NIH Public Access
Author Manuscript
Fertil Steril. Author manuscript; available in PMC 2013 September 09.

Published in final edited form as:
Fertil Steril. 2010 November ; 94(6): 2122–2127. doi:10.1016/j.fertnstert.2009.11.044.

Association of cystic fibrosis genetic modifiers with congenital bilateral absence of the vas deferens

Viktoria Havasi, M.D.[a], **Steven M. Rowe, M.D.,M.S.P.H.**[a], **Peter N. Kolettis, M.D.**[b], **Didem Dayangac, Ph.D.**[c], **Ahmet Şahin, M.D.**[d], **Ana Grangeia, Ph.D.**[e], **Filipa Carvalho, Ph.D.**[e], **Alberto Barros, Ph.D.**[e,f] **[Prof.]**, **Mario Sousa, Ph.D.**[e,f,g] **[Prof.]**, **Lluis Bassas, M.D.,Ph.D.**[h], **Teresa Casals, B.S.,Ph.D.**[i], and **Eric J. Sorscher, M.D.**[a]

[a]Department of Medicine and Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham

[b]Department of Surgery, University of Alabama at Birmingham

[c]Department of Medical Biology, Hacettepe University, Ankara, Turkey

[d]Acıbadem University, Department of Urology İstanbul, Turkey

[e]Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal

[f]Centre for Reproductive Genetics A Barros, Porto, Portugal

[g]Laboratory of Cell Biology, Institute Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

[h]Service of Andrology, Fundació Puigvert, Barcelona, Spain

[i]Medical and Molecular Genetics Center- Institute Biomedical Bellvitge (IDIBELL), Barcelona, Spain

Abstract

Objective—To investigate whether genetic modifiers of CF lung disease also predispose to CBAVD in association with CFTR mutations. We tested the hypothesis that polymorphisms of TGFB1 (transforming growth factor) (rs 1982073, rs 1800471) and EDNRA (endothelin receptor type A) (rs 5335, rs 1801708) are associated with the CBAVD phenotype.

Design—Genotyping of subjects with clinical CBAVD.

Setting—Outpatient and hospital based clinical evaluation.

Patients—DNA samples from 80 CBAVD subjects and 51 healthy male controls from various regions of Europe. One of the largest genetic studies of this disease to date.

Interventions—None.

Main outcome measures—Genotype analysis.

© 2013 American Society for Reproductive Medicine. Published by Elsevier Inc. All rights reserved

Corresponding author: Viktoria Havasi M.D., Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham, 1918 University Boulevard, Birmingham, AL 35294, USA, Tel: 1-205-934-9640, Fax: 1-205-934-7593 havasiviktoria@gmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Results—For SNP rs 5335, we found increased frequency of the CC genotype among CBAVD subjects. The difference was significant among Turkish patients vs. controls (45.2% vs. 19.4%, p<0.05), and between all cases vs. controls (36% vs. 15.7%, p<0.05). No associations between CBAVD penetrance and polymorphisms rs1982073, rs1800471 or rs1801708 were observed.

Conclusions—Our findings indicate that EDNRA polymorphism rs 5335 may be associated with CBAVD penetrance. To our knowledge, this is the first study to investigate genetic modifiers relevant to CBAVD.

Keywords

congenital bilateral absence of the vas deferens; CBAVD; CFTR; cystic fibrosis; CF; modifier gene; TGFβ; EDNRA

INTRODUCTION

Cystic fibrosis (CF) is a common autosomal recessive disorder among Caucasians, and affects one in 3500 live births in the United States (1). The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (2–4). CF is characterized by lung, pancreatic, liver and other exocrine glandular abnormalities. Approximately 98% of males with CF are infertile, and lack the vas deferens bilaterally (5). More than 1500 disease-causing CFTR mutations have been identified (6). Certain mutations have also been implicated in a variety of CFTR-related pathologic conditions such as disseminated bronchiectasis, allergic bronchopulmonary aspergillosis, diffuse panbronchiolitis, recurrent idiopathic pancreatitis, giant nasal polyposis, and congenital bilateral absence of the vas deferens (CBAVD) (7–9).

