

**Cystic fibrosis in the era of effective treatment:
Informing targeted therapy and influencing
reproductive decision-making**

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Abstract

The treatment of cystic fibrosis with small molecule therapies is heralded as a gold standard for the precision medicine era. The past decade in CF research has seen the advent of variant-specific therapies that are able to correct the underlying defect responsible for disease. The eventual goal of providing effective therapy to all individuals with CF is dependent upon the ability to estimate potential clinical improvements attributable to increases in CFTR function. Many other genetic diseases are also beginning to see advances in treatment that address the underlying disease mechanism. This, combined with the ever-increasing availability of genetic screening, means that it is vital for the medical genetics community to understand how these new therapies are affecting people's views and decisions. Taking into account a changing disease landscape is essential when developing screening and counseling protocols, as well as recommendations for carrier testing. Cystic fibrosis provides a paradigm to understand how precision treatment is being applied in the clinic and beyond.

Preface

This dissertation, and the work contained within it, were made possible by the support from so many people, too numerous to thank each by name here. But I do hope that everyone I have encountered in both my professional and personal life that has helped me throughout this journey knows that my gratitude to them knows no bounds.

Before I came to Hopkins, I worked in the lab of Dr. Erica Selva as an undergraduate researcher at the University of Delaware and I would like to thank her for being my first mentor. She taught me not only to start thinking like a scientist, but showed me what a passionate, tenacious, and dedicated scientist looks like. She worked alongside students in the lab every day doing experiments. I would only learn later how unusual that was for a PI and I'm grateful for it. During my time at UD, I was also lucky enough to be mentored academically by Dr. David Smith, who was the professor for my first genetics class and the reason I chose genetics as my field for graduate school. He not only shared my passion for genetics, but my passion for baseball as well and I cherish the memories of our long chats in his office about the Mets, the Dodgers, and baseball sabermetrics.

The Institute of Genetic Medicine has been like family to me during my time here at Hopkins and I am so grateful for all of the faculty, staff, and students for making this a great place to work for the past six years. I remember being constantly in awe of the intelligent and passionate people I was surrounded by every day, and it always pushed me to be better. The faculty were always ready to help me talk out whatever scientific question I may be facing. Sandy kept things running like clockwork and was always there for an ear to listen and a piece of candy to make me feel better after a bad day. Dr. Peter

Mogayzel and Dr. Lori Erby provided invaluable scientific and career advice as members of my thesis committee and brought perspectives that vastly improved my work.

Dr. Valle was an invaluable mentor both during my rotation and later on my thesis committee. But most importantly, he showed me from day one what a supportive place the Human Genetics program is. I knew while I was interviewing with various graduate programs that I had this sort of vague, not exactly fleshed out interest in bioethics as well as in genetics. Where other programs were somewhat dismissive of my hybrid interests, Dr. Valle and the IGM fostered them. He introduced me to Dr. Debra Mathews and that is where the idea for my interdisciplinary thesis was born.

I am lucky enough to have not just one great thesis mentor, but two. I am so incredibly grateful that Dr. Garry Cutting and Dr. Debra Mathews took a chance on me and allowed me to pursue this hybrid thesis work and forge a unique path. Garry taught me the importance of considering a question from every angle and always believed I was capable of more, even when I didn't believe it myself. His adage that "behind every variant there is a patient" kept me going during bad days and reminded me why I came to lab every day. Debra was so patient with me while I learned an entirely new skillset delving into the world of qualitative research. She lent me books, sent me papers, and was with me every step of the way. Their combined mentorship made me a better scientist and a better person.

With two mentors also came the fortuitous circumstances of two scientific homes—one in the Cutting lab and one in the Berman Institute of Bioethics—which meant twice as many fantastic people that I am so lucky to call my colleagues as well as my friends. They say that a good mentor attracts good people to their lab and that is

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My fellow graduate students and especially my classmates have been such great friends to me during my time here at Hopkins. Working on problem sets together well into the night and studying for orals together helped keep me sane during my first two years. It has been incredibly gratifying to watch my classmates grow along with me and become such great scientists. I especially need to thank Helen Schmidt, who has been not only my classmate, but also my roommate for the past six years and my closest friend during my time at Hopkins. I really treasure all of our farmer's market trips, baseball games, and long movie nights on the couch. She and our dog Cricket were the constants in my life every day and I am so grateful for that.

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Finally, of course, I would like to thank my family. My unapologetic nerdiness and love for science and baseball comes from my dad, but my relentless extroversion and need to be around people comes from my mom. I appreciate them both for instilling this combination of qualities in me, for fostering my education, and for loving me absolutely unconditionally. I would not have made it through graduate school without their unrelenting support. I would also like to thank my brother Ryan for believing in me, making me laugh more than anyone else, and not being embarrassed for having such a weird nerd around all the time. I am also incredibly grateful for my network of extended family members, who have always supported me and never failed to tell me that I have made them proud, which is really the most rewarding result of all.

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Chapter 1. Introduction

1.1 Cystic fibrosis is a single-gene Mendelian disorder demonstrating allelic and phenotypic heterogeneity

Cystic fibrosis (CF) is an autosomal recessive disorder affecting approximately 70,000 individuals worldwide.¹ It is a monogenic disease caused by variants in the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride and bicarbonate channel². CF demonstrates considerable allelic and phenotypic heterogeneity, with over 2,000 *CFTR* variants identified to date.³ CFTR dysfunction results in aberrant chloride ion transport, leading to abnormally viscous secretions in many epithelial tissues, including the airways, pancreatic ducts, and male reproductive tract. Pancreatic insufficiency is caused by loss of exocrine pancreatic function and requires enzyme replacement. Obstructive lung disease is the chief cause of morbidity and mortality in CF, responsible for approximately 80% of the deaths of individuals with CF.⁴

Despite being one of the most well-studied genes in the human genome, categorization and interpretation of *CFTR* variants remains a challenge and *CFTR* sequencing results often return variants of uncertain significance.⁵ Mutations in *CFTR* can cause disease by a variety of different mechanisms, including at the level of transcription, splicing, protein folding, protein stability, channel activity, and channel conductance.² In order to close the gap in knowledge between reported *CFTR* variants and their consequences, the Clinical and Functional TRanslation of CFTR (CFTR2) project was established, combining the assembly of clinical data from CF patients with functional assessment of *CFTR* variants, in order to perform variant classification.⁶ With this unprecedented amount of data across the phenotypic spectrum of disease, we have

not only been able to define the pathogenicity of many rare missense variants⁷, but also evaluate response to CFTR modulators, which act to augment CFTR function directly.⁸

For common and complex disorders, prediction of clinical outcomes is often difficult due to complex genetic mechanisms, variable expressivity, incomplete penetrance and environmental interactions.⁹ However, CF is a Mendelian disorder that has a single-gene etiology, is relatively common, and demonstrates both allelic and phenotypic heterogeneity, making it an excellent paradigm for genotype-phenotype studies.

1.2 Great strides have been made in CF therapeutics

Since the discovery of the *CFTR* gene in 1989, there have been many advances in therapy, from pancreatic enzyme replacement to airway clearance therapy to inhaled antibiotics.¹⁰ These improved symptomatic treatments have resulted in an increase in life expectancy from mere months when CF was first described to nearly 40 years.¹¹ But the recent advent of CFTR modulators represent the first therapeutics to directly augment CFTR function to ameliorate disease. Broadly, two classes of these compounds exist. Correctors stabilize protein folding¹², allowing more CFTR to reach the cell surface, while potentiators act upon CFTR channels already at the cell surface to increase the flow of chloride ions.¹³

In 2012, the FDA approved the potentiator Ivacaftor—the first small molecule therapy for CF.¹⁴ The corrector Lumacaftor was the second modulator therapy to be developed and while not effective enough to lead to substantial clinical benefit on its own, it was found to be effective when used in combination with Ivacaftor.¹⁵ The

combinatorial therapy Orkambi (Ivacaftor/Lumacaftor) was approved in 2015 and was followed by Symdeko (Ivacaftor/Tezacaftor) in 2018, utilizing the latest corrector compound Tezacaftor.^{16; 17} Additionally, Ivacaftor approval has since been expanded to cover additional CF-causing mutations.¹⁸ Today, over half of CF patients carry mutations eligible for modulator therapy¹⁷ and the median predicted survival age of an individual with CF born in 2017 is 46.2 years.⁴

1.3 The relationship between *CFTR* genotype and CF phenotype remains incompletely understood

The advent of small molecule therapies for CF has sparked renewed interest in understanding the nature of the correlation of *CFTR* genotype with CF phenotype, in order to evaluate the level of augmentation in *CFTR* function which may be needed to escape disease. Early work showed that individuals with CF who are homozygous for the common disease-causing variant F508del mutation were pancreatic insufficient at a higher rate than those carrying only one copy of F508del. Individuals with CF harboring no copies of F508del were pancreatic insufficient at a lower rate still than compound heterozygotes¹⁹. Similarly, the Cystic Fibrosis Genotype-Phenotype Consortium showed that F508del/R117H compound heterozygotes were more often pancreatic sufficient, had lower sweat chloride concentrations, and were diagnosed at an older age than their age- and sex-matched F508del homozygote counterparts²⁰. Subsequent studies reported that individuals with CF carrying the mild A455E variant have significantly better lung function measures than age-, sex- and CF center-matched F508del homozygotes²¹. Together, these observations established that *CFTR* genotype influenced the severity of

disease in organ systems affected in CF. As our understanding of the consequences of disease-causing variants upon CFTR function evolved, variants were grouped into classes—I through V—based on similar effects on the synthesis, processing or function of CFTR²²⁻²⁴. The variant classes allowed more detailed testing of the concept that retention of some degree of CFTR function—generally observed for variants assigned to classes IV and V—was associated with moderation in the severity of dysfunction in the pancreas, sweat gland, and—to some extent—the lung²⁵⁻²⁸. While it has been proposed that CFTR alleles conferring residual function are acting in a “dominant” fashion²⁹, due to their presence in compound heterozygosity mitigating disease severity, the actual contribution of each allele is poorly understood.

A genotype-phenotype study, a part of this work, sought to derive the CFTR function of a variety of CFTR genotypes and correlate with key clinical features (sweat chloride concentration, pancreatic exocrine status, and lung function) to develop benchmarks for assessing response to CFTR modulators in clinical trials. Additionally, we provided new insights into the contribution of each *CFTR* allele to overall disease phenotype.

1.4 The disease landscape is changing for many genetic disorders

Duchenne muscular dystrophy (DMD) and Huntington’s disease (HD) are two genetic diseases also investigated in this work that serve as comparators. Like CF, DMD is a single-gene Mendelian disorder that has an early onset, is progressive, and shortens lifespan. DMD is caused by mutations in the *DMD* gene, which encodes the cytoskeletal protein dystrophin and is inherited in an X-linked recessive fashion, affecting

approximately 1 in 3,500 live male births.³⁰ DMD is characterized by progressive muscle weakness and eventual loss of ambulation, followed by premature death caused by respiratory or cardiac complications.

The median survival rate for DMD – once in the teens and now in the late 20s – is on the rise³¹ due to advances in care. Corticosteroids are standard of care for DMD, but novel therapies are emerging that seek to correct the genetic defect. In September 2016, the FDA approved the first disease-modifying therapy for DMD, the exon-skipping therapy eteplirsen.³² Approximately 14% of DMD patients carry a mutation for which eteplirsen is applicable, but it has faced questions about its efficacy in clinical trials.³³ However, with further development of additional antisense oligonucleotides, exon skipping therapy could benefit up to 83% of DMD patients.³⁴

HD is an autosomal dominant disorder caused by a repeat expansion of the polyglutamine tract of the huntingtin (HTT) gene. Above 40 repeats results in full penetrance of the disease, below 35 repeats has a phenotypically normal result, and 36-39 repeats falls in an indeterminate zone with reduced penetrance. The number of repeats is inversely correlated with age of disease onset.³⁵ HD symptoms include motor, cognitive, and behavioral features and death typically occurs approximately 15-18 years after the onset of motor symptoms.³⁶

Like CFTR, HTT has been familiar to geneticists for quite some time. In fact, it was the very first disease gene to be mapped in 1983 and isolated in 1993. Despite years of study, no disease-modifying therapies currently exist for HD. Only 3.5% of HD clinical trials have reached the second stage.³⁷ However, RNA targeting via antisense oligonucleotides appears to be a promising avenue shown to be effective at reducing

levels of mutant mRNA and protein in animals models.³⁷ The first phase 3 clinical trial for antisense oligonucleotides in HD is currently underway and while the future is hopeful, HD is still viewed as an intractable disease. Qualitative interview data collected in this work from DMD carriers and individuals at-risk for HD in addition to data collected from CF carriers provide perspectives from people with diseases at various stages of availability of therapeutic intervention.

1.5 Genetic testing in the precision medicine era has wide-reaching impacts

For many genetic diseases, we are moving toward population-wide screening as standard of care³⁸⁻⁴⁸ and healthy carriers of genetic disease and presymptomatic individuals are increasingly being identified via disease gene panels, as well as whole exome and whole genome sequencing. As the therapeutic outlook for many diseases changes rapidly, often outpacing the availability of genetic counseling with the most up-to-date information, understanding how these changes impact individuals' understanding and use of their genetic test results is essential.

Newborn screening first became available for CF in the 1990s and currently all states offer newborn screening for CF.⁴⁹ The American College of Obstetricians and Gynecologists (ACOG) currently recommends that carrier screening for CF be offered to all women of reproductive age,⁴² which means that couples are increasingly making reproductive decisions with knowledge of their carrier status. Newborn screening is currently not universally available for DMD and is not supported by the majority of genetic counselors, but it is strongly supported by patients and families,⁵⁰⁻⁵² and carrier

screening is increasingly sought by at-risk individuals and women seeking to start families.

While previous studies in both CF and DMD have examined reproductive behavior among carriers and the impact of genetic testing on that behavior⁵³⁻⁶², the effect of novel precision therapies on carriers' views of testing and reproductive decisions has yet to be explored. A part of this work represents a qualitative assessment of the impact of the evolving disease and treatment landscape on views of genetic testing and on reproductive decision-making amongst CF and DMD carriers via semi-structured interviews.

These interviews were comprised of questions exploring carriers' reproductive decisions and whether those were affected by emerging disease-modifying therapies, but the scope of these interviews also went beyond family planning to consider other impacts of carrier status. When considering returning results of genetic testing and subsequent counseling of carriers, focusing on family planning alone does not capture the full burden of being a carrier for genetic disease. Previous work on communication of test results within families has been done in all three disease groups explored in this work.^{37; 60-71} However, this work provides an updated view which takes into account recent therapeutic advances, as well as—in the case of the HD group—long-term retrospective data. The interviews conducted in this work interrogated not only reproductive decisions, but also communication with family members, family dynamic, testing and counseling experience, support networks, conversations with children about disease or carrier status, and effects their genetic status have had on their lives outside of family planning, in order

to capture the wide range of impacts of living with genetic risk and to inform counseling protocols.

1.6 Thesis

This work sought to explore new challenges raised by this era of effective treatment, using cystic fibrosis as a paradigm, as an increasing number of genetic diseases are being screened for and are becoming treatable. This dissertation details a novel genotype-phenotype analysis to establish benchmarks to evaluate the effectiveness of CFTR modulators and explores how emerging therapies are affecting carriers' reproductive decisions, as well as other challenges faced by carriers of genetic disease.

**Chapter 2. Correlating CFTR function with clinical features to
inform precision treatment of cystic fibrosis**

2.1 Introduction

Cystic fibrosis (CF) is an autosomal recessive monogenic disorder affecting approximately 70,000 individuals worldwide¹. It is caused by variants in the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride and bicarbonate channel. The advent of variant-specific small molecule therapies, termed CFTR modulators, targeting CFTR^{72; 73} has generated renewed interest in defining relationships between *CFTR* genotype, CFTR function, and CF phenotype.

CF manifests considerable allelic heterogeneity with over 2,000 *CFTR* variants identified to date³ and broad clinical variability, rendering the relationship between genotype and phenotype difficult to fully elucidate⁷⁴⁻⁷⁶. Grouping of variants into classes based on effects on synthesis, processing, or function of CFTR²²⁻²⁴ demonstrated that retention of some CFTR function moderated the severity of dysfunction in the pancreas, sweat gland, and – to some extent – the lung²⁵⁻²⁸. As functional studies of CFTR advanced⁷⁷, estimates of the relationship between level of CFTR function and phenotype were refined⁷⁸. However, these studies relied on functional evaluation of a relatively small number of variants in each class and limited numbers of subjects, who collectively lacked adequate representation of the entire spectrum of disease. The high degree of variability in CF traits necessitates large-scale studies to achieve greater granularity in the relationships between CFTR function and clinical outcomes.

To determine which individuals with CF may benefit from modulator treatment, there has been a concerted effort to evaluate function and drug response of all *CFTR* variants occurring in three or more individuals worldwide. Consequently, we now have measurements of the function of CFTR bearing a large set of variants that span the

functional spectrum of disease^{6; 7; 79}. These data were paired with clinical and genetic data from 88,664 individuals with CF assembled by the Clinical and Functional TRanslation of *CFTR* (CFTR2) project, revealing robust correlations of CFTR function with key clinical outcomes. These relationships can provide benchmarks to inform expectations for response to CFTR-targeted therapies.

2.2 Results

Deriving CFTR function for different *CFTR* genotypes

A total of 56,871 individuals carried one of 1,835 genotypes for which CFTR function could be assigned (**Figure 2.1A**), based on functional studies of 108 *CFTR* missense or in-frame insertion or deletion variants^{6; 7; 79} and an assumption of no residual function for 245 NULL variants (**Table 2.S2**). Genotypes not considered for analysis include those with non CF-causing variants, variants for which % WT function was not assigned, complex alleles in which the combined function of two in *cis* variants is unclear, or nonsense variants known to escape nonsense-mediated RNA decay or operate by a different mechanism^{80; 81}. Of genotypes with functional assignment, 607 expected to result in no CFTR function (composed of two NULL variants) were excluded since clinical variation in these individuals is presumably due to factors other than *CFTR* genotype^{3; 82}. Collapsing the remaining NULL variants that occurred with a variant of known function into one category (since all are predicted to result in no CFTR function, thereby allowing us to consider them equivalent to each other) and removing genotypes occurring in only one or two individuals resulted in 54,671 individuals carrying one of

226 genotypes (**Figure 2.1A** and **Table 2.S3**). Genotypes were distributed across the entire range of CFTR function (**Figure 2.1B**).

Key clinical features of CF exhibit a logarithmic relationship with CFTR function

Plotting CFTR genotype function against sweat chloride concentration (sweat $[Cl^-]$) of individuals with CF revealed a non-linear relationship between these variables (**Figure 2.2A, left panel**) and curve fitting suggested that the interaction was logarithmic. Indeed, plotting CFTR function on a log scale revealed robust correlation with sweat $[Cl^-]$ (**Figure 2.2A, right panel**; $r=0.77$, $p<0.001$). In performing correlation analysis, we restricted to genotypes generating at least 0.85% WT-CFTR function, as this is the minimum function of genotypes containing the common variant F508del^(7;8), unpublished) and below which estimates of % of wild type function are considerably less certain. We also excluded genotypes generating more than 50% WT-CFTR function, as this level should be sufficient to escape disease; individuals with CF who have these genotypes likely have unidentified contributing factors.

