

Genetic Modifiers of CF: Sibling Study

Abstract:

CF (Cystic Fibrosis) is an autosomal recessive disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator gene, the primary Chloride (Cl⁻) channel in epithelial cells of numerous organ systems. In CF patients, a severe reduction in Cl⁻ transport across epithelial cell membranes results in obstructive pulmonary disease, chronic sinusitis, pancreatic insufficiency, intestinal obstruction, and male infertility. The degree of clinical severity varies tremendously among CF patients. While genotype-phenotype correlations have shown that some of the variability in clinical presentation is due to specific mutations in CFTR, considerable variability exists between patients with identical mutations suggesting factors other than CFTR genotype may contribute to disease severity. The purpose of this study is to analyze the genetic, environmental, and clinical status (at diagnosis, in the interim, and current) of twins and siblings with CF to determine possible genetic modifiers of the disease.

Specific Aims:

1. Collection of Cystic Fibrosis Twins and Siblings to determine the contribution of genetic and other factors to CF Phenotype.

The CF Foundation Registry indicates that there are approximately 120 twin pairs and 1100 sibling families with CF. This study plans to collect all twins and siblings in the United States with CF to avoid ascertainment bias. For those consenting to this study, blood will be collected from twins, siblings, and parents whenever possible. Blood will then be used for lymphoblast transformation. Plasma will be separated and cryopreserved. All patients not previously screened for CF genotype will be typed using a commercially available CF mutation strip or by sequence. Clinical data regarding the twins and siblings will be collected by the study team and/or through the CF Registry Database and will include parental health, environmental factors, familial phenotypes, birth history, and clinical parameters at diagnosis, interim, and current status. The analysis of the data will pay particular attention to birth cohort effect. Descriptive, cross-sectional, and longitudinal analysis will also be performed; in addition analyses will be stratified by CFTR genotype, environmental exposures, and other covariates or confounders as they are identified.

2. Identify biologic phenotypes that correlate with heritable CF phenotypes by the clinical study of twins and siblings. (To be conducted at the Johns Hopkins Hospitals for selected patients.) Studies of ion transport (including the Nasal Potential Difference Test, cAMP sweat rates, and sweat chlorides), fatty acid metabolism, clinical imaging studies and other assays relevant to heritable CF traits (e.g. oral glucose tolerance tests for CF-related diabetes, acquisition of chronic pseudomonas infection, induced sputum culture) will be performed on twins and siblings concordant or discordant for CF whose variance appears to have a substantial heritable component. These individuals will be asked to travel to the General Clinical Research Unit at Johns Hopkins Hospital for these additional studies. Participation in this component of the project will require additional informed consent.

3. Identify modifier genes and loci responsible for heritable CF Phenotypes by linkage, linkage disequilibrium analysis, and genome wide association studies. (To be conducted through the Institute of Genetic Medicine, Johns Hopkins Medicine)

Genome-wide single nucleotide polymorphism (SNP) maps will be obtained for 1000 twin/sibling/parent families. Outcomes of interest for linkage and/or association analyses will include both qualitative and quantitative CF related phenotypes. Allele sharing methods, which do not require the specification of a particular disease model, will be used to test for evidence of linkage; this will be assessed with the programs SIBPAL2 (SAGE 4.0, beta version 5), GENEHUNTER, and S.O.L.A.R. (sequential oligogenic linkage analysis routines). Genome-wide association analysis will be performed using transmission disequilibrium testing (TDT) and quantitative TDT as implemented in GENEHUNTER and MERLIN. Pursuit of targeted candidate gene analyses for CF related phenotypes will be performed and outcomes of interest will be prioritized according to heritability estimates from aims 1 and 2.

4. To refine estimates of the contribution of genetic and non-genetic factors to variation in CF phenotypes by prospective longitudinal analysis.