CBAVD is associated with normal spermatogenesis and obstructive azoospermia due to lack of the vas (10). A small subset of males without known CFTR defects exhibit CBAVD. However 80–97% of CBAVD subjects posses at least one defective CFTR allele, and 50–93% of individuals with CBAVD carry two variants, including class IV or V CFTR abnormalities (11– 15). CBAVD thus belongs to a group of CF related disorders - and is considered an isolated, urogenital form of CF. Although a high percentage of CBAVD patients carry mutations in CFTR, approximately one in 29 Caucasian males in the United States carries one CFTR variant but never develops CBAVD (16). The finding of a single CFTR mutation is therefore a poor predictor for involution of the vas. Other genetic or environmental factors must modify penetrance of CBAVD, but these are not yet known.

The best characterized CBAVD specific variant is the polymorphic polythymidine tract (Tn) in CFTR intron 8 (IVS8) for which length is inversely correlated with the degree of exon 9 skipping during mRNA splicing. The number of thymidines varies in this tract between 5 and 9, but extremely short alleles (with 3 or 2 thymidines) have also been described in CBAVD (17, 18). Lower numbers of thymidine residues in the tract predict an increasing proportion of nonfunctional CFTR (i.e. lacking exon 9) (15). Mak et al (19) show that a CBAVD patient with the common F508del mutation and an IVS8-5T variant produced 32% of the normal levels of CFTR in the lung (exon 9 intact; a level of expression sufficient to maintain a normal pulmonary phenotype), but insufficient full-length CFTR (26% in reproductive tissues) to allow proper structural development of the vas. The amount of functional CFTR (with exon 9) in a CF F508del/IVS8-7T carrier male (38%) in the reproductive tract was suggested to be sufficient for normal function and vas development (19). A ‘mild’ CFTR allele that maintains partial ion channel activity, R117H, is associated with the 5T allele in CF and 7T in CBAVD (20). Rave- Harel et al examined epithelial tissues from CBAVD subjects, and showed that levels of normal CFTR transcripts were

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

higher in the nasal epithelium than in epididymal epithelium (21). Therefore it has been suggested that amounts of CFTR protein required for normal function vary between different tissues (22). In general, the vas deferens has been viewed as a tissue among the most sensitive to altered CFTR activity (22).

Genetic modifiers of the CF pulmonary phenotype represent an area of intensive study. Recently, Drumm et al. (23) showed that the tissue growth factor-β1 (TGFB1) codon 10 CC genotype (rs 1982073) is associated with severe lung disease among individuals homozygous for CFTR mutations (23). This allele is linked to elevated TGFB1 gene expression and higher circulating levels of TGFB1 in human subjects (24–26). A second TGFB1 single nucleotide polymorphisms (SNP) in codon 25 (rs 1800471) may also influence TGFβ-1 protein levels (27), and has been implicated as a contributor to CF lung disease progression (27, 28).

In addition to an emerging understanding of TGFB1 as a modifier of CF severity, Darrah et al. (29) found a strong correlation between lung phenotype in CF and polymorphisms in the endothelin receptor type A (EDNRA) gene. In particular, the genotype AA at position -231 from AUG (rs 1801708) in EDNRA and genotype CC in exon eight (rs 5335) were associated with more severe lung disease in CF females (29). With reference to these results, McKone et al. (30) examined 21 tag SNPs in the EDN1 (endothelin-1), EDN3 (endothelin-3), EDNRA, and EDNRB (endothelin receptor type B) genes. The study confirmed a significant association between an EDNRA haplotype including SNP rs 5335 and CF lung disease, but no association with tagSNPs in other candidate genes (30). Interestingly, both TGFβ and endothelin play a role in extracellular matrix formation (31, 32) wound healing (31, 32), lung diseases such as asthma (33, 34) and lung fibrosis (27, 35–37). Moreover, Jain et al. (38) observed a connection between TGFβ and the endothelin-EDNRA system in idiopathic lung fibrosis, and demonstrated that endothelin-1 influences TGFβ-1 production through EDNRA.

Based on these considerations, we hypothesized that TGFB1 and EDNRA polymorphisms might play a role in penetrance of CBAVD. We designed a study to test whether codon 10 or codon 25 TGFB1 polymorphisms, or either of the two EDNRA gene polymorphisms reported to modify CF lung disease, might also contribute as genetic modifiers of CBAVD.