We performed similar analyses to evaluate correlation between CFTR function and exocrine pancreatic disease (% of individuals with the same genotype who have pancreatic insufficiency) (**Figure 2.2B**) and lung disease (FEV1% predicted of individuals with the same genotype) (**Figure 2.2C**). To account for survival and age-dependent decline, we converted lung function measures to sex- and height-matched age-specific CF percentiles transformed to z-scores (Kulich Normal Residual Mortality-Adjusted or KNoRMA)⁸³ (**Figure 2.2D**), measures which are based on a number of FEV1% predicted recordings over time (up to a three-year window; number of recordings

ranged from 1 to 145) for each individual patient. As with sweat [Cl⁻], the relationship between CFTR genotype function and each trait appeared non-linear. Plotting CFTR genotype function on a log scale revealed correlation with pancreatic status and lung function that was modest compared to sweat [Cl⁻] but statistically significant. Weighting the analysis to account for the number of individuals in each genotype group did not produce a meaningful shift in the nature or strength of the relationship between CFTR genotype function and trait (data not shown). Of note, inclusion of all data (including genotypes outside the 0.85-50% range plotted in **Figure 2.2**) generates similar degrees of correlation and significance with modest shifts in slope (**Figure 2.S1**). The logarithmic relationships indicate that increases in CFTR function have proportionally greater effect on individuals with severe disease than similar increases in function in individuals with mild disease.

Lung function of individuals of different ages and levels of CFTR function.

Since progressive lung disease is the major cause of morbidity and mortality in CF, we wanted to determine how CFTR genotype function affects lung function in individuals of different ages. We plotted cross-sectional FEV1% predicted measurements by the age at measurement for 42,924 individuals in CFTR2 stratified by groupings of CFTR genotype function. Functional grouping was based on published estimates for transition to pancreatic sufficiency (3% WT), reduction in sweat [Cl⁻] (5% WT), amelioration of lung disease (10% WT), and overlap with CFTR-related diseases (25% WT and above), acknowledging that these thresholds are approximate and likely vary amongst individuals^{7; 84-88}. Lowess smoothing revealed that higher levels of CFTR

function associated with better lung function at almost ages (**Figure 2.3A**). Exceptions were noted in individuals over 50 years of age with 3-5% function and at over 60 years of age with <2% function, presumably due to survival bias (i.e., measurements of lung function are available only from living or non-transplanted individuals, therefore potentially appearing falsely inflated). To address survival bias, we also plotted the mortality adjusted lung measure (KNoRMA) stratified by the functional groupings described above (**Figure 2.3B**). As expected, KNoRMA values increase with age, reflecting the survival of those who out-live their CF peers. Lowess smoothing revealed the same pattern observed with FEV1% predicted measures; higher levels of CFTR function associated with higher KNoRMA values at almost all ages, with most functional groups converging around a KNoRMA z-score of 2. These results also illustrate the logarithmic relationship between CFTR genotype function and lung function in that small increases in CFTR function (e.g., 2 to <3% shifting to 3 to <5%) result in substantial shifts in cross-sectional measurements at all ages, whereas less impressive (but clinically relevant) improvements occur at higher levels of CFTR genotype function (e.g., 5 to <10% moving to 10 to <25%). Finally, although limited by a small number of subjects, individuals in the CFTR2 database that have 25 to <50% WT function appear to have near-normal cross-sectional FEV1% predicted measures into adulthood.

CFTR-targeted therapies improve clinical measures to levels consistent with those observed in individuals with higher life-long levels of CFTR function

To assess the effectiveness of CFTR-targeted therapies, we compared the changes in sweat [Cl⁻] and lung function reported in clinical trials of CFTR modulators to those

predicted using the function-phenotype correlations from CFTR2 data. Pre- and post-treatment values of sweat $[Cl^-]$ and FEV1% predicted obtained in CFTR modulator trials were plotted against CFTR function derived from cell-based studies (see **Table 2.S4** and **Methods**). The resulting slopes (red dashed lines, **Figure 2.4A and B**) were compared to the slopes derived from the sweat $[Cl^-]$ and regression of FEV1% predicted of all CF subjects (black lines, **Figure 2.4A and B**). The post-treatment sweat $[Cl^-]$ observed in ivacaftor-treated individuals matched the sweat $[Cl^-]$ of individuals with higher levels of CFTR function, indicated by the overlapping slopes (**Figure 2.4A**). Post-treatment changes in lung function generated a slope that appeared to deviate from that observed at higher levels of CFTR function in the CFTR2 data (**Figure 2.4B**); however, testing using an interaction term revealed that the slopes of these lines did not differ. A mixed model approach using likelihood testing offered no advantage over simple linear regression; any apparent difference between slopes remained non-significant. To investigate if response to CFTR modulators in the sweat gland or lung changes as a function of age, clinical trial data from groups of individuals of varying ages (12 months to adulthood) were compared to CFTR2 data (mean age at PFTs 21.9 years [SD: 11.0 years]) (**Figure 4C and D**). The regressions did not differ significantly for any age cohort studied.

We also compared treatment responses of F508del homozygotes to modulator combinations in the same fashion as above. Pre-treatment values for sweat $[Cl^-]$ were comparable to values from CFTR2 subjects (**Figure 2.5A**), while pre-treatment values for FEV1% predicted were lower than those from CFTR2 subjects (**Figure 2.5B**). However, the change in each trait after treatment does not significantly differ from the slope derived from CFTR2 subjects. Analysis of responses by age group revealed no significant

differences between modulator effect in F508del homozygotes and phenotypic differences in the CFTR2 population as a result of differing levels of CFTR function (**Figure 2.5C and D**). Recognizing that functional responses to modulators tested in clinical trials were drawn from a variety of cell types and that small numbers of subjects in trials may influence correlations, we evaluated all available clinical trial data *together* to determine if acute modulator response differed from the genotype-phenotype relationship derived from CFTR2 data. Treatment effect on sweat $[Cl^-]$ or FEV1% predicted did not differ from CFTR2 data when including results from 15 trials assessing treatment response from a variety of CFTR modulators (**Figure 2.S2**). Together, these comparisons illustrate that individuals in CFTR modulator trials attain sweat $[Cl^-]$ and lung function measures that approximate those of individuals with a lifetime of higher CFTR function as a consequence of their CFTR genotype.

2.3 Discussion

The availability of functional estimates for variants associated with a range of CF disease severity facilitated robust correlation analysis between CFTR genotype function and clinical features of CF. Our observation of a logarithmic relationship between CFTR function and sweat $[Cl^-]$ is consistent with prior studies of individual sweat glands⁸⁹. Nasal potential difference (NPD) measures of CFTR-mediated chloride transport across nasal epithelium (another method of ascertaining CFTR function *in vivo*^{90; 91}) also appears to exhibit a non-linear relationship with sweat $[Cl^-]$ ^{78; 92}. Similarly, our observation of a non-linear relationship between CFTR function and severity of lung disease is supported by the non-linear correlation between level of normal *CFTR*

transcripts and FEV1% predicted in individuals carrying the 5T allele⁹³. Consistent with prior evidence, the correlation with sweat [Cl⁻] was the most robust, indicating that it is currently the best proxy measure for *in vivo* CFTR function^{92; 94-96}.

There are several plausible explanations as to why the relationship between CFTR function and phenotype appears logarithmic. Like other ion channels, CFTR efficiently dissipates gradients^{97; 98}. Thus, having a small fraction of CFTR channels operating properly may restore close-to-normal balance of ionic concentrations and the effect could be multiplied via CFTR's function as a regulator of other ion channels⁹⁸. Alternatively, an increase in CFTR abundance in a few cells may allow for increased Cl⁻ transport across epithelial tissues facilitated by intercellular ion movement via gap junctions. Co-culture studies demonstrated that 10-20% of cells in human airway epithelia expressing WT-CFTR is sufficient to correct the epithelial chloride transport defect^{88; 99; 100}. Similarly, vector delivery of WT-CFTR to a small proportion of cells was sufficient for normal chloride transport¹⁰¹ and restoration of airway surface liquid volume⁸⁷. The newly-discovered pulmonary ionocyte^{102; 103} supports the possibility that most CFTR-dependent chloride transport in airways may be restricted to a small fraction of cells that amplify transport by intercellular routes^{88; 99; 100}.

From a therapeutic perspective, a logarithmic relationship is encouraging, as it implies that modest augmentation of CFTR at low functional levels can generate substantial clinical benefit. This phenomenon is consistent with results from individuals bearing the severe G551D variant, where moderate increases in CFTR function resulted in remarkable improvement in CF clinical measures^{72; 89; 104}. Precedence for a non-linear relationship between function and phenotype is evident for other loss-of-function genetic

conditions. Severe hemophilia results from plasma coagulation factor levels below 1% of normal, while levels of 2-5% result in moderate hemophilia, and levels of 6-30% confer mild to no disease¹⁰⁵. This phenomenon is also observed with phenylketonuria, in which phenylalanine hydroxylase activity of 13-15% of normal level confers a mild presentation^{106; 107}.

Our results demonstrate that acute augmentation in CFTR function can result in improvements in clinical outcomes comparable to having a 'milder' *CFTR* genotype since conception. This result is not surprising for sweat chloride, as the sweat gland is not thought to be damaged in CF¹⁰⁸. In contrast, lung disease is progressive in CF and thought to have non-reversible components such as airway loss and fibrotic replacement¹⁰⁹. However, our analysis suggests that the fraction of recoverable lung function may be substantial. Reports of individuals with severe CF who started ivacaftor as adults and subsequently ran marathons or climbed Mount Everest¹¹⁰ illustrate this point. However, it is also possible that additional clinical trials and studies of longer duration might detect differences between lung function recovered by CFTR modulators and the phenotype that results from a lifetime of higher CFTR function.

Given the reversibility of lung disease with modulators, establishing an expected benchmark of lung function for differing levels of CFTR function is of the utmost importance: it allows more accurate predictions regarding expected lung function decline or stabilization after CFTR function augmentation. Though the CFTR2 dataset does not use individual longitudinal decline to establish these trajectories, the cross-sectional data provides insight into the relative differences amongst CFTR functional groups in a population whose vast majority of measurements occurred prior to use of modulators.

Such data helps inform how much recovery might be expected as CFTR function is increased so that a longer lifespan might be achieved. To this end, the benchmarking suggests that increasing CFTR function to greater than 10% - an accepted threshold below which life-limiting CF disease is expected - is associated with FEV1% predicted measurements above 80% into adulthood. However, even 10% CFTR function appears to result in a decrease in FEV1% predicted by age. This observation and previous reports associating variants conferring 10-25% function with variable expressivity of CF disease⁷ suggests that further CFTR augmentation beyond this level may be necessary to maintain normal lung function. The very few individuals with genotypes conferring 25-50% CFTR function limit our ability to draw conclusions at levels above 25%, but this could be further investigated by expanding function-phenotype studies to include those not meeting diagnostic criteria for CF (i.e., CFTR-related disorders). At the other end of the spectrum, our analysis suggests that smaller increases in CFTR function (e.g., <2% to 5%) could produce clinically relevant improvements in lung function.

The chief limitation of this study lies in the inherent imperfection of clinical measurements, which are prone to some error and variability. Pancreatic insufficiency was designated as a discrete variable by whether an individual was on pancreatic enzyme replacement therapy, making our data vulnerable to skewing at small sample sizes. Individual subject FEV1% predicted measurements reported to CFTR2 are a heterogeneous group and may consist of mean, median, best, most recent, or annualized values. The requirement by many clinical trials that subjects have an FEV1% predicted between 40-90% may have excluded individuals with modulator-responsive genotypes who had progressed too far in disease severity for inclusion, thereby skewing treatment

response data compared to the CFTR2-derived plot (containing individuals across the range of FEV1% predicted measurements). Finally, inter-individual variability, even within the same genotype, due to factors such as environment, modifier genes, and *cis*-regulatory variation reduce the utility of benchmarks to generalizations for groups of individuals with a certain level of CFTR function. Variance around each measure, especially for lung function, limits the confidence in predicting clinical improvements attributable to CFTR modulator therapy for an individual subject, whose response may also require assessment of CFTR augmentation on an individual level.

In summary, the extraordinary amount of data available in this large-scale study revealed clinically useful correlations between CFTR genotype function and clinical features of CF. These correlations demonstrate that individuals with severe disease as a result of very low CFTR function could benefit the most from modulator therapies, even if the increase in function is modest. Of potentially equal importance, the study generated benchmarks of CFTR function with lung flow measurements and sweat [Cl⁻] to provide points of reference for assessing CFTR modulator efficacy.

2.4 Materials and Methods

Clinical data and variant function

Clinical data from individuals with CF were collected for the CFTR2 project, which amassed data from 88,664 individuals receiving CF care in 41 countries (**Table 2.S1**), as previously described^{6; 111; 112}. When possible, a Kulich Normal Residual Mortality-Adjusted (KNoRMA) lung disease phenotype was calculated for each individual⁸³ using non-transplanted lung function measures. Variants reported in at least

three individuals in CFTR2 with clinical data were assigned a functional level based on *in vitro* studies measuring CFTR transport in Fischer Rat Thyroid (FRT) or CF Bronchial Epithelial (CFBE) cell lines^{6; 7} or their presumed production of no CFTR protein (nonsense, canonical splice, frameshift, and start-loss variants, and exon deletions or duplications; **Table 2.S2**).

Assigning genotypes, genotype function, and CF clinical trait values

Individuals with a reported *CFTR* genotype comprised of exactly two variants having functional assignment were eligible for inclusion in analysis. A %WT function for each *CFTR* variant was calculated by adjusting short circuit current measurements of variant cell lines for level of mRNA expression, and comparing to a WT standard curve, as previously described⁷. *CFTR* genotype function was determined as the sum of the functional levels of the two individual variants comprising the genotype. Each genotype group analyzed included three or more individuals. Clinical traits were analyzed only if at least three subjects within the genotype group reported clinical data for a given trait. The CF traits associated with each genotype were determined by mean value (sweat chloride, FEV1% predicted [forced expiratory volume in 1 second as a percent of predicted for age and height], KNoRMA) or % of individuals with a trait (pancreatic insufficiency).

Derivation of lung function trajectory by CFTR genotype functional grouping

Individuals in the CFTR2 dataset ages ≥ 6 years having an FEV1% predicted measurement (n=42,924) were placed into *CFTR* genotype functional groups: <2%; 2 to <3%; 3 to <5%; 5 to <10%; 10 to <25%; and 25 to <50%. Cross-sectional FEV1%

predicted measurements were plotted by age at which measurement was obtained. Lung function decrease by age was determined using Lowess smoothing or linear regression.

Derivation of the relationship between CFTR function and trait following acute modulator treatment

Treatment response comparisons to CFTR2-defined relationships between CFTR genotype function and phenotype were generated from published clinical trial data. Pre-treatment values for sweat [Cl⁻] and FEV1% predicted were used when provided; otherwise, CFTR2 clinical data were used. Post-treatment values were calculated from published data. Functional measures of CFTR with and without treatment for genotypes involving gating variants was calculated from individual variants studied in FRT cell lines^{113; 114}. For clinical trial participants bearing a genotype containing a gating variant, total genotype function was determined by summing the function of the gating variant and of F508del, which was the most commonly-reported second allele comprising the genotype in these subjects. Functional measures of CFTR with and without treatment with ivacaftor and lumacaftor for F508del was determined from CFBE cell lines⁸; homozygous F508del genotype function was derived by multiplying F508del functional levels with and without treatment by two. Baseline CFTR function for the F508del homozygous genotype treated with tezacaftor-ivacaftor and VX-445 or VX-659 was determined from CFBE cell lines⁸, and fold-change treatment response was calculated from *ex vivo* human bronchial epithelial cells from F508del homozygous donors^{115; 116}. Genotypes present in fewer than three individuals in a trial were excluded.

Correlation and statistical analysis

The relationship between CFTR genotype function and CF traits was determined by linear regression. Pearson correlation coefficient (r) was used to determine strength of correlation. Regressions using CFTR2 data and results from clinical trials were compared using an interaction term and mixed models, with $p < 0.05$ as the threshold for significance. Statistical analysis was performed using Stata (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC IC 15.0).

Figure 2.1 Subjects, genotypes, and CFTR function of individuals included in function-phenotype analysis. (A) Diagram of the filtering process used to select individuals for study. The 5,304 genotypes of 88,664 subjects from the CFTR2 dataset were provided by contributing registries and CF clinics. Genotypes including only one NULL variant were collapsed such that any NULL variant could be present in trans with a non-NULL variant of interest, and only genotype groups with at least three individuals were included in analysis resulting in 226 genotypes for study. (B) Genotype function (% WT) was assigned as the sum of the individual functions of each variant comprising the genotype. Genotype function is primarily below 10% as expected for individuals with CF, though individuals with genotype function levels above this level are present in CFTR2.

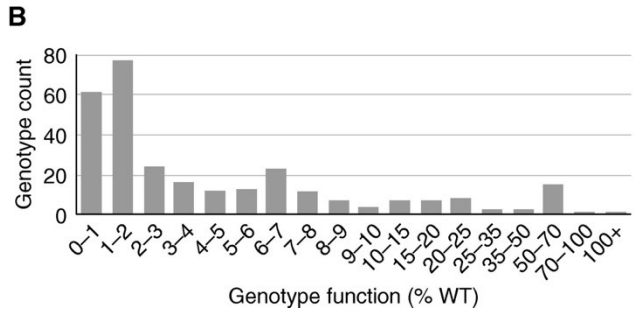
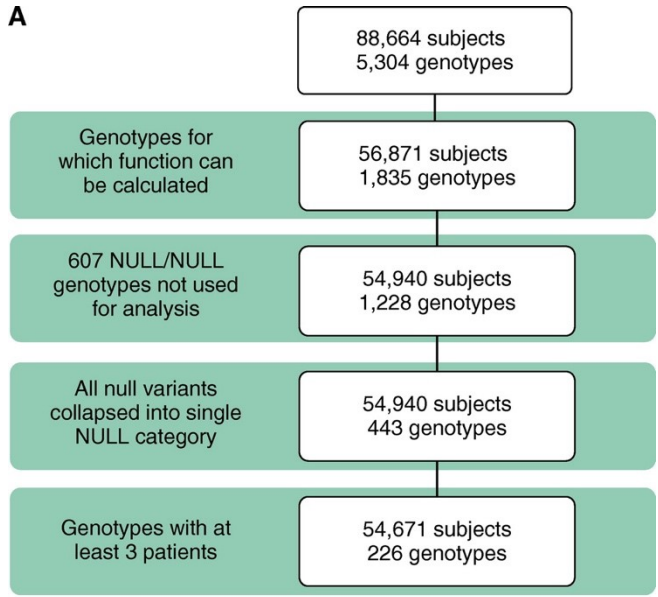


Figure 2.2 Genotype function has a non-linear relationship with CF clinical traits.

Left-hand panels for each CF clinical trait plot CFTR genotype function as a percentage of WT on a linear scale against the mean sweat $[Cl^-]$ from individuals with that genotype **(A)**, the percentage of individuals of that genotype who are pancreatic insufficient **(B)**, the mean FEV1% predicted for individuals of that genotype **(C)**, or the mean SaKNoRMA z-score for individuals of that genotype **(D)**. Best fit line is shown. Right-hand panels show CFTR genotype function plotted on a log scale. Linear regressions for right-hand panels were performed on CFTR function between 0.85 and 50% (black data points). Trait measures from CFTR genotype function outside this range are shown in grey data points. Pearson's r-value for correlation and p-value for deviation of slope from zero are shown for each trait.