Clinical data for monozygous (MZ) twins, dizygous (DZ) twins and siblings recruited by the study will be obtained annually from the CF Foundation Patient Registry. Medical records will be reviewed in cases where clinical data are not available in the Registry. Emphasis will be placed on features relevant to lung disease, nutritional status, meconium ileus, and CF-related diabetes, a

common qualitative phenotype associated with reduced survival that displays age of onset effect. Intra-pair and inter-pair variance will be computed for selected CF phenotypes, and inter-class comparisons (MZ twins vs. DZ twins/siblings) will be performed to estimate genetic and non-genetic effect.

Background and Significance

Cystic fibrosis (CF), a single gene disorder that affects 60,000 individuals worldwide, is an ideal model for the identification and characterization of modifier genes. The unfortunate commonness of CF and the presence of highly organized treatment centers around the world provide a large number of accessible patients to perform detailed phenotypic analysis necessary to identify modifier genes in humans. Disease severity and complications in CF patients vary considerably among affected organ systems. Over 1100 disease-associated mutations have been reported in *CFTR*, although one mutation, $\Delta F508$, accounts for approximately 70% of CF alleles worldwide. The commonness of $\Delta F508$ homozygosity among CF patients (approximately 50%) creates a reference population for analyzing the phenotypic consequences of variability in *CFTR* genotype. *CFTR* genotype is poorly correlated with complications and with variation in nutritional status and lung disease. These observations have led to a search for the possible causes of disease variation independent of *CFTR* genotype. Some environmental factors have been associated with worse outcome but the search for genetic modifiers utilizing case-control association analysis of biological candidates has been fraught with inconsistencies. However, two variants in a selected candidate gene encoding transforming growth factor $\beta 1$ (*TGF β 1*) were recently associated with more severe lung disease in a multi-center case-control study. *TGF β 1* genotypes have been associated with susceptibility to asthma and protection from development of chronic obstructive pulmonary disease in smokers, supporting the concept that CF modifier genes may play a pathologic role in common diseases. Variants of several other genes (*TNF2*, *GSTM1*, $\alpha 1$ -AT, mannose binding lectin) have been associated with severity of CF lung disease independent of *CFTR* genotype. In each case, the variant alleles studied had previously been shown to alter the levels of the encoded product. Interaction of genes and environment (smoke exposure) has also been associated with the negative course of lung disease in CF. Furthermore, sibling studies have revealed significant correlation in pulmonary function (%FEV1) among affected adults living in the United Kingdom and among affected, pancreatic sufficient children (10-15 years of age) living in Canada. Nutritional parameters such as height and weight percentiles differed significantly among a group of 47 African

Americans matched by age, sex, and treatment center with 188 Caucasians with the same genotype ($\Delta F508$ homozygosity). This difference in Body Mass Index may be due to differences in diet or nutritional therapy, but could also reflect differences in the genetic background of African-American and Caucasian patients with CF. Other work supports the existence of a genetic variant independent of CFTR that contributes to the development of meconium ileus in humans. As of this time, the gene involved has not been identified, but studies of larger, well characterized patient and mouse populations are likely to pinpoint the responsible gene. Therefore, CF should be an excellent model for the identification of modifier genes. High quality patient collections will be needed to verify potential associations discovered in mouse and human studies, and to enable the positional cloning of novel human modifier genes. These goals can be achieved by the in-depth study of twins and siblings with CF disease utilizing the coordinated research resources of the Cystic Fibrosis Foundation and the National Institutes of Health, and Johns Hopkins Medicine.