MATERIALS AND METHODS

Samples

We analyzed genomic DNA samples from 80 CBAVD individuals and 51 healthy male control subjects. This included 19 patient samples and 20 (non-CBAVD) controls from Spain (Medical and Molecular Genetics Center-IDIBELL), 31 CBAVD subjects and 31 controls from Turkey (Department of Medical Biology, Hacettepe University), and 30 individuals with CBAVD from Portugal (Department of Genetics, Faculty of Medicine and Laboratory of Cell Biology; Institute Biomedical Sciences Abel Salazar of University of Porto). The study therefore represents one of the largest genetic analyses to date of CBAVD, for which large patient populations are not readily available. Criteria for inclusion as a subject required known CFTR variants. Controls were defined as healthy sperm donors or other unrelated individuals with an intact vas deferens. Over 40 different CFTR polymorphisms of varying ‘severity’ were represented. Because more than 1500 CF disease associated mutations have been described previously, it is very likely that other mutations in certain subjects were present but not detected by the genotyping methods described here. The protocol was approved by the Institutional Review Board of Human Use at the University of Alabama at Birmingham and by local Portuguese, Spanish, and Turkish ethical committees.

NIH-PA Author Manuscript

Methods

A 453 bp region of the 5' end of TGFB1 gene (GenBank Accession Number: NT_011109) was amplified using 5' GAGGACCTCAGCTTTCCCTC 3' (forward) and 5' CTCCTTGGCGTAGTAGTCGG 3' (reverse) primers. This region includes both rs 1982073 and rs 1800471 TGFB1 SNPs. Conditions were as follows: predenaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 5 minutes. A region encompassing 480 bases of the promoter region of EDNRA gene (Ensembl Gene ID: ENSG00000151617), including SNP rs 1801708, was amplified using the primers 5' GTGGAAGGTCTGGAGCTTTG 3' and 5' TTCCCAGCTCTCGTCTTCTC 3'. Conditions were: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 58°C for 30s and 72°C for 40 s. The final extension step was 72°C for 7 minutes. For detection of the exon 8 SNP of the EDNRA gene (rs 5335), we used primers: 5' CTGCTGCTGTTACCAGTCCA 3' and 5' TGACCAGTTCCCATTGAACA 3'. (95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, with a final extension step of 72°C for 7 minutes).

Platinum^{R} Blue PCR Supermix (Invitrogen, Carlsbad, CA, USA), Apex^{TM} RED Taq DNA Polymerase Mastermix (Genesee Scientific, San Diego, CA, USA), or RedTaq^{R} DNA polymerase with 10× RedTaq^{R} PCR Reaction Buffer (Sigma-Aldrich, Saint Louis, MI, USA) were used for amplification. Because the EDNRA promoter constitutes a GC rich region, dimethyl sulphoxide (DMSO, 10%) was added to the PCR mixture to increase efficiency of that particular PCR. QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) was used prior to sequence analyses with BigDye Terminator v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). The sequencing products were run by standard protocols on an Applied Biosystems 3730 Genetic Analyzer with POP-7 polymer (Genomics Core Facility of the Howell and Elizabeth Heflin Center for Human Genetics, University of Alabama at Birmingham). Sequence analyses and comparisons were conducted using Chromas Lite software (39) and Clustal W Multiple Sequence Alignment software (40).

Statistical Analysis

For each SNP, an assessment was performed assuming both a dominant and non-dominant genetic relationship with the CBAVD phenotype, as the precise relationships between SNP genotype and TGFB1 or EDNRA activity are not known. Differences in the distribution of SNP genotypes were compared using χ^2 analysis. In addition, a two-sample proportion test to monitor differences in overall allelic frequencies was conducted between groups. Comparisons were performed between all cases and controls collected in the study, and subdivided by ethnicity to evaluate for population specific differences. Only historical controls were available for the Portuguese subjects; therefore these were not included in the statistical analyses. CFTR genotypes and polymorphism data for the Portuguese group are provided in Tables 1 and 4. Due to the selective nature of the candidate genes being explored, no corrections were made for multiple comparisons. All statistical analyses were done using SPSS statistical software package.