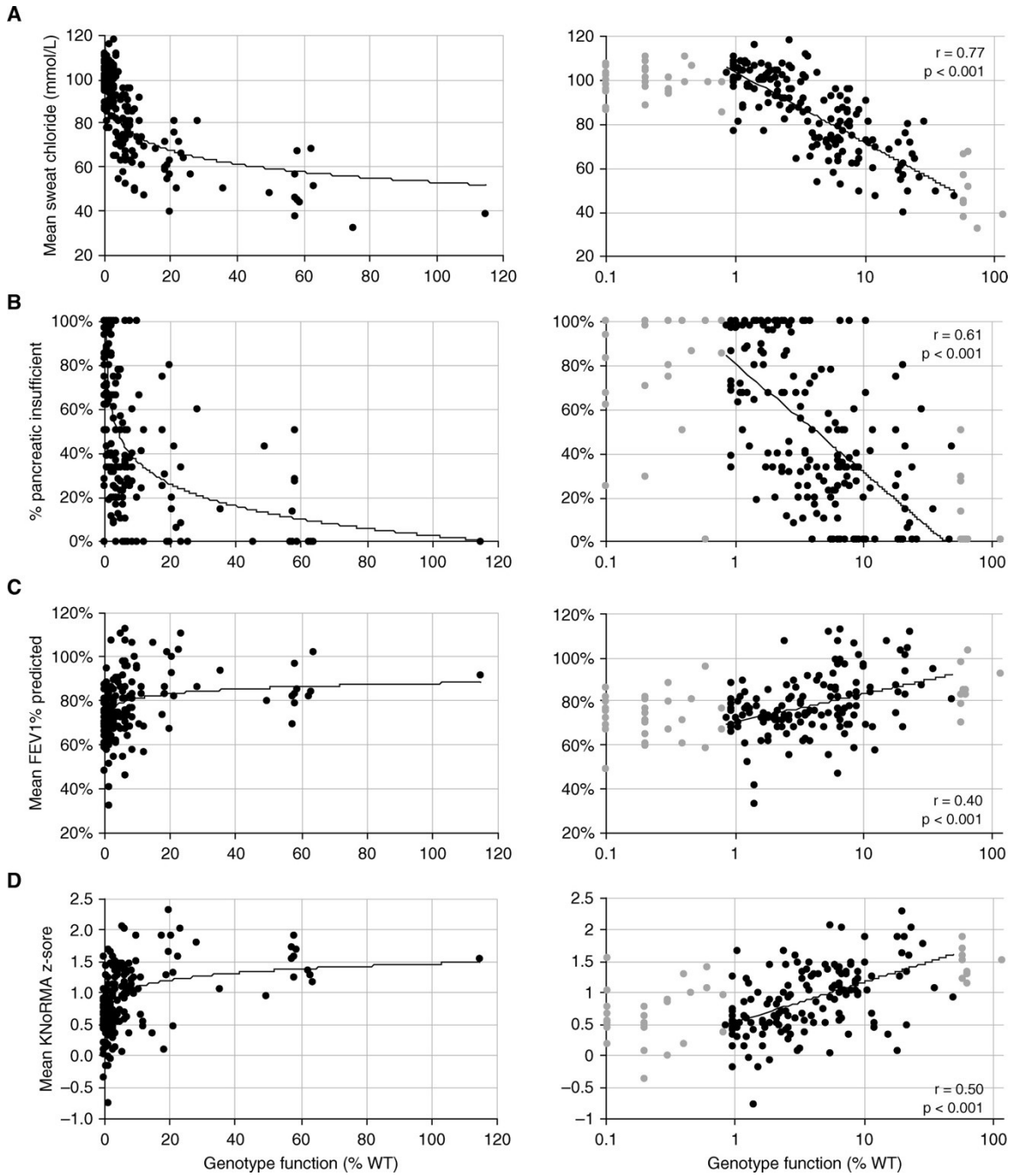
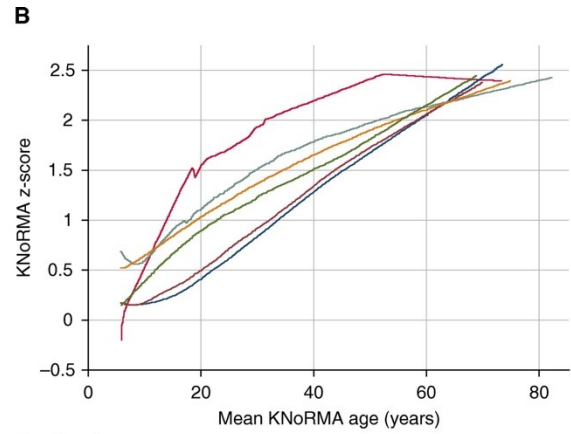
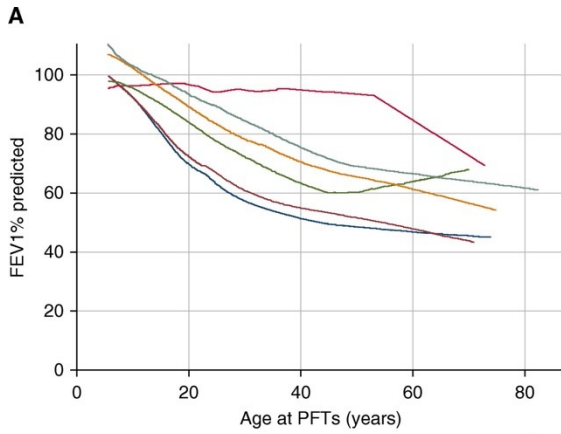


Figure 2.3 Lung function by age stratified by level of CFTR function. (A) Lowess smoothing of cross-sectional FEV1% predicted measurements from 42,924 individuals in the CFTR2 database plotted by age at which measurement was taken and stratified by CFTR genotype function. (B) Lowess smoothing of KNoRMA z-scores from 42,495 individuals in the CFTR2 database plotted by mean age at the FEV1 measures used to calculate the KNoRMA z-score and stratified by CFTR genotype function. (C) The number of patients with FEV1% predicted measurements within each genotype group at 10-year age increments are shown in the table. (D) The number of patients with KNoRMA z-scores within each genotype group at 10-year mean KNoRMA age increments are shown in the table.



Genotype functional group:

— <2% — 2 to <3% — 3 to <5% — 5 to <10% — 10 to <25% — 25 to <50%

C

Genotype functional group	Age at PFTs (years)								TOTAL
	<10	10 to <20	20 to <30	30 to <40	40 to <50	50 to <60	60 to <70	70 and older	
25 to <50%	7	3	5	6	0	1	0	1	23
10 to <25%	22	60	26	30	13	6	3	1	161
5 to <10%	118	299	228	221	194	110	45	11	1226
3 to <5%	46	118	112	80	60	20	11	0	447
2 to <3%	205	572	423	234	114	36	5	3	1592
<2%	4928	14267	12259	5509	1994	471	43	4	39475

D

Genotype functional group	Mean KNoRMA age (years)								TOTAL
	<10	10 to <20	20 to <30	30 to <40	40 to <50	50 to <60	60 to <70	70 and older	
25 to <50%	8	3	5	5	0	1	0	1	23
10 to <25%	23	62	21	30	14	5	1	0	156
5 to <10%	143	287	223	217	186	103	39	11	1209
3 to <5%	53	118	113	70	61	17	10	0	442
2 to <3%	248	583	386	217	105	26	8	0	1573
<2%	6366	14447	11257	4865	1745	381	28	3	39092

Figure 2.4 Treatment effect on gating variants mirrors the relationship between genotype function and phenotype determined using CFTR2 data. Genotype function vs. sweat $[Cl^-]$ (**A**) or FEV1 % predicted (**B**) on a semi-log plot indicates that the effect of ivacaftor treatment on individuals with gating variants does not differ from the relationship shown between these variables using CFTR2 data (solid line determined from genotypes with 0.85% to 50% function; dotted line extrapolates this relationship below 0.85% or above 50% function). Genotype function and baseline sweat $[Cl^-]$ or FEV1 % predicted of individuals tested in clinical trials with duration of either 24 or 48 weeks are represented by open red circles; following treatment, genotype function (determined by *in vitro* FRT testing) and resulting sweat $[Cl^-]$ or FEV1 % predicted are represented by filled red circles. Genotype function vs. sweat $[Cl^-]$ (**A, inlay**) or FEV1 % predicted (**B, inlay**) illustrates the non-linear relationship between these variables. Treatment effect from ivacaftor on sweat $[Cl^-]$ (**C**) or FEV1 % predicted (**D**) stratified by age cohort (colored lines) does not differ from the relationship between these variables using CFTR2 data (all ages included [mean age 22 years at time of FEV1% predicted measures]). Trials included in **C** and **D** ranged in duration from 24 to 144 weeks of treatment. All comparisons were performed using an interaction term between CFTR2 data and data from clinical trials and indicated no significant difference between the regressions for each data set. Clinical trial data plotted is detailed in **Table 2.S4** and **Methods**.

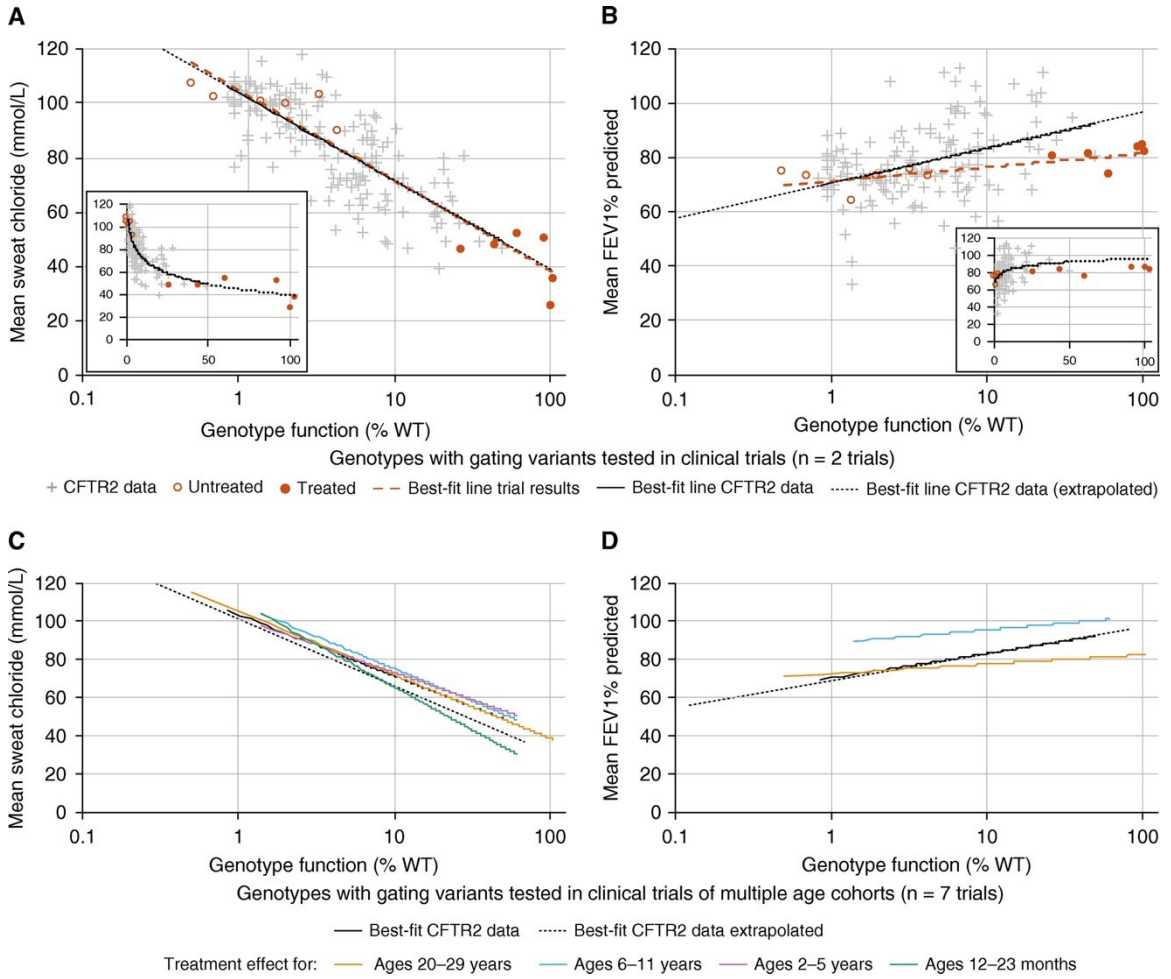
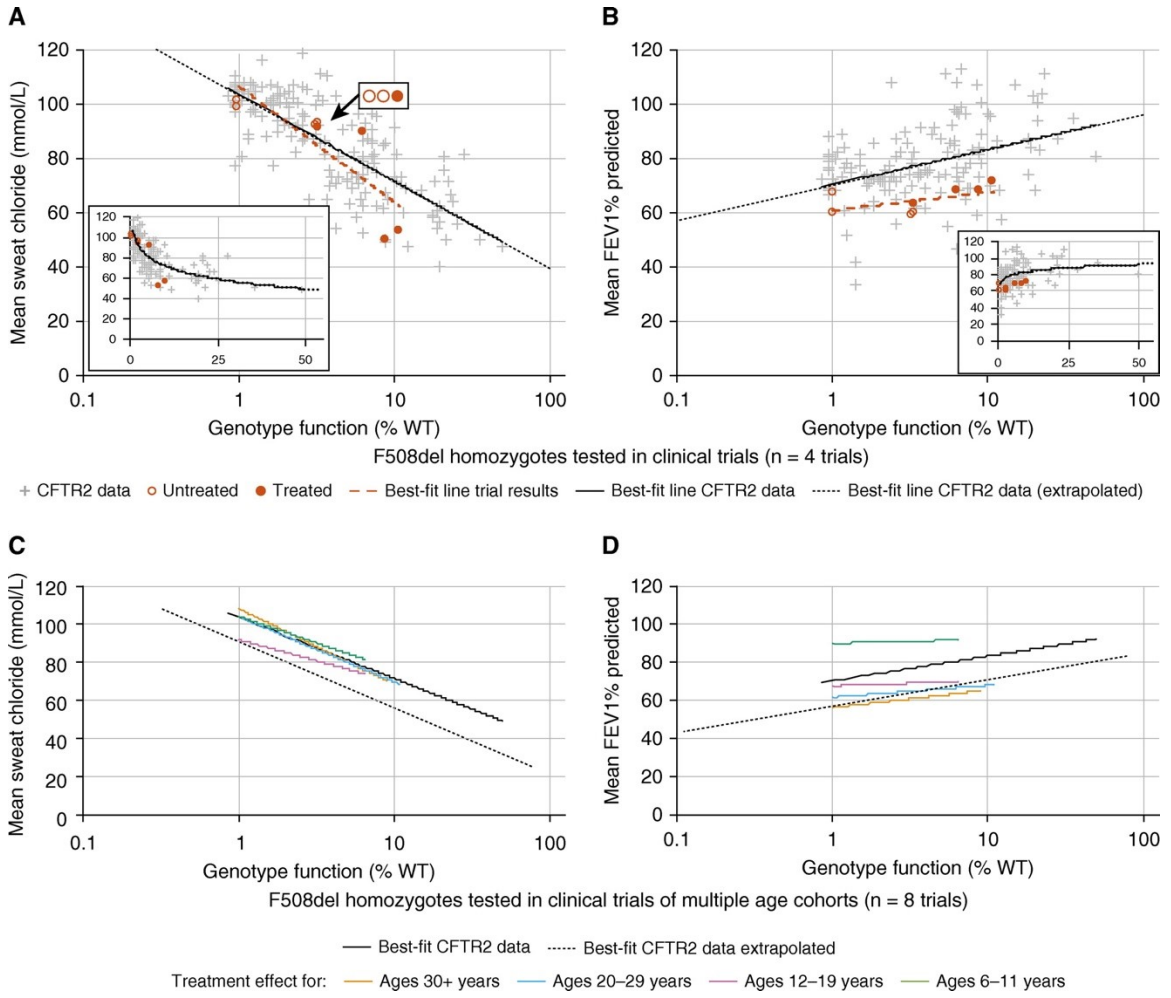


Figure 2.5 Treatment effect on F508del homozygotes variants mirrors the relationship between genotype function and phenotype determined using CFTR2 data Genotype function vs. sweat $[Cl^-]$ (**A**) or FEV1% predicted (**B**) are plotted as described in **Figure 2.4**. The treatment effect of CFTR modulators on F508del homozygotes does not differ from the relationship shown between these variables using CFTR2 data. Genotype function and baseline sweat $[Cl^-]$ or FEV1% predicted of individuals tested in clinical trials of duration 4 to 24 weeks are represented by open red circles; following treatment, genotype function (determined by *in vitro* CFBE or *ex vivo* primary cell testing) and resulting sweat $[Cl^-]$ or FEV1 % predicted are represented by filled red circles. Genotype function vs. sweat $[Cl^-]$ (**A, inlay**) or FEV1 % predicted (**B, inlay**) illustrates the non-linear relationship between these variables. Treatment effect on sweat $[Cl^-]$ (**C**) or FEV1 % predicted (**D**) stratified by age cohort (colored lines) does not differ from the relationship between these variables using CFTR2 data. Trials included in **C** and **D** ranged in duration from 4 to 24 weeks of treatment. All comparisons were performed using an interaction term between CFTR2 data and data from clinical trials and indicated no significant difference between the regressions for each data set. Clinical trial data plotted is detailed in **Table 2.S4** and **Methods**.



Chapter 3: Both *CFTR* alleles contribute to function in an additive fashion

3.1 Introduction

Cystic fibrosis is an autosomal recessive monogenic disorder caused by variants in the cystic fibrosis transmembrane conductance regulator (CFTR).² CF demonstrates a considerable amount of allelic heterogeneity, with over 2,000 *CFTR* variants having been reported to-date.³ This heterogeneity, combined with the varied mechanisms of CFTR dysfunction underlying disease, has resulted in a gap in knowledge between identified *CFTR* variants and their consequences.⁵ The Clinical and Functional TRanslation of CFTR (CFTR2) project, which combines the assembly of clinical data from CF patients with functional assessment of *CFTR* variants, was established in order to assign disease liability to *CFTR* variants.⁶

The advent of mutation-targeted therapies for CF has sparked renewed interest in understanding the specific functional consequence of every *CFTR* variant in order to achieve the goal of precision medicine for cystic fibrosis. However, understanding CFTR function at the variant level is not sufficient to properly evaluate the relationship between *CFTR* genotype and CF phenotype. Because CF is a recessive disease, every individual with CF carries at least two *CFTR* variants. In order to properly evaluate the relationship between *CFTR* genotype and CF trait, we must understand the contribution of each CFTR allele to overall genotype function.

Genome wide association studies in common disease have revealed complex disease mechanisms involving many loci, with each individually having a small effect size, but interacting in multi-faceted and often as yet unknown ways.⁹ Genome-wide transcriptomic analyses¹¹⁷, especially with the emergence of single-cell RNA sequencing technology¹¹⁸, have demonstrated widespread and dynamic allele-specific gene

expression at autosomal loci. This indicates that even for a disease with a single-gene etiology, such as CF, the contribution of each *CFTR* allele may not necessarily be straightforward.

However, it has also been shown across multiple studies^{119; 120} that mRNA transcript levels correlate only loosely with protein levels, which necessitates functional evaluation at the protein level to determine the contribution of each allele to overall genotype function. While previous studies in CF reported that having one “mild” *CFTR* allele conferring residual function is enough to avoid pancreatic insufficiency^{19; 121}, leading to the prevailing belief that mild *CFTR* alleles act in a “dominant fashion”²⁹, this idea has never been formally tested with a sufficiently large dataset. In this study, we utilize functional data of *CFTR* variants spanning the spectrum of disease severity combined with clinical data from the CFTR2 database of over 54,000 subjects to perform a quantitative assessment of the contribution of each *CFTR* allele to overall *CFTR* genotype function.