Design Methods and Statistical Analysis

Preliminary descriptive analysis will be performed on all variables collected via frequencies, χ^2 , means, and ANOVA with subgroup analyses and stratification performed as necessary. Birth cohort effects will be assessed in the siblings. Multivariate analyses will be performed incorporating biologically important variables and will be used to explore gene by gene and gene by environment interactions. Longitudinal data analyses will be performed using mixed effect models, segmented regression, and GEE as appropriate. Intra- and inter- pair differences will be determined for the continuous phenotypic values collected. Heritability will be determined using data collected from twins. Likelihood-based variance components analyses will be used to test a variety of models estimating the genetic and environmental components of quantitative phenotypes. The genetic contribution to continuous CF phenotype will be evaluated using a basic quantitative trait locus (QTL) approach. Rates of concordance and discordance, Mantel-Haenszel χ^2 , zygosity-specific odds ratios, and logistic regression models will be used to evaluate qualitative phenotypes. Estimates of heritability will be used to select phenotypes of interest. Concordance and discordance will be assessed within approximately 1,000 sibling–parent nuclear families. A genome-wide scan will be performed on the DNA of families in collaboration with other CF Researchers. Allelic sharing between sib-pairs will be evaluated for departures from expected values. Multipoint mapping will be performed to estimate map location. Regions from the genome scan that

give consistent evidence of linkage will be densely mapped using SNPs. In addition, candidate gene SNPs will be analyzed using single-marker and multi-locus TDT (focusing on the transmission of parental alleles to the siblings), and haplotype TDT analyses will be performed.

Performance Sites:

This study will be coordinated by Johns Hopkins Medicine with sponsorship from the National Institutes of Health and the Cystic Fibrosis Foundation. All CF Centers in the US, along with some centers from Australia, Israel, Canada, and Great Britain have been asked to or have agreed to participate.

Protection of patient confidentiality:

The CF Foundation Registry number, patient's name, or locally assigned study numbers will be used to mark the materials at the time of collection. Upon arrival at Johns Hopkins Medicine, all materials will have the CFF identifier number or any other identifiers removed/erased and a JHH Study number assigned. A code book will be kept so that patients can be contacted by the sponsoring organization or governmental/regulatory bodies as necessary; this code book is kept separate from any clinical or biological material, under lock and key with oversight by the Principal Investigator.

Data Sharing with other IRB Approved CF Researchers:

The information collected throughout the course of this project is unique for the study of single gene disorders; it is family based and aims for full ascertainment of siblings with CF in the US. The project has been designed for quality control of data and DNA samples, the goal of which is to establish a resource of clinical, environmental, and genetic information for use by other CF Researchers, for the purposes of CF research only. When a request for samples and/or data is made to the study PI, local IRB approval for such study will need to be obtained before any material is shared. The CF Modifier Advisory Committee (see Section: Utilization of Records below) will review and approve the proposed research projects based on its merits. Any data transferred to other researchers will be stripped of PHI. (Year of birth will replace birth date, 5 digit zip codes will be transformed to 3, and the participant's first name will be removed.)

Human Subjects:

Any patients with Cystic Fibrosis who have a twin or sibling(s) with the disease are candidates for this study. Parents will also be asked to participate for the purpose of collecting a blood sample for DNA analysis to determine patterns of heritability. The goal is to collect all CF patients with an affected twin or sibling to prevent ascertainment bias.

Recruitment:

Eligible patients will be approached during a routine CF Clinic visit, and asked to participate in the study. This may be done by the PI or his/her designee.

Informed consent:

At the time of a clinic visit, the PI or his/her designee will discuss the scope and purpose of this study with the patient/parent/guardian. If he/she agrees to participate, consent and/or assent will be obtained by the PI or designee.

Therapeutic Alternatives:

This is not a clinical trial. The only alternative to participation in this study is to not participate. There will be no penalty for non-participation.