RESULTS AND DISCUSSION

This study was designed to pursue modifier genes contributing to the CBAVD phenotype. Eighty CBAVD subjects (Table 1) and 51 controls were investigated for candidate polymorphisms in TGFB1 or EDNRA. Darrah et al. (29) previously described two polymorphisms in EDNRA associated with a more severe lung phenotype among CF subjects. Both the AA genotype of SNP rs 1801708 and the CC allele of rs 5335 were reported to occur more frequently among CF individuals with severe lung symptoms. In a

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

large cohort of CBAVD subjects and controls, we observed a notable increase of the CC allele at SNP rs 5335 in association with CBAVD (Table 2). The CC allele was significantly greater in the largest matched study cohort (i.e. Turkish patients vs. controls 45.2% vs. 19.4%, $p<0.05$ by χ^2-analysis), and between all cases vs. controls (36% vs. 15.7%, $p<0.05$). The EDNRA promoter SNP (rs 1801708) did not appear to influence the penetrance of CBAVD (p=0.22) (for either Turkish or Spanish cases vs. controls; Table 2). Similarly, studies of the rs 1982073 (TGFB1 codon 10) SNP indicated a trend towards increased T allelic frequency in all CBAVD subjects compared to controls (58% vs. 45%), although neither the subgroup analyses for polymorphism distribution by ethnicity nor genotype frequency indicated a significant association with CBAVD penetrance. With regard to TGFB1 codon 25 SNP (rs 1800471), there was no association with CBAVD for any of the analyses performed (Tables 3 and 4).

Transforming growth factor β is the best described modifier of the CF pulmonary phenotype. With reference to the present study, the human vas deferens, epididymis, and seminal vesicle develop from the Wolffian-ducts, and it is well established that TGFβ and related signaling pathways are crucial during normal Wolffian-duct development and differentiation (41–43). A rat gene expression-array suggested that during Wolffian-duct formation, androgens indirectly modify IGF (insulin-like growth factor) and TGFβ signaling pathways, both of which play an important role during epithelial-mesenchymal interactions and normal development of the vas (43). Although TGFβ and associated signaling pathways have been shown to subserve a crucial role in the normal vas, and clearly contribute to CFTR-related pathology in tissues such as lung, the significance of this pathway in atypical CF-related conditions such as CBAVD has not been studied previously.

In our experiments, we found that TGFB polymorphisms rs 1800471 and 1982073 do not impact the CBAVD clinical phenotype. The result suggests important differences in the pathogenesis attributable to altered CFTR expression in CBAVD versus pulmonary CF. For example, CF lung manifestations including polymorphonuclear cell infiltration and cytokine release are known to exacerbate CF lung injury, and TGFβ (a known inflammatory modulator) might influence the extent of pulmonary inflammation due to chronic infection. Such mechanisms may not be relevant to vas development *in utero*, and therefore cannot be invoked to account for CBAVD in the setting of CFTR deficiency.

On the other hand, results from this initial survey indicate that at least one known genetic modifier of CF lung disease (EDNRA) does appear to associate with CBAVD. Endothelin receptor type A or a close homologue have been implicated previously as important during normal formation of the mammalian nervous system, the anorectum, and cranio-facial structures such as the mandible (44–47). Our results point to EDNRA as playing a significant role during development of the vas deferens, and indicate that the gene product may contribute to loss of the vas in the setting of CFTR insufficiency. Having said this, CBAVD is likely a multifactorial disease, and a number of other modifying factors almost certainly influence disease penetrance. Further studies of larger patient cohorts, as well as genome-wide association analyses will be necessary to determine the major effectors that influence penetrance of CBAVD.

Acknowledgments

We are grateful for assistance provided by Genomics Core Facility of the Howell and Elizabeth Heflin Center for Human Genetics, UAB. We also thank the CBAVD subjects and controls who participated in this study. The work was partially supported by grant PI050804 from FIS/FEDER (ISCiii), Spain and by FCT (POCI/SAU-MMO/59997/60555/60709/2004; UMIB), NIH P30 DK 54781 and RDP 464-CR02.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