3.2 Results

To test if the combined function of two variants generated a relationship between *CFTR* function and CF trait that was different than deriving function from one variant alone, we divided the 226 genotypes into three groups (**Figure 3.1A**). The Var/NULL group encompassed 55 genotypes composed of a non-NULL variant *in trans* with a NULL variant (such as nonsense, frameshift, canonical splice, exon deletions, or exon duplications, all of which are expected to result in no *CFTR* protein production). The Var/F508del group was comprised of 101 genotypes; total *CFTR* function was derived

from a non-NULL variant *in trans* with F508del (estimated at 0.85% WT-CFTR). The third group (Var/Var) included 70 genotypes composed of 34 non-NULL variants; total CFTR function was estimated by summing the % WT-CFTR function of each variant comprising the genotype). The Var/Var group included 45 compound heterozygous genotypes and 25 homozygous genotypes. The three genotype groups were well-distributed such that correlations with clinical traits could be tested using all three approaches across the entire range of total CFTR function (**Figure 3.1B**).

The Var/Var group provided an ideal dataset on which to test whether the relationship between CFTR genotype function and phenotype differed between homozygous genotypes (in which the % WT-CFTR function contributed by each allele is the same) and compound heterozygous genotypes (in which the % WT-CFTR function contributed by each allele differs). Using an interaction term, we compared the linear regressions derived from log-transformed CFTR genotype function and phenotype for the four key clinical features assessed in this work: sweat chloride, % pancreatic insufficient, FEV1% predicted, and KNoRMA. We found that there was no statistical difference between the regressions derived from the homozygous genotypes and the compound heterozygous genotypes for all traits assessed ($p > 0.08$ for all; **Figure 3.2**). Notably, the slope of the line derived from homozygous genotypes for the KNoRMA phenotype appears to differ from the slope of the line derived from compound heterozygous genotypes, but this did not reach significance ($p = 0.089$), likely related to the rather large variation in this particular phenotype.

Given that there was no statistical difference in the relationship between CFTR genotype function and phenotype for homozygous and heterozygous genotypes in the

Var/Var group, we elected to perform all additional analysis without separating these two subsets and compared the relationship between CFTR genotype function and phenotype for all three genotype groups: Var/NULL, Var/F508del, and Var/Var. First, the relationship between CFTR genotype function and clinical trait was determined for each of the three genotype groups (**Figure 3.S1**) and comparisons of the regressions for each genotype group were then performed using an interaction term between each pair of groups (Var/NULL and Var/F508del, Var/NULL and Var/Var, and Var/F508del and Var/Var) for each trait assessed. No significant difference was found amongst the regressions for each dataset (**Figure 3.3**).

3.3 Discussion

Previous studies reported that having one “mild” *CFTR* allele conferring residual function is enough to sustain pancreatic function^{19; 121}, leading to the conclusion that mild *CFTR* alleles act in a “dominant fashion”²⁹. While our data demonstrate that the amount of function necessary to achieve pancreatic sufficiency is low, the relationship between CFTR function and sweat [Cl⁻] is the same whether the patient has one functional CFTR allele (i.e. Var/NULL) or two functional alleles (i.e. Var/F508del or Var/Var). The similarity in the correlation slopes of individuals having two identical alleles (homozygotes) and those having different alleles (compound heterozygotes) is particularly informative in this regard.

The notion that both *CFTR* alleles contribute to function in an additive fashion has important implications for small molecule therapies. If both alleles of CFTR respond to a modulator, then it is reasonable to expect that clinical effects will be greater. This

appears to have been observed in a small sample of individuals homozygous for the G551D variant, who achieve greater clinical benefit from ivacaftor than patients harboring just one G551D allele¹²². Similarly, the clinical trial assessing treatment of individuals heterozygous for F508del and a variant conferring no or only minimal function using ivacaftor and lumacaftor was halted after results indicated no clinical benefit¹²³, while trials including individuals homozygous for F508del using the same drug combination continued and showed sufficient efficacy to lead to approval by several national governing therapeutic agencies.^{16; 73; 124-129}

3.4 Materials and Methods

Clinical data and variant function

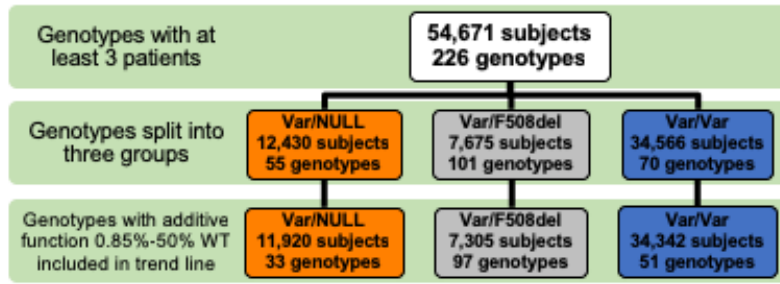
Clinical data from individuals with CF were collected for the CFTR2 project, which contains data from 88,664 individuals receiving CF care in 41 countries (**Table 3.S1**). National CF patient registries or clinical centers contributed de-identified demographic and clinical data from patient records as previously described^{6; 111; 112}. When possible, a Kulich Normal Residual Mortality-Adjusted (KNoRMA) lung disease phenotype was calculated for each individual as previously described⁸³ using non-transplanted lung function measures. Variants reported in at least three individuals in CFTR2 with clinical data were assigned a functional level based on *in vitro* studies measuring short-circuit current in Fischer Rat Thyroid (FRT) or CF Bronchial Epithelial (CFBE) cell lines^{6; 7} or their presumed production of no CFTR protein (nonsense, canonical splice, frameshift, and start-loss variants, and exon deletions or duplications; **Table 3.S2**).

Assigning genotypes, genotype function, and CF clinical trait values

Individuals with a reported *CFTR* genotype comprised of exactly two variants having functional assignment were eligible for inclusion in analysis. *CFTR* genotype function was determined as the sum of the functional levels of the two individual variants comprising the genotype. Each genotype group analyzed included three or more individuals. Clinical traits were analyzed only if at least three subjects within the genotype group reported clinical data for a given trait. The CF traits associated with each genotype were determined by mean value (sweat chloride, FEV1% predicted [forced expiratory volume in 1 second as a percent of predicted for age and height], SaKNoRMA) or % of individuals with a trait (pancreatic insufficiency).

Figure 3.1. Genotype groups included in analysis. (A) Diagram of the process used to categorize genotypes selected for study. Using a filtering process previously described (see Chapter 2, Figure 2.1), 226 genotypes reported in three or more individuals in CFTR2 were included in analysis. Genotype groupings were divided into those with a non-NULL variant of interest in *trans* with a NULL variant (Var/NULL), a non-NULL variant of interest in *trans* with F508del (Var/F508del), and two non-NULL variants of interest (Var/Var). Total genotype function (%) was assigned as the sum of the individual functions of each variant comprising the genotype. (B) The distribution of each genotype group by its total genotype function is plotted and shows representation of each genotype group at every level of total genotype function.

A



B

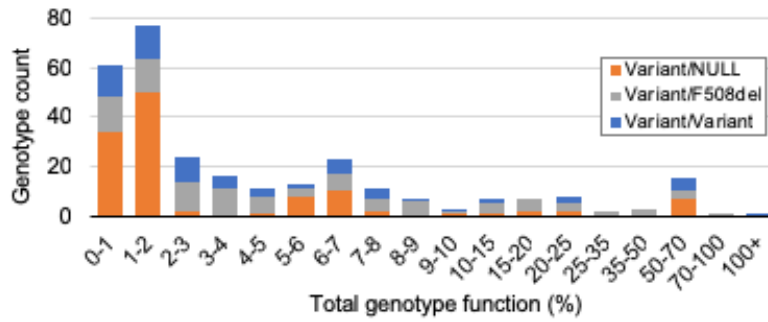


Figure 3.2. The genotype function-phenotype relationships derived from Var/Var compound heterozygous genotypes and homozygous genotypes do not differ.

Comparisons using an interaction term between the homozygous and heterozygous genotypes using log-transformed CFTR function indicates that the linear regressions for these two groups do not differ from each other for any phenotype assessed: sweat chloride (p=0.650; A), pancreatic insufficiency (p=0.685; B), FEV1% predicted (p=0.677; C), or SaKNoRMA (p=0.089; D).

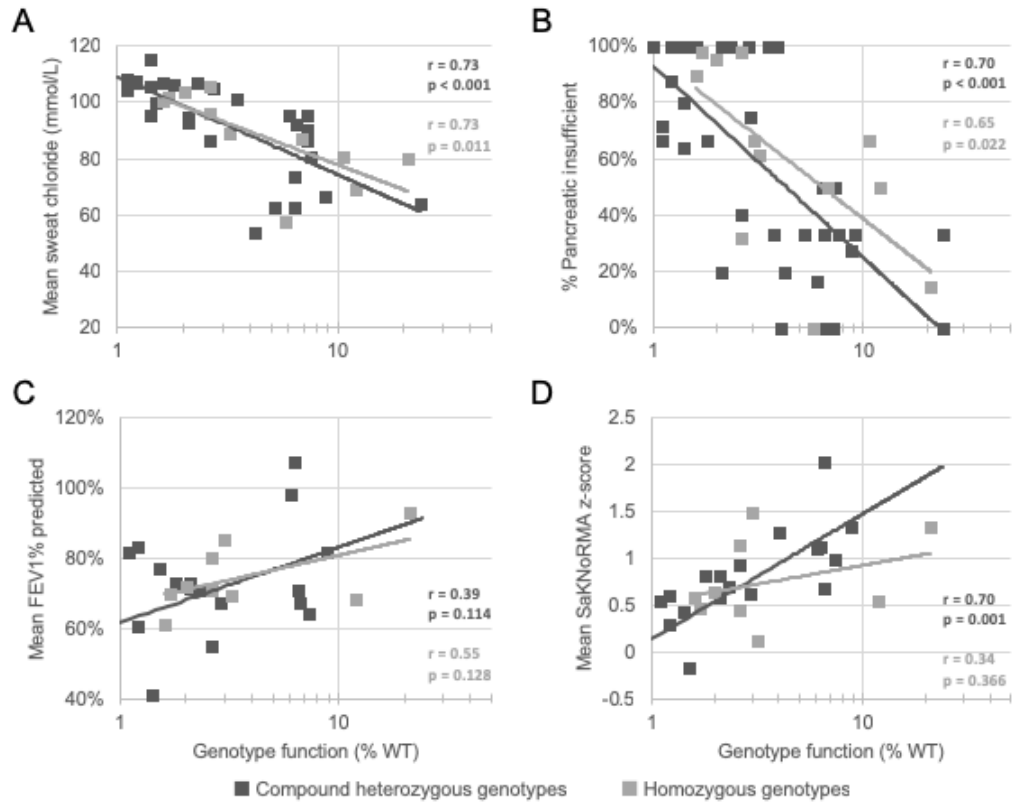
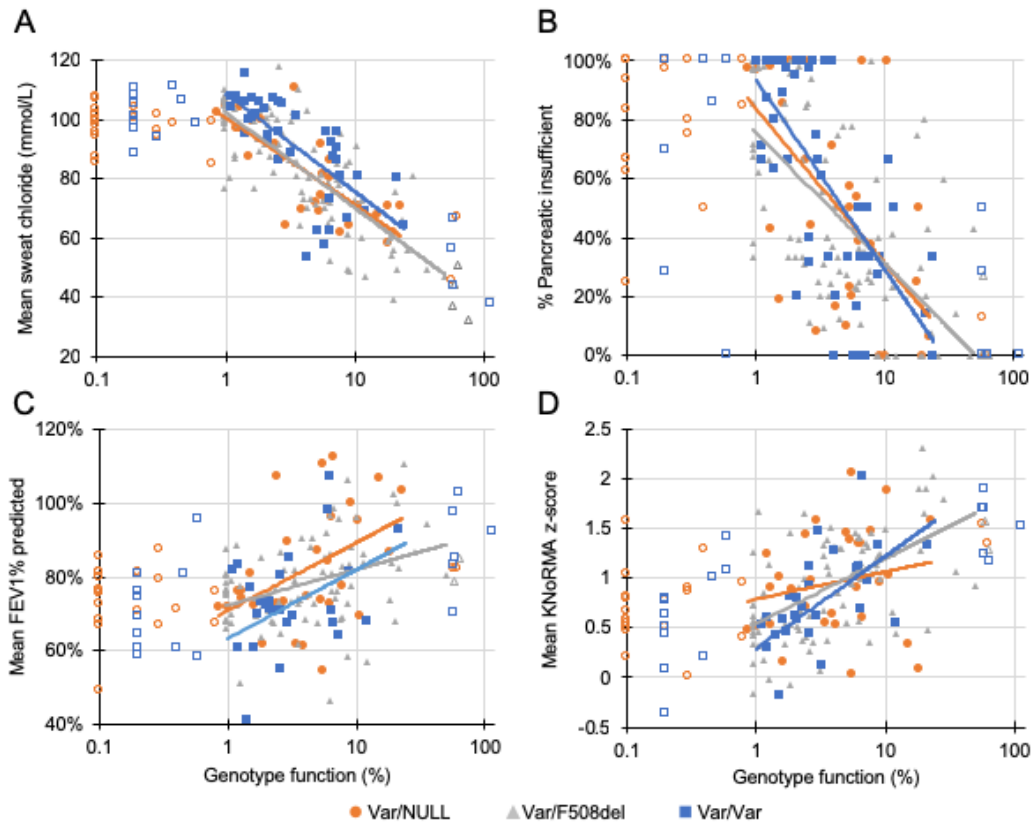


Figure 3.3. Correlations between CFTR function and CF phenotypes are similar for all three genotype groups. Composite plots of all three genotype groups with genotype function as a percentage of wild type plotted on the x-axis (log scale) against mean sweat [Cl⁻] for individuals with that genotype (A), the percentage of individuals of that genotype who are pancreatic insufficient (B), the mean FEV1% predicted for individuals of that genotype (C), or the mean KNoRMA z-score for individuals of that genotype (D). For all plots, the Var/NULL genotype group is shown in orange with circular data points, the Var/F508del genotype group is shown in grey with triangular data points, and the Var/Var genotype group is shown in blue with square data points. A line of best fit was drawn through genotypes with function between 0.85% WT and 50% WT (filled data points; open data points plotted from CFTR2 data but not included in line of best fit calculation). Comparisons of the regressions for each genotype group were performed using an interaction term between each pair of groups (Var/NULL and Var/F508del, Var/NULL and Var/Var, and Var/F508del and Var/Var) for each trait assessed; this analysis indicated no significant difference ($p > 0.05$) amongst the regressions for each data set.



**Chapter 4. The impact of disease-modifying therapies on
reproductive decision-making in cystic fibrosis and Duchenne
muscular dystrophy carriers**

4.1 Introduction

Preconception genetic testing is increasingly available and integrated into research and clinical care. Individuals are now often making reproductive decisions with genetic test results in hand. It is essential that we understand how individuals and families living with genetic risk are evaluating and using their genetic test results, to inform the medical genetics community when designing screening and counseling protocols.

While work has been done to understand presymptomatic genetic testing in the context of intractable diseases, such as Huntington's disease (HD)^{65: 130-137}, as well as diseases for which there are therapeutic and curative interventions, such as breast cancer¹³⁸⁻¹⁴⁶, little is known about the views of people facing a previously intractable disease for which treatments are rapidly becoming available. This study evaluates how views of genetic testing among those living at risk for genetic disease, such as cystic fibrosis (CF) and Duchenne muscular dystrophy (DMD), may change with a changing disease landscape.

CF is a life-limiting genetic disorder that is most often detected via newborn screening (NBS). Newborn screening first became available for CF in the 1990s and currently all states offer newborn screening for CF⁴⁹. The American College of Obstetricians and Gynecologists (ACOG) currently recommends that carrier screening for CF be offered to all women of reproductive age⁴². As recently as 1980, the median survival for CF was less than 20 years. Today, the median predicted survival age is over 40 and continues to rise¹⁴⁷, owing to the advent of new small molecule therapies that treat the underlying molecular cause of the disease⁸². In 2012, the FDA approved Kalydeco—the first small molecule therapy for CF¹⁴. Since then, two combinatorial

therapies—Orkambi and Symdeko—have received FDA approval ^{16; 17} and Kalydeco approval was expanded to cover additional CF-causing mutations ¹⁸. Today, over half of CF patients carry mutations eligible for modulator therapy ¹⁷.

DMD is also a life-limiting genetic disorder for which carrier screening is increasingly conducted. The median survival rate for DMD – once in the teens and now in the late 20s due to advances in care – is also on the rise ³¹. In September 2016, the FDA approved the first disease-modifying therapy for DMD, the exon-skipping therapy Eteplirsen ³². Newborn screening is currently not universally available for DMD and is not supported by the majority of genetic counselors, despite being strongly supported by patients and families ⁵⁰⁻⁵².

Previous studies in CF and DMD have shown that knowledge of a child’s disease status did not always cause parents to change their reproductive plans and that uptake of assisted reproductive technologies is low ⁵³⁻⁶⁰. However, parents still want to be informed of their baby’s carrier status, even if he or she is not affected with the disease ⁶⁰⁻⁶². The present study not only adds to the literature on reproductive decisions made by individuals facing genetic disease, but also explores their views on genetic testing and current practices in light of new medical advancements.

Healthy carriers of genetic disease are increasingly being identified through pre-conception and other genetic testing. For many genetic diseases, we are moving toward population-wide screening as standard of care ³⁸⁻⁴⁸. As new mutation-specific small molecule therapies are developed for a range of genetic diseases, understanding how individuals and families living with genetic disease risk view testing and how these views

change over time as genetic and medical knowledge improves is essential for genetic counselors, clinicians, ethicists, and researchers, as we try to improve care for patients.

To this end, we conducted in-depth semi-structured interviews with CF and DMD carriers, in order to assess whether a changing disease landscape has an impact on views of genetic testing and on reproductive decision-making amongst at-risk individuals and families.

4.2 Results

The reproductive choices made by our study population after learning their carrier status are summarized in **Table 4.1**. In both disease groups, a plurality of participants decided to avoid having children or to take measures to prevent having more children in the future due to their carrier status. Uptake of assisted reproductive technologies was higher in the DMD group than the CF group. However, a large number of participants cited cost as the reason they chose not to pursue pre-implantation genetic diagnosis (PGD) to avoid having an affected child. There were also a substantial number of participants in both groups who chose either to move forward with having additional children naturally or did not use any artificial or contraceptive methods to avoid having additional children. There was one reported instance of termination of an affected fetus. The representative quotes in this section are from nine DMD carriers and six CF carriers.

Effect of emerging disease-modifying therapies on reproductive decision-making

Among CF carriers, there was an even split between those who said that disease-modifying therapies had no effect on their reproductive decision-making, those who indicated that a therapy would change their reproductive planning only if it represented a

cure, and those who said that they would be more willing to risk having an affected child due to the existence of therapies. For the majority of DMD carriers, the therapies either had no effect or would change their reproductive decisions only if they represented a cure. A minority of DMD carriers responded that given the existence of an approved therapy, they would be more willing to risk having an affected child.

Some carriers were direct and unequivocal in their responses that emerging therapies have not factored into their reproductive decision-making.

“In short, I mean I think that's wonderful. Yeah, but no. I still wouldn't want to have a child with muscular dystrophy.” – DMD carrier

Other carriers stated a very clear caveat that a therapy would only potentially impact their decisions if it represented a cure or dramatic alteration of the disease.

“I don't think it's quite there yet. I realize the potential is there. I realize that potential is there to maybe, possibly, fix this disease. But it's still not going to cure it.” – DMD carrier

Even amongst carriers for whom the therapies did not have an effect on their reproductive decisions, some in both disease groups expressed hope about the future, either for their own children or for future generations of people affected by the disease.