Genetic Testing:

Approximately 20-30 ml of blood will be drawn from study participants (8-10 cc from children under 5 years of age) and sent via overnight delivery to Johns Hopkins Medicine for DNA analysis and banking. These samples will have CFF identifier numbers/local study numbers or patient names, which will be removed as described above at the time of receipt and before analysis. The results of the CFTR Genotype, if not already determined, will be shared with the patient's physician upon request (using the study code and link to patient information); this genotype testing will be verified in a CLIA approved laboratory. Parental genotype will not be ascertained. All other DNA analysis will be done under research conditions, and therefore results will not be provided to patients or providers. This further analysis will only be done to answer questions about Cystic Fibrosis. No additional permission will be asked to do these studies. If new information becomes available that may affect a patient or parents' willingness to participate in the study, such information will be provided to the patient's physician. The

blood sample will be immortalized via lymphoblast culture and stored at the Cell Core Facility at Johns Hopkins Medicine for future research in modifiers of CF.

Utilization of databases, disease registries, or member medical records:

All data retrieved for the purposes of this study will be assigned study codes. Some Personal Health Information such as first name, date of birth, and zip code will be retained in the study database located at Johns Hopkins Medicine. Additional clinical information will be obtained from the Cystic Fibrosis Foundation patient registry to facilitate longitudinal analysis and examination and data collection from patient medical records may supplement registry data. The Primary Investigator at the coordinating institution (Johns Hopkins Medicine) will delegate to appropriate staff members the responsibility of assigning study codes, reviewing and entering clinical data, and ensuring that all materials are removed of any identifiers. These individuals all have certification in Human Subject Research. The data from the Cystic Fibrosis Foundation registry is released to the CF Twin and Sibling Study only for the exact purpose for which it was requested, as outlined in a written agreement entered into with the CF Foundation in February 2006. Documentation of JHM IRB review and approval must be provided before any data is released from the Foundation. This agreement also specifies that the Data acquired from the Cystic Fibrosis Foundation Registry may not be further disclosed without the express written consent of the CFF, which it may withhold at its discretion.

The CF Foundation participates in the NIH CF Modifiers Advisory Committee whose function is to review and approve all projects that request access to de-identified clinical data or DNA collected by the CF Twin and Sibling Study. Finally, the Foundation must be acknowledged in any abstract, publication, or presentation for which CFF Registry Data was used in analysis.

Risks:

Blood sampling requires phlebotomy, which may cause minor pain, bruising, or bleeding. The collection of clinical data poses minimal risk to the patient as it is done retrospectively. As is the case in all research projects, there is a slight risk of breach of confidentiality. Every safeguard will be utilized to protect the anonymity of genetic and clinical data including study coding and anonymizing DNA samples; clinical data/test results will be anonymized as well, by removing all identifiers and replacing them with a study code and ID number. Electronic databases related to this study are password and firewall protected. All blood samples, DNA samples, and clinical data forms, previously coded, will be kept separately from each other within the office and laboratory facilities of Johns Hopkins Medicine. A

code book will be kept in a locked cabinet under the supervision of the JHM Principal Investigator in the event of having to contact or receiving contact from study subjects. In the event of a study subject wishing to withdraw his/her participation, the PI at the coordinating center will provide the patient with the contact information of Dr. Garry Cutting at Johns Hopkins. After receipt of the request in writing from the patient, Dr. Cutting will instruct the Cell Core Facility at Johns Hopkins to destroy the patients' sample (using sodium hypochlorite). Subsequently, all clinical data forms related to that patient will be destroyed, but information gathered up to the point of withdrawal may be retained in the electronic databases.

Potential Benefits:

There are no anticipated direct benefits to patients participating in this study. The overall benefit of this study is to the greater CF community, as the purpose of this study is to understand and examine other genetic modifiers of Cystic Fibrosis. Once understood, therapy could be more individually tailored with the goal of prevention of significant disease.

Expense to subject:

There will be no direct expense to the subject for participation in this study. If patients have been identified for further study, their travel to the Johns Hopkins Hospital will be supported by NIH Grant: 2 R01 HL068927. This further study may include in depth analysis of ion transport studies, imaging studies, fatty acid analysis, nasal cell brushing for determining RNA expression, and sputum analysis. Study subjects will not be paid for their participation.

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