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Return of Genetic Results in the Familial Dilated Cardiomyopathy Research Project

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Abstract

The goal of the Familial Dilated Cardiomyopathy (FDC) Research Project, initiated in 1993, has been to identify and characterize FDC genetic cause. All participating individuals have been consented for the return of genetic results, an important but challenging undertaking. Since the inception of the Project we have enrolled 606 probands, and 269 of these had 1670 family members also enrolled. Each subject was evaluated for idiopathic dilated cardiomyopathy (IDC) and pedigrees were categorized as familial or sporadic. The coding regions of 14 genes were resequenced in 311 to 324 probands in five studies. Ninety-two probands were found to carry nonsynonymous rare variants absent in controls, and with Clinical Laboratory Improvement Amendment of 1988 (CLIA) compliant protocols, relevant genetic results were returned to these probands and their consented relatives by study genetic counselors and physicians in 353 letters. In 10 of the 51 families that received results >1 year ago, at least 23 individuals underwent CLIA confirmation testing for their family's rare variant. Return of genetic results has been successfully undertaken in the FDC Research Project. This report describes the methods utilized in the process of returning research results. We use this information as a springboard for providing guidance to other genetic research groups and proposing future directions in this arena.

Keywords

dilated cardiomyopathy; genetics; family studies; return of results; genetic counseling

INTRODUCTION

The Familial Dilated Cardiomyopathy (FDC) Research Project was undertaken in 1993 to identify and characterize the molecular genetic basis of FDC. The original design was to

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identify very large FDC families (Crispell et al. 1999) for genome-wide genotyping and linkage analysis followed by gene mapping to identify rare variants in genes causing FDC. After characterizing a number of large families we began to sequence coding portions of genes implicated in FDC, such as *LMNA* (Jakobs et al. 2001; Hershberger et al. 2002) and *TNNT2* (Hanson et al. 2002). In this way we could concentrate our discovery efforts on pedigrees where genetic cause in known genes had been excluded. With the discovery of more than 15 DCM genes by 2005, the marked locus and allelic heterogeneity of genetic dilated cardiomyopathy (DCM) became apparent (Burkett and Hershberger 2005). Hence, we expanded our recruitment to families with DCM of all sizes in order to accumulate a larger genetic repository, with the rationale that such a repository could be valuable to search for additional variants of a novel candidate gene identified in one of these large pedigrees. To continue to exclude known genetic cause in our FDC cohort, and with support from the National Heart Lung and Blood Institute's (NHLBI) Resequencing and Genotyping study (RS&G), we resequenced eleven (Hershberger et al. 2008b; Hershberger et al. 2010b) DCM genes. We also resequenced the coding regions of two known (Parks et al. 2008; Li et al. 2010) and one novel (Norton et al. 2011) DCM genes in our laboratory.

While during the consent process participants are made aware that a genome-wide analysis will be performed, we only consent participants for the identification and disclosure of results relevant to DCM. Therefore, in this study all participants were consented for the possible return of DCM genetic information of relevance to them and their families. Except for an approximate 3 year period while at the Oregon Health & Science University (where we provided only *LMNA* testing results to our consented subjects with *LMNA* mutations, if desired (Parks et al. 2008)), our research laboratory has not been certified under the federal Clinical Laboratory Improvement Amendment (CLIA) of 1988. This regulation, which applies to human samples collected in the United States, prohibits the use non-CLIA compliant research results for clinical purposes, including return of research genetic information to consented participants.

The return of research results raises several issues. The first is the CLIA law that mandates that genetic information used for clinical purposes should only be provided from laboratories that are CLIA-certified (Ledbetter and Faucett 2008). This is to ensure that sample handling, processing and methods used to determine genetic sequence are rigorous, meaningful and reproducible, and with active procedures in place to reduce laboratory errors (Ledbetter and Faucett 2008). Therefore, with the discovery of what we consider meaningful genetic data regarding DCM in our (presently non-CLIA-certified) research laboratory, our approach has been to notify all consented adult members of a family that we have discovered genetic information of relevance and to recommend genetic counseling and confirmation testing in a CLIA-certified laboratory. Offering return of results as part of participation in a genetic research study has been deemed a favorable option in various studies (Kaufman et al. 2008; Murphy et al. 2008; Miller et al. 2010), especially if the results are relevant to management options (Meulenkamp et al. 2011). Our approach has been to notify all consented family members in a pedigree that we have identified a rare variant in a DCM gene that is relevant to them.