REFERENCES

1. Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. The Journal of pediatrics. 2008; 153:S4–S14. [PubMed: 18639722]
2. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science (New York, NY. 1989; 245:1073–1080.
3. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science (New York, NY. 1989; 245:1066–1073.
4. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science (New York, NY. 1989; 245:1059–1065.
5. Taussig LM, Lobeck CC, di Sant'Agnese PA, Ackerman DR, Kattwinkel J. Fertility in males with cystic fibrosis. The New England journal of medicine. 1972; 287:586–589. [PubMed: 5055208]
6. Cystic Fibrosis Mutation Database: The Hospital for Sick Children, Genetics and Genomics Biology. Toronto: 1989. Available at: http://www.genet.sickkids.on.ca/cftr/app [Accessed: August, 2008]
7. Kerem E. Atypical CF and CF related diseases. Paediatric respiratory reviews. 2006; 7(Suppl 1):S144–S146. [PubMed: 16798544]
8. Report of a joint WHO/ICF(M)A/ECFTN meeting S, Sweden, June 3, 2000. Classification of cystic fibrosis and related disorders. J Cyst Fibros. 2002; 1:5–8. [PubMed: 15473059]
9. Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros. 2008; 7:179–196. [PubMed: 18456578]
10. Anguiano A, Oates RD, Amos JA, Dean M, Gerrard B, Stewart C, et al. Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. Jama. 1992; 267:1794–1797. [PubMed: 1545465]
11. Casals T, Bassas L, Egozcue S, Ramos MD, Gimenez J, Segura A, et al. Heterogeneity for mutations in the CFTR gene and clinical correlations in patients with congenital absence of the vas deferens. Human reproduction (Oxford, England). 2000; 15:1476–1483.
12. Grangeia A, Sa R, Carvalho F, Martin J, Girodon E, Silva J, et al. Molecular characterization of the cystic fibrosis transmembrane conductance regulator gene in congenital absence of the vas deferens. Genet Med. 2007; 9:163–172. [PubMed: 17413420]
13. Ratbi I, Legendre M, Niel F, Martin J, Soufir JC, Izard V, et al. 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. Human reproduction (Oxford, England). 2007; 22:1285–1291.
14. Taulan M, Girardet A, Guittard C, Altieri JP, Templin C, Beroud C, et al. Large genomic rearrangements in the CFTR gene contribute to CBAVD. BMC medical genetics. 2007; 8:22. [PubMed: 17448246]
15. Cuppens H, Cassiman JJ. CFTR mutations and polymorphisms in male infertility. International journal of andrology. 2004; 27:251–256. [PubMed: 15379964]
16. Baskin LB, Wians FH Jr, Elder F. Preconception and prenatal screening for cystic fibrosis. MLO: medical laboratory observer. 2002; 34:8–12. quiz 4, 6. [PubMed: 12385171]
17. Radpour R, Taherzadeh-Fard E, Gourabi H, Aslani S, Vosough Dizaj A, Aslani A. Novel cause of hereditary obstructive azoospermia: a T2 allele in the CFTR gene. Reproductive biomedicine online. 2009; 18:327–332. [PubMed: 19298730]
18. Disset A, Michot C, Harris A, Buratti E, Claustres M, Tuffery-Giraud S. A T3 allele in the CFTR gene exacerbates exon 9 skipping in vas deferens and epididymal cell lines and is associated with Congenital Bilateral Absence of Vas Deferens (CBAVD). Human mutation. 2005; 25:72–81. [PubMed: 15580565]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

