneity among studies of the 5T/(TG)12_13 ($I^2 = 0.1\%$; $\chi^2 = 3.00$, $P = 0.391$), and the bias was also absent as demonstrated by the symmetric funnel plot (Supplementary data, Fig. S8) and Egger's test ($P = 0.160$).

Discussion

Numerous studies have provided evidence for a possible genetic link between *CFTR* mutations and CBAVD risk, but several factors that might affect our interpretation of the published results need to be clarified before reliable conclusions can be reached. Therefore, we have performed a systematic review and meta-analysis on a robust data set, with the aim of improving our understanding of the relationship between *CFTR* mutations and CBAVD.

The results demonstrate a high frequency of overall *CFTR* mutations, as well as heterozygous genotype F508delt/5T and F508del/R117H, in CBAVD patients and indicate a potential association of *CFTR* mutations with CBAVD. The mutation spectrum of *CFTR* in CBAVD is strikingly different from that in CF patients, who predominantly carry the homozygous defect of F508del/F508del (Claustres *et al.*, 2000; Claustres, 2005). Further, subgroup analysis by ethnic origins indicates that there are significant differences between Caucasian populations and non-Caucasian for the *CFTR* mutations associated with CBAVD. This may be partially explained by the fact that CF, primarily caused by severe mutation F508del, is common in Caucasian populations, but very rare in other nations (Bobadilla *et al.*, 2002). Additionally, our

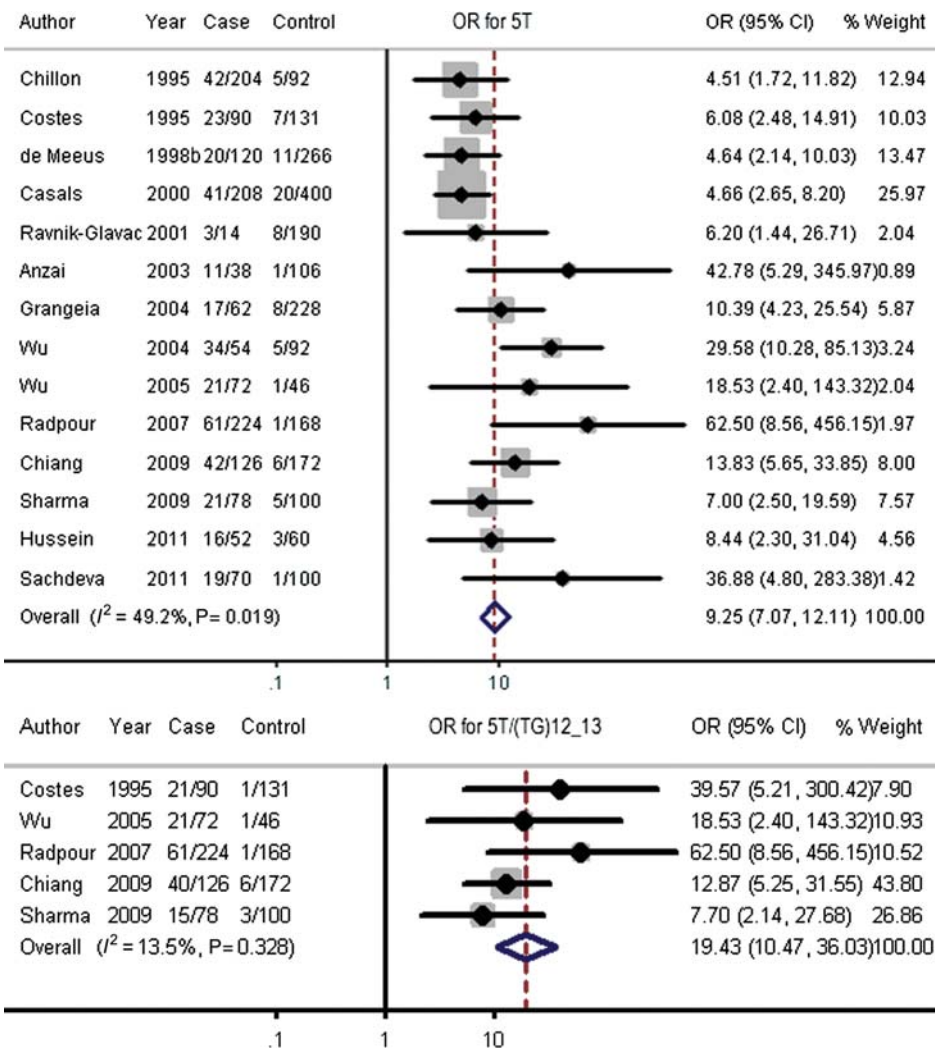

Figure 8 Forest plot for meta-analysis of ORs. Summary OR and their 95% CIs were calculated by Mantel-Haenszel method and indicated by the unshaded diamond. The solid grey square marks OR of each study, with the square size directly proportional to the weight and the horizontal lines representing the 95% CI. The dotted vertical red line indicates the overall estimate, whereas the solid black one indicates the null effect (OR = 1).

results are consistent with the assumption that severe mutations such as F508del would result in typical CF while the mild variant 5T might be responsible for atypical CF symptoms such as CBAVD (Cuppens and Cassiman, 2004; Claustres, 2005).

Since 5T (a polymorphic variant in intron 8 of *CFTR* gene) could result in less efficient exon 9 splicing and hence a reduced expression of functional CFTR proteins (Chu *et al.*, 1993), it has been taken as a pathogenic variant that was linked to CBAVD or other atypical symptoms of CF (Chu *et al.*, 1993; Mak *et al.*, 1997). Moreover, the 5T variant in combination with longer (TG)12 or (TG)13 would probably result in an increased disease risk compared with 5T itself (Cuppens *et al.*, 1998; Groman *et al.*, 2004). In accordance with this assumption, several studies have identified significant association of the 5T/(TG)12_13 combination with CBAVD (Stuppia *et al.*, 2005; Tamburino *et al.*, 2008). Similarly, our summary ORs also indicate an increased CBAVD risk for males carrying 5T allele, and even higher risk for the 5T/(TG)12_13 individuals.

However, due to lack of statistical power, we could not perform a subgroup analysis to determine the ethnic difference for the CBAVD risk of 5T individuals.

Heterogeneity and bias were observed among studies used in the summary analysis of the mutation frequency in CBAVD cases. We were unable to explain completely the reasons for them using the study characteristics reported, or by subgroup analysis, but the factors discussed below might contribute. First, the ethnic or geographical background is a great concern, since there is evidence that these affect the frequency and spectrum of *CFTR* mutations. Although we conducted subgroup analysis by ethnicity/geography, several studies included subjects with variant ethnic backgrounds or from different countries (Chillon *et al.*, 1995; Mercier *et al.*, 1995; Dork *et al.*, 1997). Such ethnic diversity may be the best explanation for the discrepancy between studies. Case heterogeneity is another concern, CBAVD cases are heterogeneous for both clinical symptoms and

genetic background. Some CBAVD cases are complicated by other urogenital abnormalities, in particular, CBAVD with renal agenesis had a lower incidence of *CFTR* mutations, and is likely to be a distinct disorder with a different genetic origin from CBAVD without renal abnormalities (Augarten *et al*., 1994; Dumur *et al*., 1996; de la Taille *et al*., 1998). Accordingly, involvement of CBAVD with renal defects might confuse the frequencies of mutant alleles observed in different studies.

Although, where the primary data permit we have excluded CBAVD cases with renal abnormalities, complete exclusion cannot be guaranteed due to the absence of information about renal abnormalities in some studies. Finally, lack of standardized scanning strategies might also contribute to discrepancies between studies. Most studies employed comprehensive strategies that are able to cover whole exons/flanking sequence, but some detected only the most common mutations and would probably slightly underestimate the overall frequency of *CFTR* mutations in CBAVD.

There are several limitations to our meta-analysis, which should be taken into consideration when considering the results. First, the observational studies included in our analysis are themselves a potential source of bias and confounding, although meta-analysis of observational studies may produce reliable answers to a clinical question (Stroup *et al*., 2000; Shrier *et al*., 2007). Second, as discussed above, confusing factors such as including cases with renal abnormalities and only scanning for the most common mutations might be biased towards lowering the reported rate of mutant alleles.

Every possible effort was made to reduce the influence of confusing factors on the summary results in order to achieve a high quality study. First, we conducted a rigorous and comprehensive literature search, which included almost all the eligible studies except for three that could not be retrieved for full text or lacked sufficient data (Bienvenu *et al*., 1997; Ravnik-Glavac *et al*., 2000; Radpour *et al*., 2006b). We have managed to clarify result discrepancies and obtain primary data in several studies via contacting the authors, although some failed to respond. Second, careful data abstraction were made, through which cases with renal abnormalities were excluded when the mutation information of the related cases was clear. Most importantly, subgroup analysis of overall mutation frequency by ethnic difference was based on only those studies that scanned whole *CFTR* sequence and concurrently performed 5T test, thus reducing the confusing influence of method variation.

CBAVD males, having detectable CFTR mutations in most of them, are able to father their children with help of assisted reproductive technologies, so CFTR mutations may be artificially transmitted to the offspring as a consequence. Therefore, the genetic testing should be offered to these CBAVD patients before undergoing assisted reproduction, which allows for better evaluation of the genetic risk for the offspring. Although no consensus has been reached regarding which mutations (most common or all) are to be evaluated in clinical practice, most experts recommend screening of all CFTR mutations (Cuppens and Cassiman, 2004; Claustres, 2005; Stuppia *et al*., 2005). Routine screening with commercial kits covers only partially most common mutant alleles, thus leaving some less frequent mutations missed.

In summary, the results of the present study demonstrate an association between *CFTR* variants and CBAVD risk in a variety of populations, although ethnic differences exist in the distribution of two common alleles F508delt and 5T. Moreover, 5T allele and 5T/(TG)12_13 contribute to the increased CBAVD risk, and 5T penetrance may be modified by concurrent presentation of adjacent (TG)12_13. Due to relatively small numbers of case–control studies involved, the interpretation of 5T and 5T/(TG)12_13 results should be taken with caution. Future large case–control studies comprising homogenous cases are required for more convincing conclusions, which should help elucidate better the genetic etiology of CBAVD.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

J.Y. performed literature search, data abstraction and drafted the manuscript. Z.C. performed literature search and data abstraction. Y.N. contributed to data abstraction and analysis and manuscript writing. Z.L. conceived the idea, developed the protocol and contributed to data analysis and manuscript writing.

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