Although formal, face-to-face genetic counseling and CLIA-compliant genetic testing are not paid for by our study, the issue of cost of confirmatory testing and formal genetic counseling is discussed during the consent process and in the results notification letters, as is the fact that the cost of these services is at their or their insurance company's expense. While we seek participation of as many family members as possible during the informed consent process, and in the results notification letters we review the risk for DCM to relatives, when we notify participants of research results we do not ask for contact information of adult family members who are not enrolled in the study to inform them of results.

A second process issue is how to transmit the information, and by whom. Since 1999 the Project has utilized genetic counselors to assist with this effort (Hanson and Hershberger 2001; Jakobs et al. 2001; Burkett and Hershberger 2005; Kushner et al. 2006; Morales et al. 2008; Parks et al. 2008; Hershberger et al. 2009a; Hershberger et al. 2010a; Morales et al. 2010a; Morales et al. 2010b). The study principal investigator, an experienced heart failure cardiologist, has provided substantial assistance with related clinical cardiovascular issues. All consented participants from families in which a relevant variant was identified were notified in writing. These letters, written by genetic counselors, provided the name of the gene that contained our finding and whether this finding was a likely or possibly disease causing mutation, as previously defined (Hershberger et al. 2008b; Hershberger et al. 2010b). These letters also reviewed how to pursue CLIA-confirmation testing, provided thorough recommendations for genetic counseling and appropriate medical follow-up related to our findings, and reviewed cardiovascular screening guidelines for those with genetic DCM or a family history of IDC. Genetic counselors have been available to explain the process and address participant concerns related to genetic test results, facilitating the process of finding a local provider to order and manage the CLIA confirmation testing, as well as identifying a CLIA-certified laboratory able to provide testing (Das et al. 2008). This role, fulfilled by genetic counselors, has been previously recognized as a key component of the process of translating research genetic testing results to the clinical setting (Das et al. 2008).

Ethical issues are also raised when potentially relevant genetic information is withheld in the name of avoiding misinformation from research results. A recent case illustrates this point. A physician scientist working on Ogden syndrome, a lethal genetic disorder, had discovered the causative gene, but could not disclose the result to the family because the results were not CLIA confirmed (Hayden 2011). In the midst of this process, a member of one of the participating families became pregnant and gave birth to an affected child, who died months after birth, coincidentally, during the same week that the research results were published (Rope et al. 2011). Since then, the principal investigator stated plans to conduct further studies under CLIA standards. He also encouraged researchers to routinely use CLIA certified laboratories to avoid similar situations. Although an ideal standard that would facilitate research and even motivate people to enroll, most research laboratories do not have the resources to implement costly CLIA protocols.

Now with over 100 rare variants identified across 92 families in our cohort from resequencing studies, return of genetic results relevant to DCM has been accomplished and is the focus of this present report. Therefore, the purpose of this study is to describe the methods utilized in the process of returning research results. While the literature on this topic is sparse, most focus on participant attitudes and beliefs. This is, to our knowledge, the first report documenting the method and experience of research genetic counselors involved in returning research results for individuals and families with DCM. We use this information as a springboard for providing guidance to other genetic research groups and proposing future directions in this arena.

SUBJECT ASCERTAINMENT AND ENROLLMENT

Institutional Review Board (IRB) approval from the Oregon Health & Science University (OHSU) and at the University of Miami Miller School of Medicine (UM) was obtained for this study. Subjects with a diagnosis of IDC and in most cases some indication of familial disease were recruited to the study, principally at OHSU, as previously reported in an interim report (Kushner et al. 2006), or more recently at UM. Enrollment of new families required a blood sample (for DNA extraction) of at least one family member with IDC. Patients were identified for this study in one of three ways: through the clinicians associated

with the heart failure and heart transplant programs at OHSU from 1993 to June 2007 (Kushner et al. 2006), UM from July 2007 to the present (Morales et al. 2010a; Morales et al. 2010b), or Tufts Medical Center from December 2010 to the present; by referral from health care professionals in North America (primarily cardiologists, genetic counselors, or geneticists); or by self-referral via the FDC project website (www.fdc.to) or telephone (Kushner et al. 2006). In the 1990's the recruitment focus was on families with FDC. Since 2000 some subjects with IDC, regardless of family history, have been recruited to this study, with an expanded focus on sporadic as well as familial disease recruitments since 2010.