19. Mak V, Jarvi KA, Zielenski J, Durie P, Tsui LC. Higher proportion of intact exon 9 CFTR mRNA in nasal epithelium compared with vas deferens. Human molecular genetics. 1997; 6:2099–2107. [PubMed: 9328474]
20. Kiesewetter S, Macek M Jr, Davis C, Curristin SM, Chu CS, Graham C, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. Nature genetics. 1993; 5:274–278. [PubMed: 7506096]
21. Rave-Harel N, Kerem E, Nissim-Rafinia M, Madjar I, Goshen R, Augarten A, et al. The molecular basis of partial penetrance of splicing mutations in cystic fibrosis. American journal of human genetics. 1997; 60:87–94. [PubMed: 8981951]
22. Claustres M. Molecular pathology of the CFTR locus in male infertility. Reproductive biomedicine online. 2005; 10:14–41. [PubMed: 15705292]
23. Drumm ML, Konstan MW, Schluchter MD, Handler A, Pace R, Zou F, et al. Genetic modifiers of lung disease in cystic fibrosis. The New England journal of medicine. 2005; 353:1443–1453. [PubMed: 16207846]
24. Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, et al. Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J Bone Miner Res. 1998; 13:1569–1576. [PubMed: 9783545]
25. Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, et al. Transforming growth factor-beta 1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97:3479–3484. [PubMed: 10725360]
26. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, et al. A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer research. 2003; 63:2610–2615. [PubMed: 12750287]
27. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation. 1998; 66:1014–1020. [PubMed: 9808485]
28. Arkwright PD, Laurie S, Super M, Pravica V, Schwarz MJ, Webb AK, et al. TGF-beta(1) genotype and accelerated decline in lung function of patients with cystic fibrosis. Thorax. 2000; 55:459–462. [PubMed: 10817792]
29. Darrah, RTV.; Smith, P.; Goddard, K.; Londono, D.; Dunn, J.; Knowles, M.; Drumm, ML. Variants in the endothelin receptor A gene associate with increased expression levels, increased cell proliferation and more severe pulmonary disease in CF females; North American Cystic Fibrosis Conference; Anaheim, California, USA. Pediatric Pulmonology; 2007. p. 73-428.
30. McKone, EFOCCM.; Rodgers, CE.; Genatossio, A.; McNamara, SGRL.; Elborn, S.; Kalsheker, N.; Gallagher, CGAM. TagSNP evaluation of the endothelin pathway demonstrates genetic association between EDNRA and CF lung disease severity; North American Cystic Fibrosis Conference; Anaheim, California, USA. Pediatric Pulmonology; 2007. p. 73-428.
31. Lawrence DA. Transforming growth factor-beta: a general review. European cytokine network. 1996; 7:363–374. [PubMed: 8954178]
32. Goraca A. New views on the role of endothelin (minireview). Endocrine regulations. 2002; 36:161–167. [PubMed: 12466016]
33. Yamaguchi M, Niimi A, Matsumoto H, Ueda T, Takemura M, Matsuoka H, et al. Sputum levels of transforming growth factor-beta1 in asthma: relation to clinical and computed tomography findings. J Investig Allergol Clin Immunol. 2008; 18:202–206.
34. Hay DW. Putative mediator role of endothelin-1 in asthma and other lung diseases. Clinical and experimental pharmacology & physiology. 1999; 26:168–171. [PubMed: 10065341]
35. Corrin B, Butcher D, McAnulty BJ, Dubois RM, Black CM, Laurent GJ, et al. Immunohistochemical localization of transforming growth factor-beta 1 in the lungs of patients with systemic sclerosis, cryptogenic fibrosing alveolitis and other lung disorders. Histopathology. 1994; 24:145–150. [PubMed: 8181807]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

36. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. The New England journal of medicine. 2000; 342:1350–1358. [PubMed: 10793168]
37. Hocher B, Schwarz A, Fagan KA, Thone-Reineke C, El-Hag K, Kusserow H, et al. Pulmonary fibrosis and chronic lung inflammation in ET-1 transgenic mice. American journal of respiratory cell and molecular biology. 2000; 23:19–26. [PubMed: 10873149]
38. Jain R, Shaul PW, Borok Z, Willis BC. Endothelin-1 induces alveolar epithelial-mesenchymal transition through endothelin type A receptor-mediated production of TGF-beta1. American journal of respiratory cell and molecular biology. 2007; 37:38–47. [PubMed: 17379848]
39. Technelysium, PL. Chromas Lite Freeware. Tewantin, Australia: 2005.
40. Thompson JDHDG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. 1994
41. Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R, Boivin GP, et al. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. Development (Cambridge, England). 1997; 124:2659–2670.
42. Stenvers KL, Tursky ML, Harder KW, Kountouri N, Amatayakul-Chantler S, Grail D, et al. Heart and liver defects and reduced transforming growth factor beta2 sensitivity in transforming growth factor beta type III receptor-deficient embryos. Molecular and cellular biology. 2003; 23:4371–4385. [PubMed: 12773577]
43. Hannema SE, Print CG, Charnock-Jones DS, Coleman N, Hughes IA. Changes in gene expression during Wolffian duct development. Hormone research. 2006; 65:200–209. [PubMed: 16567946]
44. Ruest LB, Xiang X, Lim KC, Levi G, Clouthier DE. Endothelin-A receptor-dependent and -independent signaling pathways in establishing mandibular identity. Development (Cambridge, England). 2004; 131:4413–4423.
45. Pla P, Larue L. Involvement of endothelin receptors in normal and pathological development of neural crest cells. The International journal of developmental biology. 2003; 47:315–325. [PubMed: 12895026]
46. Moore SW, Zaahl MG. Association of endothelin-beta receptor (EDNRB) gene variants in anorectal malformations. Journal of pediatric surgery. 2007; 42:1266–1270. [PubMed: 17618893]
47. Stanchina L, Baral V, Robert F, Pingault V, Lemort N, Pachnis V, et al. Interactions between Sox10, Edn3 and Ednrb during enteric nervous system and melanocyte development. Developmental biology. 2006; 295:232–249. [PubMed: 16650841]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