“[It] hasn't been enough time, kind of, gone by to see the effects of it. So at this

point, I would-- don't think it would influence my decision. But in-- you know, if I were going to be pregnant 40 years from now, when they hopefully have really amazing drugs for a lot of the mutations, and, you know, the safety and everything has been more studied, I think there's potential that I would be influenced by that." – CF carrier

"I think the biggest role [mutation-specific drugs] play is the option hopefully to extend the lives, not with my son, but of all the other boys that are affected... I don't think I would still risk, you know, I would say, 'Oh, let's just have another kid and see because the research is coming along.' I definitely would not do that – like that seems like a really risky move. But just the hope that I have, I think, for the son we do have is the biggest thing." – DMD carrier

In the DMD group specifically, there were some carriers for whom the existence of therapies and current research did not increase their willingness to have an affected son, but did increase their willingness to risk having a carrier daughter.

"So I guess maybe it swayed me a little bit as far as having a carrier daughter because again, at first I was like, no way, I would never do that, but now I kind of feel like it would be okay because she might – I mean, I would hope that she has a lot more options than even I have at this point by the time she'd be ready to have kids." – DMD carrier

Multiple DMD carriers specifically referenced cost and availability of therapies when evaluating the impact on their perspective and decisions. These concerns were not raised in the CF group. One DMD carrier stated, “[Eteplirsen] does so little for so few people, and it's not available.”

However, for a subset of participants in both groups, disease-modifying therapies did increase their willingness to risk having an affected child.

“I know with like the genetic testing and the research that's coming out, and the things that are being done with DMD, I am more – I lean more towards, you know, maybe I – we will have another child just because the research has come such a long way. Even since last year during it, when he was diagnosed, it's come even further. And, you know, I think it's affected our decision and maybe we -- maybe we'll chance it and maybe we will have another one. And if they have DMD then the research is there.” – DMD carrier

“Yeah, I think ultimately, learning more about CF – and then, we found out our genetic mutations for the baby, and we found out that one of his genetic mutations made him eligible for a new drug, which helps to extend life expectancy. So, we felt like we had a better chance. Before then, I feel like we were kind of 50/50 on what we were going to do in terms of ending the pregnancy or continuing. But after finding that out, I think we both felt confident moving forward.” – CF carrier

One carrier in each disease group said they probably would have made the same decision regarding children, but the existence of therapeutic intervention may have lessened their anxiety surrounding that decision.

“I probably would have still had the six children even if we still had the life expectancy that [oldest child] was first given and we didn't have Orkambi, because like I said, we approached it kind of like that prayerful approach where we were going to live with hope and be open to life and use that sort of message. So I probably would be in the same boat, but it has definitely eased any anxiety I've had, and it definitely made me feel more optimistic and more encouraged.” – CF carrier

“I mean, I might have made the same decision, personally, but I also know—you know, it wouldn't have felt like—it would've felt like I hadn't lost as many choices. It wouldn't have felt burdensome... if I knew that if I had a child who had Duchenne, but that they could be treated, then it wouldn't be nearly as negative a thing to think about.” – DMD carrier

Views regarding therapies and reproductive decision-making were influenced by numerous factors, including whether the couple had affected children and the severity of their child's disease, their personal experience with and knowledge of the therapies, family history of the disease, and personal religious views. A greater proportion of carriers without affected children said that the emergence of new therapies would have an

effect on their family planning than carriers with at least one affected child. Direct experience with the therapies—having an affected child on the therapy or in a trial—made it more likely that the therapies would impact their decisions.

“[My son] was in the [Orkambi] trial for about six months when we finally started to go after another. So yeah, it definitely played a role, because he was responding so well and I don’t know if we would have not if he wasn’t doing well on it, but he definitely made us less hesitant to go after another child.” – CF carrier

However, there were two participants in the CF group, both of whom had only unaffected children, who felt they had insufficient information about available disease-modifying therapies to reach a conclusion on whether such therapies would affect their decision-making.

Out of seven participants who mentioned a personal religious belief or conviction, six said that the therapies would have no effect on their reproductive decisions. A larger proportion of carriers who had a family history of the disease prior to learning their carrier status said that the therapies would affect their reproductive plans than those who had no family history of the disease.

Views on genetic testing and current practices

Participants were asked about their views on preconception genetic testing generally and how they felt about the increasingly widespread use of this testing. Both disease groups

had a generally positive view of preconception testing, but felt it was an individual decision.

“I guess I’d say I highly recommend it so people can go in eyes wide open with the data. They’re armed with information.” – CF carrier

“Oh, I think it’s a great thing. Yes, I think it’s awesome, and I think it just gives people the tools they really need to make an informed decision, a good decision, a decision that will work for their family and the way they want to live life and everything like that [...] I think the more knowledge people have, the better.” – DMD carrier

Some carriers expressed that if they had been offered screening before having children, they may have made different reproductive decisions.

“So, yeah, that very much would’ve influenced us, and I very—I’m very much in support of the pre-pregnancy genetic testing. And I tell anybody that’s thinking about pregnancy, ‘You know’—I don’t, obviously, tell them, ‘You should definitely do this,’ but I’m like, ‘You know, might want to think about this.’ And I even told my OB, you know... she was phenomenal, and I was just like, ‘You know, they offered this testing when I was like 10 weeks pregnant, and I declined it.’ And again, it wouldn’t have changed, even if it had come back and I found out that way. It wouldn’t have changed anything. I was not going to terminate a

pregnancy over it. But, you know, I'm like, 'They should really offer it to people that are thinking of getting pregnant.'" – CF carrier

However, a minority of carriers expressed reservations about carrier screening, including concern about the undue burden testing may place on couples, especially those for whom the outcome of the test would not influence their reproductive decision-making. Couples may “get freaked out,” as one participant put it, especially if the test covers many different diseases. Participants also emphasized the need for physicians and other medical professionals disseminating the information to be properly educated about the disease and the implications of the test results.

“If someone wants to [have preconception genetic testing], they should have freedom of choice. I always would agree with that. But to make [DMD] a standard thing that you would test for, no. I personally don't want to see that happen. Because I think that – how many things then do we test for? You know what I mean? The list could go on for miles.” – DMD carrier

“I guess it depends on the people. I think maybe in hindsight I might've not gotten this test, because when we got pregnant I wasn't going to abort her or abort the pregnancy because of it, so I guess if you're in the mindset that, 'Oh, if there's something wrong with the baby I'm not going to have it,' then maybe you should do it, but no matter what, we weren't going to do that, so I probably in hindsight wouldn't have gotten all this testing done.” – CF carrier

Even some carriers who generally supported the idea of preconception screening advocated for education in the medical community about the most up-to-date information about the disease, given the context of new therapies and interventions.

“I think now the cystic fibrosis people are living longer, and we’re seeing—I don’t know if we’re seeing an increase in the number of patients with cystic fibrosis, but they’re certainly living longer, I think it’s important for the medical community to be really, if they’re going to do the genetic testing, to be very aware of the results, how to perform the test and what those results mean. So have some good, current data on CF. It’s not the same disease as my 60-year-old obstetrician learned about when he was doing his residency.” – CF carrier

Participants also repeatedly brought up the issue of cost, not only of the therapies themselves, but of assisted reproductive technologies.

“... my concern is that once a person gets the idea that they have, you know, a genetic issue that they don't really have any answers to that. You know, you can move forward naturally and just pray and hope or, you know, you can try to spend a bunch of money [exploring and using assisted reproductive technologies] and figure out how you want to handle it, but there's no insurance help for... And so I think there's a big disconnect there. If we're going to be providing people with information then we need to also be able to say well, you know, to stop this from moving forward, insurance companies can provide financial help in these

sorts of situations, you know?” – DMD carrier

Participants were also asked whether they agree with current guidelines and practices regarding genetic testing for their family’s disease. CF carriers generally agreed with the recommendation from ACOG that carrier screening be offered to all women thinking about pregnancy. DMD carriers expressed a desire for genetic screening to become more widely available.

“Right. You know, when they have the talk about Down syndrome or cystic fibrosis, these are things— why is this not included? It should be.” – DMD carrier

“So in an ideal world with perfect humans <laughs> then I would advocate for universal genetic screening for Duchenne... The only thing we have right now is knowledge. So I think it's important to include it in that heel prick and then when a kid is identified with any of those disorders, to have somebody from that disorder, you know, a specific organization, whether it's PPMD [Project Parent Muscular Dystrophy] or the MDA [Muscular Dystrophy Association] or, you know, whatever, the Cystic Fibrosis Foundation or whatever, to have somebody from that culture give you a call and say, ‘Hey, I just, you know, they sent me the data because that's my job. And I wanted to let you know I'm here for you. I have a bunch of information.’ “ – DMD carrier

4.3 Discussion

While great strides have been made in fixing the underlying defects that cause CF and DMD, the current interventions are not a cure for either disease. These two diseases are in a period of rapid evolution when it comes to new therapies and clinical trials. Therefore, understanding how CF and DMD carriers take into account a changing disease landscape when making reproductive choices is important for helping genetics professionals and patients navigate this period of transition from untreatable to treatable disease. This study represents a novel evaluation of how individuals facing genetic disease consider the treatability of the disease in family planning and their views on genetic testing.

A majority of participants in our study changed their reproductive plans as a result of knowing their carrier status, which is a greater proportion than reported in many previous studies⁵³⁻⁶⁰. This difference may be due to increased availability of preconception and prenatal testing. However, uptake of *in vitro* fertilization with pre-implantation genetic diagnosis was still relatively low in this cohort, with prohibitive cost being a major determining factor.

Perhaps predictably, a changing disease landscape produces a spectrum of opinions regarding the role of emerging disease-modifying therapies in reproductive decision-making amongst carriers. For some carriers, therapies did not enter into reproductive decisions at all. For others, therapies may not have had an appreciable impact on their decisions, but they foresaw a future in which these therapies—if curative and widely available—could perhaps influence their views. Some DMD carriers expressed more willingness to risk having a carrier daughter with hopes that by the time

she was making her own reproductive decisions, she would have more options and the therapeutic landscape would be more advanced. A subset of carriers from both disease groups felt more willing to take a chance and risk having an affected child, given the existence of disease-modifying therapies.

The cost and availability of therapies was a concern specific to the DMD group, which makes sense given the context of the troubled FDA approval process for Eteplirsen, the exon 51 skipping therapy for Duchenne ¹⁴⁸. Eteplirsen was approved by the FDA in late 2016, despite concerns about its efficacy, due to a strong push from the Duchenne community. However, the drug is costly and due to the efficacy concerns, not all insurers cover Eteplirsen. Additionally, only about 14% of Duchenne patients carry a mutation eligible for the drug. The new FDA-approved small molecule therapies for CF, while also very expensive, are covered by insurers for eligible mutations, but the combination therapy, Orkambi, has been facing challenges over cost as well ¹⁴⁹. However, therapy-eligible mutations represent a greater proportion of CF patients than DMD patients ¹⁴⁹.

A number of factors influenced how carriers in both disease groups made reproductive decisions in the context of disease-modifying therapies, including experience with the disease in the form of family history or an existing affected child, familiarity with and eligibility for therapies, and religious views. Carriers with affected children were less likely to say that disease-modifying therapies had an impact on their reproductive decisions than carriers with no children or with only unaffected children. This makes sense, as the former are already dealing with the implications of having a child with a serious genetic condition. However, the severity of the child's disease also

plays a role; parents of children who are very ill were less likely to be optimistic about the outcome, should they choose to risk having another child. Similarly, knowing that their family's mutation is already eligible for a therapy or seeing their child improve while on a therapy is an experience that will inevitably shape carriers' views about the impact of therapies on their choices, while for carriers whose mutations are not therapy-eligible, questions about impact are more theoretical.

Of the carriers who expressed some form of religious belief, all but one said that the therapies would not have an effect on their reproductive decisions. A small amount of work has been done examining the role of religious beliefs when considering intervention for genetic disease specifically. Qualitative research suggests that religious members of the lay public often struggle to balance religious doctrine or teaching—on which it was often difficult to obtain information—with their own personal experiences¹⁵⁰ and that the idea of PGD created “moral dilemmas” for them¹⁵¹. Religious scholars in the Jewish, Islamic, and Buddhist faiths generally have an accepting and positive view of PGD, but Christian scholars are “very skeptical” of the implications of the long-term use of PGD^{152; 153}. All participants in the current study who revealed a specific religious affiliation self-identified as Christian. However, participants were not asked about their religious beliefs directly as part of the interview and we have only reported findings where religion was explicitly stated as a factor in reproductive decision-making.

Overall, our cohort had a very positive view of preconception carrier screening for genetic disease. The vast majority of carriers felt it should be an individual choice, but it should be made widely available. Some carriers expressed reservations about excessive burden posed by carrier testing for a large number of genetic conditions, especially if the

test results would not change one's reproductive choices. The CF group overwhelmingly supported the current ACOG recommendation that all women of reproductive age be offered CF screening. The DMD group expressed a desire for screening to be more widely available than it is at present, including both preconception screening and newborn screening, which supports conclusions drawn in prior literature⁵⁰⁻⁵².

This study has several limitations. These results are qualitative data from a relatively small number of interviews with CF and DMD carriers. Additionally, this cohort is entirely female. The DMD carrier group was expected to be all female, due to the X-linked inheritance pattern of DMD. While CF carrier couples were recruited, responses were overwhelmingly female, and everyone who made it through to interview were women. While carriers were asked during the interview about their partners' perspectives on their reproductive decision-making process, the perspective of male CF carriers is lacking in this study and warrants further examination. The trends regarding religiosity's role in attitudes toward PGD and reproductive decision-making also warrant further study, as demographic data regarding religion was not explicitly collected and trends were observed simply based on the group of individuals who expressed personal religious beliefs. Additionally, it is worth noting that while participants were asked about their reproductive decisions, they were not specifically asked about pregnancy termination, rendering us unable to accurately draw any conclusions about decisions surrounding termination in this cohort.

Our results demonstrate that some DMD and CF carriers are beginning to consider emerging disease-modifying therapies while making reproductive decisions and that there is support for widespread access to preconception carrier screening. As

mutation-specific therapies become available for an increasing number of genetic diseases, knowledge of one's own disease mutation becomes vital, as eligibility for therapy may influence the reproductive choices of some couples. A greater understanding of how rapidly changing treatability of a disease plays a role in individuals' desire for testing and how they will use their test results is essential for the medical genetics community as it designs screening protocols and counseling procedures. This study was a first look into how the changing landscape of genetic disease may affect how individuals think about genetic testing and using that information in reproductive decision-making. Larger scale follow-up studies should be conducted as therapies become more widely available and cover a greater percentage of disease mutations.

4.4 Materials and Methods

Study Sample

Thirteen (13) confirmed CF carrier women (ages 29-49), whose partners are also carriers, were recruited via the Johns Hopkins Cystic Fibrosis Center, the Johns Hopkins All Children's Hospital, and genetic counseling email listservs. Nineteen (19) confirmed DMD carrier women (ages 25-43), were recruited via Project Parent Muscular Dystrophy – a muscular dystrophy patient advocacy group. Semi-structured interviews were conducted with both carriers who did not have any affected children and carriers who already had a child/children with the disease (Table 1). Eligibility for the study was limited to carriers who either had no children or who had at least one child under 10 years of age, in order to target individuals currently making reproductive decisions or individuals who have made reproductive decisions in the very recent past.

This study was reviewed and approved by the Johns Hopkins Institutional Review Board and all participants gave informed consent.

Study Design

A letter explaining the study was given to the Johns Hopkins Cystic Fibrosis Center, Johns Hopkins All Children's Hospital, genetic counseling email listservs, and Project Parent Muscular Dystrophy to distribute (either via paper mail or electronically) to potential study participants. Potential participants were asked to contact the study team via post, email, or phone, at which time a phone or Skype interview was scheduled. Semi-structured interviews (~45 minutes in duration) were conducted, focusing on participants' views on genetic testing and reproductive decision-making and how availability of disease modifying therapies affects these views (See Supplemental Materials). Interviews were recorded and transcribed.

Interviews covered the following domains:

- The participant's experience with genetic testing;
- The participant's experience (or lack thereof) with the disease prior to testing;
- If and how genetic test results were communicated within the family;
- Reproductive decision-making and how genetic test results informed those decisions (if at all);
- The participant's views on genetic testing and how the availability of disease modifying therapies has affected these views and reproductive decision-making (if at all); and,

- If and how the participant's experience with genetic testing has affected their life outside of reproductive decision-making.

Data Collection and Analysis

Transcribed interviews were stripped of identifying information and coded independently by two researchers using the codebook to capture thematic information. The codebook was developed by three members of the research team and revised iteratively as more transcripts were coded. All transcripts were analyzed with the final version of the codebook. Any conflicts between the two coders were discussed and reconciled. Final codes were entered into QSR International's NVivo 11 qualitative analysis software. NVivo 11 was used to create code reports, which were then analyzed to determine the spectrum and distribution of responses to interview guide questions.

Table 4.1: Characteristics of participants

Characteristic	CF	DMD
No children	0	6
Only unaffected children	3	3
One affected child	7	6
Multiple affected children	3	4
Learned carrier status through pre-conception screening	2	12
Learned carrier status through prenatal testing/diagnosis	8	0
Learned carrier status through child's diagnosis	3	7
Chose not to have children/to stop having children	5	6
Pursued IVF/PGD	1	6
Did not intervene/change reproductive plans	6	3
Pursued sperm or egg donation	1	0
Pursued adoption	0	2
Pursued sperm sorting	-	2
Terminated an affected pregnancy	0	1

Chapter 5: “I feel like we've sort of been ignored for a long time”: A qualitative assessment of challenges faced by cystic fibrosis and Duchenne muscular dystrophy carriers

5.1 Introduction

Due both to advances in our understanding of the molecular mechanisms underlying an increasing number of genetic diseases, the development of mutation-targeted therapies, and to the increasing ease and availability of genetic testing in clinical care, research, and direct to consumer, more carriers of a variety of genetic conditions are being identified. Where carrier screening was previously restricted to single disease or gene tests, generally based on ancestry, expanded carrier screening is multi-disease and pan-ethnic¹⁵⁴. Additionally, for many genetic diseases, we are moving toward population-wide screening as standard of care³⁸⁻⁴⁸. The rapid expansion of carrier screening raises new and complex challenges for how these individuals, who face a wider range of challenges than is generally appreciated, should be counseled.

Generally, when returning results for preconception carrier screening, the focus on how the information will be used has centered around reproductive planning. While much work has been done to understand preconception genetic testing and counseling in terms of reproductive decision-making^{53; 55-57; 59; 155-157}, focusing on family planning alone does not capture the full burden of being a carrier for genetic disease. We did explore reproductive decision-making in this cohort (manuscript in preparation). However, our interviews spanned a wide range of topics in an attempt to capture the participants' full experience. In this study, we focused on two recessive conditions—one autosomal and one X-linked—which are progressive and life-shortening, have childhood onset, have a relatively high incidence for a genetic disorder, and for which preconception screening is readily available. In both of these disorders, rapid progress is

being made in the availability of mutation targeted therapies that are now commercially available and improving outcomes for patients.

Cystic fibrosis (CF) is a multi-system disorder that results from loss of function mutations in the cystic fibrosis transmembrane conductance regulator which lead to defective ion transport, resulting in disease ⁸². With the advent of precision treatment, the life expectancy for individuals with CF is rising rapidly ¹⁴⁷. Newborn screening first became available for CF in the 1990s and is currently offered in all 50 states and the District of Columbia ⁴⁹. The American College of Obstetricians and Gynecologists (ACOG) currently recommends that carrier screening for CF be offered to all women of reproductive age ⁴² and CF is the first disease for which universal screening became a standard of care for prenatal patients ¹⁵⁸.

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by loss of function mutations in the dystrophin protein, which result in progressive muscle fiber damage ¹⁵⁹. The median survival rate for DMD—once in the teens and now in the late 20s due to advances in care—is also rising ³¹. Newborn screening is currently not universally available for DMD and such screening is not supported by the majority of genetic counselors, despite being strongly supported by patients and families ⁵⁰⁻⁵². However, carrier testing is available to at-risk females with a family history and is often offered preconceptionally. Unlike in CF, where carriers are asymptomatic, about one fifth of carrier females of DMD manifest symptoms of the disease, such as muscle weakness and dilated cardiomyopathy ¹⁶⁰.

In this study, we conducted in-depth semi-structured interviews with CF and DMD carriers, exploring not only their reproductive decisions, but also communication

with family members, family dynamic, testing and counseling experience, support networks, conversations with children about disease or carrier status, and other effects their carrier status have had on their lives outside of family planning.

5.2 Results

In the DMD group, we were able to interview both parents and child-free individuals. While the perspective of people without children is missing in the CF group, we spoke to three individuals who do not have any children with CF. In both disease groups, we were able to interview both individuals who knew of their carrier status before having children and individuals who learned of their carrier status after becoming pregnant or after having children. The 41 representative quotes below are from nine participants who are CF carriers and fifteen participants who are DMD carriers.

Lack of support from the medical community

Many participants in both disease groups faced a wide range of challenges resulting from insufficient support or counseling on the part of the medical community. Less than half of individuals who carry CF mutations and just over half of individuals who carry DMD mutations reported being offered genetic counseling and of those who had counseling, only two thirds of the CF group and less than half of the DMD group found the counseling helpful.

A substantial minority of both groups reported feeling that this lack of support in the immediate aftermath of learning their status was a result of their doctors or other medical professionals not having enough experience with the disease.

“I just feel lost in a lot of ways because there's a lot of information that I wish that I had that I don't. In our area there are no genetic counselors that could sit down and talk to me. My OB office, like I was saying, didn't even know about [IVF with pre-implantation genetic diagnosis]” – CF carrier

“I felt completely like I was drowning and no one understood, and the doctors and the professionals in my area had no idea what I was talking about, and I think that was like the building foundational steps of like, ‘You know what, no one knows about this, and if this is going to change my life you sure bet I'm going to make my voice heard so that these people now know what this is,’ because I was just kind of like really frustrated. I'm like ‘Okay, well, we need to get to these important centers in [city], in [city], because these people know how to handle it, and no one here does.’ And I kind of still feel that way. We have a few families up here, and none of us see anybody here. It's always worse then when he gets sick or something happens. I'm like ‘They have no idea what we're dealing with here.’ “ – DMD carrier

Inattention to symptoms in manifesting carriers

In the Duchenne group specifically, multiple participants cited difficulties getting testing for their daughters and an underappreciation of the fact that females can manifest symptoms of the disease. They stated that knowledge of one's carrier status as a female is important, not only for family planning, but also for greater understanding of one's own symptoms, which they say are not discussed enough in the medical community.

“Well, I shopped around until I found a doctor who was willing to say ‘Oh, no, we’ll go ahead and write the order and have her tested so that you can raise her with this knowledge like you want to do,’ and it was a little bit clandestine. Yeah, it was a little bit outside of the system. There was a lot of resistance, and it was actually later that I found out that many people were being denied testing because of this, in my opinion, misguided ethical idea so that these girls would grow up with brothers with Duchenne and would not know whether they were carriers or not, would live with this uncertainty until they were like mid-teens, and then they would be allowed to get tested, and then they would find out, so not a good family dynamic.” – DMD carrier

This interviewee went on to say that she “strongly believed” every girl should have access to testing as early as possible and that it is “absolutely essential that in the Duchenne community we recognize that girls are not unaffected carriers.”

“So [my mother’s] been through-- she went through so many doctors that did-- nobody ever mentioned muscular dystrophy to us or the fact that it-- that women could actually have symptoms from this because it’s so rare. They really weren’t sure. That’s the other thing. I feel like carrier testing and symptoms in a woman is not so much talked about. I think it’s kind of brushed under the rug a little bit, and I feel like that’s a big deal. So if they had thought about it more, maybe my mom would’ve gotten diagnosed sooner as a carrier, so we would’ve know that ahead of time before having children, you know?” – DMD carrier

“And the fact even more and more people are starting to look at carriers and trying to figure out just these kinds of things because I feel like we’ve sort of been ignored for a long time.” – DMD carrier

Downplaying the risk

A common theme seen in the CF group when asked about their testing experience was a feeling that medical professionals were downplaying the importance of testing or the risk of their partners also being CF carriers.

“So, the whole time I was pregnant, it was repeated to us multiple times, by multiple doctors, even after switching care, that the chances that [son] would have CF were so remote that we shouldn’t even worry about it. And I asked my OB about doing an amniocentesis to find out, because I was having a lot of worries and concerns. And her exact response to me was, ‘Well, if it does turn out that there is CF, would you terminate your pregnancy?’ And we both said, no, we wouldn’t. She said, ‘Well, then there’s no point in doing an amnio.’ “ – CF carrier

“And the nurse was kind of like, ‘Yeah, unless Dad’s a carrier, it really doesn’t matter, and if you want to have Dad tested, and you already have one kid and he’s fine.’ “ – Ppt. 43, CF carrier

Judgement from others and social isolation

When asked about sources of support, all but eight individuals in the DMD group and one individual in the CF group cited a lack of support in some fashion or another.

Cited sources of support included family, friends, the disease community or disease-specific support groups, and church or faith-based groups. However, many participants stated that they felt judged for their choices by medical professionals, family members, or even other members of their disease community.

“Yeah, and then I have other friends that think you have one child that's healthy you should be done. Don't have any more children. Even my OB kind of gave me his personal opinion and said that if it were him he would just be thankful that they had one child and then that's it...” – CF carrier

“My one uncle said I was stupid. <laughs> And I'm, like, whatever. You don't know. He just didn't get it. I mean, that's-- we don't-- I'm not one to hold things against people, whatever. I'm just, like-- when someone says something like that, you just know they don't understand the whole situation. And especially-- I mean, for me, about to have an abortion when I don't believe in it, you can't judge someone until you're in their position. You have no idea. You can plan your whole life thinking you'll make the decision this way, but then it happens and you do something different. You truly don't know 'til you're there. So, I mean, that's kind of what I think.” – DMD carrier

“All the shaming that came with getting pregnant again when you know you carry cystic fibrosis. So between finding out I was pregnant-- and we didn't keep it a secret. My first and second, we did, until I had passed my first trimester. But the third one, there was no hiding it. I just lost my mind. So everyone knew I was pregnant and there was shaming, a lot of shaming on everyone else's part.” – CF carrier

The CF group in particular noted a very powerful and stark divide within the community surrounding reproductive choices and pre-implantation genetic diagnosis.

“Yeah. And to the point of where I went to the Breath of Life Gala in 2015, and I was about, oh, 28 weeks’ pregnant with the twins, so I was 5 months’ pregnant. And I was sitting at the CF people table, <laughs> and I could feel the questions and the kind of like... shock, you know? Because it’s such a small community, and we all get to know each other, and... <laughs> ... So, yeah, there was a lot of-- and people are not afraid, in the CF community, to ask you those personal questions, either. They’ll straight-up be like, ‘So, I see you decided to have more kids.’ <laughs>” – CF carrier

“I know that the community-- the CF community in particular is really split in terms of reproductive decision making when it comes to CF. That’s been a challenge for me, because a lot of my friends believe that CF is a blessing in a sort of way and that we really shouldn’t control for it in the population in terms of testing. And so when they found out that not only I decided to get pregnant again, but that I was getting tested to find out if the baby was going to have CF and that it might affect my decision, I think a lot of people were kind of offended in a way, like something’s wrong with my son, so I wouldn’t want another one like him.” – CF carrier

“I think the CF community in general needs to talk more about PGD, it seems to be like this dirty secret or something, there’s like a group of people who’ve done it and we’re really for it and we wish other people would do it but I’m always afraid someone is going to take offense or-- even people with CF sometimes take offense because they say, ‘Oh, I

wouldn't be alive.' But the other people say, even with CF say, 'No, we need to get rid of this disease and this is the only way.' It's like a taboo subject and I really wish it wasn't."

– CF carrier

While a majority of interviewees in both groups cited their disease communities as an immense source of support as a community of people that understand what they are going through, some reported that online forums in particular were at times toxic environments.

"Because probably I guess the judgmental side of other CF moms, like 'Oh, my kids never miss a treatment. If your kid misses a treatment, you're not doing it right.' And it's like, 'Nobody is 100 percent perfect, and if you're trying to tell me that you've never missed a treatment, then I think that maybe there's some untruth there.' That kind of turned me off. While it claims to be a support group, it's not always a support group. People have different opinions about the best way to do their treatments. And it just was not the right environment for me. I felt like it wasn't open to people having different opinions about things. Then it's like, 'I'm not gaining anything from this anymore.' " –
CF carrier

This is especially notable for the CF community, for whom social isolation is particularly acute; not only do they face a unique situation that family and friends often do not relate to, there is the additional risk that an affected child may catch an infection in social situations. Multiple CF parents reported losing friends or negative impacts on relationships with family members due to their child's situation.

“... I think we found, as time went on, that we lost a lot of friends because people didn't really understand why we weren't coming and showing up to events or bringing the baby and letting everybody hold her, and why we weren't going to parties and why we had distanced ourselves from everyone. I think they were confused, because when you have a baby, I think everybody wants to partake in holding the baby and celebrating the baby, and actually we were just the opposite.” – CF carrier

“Oh my gosh, it's definitely-- isolating is a great word. Even from family I think, like, we declined to go to a family event a couple of months ago because somebody was sick and they were still going, and nobody understood why we would keep him away. And, I think you just kind of shut down, because you've become a broken record and nobody knows it from your point of view, even if they're close family.” – CF carrier

“I think they try to be understanding but I don't think they can fully understand it. I'm not as close to a lot of my friends anymore just because our lives are just so different and I know some of them just don't know how to talk to me anymore, it's easier not to talk to me.” – CF carrier

Participants also discussed times when people in their social networks would make comments that were unintentionally hurtful, especially for women who are carriers of DMD mutations.

“Just a little bit because I felt like it was so unfair. But I didn't talk to the doctor about everybody <laughs>, you know, there will be families of—in our area there's church,

there's lots of families that have like six to nine children and you just have people that could just so easily have children and they would just say the wrong things especially, you know, like oh, especially when we're adopting, 'Oh, you're so lucky. You don't have to have stretch marks. You don't have to have morning sickness and all that stuff.' And I'd be like, 'I would trade you in an instant.' “ – DMD carrier

“Obviously I went through fertility treatments so I was desperate to be a mother but-- people used to even say, 'Maybe god was trying to tell you not to have kids.' “ – DMD carrier

Family communication and discussing genetic disease with children

When a person learns that he or she is a carrier for genetic disease, it does not just have implications for that person, but for that person's entire family as well. Many interviewees in the CF and DMD groups discussed the challenges involved with communicating genetic information with their families and the impact their carrier status had on their family dynamic.

Some participants had difficulties educating their families about the disease or reported that they felt family members did not understand the seriousness of the disease or the importance of testing.

“Versus my husband's family was more, like they've never had in their family to my knowledge a human being with special needs, so it's kind of, I felt like it was a totally different exposure trying to explain to them what was going on. And there's still a lot, even when we had that conversation, there's still a lot we didn't know, a lot that we don't

know, like it's so hard to try and explain it. I feel like I'm constantly repeating and people will make comments, like my son he actually just this weekend just started pulling to a stand. At about 2 ½ years old. And so there's a couple of instances with my stepfather where he's just like, 'Oh, you can stand up. It's so easy!' And it just like, almost crushes my soul—because you don't, like it's so hard, you don't understand, like it's not that easy. That's just not true. Like I called my husband that day and I was like so mad at him because I'm like, 'I can't understand how he doesn't understand.' But, it's hard.” - DMD carrier

“[My family members] know all about all this kind of stuff, and I wrote them a lengthy thing explaining everything, explaining the importance of them getting tested, and even if they aren't thinking about having more kids or whatever that just for health reasons too for the cardiac issues that carriers can have and that kind of stuff, and no one listened to me, and no one cared, so I was just like-- but a lot of them-- my whole family's from [state]. They're [...] very religious, and I think that played into it. Their sort of mindset is 'Well, if that's the case then that's God's will, and that's how it's supposed to be, so we don't need to get tested' or "We don't care about getting tested" and things like that, which-- that also made me angry. I had some anger issues over that too, but to each his own. I can't force them to do it.” – DMD carrier

“So, those boys are college-educated, and I think it's outright denial. It also doesn't help that their father, who's not college-educated, but he's really a quite smart gentleman, he has come outright and said that he's not a carrier at all, which would be impossible, since his son is a carrier. And I've tried to explain the genetics to them several times,

<laughs> and they won't accept. They feel like, if they accept it, they're taking blame of some kind. And it doesn't matter how I try to explain to them, 'Look, it has nothing to do - it's nobody's fault, but I don't want you to have a grandbaby that's affected, and not treated, because we're in denial.' You know?" – CF carrier

"So yeah, it just was awkward because they didn't understand, like they don't believe in IVF, so they're like, 'Well, why are you doing this?' And my other-- one of my aunts, too, didn't understand how serious muscular dystrophy is because she said something like, 'Oh, my friend has a baby with Down syndrome and he's so cute.' So she just doesn't really understand. And it was hard explaining to them, like because I have a science background, so I felt like I understood everything really well and explaining it to them was it just really challenging. They didn't seem to understand it very well." – DMD carrier

In-laws of some interviewees who are carriers of CF mutations went as far as to describe the disease as "bad blood" or a "curse" and placed blame entirely on the woman.

"But when she found out about [name of daughter], she blamed me, she asked me if I knew that I had bad blood when I married her grandson." – CF carrier

"I don't know, [my husband's] family deals with things in kind of an odd way. His parents are much older than my parents, so his mother was elderly and she-- well, she wasn't elderly then, I guess, but she definitely has this old-fashioned—'This is just a curse on the family and I'm not going to entertain a curse.' I'm like, 'No, it's not really a curse.

It's actually science and genetics.' 'Oh, no, it's a curse.' Like, 'I really don't think it's a curse.' “ – CF carrier

“...So no, I did talk to my aunt and my dad was super supportive. He was like, 'I've got you, you'll be fine. We're in this together.' So my family was just really, really strong and rallied. My husband's family did not and they insisted that since it's so rare in Asians that I must have cheated on him so...we don't really speak-- yeah, pretty bad. We don't really talk to them anymore.” – CF carrier

Given that many participants who are carriers of DMD mutations had a family history of the disease and therefore knew they may be carriers from an early age, the DMD group specifically referenced a lot of feelings of guilt on the part of carrier females and unaffected females, as well as often complex and fraught relationships with family members.

*“And I remember having to tell my [affected] brothers and it was the hardest conversation I've ever had. It sucked really bad. <chokes up> Because I remember telling them that I was pregnant and I was, like, excited but nervous and not sure and, you know, having to go and tell them that, “Hey, guess what? I'm pregnant,” and they were pissed. They were angry. They said not nice things. They were like, ‘Why would you do this to a child? You've seen us grow up your whole life like this and it f*****g sucks?’ Like, ‘Why would you do this to us?’ and they were just very, very angry at me.”*
– DMD carrier

“There was only one sister who was not a carrier and, you know, it wasn't like there was any problem with that. It was just the way it was and it wasn't a bad thing and I don't think it influenced our relationships at all. I think it was harder for my brother who did not have Duchenne's. I think it was harder for him than anybody because he just couldn't understand why [my brother] and not me.” – DMD carrier

“I mean, she was, like-- my mom is a very open person, but I think this was just the one thing in her life she just could not talk about. I think a big part of that because she was the only girl and her parents were working all the time. So she was the one that took care of her brothers. I think their passing was, like, particularly hard, as it was for everyone. So we didn't really talk about it very much.” – DMD carrier

This participant went on to say that “if our family communication style had been much more conversive I think that might have made me get more information sooner.” However, some interviewees did discuss positive impacts dealing with genetic risk in the family had on their family dynamic.

“I feel like it's pulled my immediate family closer together, too. I feel like my relationship with my sister is better. There's this... yeah. <laughs> I feel like we're even closer than we were before, because we share this. And I know I've seen this in my niece, where she feels this-- you know, she's so close to her brother, and feels this-- and I think knowing that she can look to her grandmother, her aunt, her mother, and say, “Oh, they all have this genetic disorder, too, they're all carriers, too,” I think that has given her a sense of

kind of strength, knowing that she's not different or alone; that this is something that unites her family.” – DMD carrier

“I think it's brought our family closer, I mean our immediate family, my husband and I, it's definitely brought us closer.” – CF carrier

For parents of affected children, there is an extra challenge of speaking with them about the reality of their disease, as well as with their unaffected siblings, which many participants in both disease groups discussed.

“Maybe you shouldn't talk that much about him with those things but he asks about death and God and heaven and all these things. So it's, yeah, it can be very tough.” – DMD carrier

“And I know... there's been some upsetting times. I can remember distinctly-- that was the day before his fifth birthday, because, ‘Mommy, am I going to have CF when I turn five?’ Just broke my heart.” – CF carrier

“So he had this really big year, with all these emotional things on top of his CF, and he had two hospitalizations in that timeframe. So, what started happening was, he started saying things like, “I wish I were dead.” And to hear that come out of your five-year-old's mouth is earth-shattering and shocking.” – CF carrier

“So my husband and I agreed that we wanted to normalize this idea of having Duchenne in the family. We didn't want this to be, like, you know, some big, awful family drama. We wanted this to be, you know, ‘Hey, you've got dimples and you've got brown eyes and you also [are a] Duchenne carrier. Our family has the Duchenne.’ So when we got [name of daughter] tested—she was 3-years-old and we-- so my daughter, basically, we were just trying to tell her, you know, ‘Hey, this is part of you. This is normal.’ And so we had these conversations, you know, very early.” – DMD carrier

Wide-ranging impacts

When asked about what effects knowledge of their carrier status and dealing with a genetic disease in the family had on their lives outside of decisions surrounding family planning, interviewees gave a wide range of responses.

Positive effects included learning of one's inner strength, renewed focus on life's important things, more understanding of the plights of others, and a sense of community.

“You get to Duchenne, and then you navigate that, and then you get a divorce and you navigate that, and when you're asked to do things that you don't think you can handle but you still have to do it and then you get through that it's like this strength that you're like “I can handle anything. I am not scared. Come at me.” I'm not scared anymore, and so I would say too it's just so changed my life. I used to be a very quiet, kind of spineless-- I was shy, didn't raise my hand in school. I'd become beet red. And now I'm in this grad program, and they call me leader, and I'm in all these leadership roles, and I run organizations, and I am an advocate, and I fight in the community, and I don't stop. I don't really sleep much. It's turned my entire life around. It's made me realize I have a

voice. I can make a difference. This was given to me for some reason. I'm going to make the most of this.” – DMD carrier

“And I don't know, I feel like I have a focus that I didn't have before that's not just work. I don't know how to explain it. I'm not just running around at home doing little things. I feel like we have a greater purpose, like we're working toward something of the group, as a family and even with just the rest of the CF community, and I kind of like having that outside purpose, I guess. Not that I like that our kids have CF, but it's nice to feel like a part of a community. I didn't have that before.” – CF carrier

“Yeah. They do-- I mean, that was one of the first times I felt like, oh, there's a place for me. I am a carrier and there are real issues. Oh, my goodness, other people have this too? Like they have-- they feel this? Like, you know, I know there's a lot out there for kids and adults, young adults with muscular dystrophy but I didn't really know there was a lot for carriers. So that was really welcoming for me, just to also have a familiarity and a relationship with people that-- Or even just reading their posts. Like, "Oh, she's thinking what I'm thinking. Okay. This is normal. I'm okay.” – DMD carrier

However, participants cited unanticipated negative impacts as well. One individual who is a manifesting DMD carrier discussed how dealing with symptoms changed her career path.

“... I was thinking when I went to college what I was going to do with my career. Basically it just affected a lot of my future plans [...] before my senior year I had been

talking to college coaches, had been looking at going to college for sports. And then when I found out, that kind of changed all my plans. I had no idea what to do basically. Because my plan was to maybe train for like maybe the Olympics and then be like a personal trainer on the side. And that kind of changed that because I couldn't do any of that. And then as far as career, yeah. So for me I had to find a completely new direction in my life.” – DMD carrier

One participant in the CF group said she wished she had gotten prenatal testing because of unanticipated challenges beyond the decision of whether to terminate that she felt ill-prepared for prior to the birth of her child.

“Now, I wasn't thinking in terms of preparing myself for what kind of medical leave I would need. I wasn't using it in terms of thinking about what kind of help after having the baby I would need, which is more of like-- I wish I would have known that before I said no to the genetic testing. But I was thinking, ‘I'm not going to terminate my pregnancy no matter what.’ That was immediately what my thought was, ‘I'm not going to terminate the pregnancy no matter what, so I don't care what the result says.’ However, I wasn't thinking, ‘Oh, your child might have to have surgery right away and you might need to-- instead of that nine-week maternity leave that you were thinking about taking, you're actually going to have to take 12 weeks.’ I wish I had been more prepared, because I would have saved more vacation time. And then also even care, like we had assumed our son would have gone into daycare.” – CF carrier

Interviewees in both disease groups discussed how they often put their own feelings and challenges aside because they felt the challenges of affected children and finding a cure for the disease were more important than the difficulties they themselves were facing.

“Yeah. I feel like it's something I've been very-- I don't know. Not like shameful is the word, but I've been very shy to pursue asking those questions because I feel almost silly talking about my symptoms when the real issue is that there are people out there with actual muscular dystrophy.” – DMD carrier

“Oh, it's a huge part of the future of treating the community, right? Because the focus is all on the disease, and less on the community, as a whole, and there are moms out there that are just floundering. They don't know how to advocate for themselves. They've never had to before. They want to; they just don't know how. They don't know what to do. They don't have enough experience with it, you know?” – CF carrier

5.3 Discussion

When it comes to carrier testing for genetic disease, the focus has largely been on reproductive decision making. While we have studied that in this cohort (manuscript in preparation), we also learned a great deal about the myriad of other challenges carriers face today.

The two broad challenges faced by our cohort outside of reproductive decision-making were a lack of sufficient support—either on the part of medical professionals and counselors or social support—and communication of genetic information to other at-risk relatives or carrier or affected children. The fact that less than half of CF carriers and just

a little over half of DMD carriers in our cohort saw a genetic counselor upon learning their carrier status and that only a subset of those cited adequate support is concerning. However, this is consistent with the findings of another recent qualitative study of women who had DMD or Becker muscular dystrophy (BMD) carrier testing as adolescents ¹⁶¹. This suggests that many carrier females with a family history of DMD may need follow-up counseling, as they are often tested at a younger age when they are not yet considering having children.

Meanwhile, a prevailing theme among some individuals in our cohort across both disease groups was the dearth of medical professionals well-versed enough in the disease to provide adequate assistance. Diseases with substantial allelic heterogeneity and variation in disease mechanisms, such as CF, require a level of expertise likely only to be available through a specialized genetic counselor ¹⁶². Prenatal counselors often do not feel knowledgeable enough to discuss the most recent therapies ¹⁶³. There is a well-known shortage of trained genetic counselors ¹⁶⁴ and access to a specialist might be even more scarce. However, for individuals who are carriers of DMD mutations in particular, who manifest symptoms of the disease, an absence of counseling is consequential, as multiple interviewees in our cohort admitted prior ignorance of the symptoms faced by carrier females and emphasize that symptomatic carriers' challenges are rarely discussed in the community. And indeed, previous work demonstrates that once a DMD carrier is aware of her own carrier status, she is up to twice as likely to have an echocardiogram ¹⁶⁵ – an important screening test for this population.

Previous qualitative work in CF showed that couples were unprepared for a carrier couple result and devastated upon learning their status ¹⁵⁷. Our study supports this

finding, but also demonstrates that a lack of preparedness to handle a positive result may be due, in part, to downplaying the likelihood of both partners being CF carriers. While this may be done in an effort to ease anxiety on the part of the individual, for couples who receive a positive result in both partners, this approach can backfire, leaving them feeling shocked and blindsighted.

Previous work with CF newborn screening cohorts showed that parents still want to be informed of their baby's carrier status, even if he or she is not affected with the disease⁶⁰⁻⁶², which was a phenomenon also observed in this cohort. Uptake of carrier testing after the diagnosis of a relative has been found to be low, and many families in previous studies express frustration and uncertainty regarding communicating this information to their relatives⁶⁰⁻⁶⁴. Our observation that family conversations mostly center around care for the affected child, rather than genetic risk is consistent with previous work⁶⁴ and carriers in both disease groups of this study also express frustration with relatives for lack of understanding or acceptance.

Parents of affected children or children at risk for being carriers have the extra burden of having to discuss the child's disease or carrier status, which often led to emotionally difficult conversations about physical limitation, death, and reproductive choices. Most parents adopted a strategy of answering their child's questions openly and honestly in an age-appropriate way, while maintaining a hopeful attitude, which has been shown to be the way adolescents at-risk for other genetic diseases prefer genetic information be communicated to them¹⁶⁶. Previous studies of communication between mothers and daughters has shown that most mothers preferred a gradual style of communication^{60; 66}, which is consistent with what we see in our cohort.

While deeply-held feelings of guilt are nothing new among females who are carriers of DMD mutations¹⁵⁶ and are also seen in a subset of participants in this study, further examination of how that guilt affects family communication style and the downstream impact on the likelihood of female family members to see information and testing is warranted.

Particularly in the cystic fibrosis group, there were strong feelings of social isolation among parents and many reported losing friends or strained relationships with family members due to fear of infection of the affected child. While some work has been done on the psychosocial impact of cystic fibrosis on the part of affected individuals¹⁶⁷, the impact of such social isolation on parents remains underexplored. For the CF community, this phenomenon has resulted in increased reliance on online communities as sources of support¹⁶⁸. However, while the majority of carriers of CF mutations view the community as a source of support and comfort, multiple interviewees cited feelings of judgement and shame—particularly online, where relative anonymity often breeds excessive cruelty—regarding parenting styles or reproductive choices; this is something that merits further attention.

Across both disease groups, there was a tendency for carriers to put aside their own psychological and medical needs in favor of affected children and the desire to fight for a cure for the disease. However, this study demonstrates that carriers of recessive genetic conditions face varied and far-reaching challenges independent of just family planning that require the attention of the medical and counseling communities.

This study has several limitations. These results are qualitative data from a relatively small number of interviews with individuals who carry CF or DMD mutations.

Additionally, this cohort is entirely female. The DMD group was expected to be all female, due to the X-linked inheritance pattern of DMD. While CF carrier couples were recruited, responses were overwhelmingly female, and everyone who made it through to interview were women, all of whom had children. The perspective of males who carry CF mutations and individuals without children who carry CF mutations warrants further study. Larger-scale, survey-based studies as well as studies of other genetic disorders are needed before drawing generalizable conclusions beyond this cohort.

Other limitations include those of human memory and bias. The interviews in this study involved lengthy discussion of interactions between individuals and medical professionals, which have been interpreted through the lens of that individual and are subject to selective recollection and bias, as are all humans. Follow-up studies with the counseling and broader medical community are warranted to examine why interviewees came away with these experiences and feelings and what can best be done to better serve them moving forward.

In an era where genetic testing is more available and more important than ever due to rapid advances in precision treatment and the choices available to families, it is important that we keep up with the full range of challenges faced by carriers of genetic disease. Both CF and DMD carriers cited lack of adequate support and counseling to deal with knowledge of their carrier status, challenges communicating this information to family members, and feelings of social isolation and judgement, which are often not acknowledged or addressed by medical professionals. Carriers discussed a wide range of impacts of their genetic status outside of family planning and those impacts warrant further exploration, and possible remedies, such as revised counselling protocols.

5.4 Materials and Methods

Study Sample

Thirteen (13) confirmed CF carrier women (ages 29-49), whose partners are also carriers, were recruited via the Johns Hopkins Cystic Fibrosis Center, the Johns Hopkins All Children's Hospital, and genetic counseling email listservs. Nineteen (19) confirmed DMD carrier women (ages 25-43), were recruited via Project Parent Muscular Dystrophy – a muscular dystrophy patient advocacy group. Semi-structured interviews were conducted with both carriers who did not have any affected children and carriers who already had a child/children with the disease (Table 5.1).

This study was reviewed and approved by the Johns Hopkins Institutional Review Board and all participants gave informed consent.

Study Design

A letter explaining the study was given to the Johns Hopkins Cystic Fibrosis Center, Johns Hopkins All Children's Hospital, genetic counseling email listservs, and Project Parent Muscular Dystrophy to distribute (either via paper mail or electronically) to potential study participants. Potential participants were asked to contact the study team via post, email, or phone, at which time a phone or Skype interview was scheduled. Semi-structured interviews (~45 minutes in duration) were conducted and interviews were recorded and transcribed, as previously described (cite reproductive decisions paper).

Interviews covered the following domains:

- The participant's experience with genetic testing;
- The participant's experience with the disease prior to testing;
- If and how genetic test results were communicated within the family;
- The participant's access to support and counseling;
- Reproductive decision-making and how genetic test results informed those decisions (if at all);
- If and how the participant's experience with genetic testing has affected their life outside of reproductive decision-making.

Data Collection and Analysis

Transcribed interviews were stripped of identifying information and coded independently by two researchers (AM and KS) using the codebook to capture thematic information, as previously described (cite reproductive choices paper). A codebook was developed and revised iteratively. All transcripts were analyzed and any discrepancies between the two coders were discussed and reconciled. Final codes were entered into QSR International's NVivo 11 qualitative analysis software. NVivo 11 was used to create code reports, which were then analyzed as previously described (See Chapter 4).

Table 5.1: Characteristics of participants

Characteristic	CF	DMD
No children	0	6
Only unaffected children	3	3
One affected child	7	6
Multiple affected children	3	4
Learned carrier status through pre-conception screening	2	12
Learned carrier status through prenatal testing/diagnosis	8	0
Learned carrier status through child's diagnosis	3	7

**Chapter 6: Over Thirty Years of Presymptomatic Genetic
Testing for Huntington's Disease**

6.1 Introduction

Huntington's disease (HD) is the first disorder of adult onset for which a predictive genetic test was developed and offered to asymptomatic people at risk.¹⁶⁹⁻¹⁷¹ Since there is no cure or highly effective treatment for HD, and repeat lengths 40 and above are completely penetrant, predictive testing for HD is, in some ways, a worst-case scenario. If we can provide presymptomatic testing for HD in such a way as to minimize long-term negative outcomes and maximize personal growth and happiness, it may be a model for predictive genetic testing for other diseases.¹⁷²

Much work has been done to explore the psychological and social consequences of DNA testing for HD.^{170; 173; 174} However, there are four major limitations of the previous studies. First, many of them followed very small samples of mutation-carriers, often fewer than 30.^{130; 175; 176} Second, the period of follow-up has typically been very brief. Even those studies that purport to assess the "long-term" consequences of genetic testing describe persons being followed for an average of less than 5 years.^{177; 178} Third, many of the studies that find persons with the gene mutation having more prevalent psychological symptoms or disorders, or a higher incidence of these morbidities over time, are studying persons who are likely already clinically affected with HD.^{179; 180} Thus, the effects of the disease are difficult to disentangle from the effects of disclosure of test results. Finally, most of the previous studies have focused on important but isolated outcomes, such as suicide attempts,^{179; 181} specific psychological test scores,^{178; 180} or incidents of discrimination.^{182; 183} Fewer studies have examined more global, difficult-to-quantify quality of life outcomes, such as increased spirituality, deeper appreciation of relationships, and other effects.

Johns Hopkins was one of the first institutions to provide presymptomatic testing to at-risk individuals. This testing occurred in the context of a research study conducted at the Johns Hopkins Huntington's Disease Center. Following extensive pre-test counseling and psychological testing, outcomes of persons tested presymptomatically through the program were assessed immediately following testing¹⁸⁴⁻¹⁸⁸, as well one year after testing¹⁸⁸ and up to seven years following testing for a subset of individuals.¹⁸⁷ The current study is the longest-term follow-up study to date of individuals at risk for HD who underwent presymptomatic genetic testing. We conducted semi-structured interviews of 41 individuals who were enrolled in the Johns Hopkins HD presymptomatic testing protocol between 1986 and 1998. We also conducted 12 interviews of spouses and children of these probands. Interviews covered a broad range of topics, including reasons for testing, memories of the testing process, how HD risk and test results were communicated in the family, how the test result was used, and long-term impacts of presymptomatic testing. This qualitative assessment is a retrospective analysis almost 30 years later of a well-characterized cohort who pioneered presymptomatic testing of Huntington's disease.

6.2 Long-term impact of presymptomatic genetic testing for HD: Family narratives

Prior studies on presymptomatic genetic testing have focused on the impact of testing on at-risk individuals over years, not decades, and have focused almost exclusively on the individual being tested, with little to no attention being paid to effects on family members. Our study focused on communication and family relationships within families of tested individuals.

Of the 41 probands who participated in our interview study, 39 individuals provided one or more reasons for their decision to pursue presymptomatic testing for HD. Reported reasons for testing fell into fourteen distinct categories. Eight of the fourteen categories had implications for the greater family while six categories reflected indications for testing that were personal to the proband. The eight family focused reasons for testing, in order of frequency, include: decision about having children/additional children (17 probands), financial planning (14), learning information for the sake of the children (13), decisions about romantic relationships (7); work/professional reasons (6); planning long-term care (2); wanting information for the sake of the extended family (2), and planning long-term care for other family members (1). Personal reasons for testing mainly focused on easing anxiety and a need to know. The six categories offered were: need to eliminate uncertainty (16); concerned about symptoms/symptomizing (9); not wanting to act like an affected parent (2); make treatment decisions/participate in research (2); clean up act (1); and to plan to end life when affected (1). Of the 39 probands who provided an answer, 32 offered both family and personal reasons for testing while six probands offered only personal reasons. The two individuals who did not offer a response were unable to answer the question due to the advanced stage of their disease.

Probands were also asked about the impacts of testing, including positive, negative, and unexpected effects. The most commonly cited positive effect (22) was peace of mind knowing the result. However, a majority of probands cited positive effects of testing having to do with family, including future planning (13), becoming a focal point for family members for knowledge of testing/genetics (7), and decision-making

surrounding relationships or children (14). Two probands reported getting testing not for themselves, but solely so that their family members would know the result.

Twenty-eight probands reported some sort of negative effect of testing. These included effects of testing positive/having HD (7), more depressed (1), feelings of guilt about a good result (3), stress incurred during the testing process (3), negative impacts on family relationships (6), and other negative effects (3). Of the interviewees who did not report any negative effects, most had a normal result (10/13). Feelings of survivor's guilt and negative impacts on family relationships were negative effects specific to the family and these negative impacts included feeling different than other family members after receiving results and resentment directed at them by family members who disagreed about their decision to be tested.

Both probands with expanded repeats and probands with normal repeats reported unexpected effects of testing, most of which were related to family. Unexpected effects reported by multiple probands included that the process was difficult on their kids, that the result affected family relationships, and that the result impacted their decision to get married.

Probands were asked specifically about impacts of testing on familial relationships. Surprisingly, a number of probands (8) did not report that the testing had any effect on their familial relationships. Another 7 did not answer the question or provide any feedback. But, for probands who did report effects, they were greatly varied. A subset of interviewees reported that testing made their family closer in some way. But for others, the exact same factors made the family more distant or caused conflict. Seven probands said that the testing negatively impacted their family either because it caused

family members to drift apart or because members resented other members for their choice to get tested (or not). In fact, four probands reported specifically that there was a lack of understanding between family members who felt differently about testing. Other impacts on family included the proband becoming a focal point for the family about HD/testing (3) and stating that they understood their family members better after testing ("now I know why Dad was the way he was") (4). Feelings of survivor's guilt among siblings were common (5).

When asked to reflect back upon their testing experience, all probands who offered a comment said that they would take the test again; this was true both for probands who received a normal result and for probands who tested positive for an expanded repeat. Participants in both groups also said that their presymptomatic test results continue to impact their lives. A total of ten ways in which the HD test result continued to impact their lives were reported. Of these ten, seven were family-related. While both probands with expanded repeats and normal repeats reported factors that were family related, all factors that were not family-related were reported by individuals with normal repeats. Participants were also asked whether testing continued to impact their lives. Answers to this question varied greatly and the only answer that was reported by more than two probands was improved ability to support other family members at-risk/affected by HD. Five probands reported that their result did not continue to impact, two probands reported that their result continued to impact their lives but gave no further explanation, and 18 probands did not answer this question.

The decision to get tested for HD presymptomatically is an important one that has wide-ranging impacts on both the individual being tested and their family members over

the course of their lives. While participants cited both positive and negative effects of pursuing testing, overwhelmingly, our participants reported the testing was useful and they would do it again if they had it to do over. This is due, in large part, to the benefit the knowledge has for family members, as well as for the individual. These data demonstrate the degree to which at-risk individuals focused on the impact of this testing not for themselves, but for their families. This highlights a need to improve and develop new guidance for how we talk to and about family members in the context of genetic testing and research.

6.3 Perspectives on Genetic Testing and Return of Results from the First Cohort of Presymptomatically Tested Individuals At-Risk for HD

In addition to questions regarding reasons for testing, how HD risk is communicated in the family, memories of the testing process, how the test result was used, and long-term impacts of presymptomatic testing, participants in our interview study were also asked open ended questions about their opinions on the importance of autonomy in the decision to be tested, whether a formal testing protocol is necessary, whether physician ordering is acceptable without a formal protocol, whether online direct-to-consumer (DTC) genetic testing for HD is acceptable, and whether incidental/secondary findings should be returned in the context of whole exome/genome sequencing.¹⁸⁹

The vast majority of participants believed it is very important for an individual to decide for him or herself whether and when to pursue presymptomatic testing. However, a minority of participants cited a belief that all individuals should know their genetic status before having children.¹⁸⁹

All individuals who went through presymptomatic testing as part of the Johns Hopkins program were required to participate in a formal protocol before being tested or receiving their test result. This protocol included extensive pre-test counseling and psychological testing and follow-up visits after disclosure. The majority of interviewees in this study said that a formal protocol similar to the one they went through should be required before learning one's test result. Others said that some sort of protocol should be required, but the protocol should be shorter than the Hopkins protocol. And there were still others who stated that every individual should decide for themselves whether to participate in a formal protocol before receiving their result.

A majority of respondents said that it would be unacceptable for a physician to order a test for HD without having a patient go through a formal testing protocol, due to the absence of education and counseling. However, a small number of participants said they believed physician ordering was acceptable—stating that a protocol involving counseling should be encouraged, but ultimately the choice should be up to the patient. Similarly, a majority of participants told us that direct to consumer genetic testing for HD is unacceptable.¹⁸⁹

Participants were also asked what doctors should do in the case of an incidental finding—when a genetic test is performed and a result is found that is unrelated to the reason the individual is seeking testing—both for if the finding was an HD expansion and if the finding was a different condition for which interventions are available, such as increased risk for heart disease. About half of participants said that the doctor should ask the patient prior to doing any genetic test if they wish to know about any incidental findings. Many other interviewees stated that the doctor should simply tell the patient

about an incidental finding of HD. Only one participant did not think the doctor should inform the patient of an incidental finding of HD. Similarly, only two participants believed the doctor should not tell the patient about an incidental finding of increased risk for heart disease or cancer.¹⁸⁹

This study represents the opinions of a cohort that is extremely well-informed about these issues, being one of the first cohorts presymptomatically tested for genetic disease and having lived with the results for 20-30 years. These results should be considered by policy makers, genetic counselors, and other medical professionals when designing testing protocols, considering the availability of genetic testing, and making decisions about return of results.

6.4 Risk perception before and after presymptomatic genetic testing for Huntington's disease: Not always what one might expect

As part of the presymptomatic HD testing program at Johns Hopkins, at-risk individuals were asked to report their risk perception (RP) on a visual analog scale at sixteen different timepoints both before and after testing. Participants indicated their perceived risk by putting a vertical slash along the horizontal line where 0% was “absolutely certain that I will not develop HD” and 100% was “absolutely certain that I will develop HD.” We investigated long-term changes in RP, utilizing these recorded RP scores, contemporaneous research clinical notes, and information about perceived risk from our semi-structured follow-up interviews of probands.¹⁹⁰

While the RP scores of most individuals changed in the way one would expect following a presymptomatic test result (a decrease in perceived risk following a normal result or an increase in perceived risk following a result of an expanded repeat), 27% of

individuals demonstrated unexpected changes in RP following testing.¹⁹⁰ A significantly higher proportion of individuals who received an expanded repeat result had unexpected changes in RP, compared with those who received a normal repeat result, uninformative result, or were undisclosed.

There were a number of factors that appeared to influence risk perception. These included symptomizing (worry that every day failures are symptoms of HD), the inability to accept a normal test result, denial or inability to accept an expanded repeat result, the feeling that test results of family members are predictive of one's own result, physical or personality resemblance to a family member as a reflection of one's likelihood of sharing their status, hope for a cure, linkage test accuracy or belief in lab mistake, optimism that one will not develop HD, despite an expanded repeat result, and misunderstanding of genetic test result or HD risk, and the age of parental onset.¹⁹⁰

This surprising finding in our data of a higher than expected proportion of individuals with an unexpected change in RP demonstrates that RP is complex and influenced by a variety of factors that go beyond one's understanding of genetics and interpretation of a test result. RP is often connected to an individual's past experiences and beliefs and in some cases may be a manifestation of coping mechanisms employed in the face of a difficult result. This study suggests that follow-up or additional interventions may be necessary after testing to ensure individuals are effectively processing their result, including additional post-test counseling and disease-specific education.

6.5 A qualitative examination of family communication of Huntington's disease risk and test results

We also performed an in-depth examination of family communication patterns, factors that influenced family communication patterns, and the impact of family communication patterns on family relationships in our cohort (manuscript in preparation). While previous studies have explored family communication of genetic information^{65; 67; 70; 191-193}, none have done so over a period of decades after testing or assessed how family relationships changed over time.

All participants with a partner at the time of testing communicated disease risk, decision to pursue testing, and test result with their partner and cited that person as a primary source of support. A subset of probands reported that their disease risk and/or test result did not impact their relationship, but the majority of probands did report some sort of impact. Of the probands who reported some sort of impact, the overwhelming majority cited positive impacts, saying that communication of HD risk and test result information strengthened the relationship.

All participants with children reported informing their children of their test result, regardless of mutation status. However, the timing and extent of communication was highly dependent on mutation status, age of the child, and the child's experience with HD. Some children had experiences with affected family members from a young age and therefore were aware of HD, but learned more about the genetic nature of the disease and their own risk as they grew older. However, most children were informed by the time they reached their teenage years. Individuals with expanded repeats had more thorough and frequent conversations about HD than individuals with normal repeats. In the only three cases in which participants reported that communication negatively impacted their

relationship with their child, the child found out about his or her HD risk from a source other than their parent.

In almost all cases, the proband communicated about their HD risk with family members beyond spouses and children. However, the frequency and extent to which this was done varied greatly. A majority of probands communicated their test result immediately to family members with whom they were closest, usually a sibling or parent, and this communication either had no effect on the relationship with that family member or strengthened the relationship. Test results were then communicated to other family members over time, either passively or in response to a particular event, such as the death of a family member or HD symptom onset. Reported reasons for sharing results with family members included a sense of responsibility to inform other family members about HD risk and testing, obtaining emotional support, preparing others for one's own symptom onset, and relieving relatives of worry in cases of a normal-repeat result. However, lack of communication surrounding HD risk and/or test results was strongly tied to both denial regarding HD in the family and family trauma (both related and unrelated to HD).

These results indicate the importance of communicating genetic risk with both significant others and children, as open and honest communication generally had a positive impact on relationships, despite the often-distressing nature of the situation. Understanding the potential impacts of family communication patterns on familial relationships and underlying factors such as trauma and denial that may be influencing these patterns is essential for medical professionals to best assist at-risk individuals in navigating the communication of risk and test results to their family members.

Chapter 7: Conclusions

Many genetic conditions are beginning to see therapeutic advancements that address the underlying mechanism of action of the disease that will lead to substantially improved outcomes for patients. With the emergence of mutation-targeted therapies, it is of critical importance both to establish benchmarks to assess their effectiveness and to evaluate how these new therapeutic options are affecting the way people think about genetic testing, communicate genetic information, and make reproductive decisions. Using cystic fibrosis as a paradigm, this work explored new challenges raised in this era of precision treatment for genetic disease.

We were able to utilize the unprecedented amount of clinical data available in the CFTR2 database to conduct a large-scale genotype-phenotype study, which revealed a logarithmic relationship between CFTR function and clinical features of cystic fibrosis. These correlations not only revealed that individuals with severe disease are in a position to benefit the most from mutation-specific therapies, but also established benchmarks to evaluate the efficacy of these therapies. This work demonstrated that acute augmentation of CFTR function via modulator therapy can achieve similar improvement in clinical phenotypes as one would see with a milder cystic fibrosis causing variant over the course of a lifetime.

Through further analyses of this data, we were also able to show that the logarithmic relationship between CFTR function and CF phenotype is similar whether total genotype function is derived from one *CFTR* allele (in the case of a missense variant *in trans* with a null variant) or from both *CFTR* alleles (in the case of two missense variants *in trans*), leading to the conclusion that both *CFTR* alleles contribute to function in an additive fashion. This has important implications for mutation-specific therapies, as

it implies that both alleles should be targeted by modulator therapy and that this approach would result in even greater benefit than targeting either allele alone.

This work moved beyond the bench to assess how couples who are carriers for cystic fibrosis are taking information about targeted therapy into account when making reproductive decisions and how a changing disease landscape affects their views and raises new challenges. To this end, we conducted interviews with individuals who carry CF-causing mutations, as well as individuals who carry DMD-causing mutations, in order to assess what patterns may be unique to CF and what patterns may be generalizable beyond CF to other genetic diseases for which new therapies are emerging.

We found that for a subset of both disease groups, currently available therapies did substantially influence their reproductive decision-making. We also found that carriers in both disease groups overwhelmingly supported preconception screening. The information garnered from this study is vital for the medical genetics community as it designs screening protocols and counseling procedures. Many factors influenced individuals' reproductive decisions, including experience with the disease through family history or an existing affected child, familiarity with and eligibility for therapies, and religious views. However, there were also considerations that were specific to a certain disease group, such as concerns about the cost of mutation-specific therapy among DMD carriers, owing to the fact that these drugs are often not covered by insurance providers.

Analysis of the data from this interview study went beyond family planning and also explored other related challenges, which were numerous and wide-ranging and have been previously understudied. Both disease groups cited lack of adequate support and counseling to deal with knowledge of their carrier status. Both groups also faced

challenges communicating information about risk and testing to family members, as well as communicating with their children about their carrier status or disease. There were feelings of social isolation and judgement for reproductive choices, which were particularly acute for CF parents whose children often must be kept away from social gatherings due to infection concerns. There were also feelings of guilt for passing on a genetic disease, which were especially prevalent among DMD carrier women. Many of these wide-ranging psychosocial impacts were not acknowledged or addressed by medical professionals and this work emphasizes the need to consider challenges that go beyond family planning when counseling carriers.

Part of this dissertation consists of a separate qualitative interview study of individuals at-risk for Huntington's disease—still seen as intractable from a therapeutic perspective—who were tested at Johns Hopkins as part of the first cohort of individuals to receive presymptomatic testing for HD. This study also revealed robust themes surrounding communication within families and family relationships. We found that many individuals took family reasons into account when considering whether to pursue testing and also found that many of the downstream impacts of presymptomatic testing were centered around the family. Open and honest communication surrounding HD generally had a positive impact on family relationships.

Our qualitative assessment also provides vital information to the medical genetics community in the areas of risk perception and testing protocols. The majority of participants said that people wishing to learn their HD status should go through a formal counseling protocol and that physician ordering of a test or a direct-to-consumer test absent formal counseling were not acceptable. Most participants also said that physicians

should inform patients of an incidental finding of HD. Interestingly, about a quarter of participants had unexpected changes in risk perception following their test result, which demonstrates the complexities involved in risk perception that go beyond understanding of genetics and interpretation of a test result and further support the notion that a formal and robust counseling protocol with multiple follow-ups may be required for individuals dealing with these distressing situations.

As we enter the precision medicine era, we are navigating a transition from untreatable to treatable disease for many genetic conditions. Concurrently, more and more conditions are being tested for and testing is increasingly available and affordable. With this work, we have been able to not only identify which individuals can benefit the most from precision therapies and establish benchmarks to assess the effectiveness of these therapies with population data, we have also taken a first look at what impact these therapies are having on the views of individuals dealing with genetic risk. For diseases like cystic fibrosis, emerging mutation-specific therapies are effective enough that for many individuals, they are having a direct impact on consideration and use genetic test results. The cystic fibrosis genotype-phenotype study in this work can serve as a paradigm for other diseases, like Duchenne muscular dystrophy, which is beginning the same journey from untreatable to treatable disease and is seeing the same subsequent change in views amongst carriers as well as new counseling challenges. While no FDA-approved precision treatment exists for Huntington's disease, advancements are still being made, and 30 years of presymptomatic testing has yielded many lessons in how counseling and testing protocols can be updated to better serve the needs of patients.

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Curriculum Vitae

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Education

- February 2019 Doctor of Philosophy in Human Genetics, Johns Hopkins University School of Medicine, McKusick-Nathans Institute of Genetic Medicine, Baltimore, MD
- May 2013 Bachelor of Science in Biological Sciences with a concentration in Cell and Molecular Biology & Genetics, University of Delaware, Newark, DE
Minors: Italian and Political Science

Research Experience

- August 2013-February 2019 Doctoral Researcher, Johns Hopkins University School of Medicine, McKusick-Nathans Institute of Genetic Medicine
Thesis Advisors: Dr. Garry Cutting and Dr. Debra Mathews
- Created stable cell lines bearing various *CFTR* mutations in order to evaluate the relationship between *CFTR* function and cystic fibrosis disease severity.
 - Data resulted in the ability to make functional calls and classifications of *CFTR* variants for the CFTR2 website, used by researchers, clinicians, and patients and families.
 - Investigating the role of emerging disease-modifying therapies in views of genetic testing and reproductive decision-making via semi-structured interviews of individuals living at risk for genetic disease.
 - Cleaned, coded, and entered interview transcripts into qualitative research software and analyzed qualitative data.
- January 2011-May 2013 Undergraduate Researcher, University of Delaware, Department of Biological Sciences
Thesis Advisor: Dr. Erica Selva
- Investigated the role of N-linked glycosylation during *Drosophila* development.
- Summer 2011, Summer 2012 Summer Scholars Program Participant
- HHMI Undergraduate Research Scholar (Summer 2012)

Teaching and Mentoring Experience

March 2018	Invited guest lecturer, seminar course, The Johns Hopkins University/National Human Genome Research Institute Genetic Counseling Training Program
Summer 2017-present	Organizer, Women in Genetics seminar series <ul style="list-style-type: none"> • A quarterly lunch seminar in which female faculty in the Human Genetics department speak to female Human Genetics graduate students about their experience as a woman in science.
Summer 2016-present	Graduate student peer mentoring program leader <ul style="list-style-type: none"> • Assisted in creating a graduate student peer-mentoring program in the Human Genetics department. • Held monthly meetings with a small group of students in order to discuss any challenges they may be facing and acclimate new students to the program. • Held practice sessions for students for their comprehensive exams.
August 2015-September 2015	Teaching Assistant, The Concept of a Gene: The Evolution of Our Understanding of the Unit of Heredity <ul style="list-style-type: none"> • Assisted students in the creation of class presentations, giving feedback and guidance. • Facilitated class discussions.
Spring 2012-Spring 2013	Founder and Co-Coordinator of UDiscover: Undergraduate Research Mentors <ul style="list-style-type: none"> • Worked to spark interest in undergraduate research as well as provide upperclassmen peer mentors for underclassmen getting involved in research.
October 2012-May 2013	Volunteer Tutor, Tri-Beta Biological Sciences Honor Society <ul style="list-style-type: none"> • Tutored introductory biology and chemistry courses, as well as organic chemistry, cell biology, molecular biology, microbiology, physiology, and genetics.

Professional Memberships and Activities

Fall 2018-present	Trainee representative, American Society of Human Genetics Policy and Advocacy Advisory Group <ul style="list-style-type: none"> • Advises ASHG's Board on the Society's policy and advocacy priorities and activities, and provides governance oversight and feedback to staff on related ASHG goals and programs. • Promotes the value of genetics advocacy to the wider ASHG membership and the field at large.
March 2018	Judge, American Society of Human Genetics DNA Day Essay Contest <ul style="list-style-type: none"> • Evaluated and scored essays submitted by high school students about direct-to-consumer genetic testing.
April 2017	Attendee, American Society for Biochemistry and Molecular Biology Hill Day <ul style="list-style-type: none"> • Met with policymakers on Capitol Hill in order to promote scientific research

December 2017-present	Contributing writer, “The Nascent Transcript” <ul style="list-style-type: none"> • American Society of Human Genetics quarterly trainee newsletter • Writes feature articles focused on science policy-related topics and issues.
January 2017-present	Project leader, Johns Hopkins Science Policy Group <ul style="list-style-type: none"> • Participated in advocacy training as well as advocacy events, such as sessions at which students call or write members of Congress about relevant issues. • Spearheaded a Science Policy Town Hall event on campus.
Fall 2015-present	Member, American Society of Human Genetics

Publications

- **Allison F. McCague**, Karen S. Raraigh, Matthew J. Pellicore, Emily F. Davis-Marcisak, Taylor A. Evans, Sangwoo T. Han, Zhongzhou Lu, Anya T. Joynt, Neeraj Sharma, Carlo Castellani, Joseph M. Collaco, Mary Corey, Michelle H. Lewis, Chris M. Penland, Johanna M. Rommens, Anne L. Stephenson, Patrick R. Sosnay, Garry R. Cutting. Correlating CFTR function with key clinical features to inform precision treatment of cystic fibrosis. *Am J Respir Crit Care Med.* 2019 May 1;199(9):1116-1126
- **Allison F. McCague**, Kelsey M. Stuttgen, Juli M. Bollinger, Rachel L. Dvoskin, Debra J.H. Mathews. The impact of disease-modifying therapies on reproductive decision-making. Submitted to *Prenatal Diagnosis* May 2019.
- **Allison F. McCague**, Kelsey M. Stuttgen, Juli M. Bollinger, Rachel L. Dvoskin, Debra J.H. Mathews. “I feel like we've sort of been ignored for a long time”: A qualitative assessment of challenges faced by cystic fibrosis and Duchenne muscular dystrophy carriers. Submitted to *Journal of Genetic Counseling* April 2019.
- Karen S. Raraigh, Sangwoo T. Han, Emily Davis, Taylor A. Evans, Matthew J. Pellicore, **Allison F. McCague**, Anya T. Joynt, Zhongzhou Lu, Melis Atalar, Neeraj Sharma, Molly B. Sheridan, Patrick R. Sosnay, Garry R. Cutting. Functional Assays Are Essential for Interpretation of Missense Variants Associated with Variable Expressivity. *Am J Hum Genet.* 2018 Jun 7;102(6):1062-1077
- Sangwoo T. Han, Andras Rab, Matthew J. Pellicore, Emily F. Davis, **Allison F. McCague**, Taylor A. Evans, Anya T. Joynt, Zhongzhou Lu, Zhiwei Cai, Karen S. Raraigh, Jeong S. Hong, David N. Sheppard, Eric J. Sorscher, and Garry R. Cutting. Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators. *JCI Insight.* 2018 Jul 25;3(14).
- Stuttgen KM, Bollinger JM, Dvoskin RL, **McCague A**, Shpritz B, Brandt J, Mathews DJH. Perspectives on Genetic Testing and Return of Results from the First Cohort of Presymptomatically Tested Individuals At-Risk for HD. *J Genet Couns.* 2018 Jul 2.
- Stuttgen KM, Dvoskin RL, Bollinger JM, **McCague A**, Shpritz B, Brandt J, Mathews DJH Risk perception before and after presymptomatic genetic testing for Huntington's disease: Not always what one might expect. *Mol Genet Genomic Med.* 2018 Nov;6(6):1140-1147
- Neeraj Sharma, Taylor A. Evans, Matthew J. Pellicore, Emily Davis, Melis A. Aksit, **Allison F. McCague**, Anya T. Joynt, Zhongzhu Lu, Sangwoo T. Han, Arianna F. Anzmann, Anh-Thu N. Lam, Abigail Thaxton, Natalie West, Christian Merlo, Laura B. Gottschalk, Karen S. Raraigh, Patrick R. Sosnay, Calvin U. Cotton, Garry R. Cutting Capitalizing on the heterogeneous effects of CFTR nonsense and frameshift variants to

inform therapeutic strategy for cystic fibrosis. *PLoS Genet.* 2018 Nov 16;14(11):e1007723

Presentations and Conferences

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| October 2018 | American Society of Human Genetics Annual Meeting – poster presentation, Reviewer’s Choice abstract <ul style="list-style-type: none">• “The role of emerging disease-modifying therapies in reproductive decision-making for cystic fibrosis and Duchenne muscular dystrophy carrier couples” |
| October 2017 | American Society of Human Genetics Annual Meeting – platform presentation <ul style="list-style-type: none">• “Correlating CFTR function with key clinical features to inform targeted treatment of cystic fibrosis” |
| February 2015 | MD-GEM Genetics Research Day, Baltimore, MD – poster presentation <ul style="list-style-type: none">• Honorable mention in Doctoral Students category |
| October 2012 | 15 th Annual UMBC Undergraduate Research Symposium, Baltimore, MD – poster presentation <ul style="list-style-type: none">• 2nd place award in biological sciences |
| April 2012 | Experimental Biology (EB) 2012 Meeting, San Diego, CA – poster presentation <ul style="list-style-type: none">• Selected to give a platform talk in glycobiology |
| April 2012 | Thomas Jefferson Sigma Xi Research Day, Philadelphia, PA – poster presentation |
| October 2011 | 14 th Annual UMBC Undergraduate Research Symposium, Baltimore, MD – poster presentation |

Honors and Awards

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| Summer 2012 | Adair B. Gould Memorial Award <ul style="list-style-type: none">• \$300 scholarship given to an outstanding female biological sciences major who is actively involved in undergraduate research with plans to pursue graduate studies in biological sciences. |
| Summer 2012 | Richard M. Johnson Jr. Award <ul style="list-style-type: none">• \$300 scholarship given to the junior in biological sciences who best exemplifies the ideals of sound scholarship and intellectual leadership, actively pursues truth and appreciates the significance of science to mankind. |
| Spring 2012
Diego | ASBMB Travel Award for Experimental Biology 2012, San |
| Spring 2012-Spring 2013 | Phi Beta Kappa Honor Society |
| Fall 2009-Spring 2013 | Tri-Beta Biological Sciences Honor Society |