Subjects were asked to complete standardized screening questionnaires and/or answer questions orally regarding their diagnosis, duration, symptoms and etiology of HF, and family history of cardiac problems, as previously reported (Kushner et al. 2006; Morales et al. 2010a). All subjects participating provided a signed release for medical records and/or death certificates and autopsy reports to document diagnoses, and a blood sample or other specimen for DNA extraction.

Informed consent was obtained from all subjects who participated in the study. All subjects were consented for ongoing follow up and recontact. Recontact occurred via telephone, letter, email, newsletters, or contact during usual clinic visits for those receiving care from program physicians. Newsletters, distributed tri-annually since January 2000 and also available online through the project website, are sent to all consented family members (unless otherwise requested, due to, for example, sharing the same address). All newsletters also encouraged subjects to provide updated personal and family cardiac status and contact information. When indicated, medical records were collected for changes or updates in cardiac status. All subjects were consented for possible return of relevant genetic findings impacting their risk for DCM.

Definitions of DCM, IDC and FDC

For this publication, the term 'dilated cardiomyopathy' or 'DCM' is used morphologically; that is, DCM has been defined to mean dilated cardiomyopathy from any cause (ischemic or any other etiology). IDC is defined as left ventricular enlargement (LVE) accompanied by systolic dysfunction after all known causes of dilated cardiomyopathy, except genetic, have been excluded, as previously described (Kushner et al. 2006; Hershberger et al. 2010a). A history of coronary artery disease, valvular heart disease, rheumatic heart disease, hypertensive heart disease, congenital heart disease, hemochromatosis, toxic or drug induced cardiomyopathies such as adriamycin, or any other potential cause of DCM precluded a diagnosis of IDC (Kushner et al. 2006).

All IDC diagnoses required confirmation by a review of medical records. FDC was defined as IDC diagnoses, confirmed by medical records, in at least two family members (Kushner et al. 2006). Those families in which FDC was not confirmed, based on family history and/or available medical data, were categorized as 'probable FDC' or 'possible FDC' based on the weight of the evidence, as previously defined (Kushner et al. 2006). Pedigrees without FDC were referred to as IDC, defined in most cases based upon a negative family history; only a minority of family members of IDC probands underwent clinical screening (ECG, echocardiogram, history and exam).

After review of all relevant medical information, status assignments were provided for all consented subjects by a research team cardiologist in conjunction with a study genetic counselor or nurse. Individuals deemed to have IDC were classified as affected, those with a cardiovascular evaluation with no abnormalities were classified as unaffected, and those with some cardiovascular abnormality not meeting criteria for IDC were classified as unknown. Individuals with limited data or confounding risk factors noted above were

classified as indeterminate, as previously described (Kushner et al. 2006). Occasionally, upon inspection of medical records, consented probands or their family members were found to have cardiomyopathy diagnoses other than DCM (e.g., ARVD, HCM, RCM, valvular, toxic). These probands and families were included as indeterminate in the database.

A pedigree was constructed for each proband in Progeny (Progeny Software, Delray Beach, FL) and all clinical and molecular genetic data were entered into Progeny, as previously reported (Kushner et al. 2006).

A genetic finding was considered reportable if a possibly or likely disease causing rare variant was found in one of the following 14 resequenced genes (*BAG3, CSRP3, LDB3, LMNA, MYBPC3, MYH6, MYH7, RBM20, SCN5A, TCAP, TNNC1, TNNI3, TNNT2* or *TPM1*) but not in control DNAs, as previously reported (Hershberger et al. 2008b; Parks et al. 2008; Hershberger et al. 2010b; Li et al. 2010; Norton et al. 2011). If the above criteria were met, notification letters were sent to consented subjects in the family who were living and had been consented as adults, and for whom contact information was available. Results of variants that were identified in controls or published as unlikely to be disease causing were not returned to participants. All oral and written contacts with research subjects were logged into our research database as a progress note describing the nature and outcome of the interaction. The research files of all participants who received notification of genetic findings prior to May 2011 (51 of 92 families) were reviewed for any evidence of CLIA confirmation testing.

In the results notification letter, the name of the gene of interest was disclosed to the consented subjects, however, the specific rare variant of interest in a given family was not disclosed, and a given individual's variant status (positive or negative) was not provided. Written recommendations were provided, including the option to undertake CLIA-compliant molecular genetic testing with appropriate genetic counseling. When requested, with proper release of records permission from the research participant and coordination by an appropriate healthcare provider, the specific variant was disclosed to a CLIA-certified laboratory for confirmation testing. On occasion and with written permission from the subject, a DNA sample was provided to the testing facility to serve as a positive control.

SUMMARY OF STUDY RESULTS

Data were analyzed from 606 probands recruited from 1993 to June 2011 (438 at OHSU, 161 at UM, and 7 at Tufts Medical Center). Of these 606 probands, 269 had 1670 family members also recruited to this study from 47 of the 50 US states, Canada, Puerto Rico, Australia, the Cayman Islands and Japan.

The demographics of the probands and family members are given (Table 1). A total of 606 probands have been recruited at the time of this study, of which most were Non Hispanic White. Similar distributions were observed for the 1,670 consented family members. The range of ages of consent, current ages and ages at death varied from infants to the very elderly, consistent with the multi-generational family-based nature of the study. A total of 21 probands and 431 family members were less than 18 at the time of first consent.

FDC, IDC and Other Database Assignments

Of the 606 total probands recruited, 433 probands and their 1548 family members have had adequate data obtained, reviewed and categorized to achieve classifications of sporadic (IDC) or familial disease (FDC; Table 2). Of the 433 proband assignments, 160 have been assigned to FDC and 273 to IDC, and the remaining 173 consented probands comprised a variety of database assignments (Table 2).

Resequencing Study Results

Research genetic testing was accomplished in up to 324 probands, as previously reported (Hershberger et al. 2008b; Parks et al. 2008; Hershberger et al. 2010b; Li et al. 2010; Norton et al. 2011), with results availability notification provided for 14 genes (Table 3). Of the 107 rare variants associated with DCM in these reports, five were classified as unlikely to be disease causing (2 in *SCN5A*, 1 in *TCAP*, 1 in *LDB3*, and 1 in *MYH6*), and thus return of results was deemed unnecessary (Hershberger et al. 2008b; Hershberger et al. 2010b). The remaining 102 rare variants in 92 families were eligible for return of results to research subjects. In 12 of these families, multiple mutations were identified (Hershberger et al. 2008b; Hershberger et al. 2010b; Li et al. 2010; Norton et al. 2011). Return of results availability was accomplished for all living consented participants age 18 or older within these 92 families for whom contact information was available (Table 3), totaling 353 notification letters. In the 9 families in which the proband was a minor at first consent, results notification was provided to the consenting parent. Sixteen probands were deceased at the time of results disclosure. In 10 of these families, notification was sent to other consented family members. Another case included a proband with two mutations. Notification was sent regarding the first mutation; however, the proband was deceased at the time that the second mutation was identified. In this case, as well as in the remaining 5 cases in which the proband was deceased at the time of results, no additional family members were enrolled in the study, and thus notification could not be provided.

We have not actively and systematically surveyed all individuals and families who received notification of DCM genetic findings to assess whether CLIA confirmation genetic testing was undertaken, or to gauge attitudes, beliefs and knowledge regarding genetic testing. However, in the 51 families who received notification prior to May 2011, we do have knowledge of 23 individuals among 10 families that have pursued and completed CLIA confirmation testing of their research result in our study (Table 4). Our knowledge of this is partially due to the CLIA-confirmation process, because we needed to provide the CLIA-certified laboratory with our findings for the initial person in the family who sought CLIA confirmation testing. In addition, our research study genetic counselors have often been contacted as a resource for understanding the clinical application of genetic testing and the testing process. We have documentation of additional families who have explored CLIA confirmation testing, although we do not yet have information regarding completion of the process. In addition, it is possible that some subjects had knowledge of their family mutation or rare variant from another source and sought clinical genetic testing independent of our research study.

DISCUSSION

The purpose of this research project, undertaken in 1993, has been to discover the molecular genetic causes of FDC (Kushner et al. 2006). Our study has successfully recruited 606 probands and their 1670 family members to this FDC research project, and 433 probands had sufficient cardiovascular data to confirm LVE with systolic dysfunction and exclude all known causes of DCM, except genetic. During this discovery effort we have also successfully identified genetic cause in some of the 324 probands who were resequenced for 14 genes (Table 3) (Hershberger et al. 2008b; Parks et al. 2008; Hershberger et al. 2010b; Li et al. 2010; Norton et al. 2011).

As noted above, we have documentation of 23 consented subjects from 10 families spanning 5 genes who have participated in this process. From its inception this study has intended to return molecular genetic information to the consented probands and family members if it would be clinically relevant for genetic risk assessment of DCM. Genetic DCM offers the opportunity for medical and/or device intervention in early disease that may favorably affect

the natural history of this condition (Burkett and Hershberger 2005; Hershberger et al. 2009a; Hershberger et al. 2009b; Hershberger et al. 2010a). Hence, presymptomatic molecular genetic testing of at-risk relatives should be considered after a genetic DCM diagnosis has been established, as we have previously suggested (Burkett and Hershberger 2005; Hershberger et al. 2009a; Hershberger et al. 2010a; Hershberger and Siegfried 2011) and has also been recently recommended in guidelines developed by US, Canadian and European cardiovascular societies (Hershberger et al. 2009b; Ackerman et al. 2011; Gollob et al. 2011).

A larger issue beyond establishing CLIA-compliant protocols is deciding which results merit communication to our consented subjects. The usual standards we have employed for Mendelian disease (Hershberger and Siegfried 2011) are similar to those used by molecular geneticists for clinical diagnostic genetic testing (Richards et al. 2008) and those discussed previously by others for rare variant cardiovascular disease (Ho and MacRae 2009; Caleshu et al. 2010). This includes prior evidence that the variant is pathogenic, including the type of variant (nonsynonymous, synonymous, intronic, etc), rarity in the population, segregation of the variant with the disease phenotype ideally in one or more large families, and relevant functional data when available (Table 5), augmented by established criteria that the results are meaningful and actionable (Bookman et al. 2006; Hershberger et al. 2009b; Fabsitz et al. 2010).

The stringency of our standards for returning results and our level of certainty in the pathogenicity of the result is based in part upon whether a gene has been previously implicated in DCM cause or whether it is novel (Table 5). Our earliest return of results occurred with two very large families harboring rare nonsynonymous variants in *LMNA* (Jakobs et al. 2001), which raised no significant issues in this regard based upon the typical features of lamin A/C cardiomyopathy as had been previously reported and well-established (Fatkin et al. 1999; Hershberger et al. 2008a) (arrhythmia and conduction system disease, followed by DCM and heart failure) coupled with complete segregation of the *LMNA* rare variants in multiple affected subjects. Further, one variant, E203K, affected the same amino acid as previously reported (E203G) in a different family (Fatkin et al. 1999), and the variant in the second family was a nonsense variant (R225X), which we considered to strengthen its evidence of causality. Collectively, we considered the data to be very strong that both were disease-causing in these families.

However, to consider return of results with a novel DCM gene discovery we require a higher standard than with a previously identified DCM gene, ideally including rare variants in multiple families accompanied by functional data or tissue studies to augment the evidence of relevance (Table 5). For novel genes we await acceptance for publication prior to notification. In the recent case of *BAG3*, a gene not previously described as causing DCM, we utilized a large family with excellent segregation of a *BAG3* 8.7 kb deletion, four smaller families showing segregation, and three patients with IDC and no family members available for testing; all seven had unique nonsynymous rare variants not identified in more than 1300 controls. Evidence that disruption of *BAG3* was relevant for DCM was augmented with functional data in a zebrafish model (Norton et al. 2011).

More difficult cases to consider for return of results are those of novel, nonsynonymous rare variants without precedent in the literature that are observed in a well established DCM gene, but only in a singleton case of IDC with no family members available for testing. These issues are well known to us for DCM (Burkett and Hershberger 2005; Hershberger et al. 2009a; Hershberger et al. 2010a) and have been discussed for the genetics of cardiovascular disease in general (Ho and MacRae 2009; Caleshu et al. 2010), as well as having received formal review by the American College of Medical Genetics (Richards et

al. 2008). We identified numerous rare nonsynonymous variants meeting these criteria in conducting our resequencing studies. In an effort to report back meaningful information to our consented subjects harboring such rare variants, while yet respecting the notion that some nonsynonymous rare variants may be benign, we developed the terminology of 'possibly' or 'likely' disease-causing for our two resequencing data sets comprising coding sequence from 11 genes (Hershberger et al. 2008b; Hershberger et al. 2010b). We applied the 'likely' term to variants meeting all usual clinical criteria to be attributed as disease-causing, including prior reports of its association with DCM, and other criteria (Table 5). The 'possibly' disease-causing category required all of the former conditions except that segregation could not be assessed, as by definition, only one affected family member was available for testing. Based on our experience, these, and other considerations that research staff must consider at the time of disclosure of research results have been summarized (Table 6).

Future Directions

While such accommodations may have been reasonable for past studies, as mentioned above, the field is changing rapidly as next generation sequencing (NGS) has dramatically improved sequencing throughput. Perhaps the greatest impact is the availability of thousands of 'control' sequences, as the NHLBI's Exome Sequencing Project (ESP) has deposited thousands of exome sequences into a public database. The utility of such databases will be greatly improved if they are annotated with clinical and disease phenotype information, and ideally with notation of the credibility of the disease phenotype data (e.g., data collected with careful medical records review in a research study versus data collected from a requisition form filled in by a patient). Further, clinical guidelines for the cardiomyopathies need to be updated to consider when NGS-facilitated testing for panels of 10–60 genes may be appropriate.

NGS has also led to the use of whole exome sequencing (WES) for genetic discovery, which we have recently utilized for one family (Norton et al. 2011), and we have WES of other families underway. Each genome itself contains ~7000 nonsynonymous variants, of which several hundred (usually ~400) will be absent in currently available databases. While WES has already shown its power and utility for gene discovery, it will also detect medically relevant rare variants in genes beyond DCM, raising new issues for research testing (Trinidad et al. 2011). Our commitment to 'give back' as much knowledge as possible to the hundreds of probands who have entrusted their genetic material to this research project now takes on a new dimension. Our current informed consent recognizes the intent to perform exome or whole genome sequencing, and for NIH-funded studies, to place this genomic data into publicly available databases. Public opinion also appears favorable on this issue (Trinidad et al. 2011). Since 2009 we have included information in our informed consent document that states that we may acquire genetic data beyond that related to DCM, but we have not explored the knowledge or preferences of our consented study participants regarding return of results beyond those relevant for DCM. As this study and many others like it move into the NGS era, these and related issues will need to be addressed.

We note that 84 families (~14%) consented to our study did not meet criteria as IDC or FDC based on our stringent protocols for classification, despite passing our initial screening for IDC and/or being referred in most cases by a cardiologist with knowledge of our eligibility criteria. This observation reflects the difficulty and complexity of systematic phenotyping in clinical research, and emphasizes the importance of careful review of high quality medical data, as inaccurate phenotyping will invariably confound genetic analysis. We also use this observation to reemphasize the importance of high quality phenotype data in the publicly accessible sequencing databases. An important area for future research is the systematic collection of psychosocial issues relevant to participation in genetic research

studies and the return of research results. Topics for exploration include: 1) family communication issues; 2) results disclosure to minors; 3) participant's understanding of the information discussed; 4) barriers associated with confirmation of a research result; and 5) overall participant satisfaction with their research involvement, regardless of identification of results, among others. While our study did not systematically collect this data, we can share anecdotal experience. Unlike the clinical genetic counseling model, in which a detailed agenda covering all conceivable issues that may arise is followed, our research genetic counseling model is problem-focused. A recurring issue brought up by our participants relates to family communication issues. For example, during a phone call to one of our genetic counselors, a participant with a known disease-causing mutation shared her concern that her family dismissed the confirmed research finding as not relevant to her or any of the family member's health. On the other hand, an important issue that has not been brought up to the attention of the study genetic counselors is the disclosure of genetic results to minors. In our study, results on minors were provided to parents, who in most instances were also enrolled in the study. Understandably, in an unaffected minor, the disclosure of a positive result would be more complicated from a psychosocial standpoint. Furthermore, determining the upper bounds for normality in minors is more problematic than in adults because of the variable and rapid cardiac growth during childhood and adolescence creates wide confidence intervals. While the obstacles that we encountered as we moved through the confirmatory process are similar to those of any longitudinal study (for example, death or change of contact information) in our experience, the disclosure process has worked smoothly. This may owe in part to the fact that, during the consent process we strongly emphasize that participants should not expect any results, and if they do, they will need to have their result confirmed in a CLIA laboratory (that usually also will include a repeat phlebotomy and self payment if not covered by their insurance).

While we have had limited funding from research sources for follow up counseling for study participants, research participation has allowed subjects to receive counseling from our study genetic counselors and non-CLIA based research testing at no cost to the subject. For research participants who desire genetic testing once a DCM-causing mutation has been identified in our laboratory, targeted confirmatory testing has been recommended, which involves a much lower cost than sequencing a panel of DCM genes. Regardless of the financial situation of our research participants, we devote substantial time addressing these issues during the consent process.

Our consent process also involves discussing the implications of positive results, including the value of sharing confirmed results with at-risk family members. However, because we consider our research results preliminary until CLIA confirmed, we hesitate to use non-CLIA confirmed results as a basis for the duty to warn. While we have not yet encountered such a situation, if a proband were unwilling to share knowledge of a high impact CLIA-confirmed result with their family, we would consider warning at-risk family members about risks for disease onset or sudden cardiac death. We recognize that the duty to warn concept in medical genetics entails high complexity. Many factors would need to be considered before activating this approach.

Limitations

Limitations of this study include the lack of systematic investigation into the beliefs, knowledge, attitudes and desires of our consented subjects regarding receipt of genetic information, as well as a lack of systematic follow-up regarding pursuit of clinical genetic testing. However, we have established long term follow up and have acquired considerable information intermittently in some of these families regarding their pursuit of CLIA-confirmation genetic testing, as noted above. We also note that our long term follow up provides a ready cohort to undertake such a study. We also acknowledge that some of these

rare variants assigned as possibly or likely disease causing may need revision as new information becomes available. However, we have obtained functional data (Hershberger et al. 2009c; Cheng et al. 2010; Cowan et al. 2010) for some of these rare variants, and in almost all cases their likely pathogenic nature has been validated.

Conclusion

The return of genetic testing results from our research laboratory utilizing CLIA-compliant protocols has been successfully accomplished for this study. We now outline considerations for research personnel involved in returning research results (Table 6), based on this experience returning research results for DCM. Future efforts are needed to evaluate the impact of this effort, and to determine preferences for return of genetic information beyond that relevant only for DCM.

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Table 1

Demographics of Probands and Family Members

		Number of Consented Probands	Number of Consented Family Members
All		606[a]	1670
Ethnicity	Not Hispanic	529	1588
	Hispanic	61	53
	Unknown	16	29
Race	White	505	1334
	Black	70	270
	Asian	5	3
	More than one race	8	19
	Native American	3	6
	Pacific Islander	0	0
	Unknown	15	38
Gender	Male	354	769
	Female	252	901
Age, first consent	n, mean±SD, median (range)	606, 47±14.6, 49 (0–82)	1599, 35+20.7, 36 (0–90)
Current Age	n, mean±SD, median (range)	544, 53±14.6, 56 (2–88)	1535, 44±19.6, 44 (1–101)
Age if deceased	n, mean+SD, median (range)	56, 52±17.8, 53 (11–80)	63, 57±20.1, 58 (1–92)

[a]For 10 families, proband consent is still in process or was never accomplished but at least 1 family member is enrolled

Table 2

Characteristics of Probands and Family Members by Proband Status Assignment

	Number of Consented Probands	Number of Consented Family Members
FDC	160	1353
All IDC	273	195
IDC probable FDC	73	77
IDC possible FDC	101	76
IDC	99	42
Total, FDC and IDC	433	1548
Other Database Assignments		
In Process, DCM likely	89	58
Ischemic Dilated Cardiomyopathy	13	2
DCM with mixed phenotypes[a]	4	9
Other types of cardiomyopathy[b]	3	8
No DCM, DCM from other causes, DCM with confounding risk factors, or inadequate data[c]	64	45

[a]This includes three families with DCM and HCM, and one with DCM and ARVD.

[b]This includes an arrhythmogenic right ventricular cardiomyopathy (ARVC), a restrictive cardiomyopathy (RCM), and an X-linked dilated cardiomyopathy.

[c]This includes two probands with chemotherapy-induced DCM, two with valvular cardiomyopathy, a substance abuse DCM; the remainder had other unclassifiable cardiomyopathies, and/or did not have LV enlargement, or did not have LV measurements available to assign a DCM diagnosis.

Table 3

Research Molecular Genetic Testing and Notification of Results

	Genetic Testing				Notification of Results			
	Number of Probands Tested	Number of Probands Positive for Rare Variants[a]	Number of Family Members Tested	Number of Family Members Testing Positive for Family Rare Variant	Number of Families Notified of Rare Variant[a]	Number of Adults Notified[b]	Family Notification Not Possible (Deceased or No Contact Information)	Family Notification Not Indicated
LMNA Study (Parks et al. 2008)	324	19[c]	149	55	19	124		
6 Gene Study (Hershberger et al. 2008b)	313							
LDB3		3[c]	4	2	2	6	0	1
MYH7		13[c]	37	14	11	40	2	0
SCN5A		8	49	15	5	22	1	2
TCAP		3[c]	3	3	2	3	0	1
TNNT2		9[c]	26	10	8	20	1	0
CSRP3		1	0	0	1	1	0	0
5 Gene Study (Hershberger et al. 2010b)	312							
MYBPC3		13[c,d]	32	11	12	28	1	0
MYH6		10[c,d]	9	5	8	15	1	1
TNNC1		4[c]	15	3	4	11	0	0
TNNI3		2	6	3	2	6	0	
TPM1		6[c]	10	7	5	9	1	0
RBM20 Study (Li et al. 2010)	312	6[c]	51	18	5	23	1	0
BAG3 Study (Norton et al. 2011)	311	8[c]	27	15	6	45	2	0

[a] Number of families notified is less than the number of probands positive because the notification was not possible or not indicated.

[b] Number of adults notified refers to both probands and family members, excluding minors.

[c] Probands identified with a second mutation in a different gene. The number of probands (indicated in parenthesis) with additional mutations is as follows: *LMNA* (3); *LDB3* (2); *MYH7* (2); *TCAP* (1); *TNNT2* (2); *MYBPC3* (2); *MYH6* (3); *TNNC1* (2); *TPM1* (1); *RBM20* (2); *BAG3* (2).

[d] Two mutations in the same gene were identified in the same individual.

Table 4

Outcomes of Research Test Results Notification

Gene	Number of Families with CLIA Certified Results	Number of Individuals with CLIA Certified Results
LMNA	5	15
MYBPC3	1	2
TNNC1	1	2
TNNI3	1	1
TNNT2	2	3

Table 5

Criteria Used to Decide Relevancy for Return of Results for Rare Variants in DCM Genetic Research

	Established DCM Gene	Novel DCM Gene
Variant previously published as disease causing	x	NA[a]
Rare	x	x
Nucleotide and/or amino acid conserved across species	x	x
Type of variant (.e.g, nonsynonymous, nonsense, splice site, etc)	x	x
Familial segregation	x	x
Multiple rare variants associated with disease, ideally segregating in more than one family		x
Relevant functional data	x[b]	x
Histological evidence of key protein/protein disruption in human tissue specimens		x

[a] NA is not applicable.

[b] The availability of functional data for specific variants identified in established DCM genes adds to certainty, especially for novel variants in sporadic disease, as discerning pathologic versus neutral variants can be challenging without evidence of segregation.

Table 6

Considerations for Research Personnel Involved in Returning Research Results

1	The plan for notification of research results must be part of the research protocol and approved by the site's Intuitional Review Board (IRB).
2	Adjudication of the mutation pathogenicity must be conducted, with the principal investigator's approval, before reporting.
3	The consent process and documentation must be reviewed before returning results. Specifically:
	a. Research personnel returning research results should be confident that enrollment occurred voluntarily, with ample time for discussion;
	b. Participant must be made aware of the plan for results disclosure (if any), including need for confirmatory testing and cost;
	c. If the consent form has changed, or if the individual was a minor at first consent, attempt should be made to re-consent the individual with the most recent consent form.
4	For conditions with increased risk for death, the individual obtaining consent should consider consenting as many family members as possible, or at a minimum, an alternate family member to whom results availability may be communicated in case the proband is deceased.
5	The notification letters must be readable by both patient and provider and must avoid ambiguity as much as possible.
6	The individual's research genotype may not be reported so it cannot be used for or construed as contributing to clinical care.
7	Participants should optimally be referred to cardiovascular centers staffed by genetic professionals for discussion of the research findings and assistance with confirmatory testing.
8	Once a participant expresses interest in confirming their research result:
	a. Research laboratory personnel must obtain release from the patient before sharing any personal information from the participant with confirmatory laboratory.
	b. A fresh blood sample must be drawn at the participant's doctor's office (preferably a cardiovascular genetics center, as noted in #6).
	c. The research laboratory provides the confirmatory laboratory with the individual's genotype to be confirmed.
	d. Primer sequence, an anonymous positive control sample (sample with a known mutation), and other technical information may be required by the confirmatory laboratory and may be facilitated by the research laboratory.
9	For multiple family members, it is best practice to confirm research results in the same laboratory.
10	The use of a newsletter or another ongoing form of contact with participants should be considered, as it helps to remind subjects of the study protocol.
11	A genetic counselor, ideally one involved in the specific research study relevant to the participant, should be available to address questions and concerns from participants who receive research results, and their providers.
12	Variant interpretation may change over time. Research participants should be informed of this, and depending upon the study plan and consent discussion, research personnel should have plans in place, including possible recontact as new information becomes available.
13	Detailed progress notes of all discussions are essential.