Distribution of CFTR mutations among CBAVD samples

Portuguese	CFTR alleles	Spanish	CFTR alleles	Turkish	CFTR alleles
5T	22	F508del	11	5T	20
F508del	14	5T	9	D1152H	14
R334W	5	D443Y [a]	3	D110H	3
R117H	3	G576A [a]	3	F508del	2
S1235R	3	R668C [a]	3	3041-11del7	2
N1303K	2	G542X	2	1767del6	2
P205S	2	R117H	2	2789+5G>A	2
D614G	2	V232D	2	CFTRdele2(ins186)	2
G542X	1	L997F	1	3120+1G>A	1
L206W	1	H609R	1	G1130A	1
V562I	1	N1303H	1	M952I	1
I507del	1	L206W	1	365insT	1
3272-26A>G	1	3272-26A/G	1	E585X	1
2789+5G>A	1	L15P	1	2752-15 C>G	1
G576A [a]	1	R347H	1	R334Q	1
R668C [a]	1	2689insG	1	R347H	1
CFTRdele2,3	1	R1070W	1	E831X	1
L1227S	1	I 1027T	1	R1070W	1
E831X	1			3272-26A>G	1
				L997F	1
				I853F	1
				A349V	1
				6T	1

[a] in *cis*

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 2

EDNRA genotype distributions in CBAVD patients and controls

EDNRA polymorphisms			Patients		Controls	
Exon 8 (rs 5335)	*Turkish*	CC [a]	14	45.2%	6	19.4%
		CG	12	38.7%	20	64.5%
		GG	5	16.1%	5	16.1%
	Spanish	CC	4	21.1%	2	10%
		CG	11	57.8%	13	65%
		GG	4	21.1%	5	25%
	Total [b]		**50**			**51**
Promoter (rs 1801708)	*Turkish*	AA	5	16.1%	8	25.8%
		AG	18	58.1%	10	32.3%
		GG	8	25.8%	13	41.9%
	Spanish	AA	0	0%	2	10%
		AG	7	36.8%	8	40%
		GG	12	63.2%	10	50%
	Total		**50**			**51**

[a] $p < 0.05$ for Turkish population, χ^2 analysis

[b] $p < 0.05$ for all subjects shown, χ^2 analysis

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 3

TGFB1 genotype distribution in CBAVD patients and controls

TGFB1 polymorphisms			Patients		Controls	
Codon 10 (rs 1982073)	*Turkish*	CC	8	25.8%	13	41.9%
		CT	12	38.7%	8	25.8%
		TT	11	35.5%	10	32.3%
	Spanish	CC	4	21.1%	6	30%
		CT	6	31.6%	10	50%
		TT	9	47.4%	4	20%
	Total		**50**			**51**
Codon 25 (rs 1800471)	*Turkish*	GG	26	83.9%	28	90.3%
		GC	5	16.1%	3	9.7%
		CC	0	0%	0	0%
	Spanish	GG	19	100%	17	85%
		GC	0	0%	3	15%
		CC	0	0%	0	0%
	Total		**50**			**51**

Table 4

Allelic frequencies of EDNRA and TGFB1 SNPs

EDNRA exon 8 SNP (rs 5335)

C allelic frequency	Ethnicity	Patients	Controls
	Turkish	64.5%	51.6%
	Spanish	50%	42.5%
	Portuguese	33.3%	ND [a]

EDNRA promoter SNP (rs 1801708)

A allelic frequency	Ethnicity	Patients	Controls
	Turkish	45.2%	30%
	Spanish	18.4%	41.9%
	Portuguese	26.8%	ND [a]

TGFB1 codon 10 SNP (rs 1982073)

T allelic frequency	Ethnicity	Patients	Controls
	Turkish	54.8%	45.1%
	Spanish	63.2%	45%
	Portuguese [b]	55%	44.4%

TGFB1 codon 25 SNP (rs 1800471)

G allelic frequency	Ethnicity	Patients	Controls
	Turkish	91.9%	95.2%
	Spanish	100%	92.5%
	Portuguese [b]	95%	92.5%

[a]ND = not done

[b]Control data from Alves H, Histocompatibility Center, University of Porto, Portugal

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript