ARTICLE

Evaluation of Recipients of Positive and Negative Secondary Findings Evaluations in a Hybrid CLIA-Research Sequencing Pilot

Julie C. Sapp,[1] Jennifer J. Johnston,[1] Kate Driscoll,[1] Alexis R. Heidlebaugh,[2] Ane Miren Sagardia,[2] D. Nadine Dogbe,[2] Kendall L. Umstead,[2] Erin Turbitt,[2] Ilias Alevizos,[3] Jeffrey Baron,[4] Carsten Bönnemann,[5] Brian Brooks,[6] Sandra Donkervoort,[5] Youn Hee Jee,[4] W. Marston Linehan,[7] Francis J. McMahon,[8] Joel Moss,[9] James C. Mullikin,[10] Deborah Nielsen,[7] Eileen Pelayo,[3] Alan T. Remaley,[11] Richard Siegel,[12] Helen Su,[13] Carlos Zarate,[8] NISC Comparative Sequencing Program,[10] Teri A. Manolio,[14] Barbara B. Biesecker,[2,15] and Leslie G. Biesecker[1,*]

While consensus regarding the return of secondary genomic findings in the clinical setting has been reached, debate about such findings in the research setting remains. We developed a hybrid, research-clinical translational genomics process for research exome data coupled with a CLIA-validated secondary findings analysis. Eleven intramural investigators from ten institutes at the National Institutes of Health piloted this process. Nearly 1,200 individuals were sequenced and 14 secondary findings were identified in 18 participants. Positive secondary findings were returned by a genetic counselor following a standardized protocol, including referrals for specialty follow-up care for the secondary finding local to the participants. Interviews were undertaken with 13 participants 4 months after receipt of a positive report. These participants reported minimal psychologic distress within a process to assimilate their results. Of the 13, 9 reported accessing the recommended health care services. A sample of 107 participants who received a negative findings report were surveyed 4 months after receiving it. They demonstrated good understanding of the negative secondary findings result and most expressed reassurance (64%) from that report. However, a notable minority (up to 17%) expressed confusion regarding the distinction of primary from secondary findings. This pilot shows it is feasible to couple CLIA-compliant secondary findings to research sequencing with minimal harms. Participants managed the surprise of a secondary finding with most following recommended follow up, yet some with negative findings conflated secondary and primary findings. Additional work is needed to understand barriers to follow-up care and help participants distinguish secondary from primary findings.

Introduction

While the debate surrounding the return of secondary findings from clinical exome sequencing and genome sequencing (ESGS) has settled, the handling of these findings in clinical research has not yet moved to consensus. While there is no general obligation to return such findings in a research context,[1,2] there is a growing recognition that there is overlap of research and clinical activities. However, it is ambiguous as to whether clinical care or research ethics should apply.[3] Although the ethics debate is unresolved, research participants are often interested in learning primary and secondary results, and researchers express interest in returning them, but few researchers actually do so.[4–6] There are many questions surrounding return of results from ESGS to research participants, including the necessity or obligation to do so, mechanisms for how this could be accomplished, regulatory issues, actual participant uptake of such results, and how participants would react to and use such results.[2,4,7]

We developed a hybrid, research-clinical process for analysis and return of secondary findings from translational genomics research and assessed participant responses to receiving such results to inform the debate with evidence. This hybrid included generation of next-generation sequencing (NGS) CLIA-validated data to be used in a secondary finding analysis process that enabled clinical secondary finding reports for all participants. A notable component of our process was that the NGS CLIA sequencing was coupled to generation of non-CLIA research exome data used by investigators studying heritable disorders. Because we CLIA-validated the exome sequencing of the ACMG secondary findings genes, this allowed the return of negative secondary finding reports to research participants and

[1]Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, Bethesda, MD 20892, USA; [2]Social and Behavioral Research Branch, National Human Genome Research Institute, Bethesda, MD 20892, USA; [3]National Institute of Dental and Craniofacial Research, Bethesda, MD 20892, USA; [4]Section on Growth Development, National Institute of Child Health and Human Development, Bethesda, MD 20892, USA; [5]Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurologic Disorders and Stroke, Bethesda, MD 20892, USA; [6]Office of the Clinical Director, National Eye Institute, Bethesda, MD 20892, USA; [7]Urologic Oncology Branch, National Cancer Institute, Bethesda, MD 20892, USA; [8]Human Genetics Branch, National Institutes of Mental Health, Bethesda, MD 20892, USA; [9]Laboratory of Translational Research, National Heart, Lung, and Blood Institute, Bethesda, MD 20892, USA; [10]NIH Intramural Sequencing Center, National Human Genome Research Institute, Bethesda, MD 20892, USA; [11]Lipoprotein Metabolism Laboratory, National Heart, Lung, and Blood Institute, Bethesda, MD 20892, USA; [12]Office of the Clinical Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD 20892, USA; [13]Human Immunological Diseases Section, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA; [14]Division of Genomic Medicine, National Human Genome Research Institute, Bethesda, MD 20892, USA
[15]Present address: RTI International, Research Triangle Park, NC, USA
*Correspondence: lesb@mail.nih.gov
https://doi.org/10.1016/j.ajhg.2018.07.018.

provided us with an opportunity to study the outcomes of returning such reports. Here, we describe our process, the sequencing of 1,197 individuals, the secondary findings that resulted from this, the reactions of the participants to the receipt of both positive and negative secondary finding reports, and the follow-up health care undertaken by the recipients of the positive reports.

Subjects and Methods

Clinical-translational investigators in the NIH Intramural Program accessed the process we developed through the Clinical Center Genomics Opportunity (CCGO) program and consented their participants to return of secondary findings. The primary sequencing activities and return of secondary findings were reviewed and approved by 5 Institutional Review Boards for the 11 principal investigators whose projects were supported by CCGO. This social and behavioral sub-study, under which these interviews and surveys were conducted, was approved by the National Human Genome Research Institute IRB (16-HG-0017).

Exome sequencing was performed on samples submitted by intramural investigators. Genomic DNA and whole blood were accepted for testing following an order placed in the NIH Clinical Center electronic medical record (EMR) system. DNA was isolated from whole blood using the salting-out method (QIAGEN, Gentra) and all DNA was phenol-chloroform extracted before sequencing. Exome capture and sequencing was performed using standard techniques. Research data (complete exome data) were returned to the investigator and in addition, variants in the then-currently recommended 56 genes[8] for secondary variant analysis were interrogated by the CCGO clinical analysis team. Briefly, variants in coding regions and canonical splice sites (+1, +2, −1, −2) were analyzed to determine potential pathogenicity (bed files in Table S1). Variants were initially filtered for quality and for minor allele frequency ($\leq$0.01 in ESP6500). Alamut Visual (Interactive Biosoftware) was used to identify relevant information for variant assessment. For all variants, the frequency of the variant in control databases (ExAC)[9] was expected to be below the frequency of the associated disease in order for the variant to be considered for likely pathogenic or pathogenic status. Variants that met this criterion were considered rare variants for this analysis. For rare variants, ClinVar and HGMD were queried to identify relevant information. Missense variants that were not present in ClinVar or present in HGMD were not further considered. For the remaining variants, relevant information from published literature and ClinVar submissions was considered to assess pathogenicity according to the ACMG/AMP guidelines.[10] Variants assessed as pathogenic and likely pathogenic were returned for all genes except for *MUTYH* (MIM: 604933) where variants were returned only if found in the biallelic state. For pathogenic and likely pathogenic variants, BAM files were examined for read quality and BLAT (UCSC Browser) was used to confirm that the sequence was unique in the genome browser. Variants that passed both of these checks were returned to participants as preliminary and a saliva sample was requested for confirmation testing, which was performed using Sanger sequence analysis. All sequenced individuals received either a positive secondary findings report or a negative secondary findings report.

For each secondary variant returned by a CCGO genetic counselor, a genetics consultation note for the positive result recipient was entered into the EMR and a lay summary letter was sent to the participant (see Supplemental Data for example letter). Patient referrals were made to a local genetic counselor or another expert clinician at a center that offered specialty services for the secondary finding disorder (see Supplemental Data for genetic counseling outline). A local health care provider was directly contacted to review the indication for the referral and transfer ongoing care for the secondary finding recipient.

A subset of participants was recruited to join this sub-study, which included surveys of recipients of negative secondary finding reports and interviews of recipients of positive secondary finding reports. Positive secondary finding recipients who were eligible for this study included those whose results were returned to them by a CCGO genetic counselor. Recipients of positive findings were interviewed by telephone by one of the authors (B.B.B.). For minor probands, a parent or legal guardian was invited to be the respondent.

Negative secondary findings reports returned as part of the sub-study were mailed directly to participants with a cover letter that explained and defined actionable variants and outlined the limitations of sequencing. Recipients of negative findings were invited to take a survey administered 4 months after receipt of results. Surveys of negative secondary findings recipients included five scales with 3–20 items each. Knowledge about sequencing was assessed using a valid and reliable 12-item measure that included two subscales for sequencing benefits and sequencing limitations. Scores on each of the subscales ranged from 0 (low knowledge) to 10 (high knowledge)[11] ($\alpha = 0.73$). A four-item scale[12] assessed the perceived benefits of receiving secondary finding (e.g., "Secondary findings may be valuable for maintaining one's future health"); possible scores on this scale ranged from 1 to 5 ($\alpha = 0.81$). Depressive symptoms were assessed using the CES-D-20, a widely used depression screening tool with scores ranging from 0 to 60; scores > 15 are considered to be indicative of increased risk for depression in the general population[13] ($\alpha = 0.90$). The PROMIS-Global Health, a ten-item scale, assessed perceived physical, mental, and general health with the US general population as the reference population[14] ($\alpha = 0.76$; raw scores are converted to T-scores with a mean of 50 and a SD of 10). A three-item version of the Life Orientation Test-Revised (LOT-R) scale[15] was used to assess dispositional optimism with possible scores ranging from 0 to 4 ($\alpha = 0.88$).

Recipients of negative secondary finding were asked five open-ended questions, which they responded to by completing an open field. The first three questions were designed to assess participants' perceptions of the meaning, value, and utility of receiving secondary finding. Two additional questions assessed expectations of receiving a secondary finding and feelings of reassurance or disappointment after receipt of negative findings (see Table 1). The data were scored to describe the understanding and expectations of these participants for receiving secondary finding. Open-ended responses were independently coded (D.N.D. and A.R.H.) and semantic discrepancies were resolved. The coded data were analyzed for themes.

Interviews of recipients of positive reports were conducted by a single investigator, audio-recorded and transcribed. In one instance, field notes were taken when audio-recording was not possible. Interviews explored psychological responses to learning the result, dissemination of the information to relatives and health care providers, and use of recommended health care services. A codebook was developed based on the interview guide and expanded to code the data for subsequent thematic analysis.

Table 1. Open-Ended Questions from Survey of Recipients of Negative Secondary Findings Results

If you had received a secondary finding, what would it have meant for your health and well-being?
In what ways do you think learning a secondary finding may have been useful to you?
How valuable do you believe this information may have been to you and your family?
What are your feelings about not receiving a secondary finding?
If you felt reassured, please describe in what ways it is reassuring?
If you felt disappointed, in what ways is it disappointing?
Did you expect to receive a secondary finding?
If so, was your expectation based on your family history or known health risks in your family?
If not, why?

The data were independently coded by two coders (A.M.S. and E.T.) and semantic discrepancies resolved. The overall unweighted Kappa co-efficient was 0.86, indicating high inter-rater reliability.

Results

Sequencing Results and Variant Analyses

A total of 1,197 samples were submitted for analysis of the ACMG 56 gene list[8] with 1,620 unique variants (Tables 2, S2, and S3) passing initial quality and frequency filters. Clinical review was performed on 1,008 variants using data from ClinVar, HGMD, and the literature. After frequency filtering, all putative loss-of-function variants in ACMG "expected pathogenic" genes and all missense variants in all 56 genes with ≥ 1 pathogenic assertion(s) in ClinVar or a "DM" assertion in HGMD underwent an evaluation of ACMG/AMP criteria[10] (141 variants). A total of 28 variants were assessed as pathogenic or likely pathogenic. Of these, 4 were not returned because they were monoallelic variants for a recessively inherited disorder (*MUTYH*), and 10 variants were primary findings and returned separately (Table S3), leaving 14 secondary findings (Table 2) among an estimated 1,678 unique chromosomes, for an overall yield of 1.9%, taking into account relatedness of samples.

Interviews of Positive Secondary Finding Recipients

14 families, involving 19 individuals, received a secondary finding from CCGO. 13 individuals (10 adults and 3 parents from 11 families) participated in this interview sub-study. All but two results disclosures were made by phone; two were done in person. The disclosures took an average of 55 min (range 36–90 min). At the time of secondary finding disclosure, one participant (a 52-year-old woman with a pathogenic *APOB* [MIM: 107730] variant) had understood that high cholesterol ran in her family but did not know that this disorder had a name or that specific genes were associated with it. The remaining participants were previously unaware of any personal or family history risk related to their secondary finding. In seven cases, additional personal or family history of symptoms of the secondary finding disorder were provided by a patient during the disclosure sessions.

13 positive secondary finding recipients were interviewed 4 months after receipt of their secondary finding. Interviews took an average of 25 min (range 14–48 min). 10 of the 13 participants reported being surprised by their secondary finding result. Participants described their initial reactions as disbelief and shock but also recounted an evolution in their responses over the intervening 4 months to arrive at feelings of relief and gratitude for the opportunity to learn health risk information that they would otherwise not know (see Table S4 for representative quotes). Two participants explicated the advantage of learning about the pathogenic variant while they were asymptomatic, in contrast to learning it at the time of being diagnosed with a life-threatening condition. Several offered that they were still processing what the results meant for them 4 months after learning them.

In describing their healthcare use at 4 months after disclosure, 7 of 13 had told their (or their child's) primary care physicians their results (see Table 3). 9 of 13 followed up with a specialist as recommended by the CCGO team and 1 saw a second specialist. 9 of 13 reported taking a healthcare action (such as recommended screening or diagnostic evaluation). 4 women with pathogenic variants opted for prophylactic surgeries to reduce their cancer risk. 4 interviewed participants had not followed up with the recommended specialist or taken any healthcare actions at 4 months.

11 of the 13 participants shared their secondary finding with their relatives. Most participants did not know whether their relatives had followed up on the information with their health care provider, to pursue genetic testing (for those at risk) and/or undergo clinical evaluations. The reason offered by the two respondents who did not tell their relatives and by two who had told only a few relatives was that they had been focused on learning more about their own health risks. Another reason noted by a parent for a lack of focus on relatives' risks was primary concern about their child enrolled in an NIH study. Nevertheless, most shared the information with their close relatives.

When asked whether they had regrets about learning a secondary finding, 11 of 13 reported no regret. They cited the importance of having the information to make decisions going forward. Two participants expressed concern about knowing their secondary finding, wishing they were not at risk and did not have to worry. Both interviewees added that regardless, they would rather know than not know. In one case, a participant expressed anxiety about his son's future access to health insurance and another participant worried about her job security due to her diagnosis of arrhythmogenic right ventricular dysplasia. Although not expressing

Table 2. Secondary Findings Variants Identified in the Clinical Center Genomics Opportunity Project

Variant Genomic Position GRCh37	Gene Name (MIM)	Variant cDNA	Predicted Protein Change	ACMG Scoring[a]	Pathogenicity Assessments
chr1:g.17371319C>T	*SDHB* (185470)	NM_003000.2; c.137G>A	p.Arg46Gln	PS3_Moderate, PS4_Moderate, PM5, PM2_Supporting, PP3, PP5	ACMG, likely pathogenic; returned as pathogenic
chr2: g.21229160C>T	*APOB* (107730)	NM_000384.2; c.10580G>A	p.Arg3527Gln	PS3, PS4, PM1, PM5, PP1, PP5	ACMG, pathogenic; returned as pathogenic
chr11: g.2591868del	*KCNQ1* (607542)	NM_000218.2; c.488del	p.Leu163Argfs*74	PVS1, PS4_Moderate, PM2, PP5	ACMG, pathogenic; returned as pathogenic
chr13: g.32903606_32903607del	*BRCA2* (600185)	NM_000059.3; c.658_659del	p.Val220Ilefs*4	PVS1, PS4, PP5	ACMG, pathogenic; returned as pathogenic
chr13: g.32907126_32907127del	*BRCA2*	NM_000059.3; c.1511_1512del	p.Ser504Tyrfs*9	PVS1, PS4_Moderate, PM2, PP5	ACMG, pathogenic; returned as pathogenic
chr13: g.32914438del	*BRCA2*	NM_000059.3; c.5946del	p.Ser1982Argfs*22	PVS1, PS4, PP1, PP5, BP2	ACMG, pathogenic; returned as pathogenic
chr13: g.32953959_32953963del	*BRCA2*	NM _000059. 3; c. 9026_9030del	p.Tyr3009Serfs*7	PVS1, PS4, PM2_PP, PP5	ACMG, pathogenic; returned as pathogenic
chr13: g.32969004_32969005del[b]	*BRCA2*	NM_000059.3; c.9435_9436del	p.Ser3147Cysfs*2	PVS1, PS4, PM2_Supporting, PP5	ACMG, pathogenic; returned as pathogenic
chr14: g.23886383G>A	*MYH7* (160760)	NM_000257.3; c.4498C>T	p.Arg1500Trp	PS3_Moderate, PS4_Moderate, PM2, PP1, PP2, PP3	ACMG, likely pathogenic; returned as likely pathogenic
chr17: g .41276047_41276048del	*BRCA1* (113705)	NM_007294.3; c.68_69del	p.Glu23Valfs*17	PVS1, PS4, PS3_PM, PP1, PP5	ACMG, pathogenic; returned as pathogenic
chr18: g.28662344G>A	*DSC2* (125645)	NM_024422.3; c.1123C>T	p.Arg375*	PVS1, PM2_Supporting	ACMG, likely pathogenic; returned as likely pathogenic
chr18: g.29101208T>C	*DSG2* (125671)	NM_001943.3; c.523+2T>C	p.?	PVS1, PS4_Moderate, PM2_Supporting, PP5, BP2	ACMG, pathogenic + BP2; returned as likely pathogenic
chr18: g.29111043dup	*DSG2*	NM_001943.3; c.1109dup	p.Thr371Tyrfs*19	PVS1, PM2	ACMG, likely pathogenic; returned as likely pathogenic
chr19: g.38986905C>T	*RYR1* (180901)	NM_000540.2; c.6599C>T	p.Ala2200Val	PM1, PS4_Supporting, PP2, PP3	ACMG, VUS; returned as likely pathogenic

[a]ACMG: American College of Medical Genetics/AMP pathogenicity criteria and pathogenicity assertion. See Richards et al.[10] for details.
[b]This variant was disclosed by the participant's primary team and this participant was not included in the social and behavioral sub-studies. Setting aside this variant, there were 13 variants returned to 18 research participants.

regret, three participants expressed concern about their children being at risk. Three participants spontaneously described the receipt of the information as empowering as they felt they could act on the information and prepare for the future. Two stated that they were pleased that so far nothing had been found in their follow-up evaluations.

Surveys of Recipients of Negative Secondary Finding Reports

There were a total of 1,197 individuals sequenced in CCGO; 687 were available for consent to this sub-study and 384 were enrolled. Negative secondary findings reports were returned to 318; of these, 235 received a survey invitation and 107 completed it (see Table 4 for demographic information). Objective knowledge of the benefits and limitations of genetic sequencing was measured by two sub-scales. The limitations sub-score mean was midrange; 6.2 (2.7 SD, scale range 0–10). The benefits sub-score mean was also midrange; 4.9 (2.3 SD, scale range 0–10). On a specific tool that measured benefits of secondary findings in particular, the mean score was at the high end of the scale at 4.3 (0.5 SD, scale range 0–5). The PROMIS global health score mean was 34.3 (5.4 SD), which is between one and two standard deviations below the U.S. general population mean of 50. Their mean depressive symptoms score was low (mean 7.0; 8.0 SD, scale range 0–60). The mean optimism score was above the middle of the range at 2.8 (SD 0.8, scale range 1–4).

We assessed participants' understanding of the nature of secondary finding results by asking several related questions about the perceived meaning, value, and utility of receiving a secondary finding; most survey respondents answered at least one of these. When responding to a question about what receiving a secondary finding would have meant for their health and well-being, 12 of 102 participants (12%) indicated that a secondary finding would allow for a better understanding of the primary condition. In their responses, 10 of 99 (10%) participants cited a better understanding of their current, primary condition

Table 3. Participants' Reports of Actions Taken in the 4 Months after Receipt of a Positive Secondary Finding

Participant	Gene with Secondary Finding and MIM	Actions Recommended during Results Disclosure	Participant Follow-up Appointments/Actions	Participant Follow-up Tests/Actions Performed	Participant to Family Communication
1	*BRCA2* (600185)	contact GC at HBOC clinic	HBOC clinic, breast surgeon	breast MRI	husband, children
2	*DSG2* (125671)	contact GC at CG clinic	none	none	male cousin
3	*RYR1* (180901)	contact specific malignant hyperthermia specialist, inform surgeons immediately	geneticist	put result on registry	husband, children, four siblings
4	*BRCA2*	contact GC at HBOC clinic	HBOC clinic	breast MRI, oophorectomy scheduled	husband, children
5	*BRCA2*	contact GC at HBOC clinic	genetic counselor, followed by oncologist	Salpingo-oophorectomy, MRI (type not specified), CT scan	mother, cousins
6	*SDHB* (185470)	contact GC at endocrine cancer clinic	endocrinologist	endocrine testing, CT scan	child
7	*BRCA2*	contact GC at HBOC clinic	none	none	husband, mother, sibling
8	*DSC2* (125645)	contact GC at CG clinic	none	none	none
9	*APOB* (107730)	contact specific familial hypercholesterolemia specialist	none	none	none
10	*BRCA1* (113705)	contact GC at CG clinic	genetic counselor	mammogram	family (no specifics)
11	*KCNQ1* (607542)	contact GC at CG clinic	cardiologist	heart monitor, echocardiogram, EKG	children
12	*MYH7* (160760)	contact GC at CG clinic	cardiologist	echocardiogram	husband, parents, sibling
13	*DSG2* (125671)	contact GC at CG clinic	cardiovascular genetics clinic	cascade evaluations and testing of parents	aunts and uncles

Abbreviations: HBOC, hereditary breast and ovarian cancer; GC, genetic counselor; CG, cardiovascular genetics clinic.

as a reason why receiving a secondary finding would be valuable. When the question was subtly rephrased, asking how receiving a secondary finding would have been useful, 17 of 98 (17%) participants indicated that receipt of such of finding would have provided a better understanding of their current primary condition (see Table S4 for representative quotes).

Participants were asked to reflect on feelings of reassurance or disappointment after receiving a negative report. A majority of participants (64%) reported feeling reassured by not having received a secondary finding. The most frequently cited reasons included having additional information about one's health (33%) and a sense of ease and/or relief at not having secondary findings (22%). Close to a third (30%) of participants were disappointed to not receive a secondary finding. Reasons for disappointment included a lack of insight into the participant's primary diagnosis or medical condition (25%) and frank statements of different expectations from the study expressed by two participants (2%). Of 45 respondents who answered this question, only 10 (22%) indicated that they did not expect to receive such a finding. 17 (38%) participants mentioned anticipating receiving secondary findings based on their family history of medical problems.

Discussion

This study describes the results of a pilot of an approach to the integration of secondary genomic findings into clinical research. This approach to secondary findings analysis and return in research required minimal investment of time and resources on the part of the research groups as they were required only to obtain informed consent for the sequencing and secondary findings, order the clinical secondary findings analysis, and provide contact information for follow-up of positives. The approach we describe here is distinct from that which we have previously described.[16] In our previous work, we described a process whereby secondary findings from research NGS data could be validated with *post hoc* Sanger testing of variants. In the present work, the secondary findings were identified in an NGS test that is itself part of the CLIA license. That the secondary findings were CLIA-valid as NGS data provided us with an opportunity to study the consequences of negative results reporting, which is possible with CLIA-NGS, but not with *post hoc* Sanger testing. All sequenced individuals (positives and negatives) had a secondary finding clinical laboratory test report returned to their EMR. We undertook this study to perform an initial evaluation of this approach to secondary findings by evaluating the yield, interviewing

Table 4. Demographics of 107 Surveyed Recipients of Negative Secondary Findings Reports

	Adult (n = 93) (%)	Parent (n = 14) (%)
Gender		
Female	47 (51)	12 (86)
Male	45 (48)	2 (14)
Race		
White	79 (85)	12 (86)
Black or African American	7 (8)	1 (7)
Asian	3 (3)	1 (7)
Mixed	2 (2)	–
Ethnicity		
Hispanic or Latino	6 (7)	1 (7)
Not Hispanic of Latino	85 (91)	13 (93)
Education		
Less than high school	0 (0)	2 (14)
High school	8 (9)	1 (7)
Technical school	2 (2)	0 (0)
Some college	18 (19)	4 (29)
College graduate	22 (24)	6 (43)
Post-graduate	43 (46)	1 (7)
Relationship Status		
Divorced	8 (9)	2 (14)
Married	64 (70)	9 (64)
Single	17 (19)	3 (21)
Widowed	2 (2)	0 (0)
Age, years (mean, SD)	50 (15)	11 (5)
NIH Institute Conducting Primary Study[a]	**Number of Survey Respondents**	
NIMH	4	–
NIAMS	8	3
NINDS	19	5
NHLBI	19	5
NICHD	3	1
NIDCR	8	–
NCI	32	–
Total	93	14

[a]See Table S5 for details on the Primary Study Protocols.

recipients of positive secondary finding reports, and surveying individuals who received negative secondary finding reports.

The interviews with the recipients of a positive secondary finding report showed that they held generally positive views of learning the results. At the time of the interviews, no participants reported psychologic distress or regret at undergoing secondary finding testing. None of the 13 participants expressed regret at undergoing the secondary finding testing, an important extension of existing work indicating that research participants prefer to receive findings of this nature.[5] Two did express a form of regret in stating that they wished that they did not have the trait or disorder, although they then went on to say that even though they regretted having the condition, they would rather know about it than not know. This raises an important question surrounding the secondary findings debate, as these individuals are clearly articulating a distinction between their status of being at risk, which is aversive or regretful to them, and the secondary finding process itself. These individuals did not conflate these two related concepts and future research in secondary findings should explore this further.

Although 9 of 13 followed through with a recommended specialist, we were concerned that 4 of 13 (>30%) had not done so at the time of our interview. It is possible that with a family member sufficiently ill to be in a NIH research study, they may be logistically challenged to address a secondary finding. One participant was in her second trimester of her first pregnancy when she received her secondary finding. Two of the four individuals likely did not follow up because they minimized the secondary finding—one felt that the elevated cholesterol was already recognized and one was reluctant to undergo testing that might confirm a cardiomyopathy. This is interesting in light of the result above that all 13 expressed no regret with respect to learning their secondary finding. Of 13, 11 of these participants communicated results to at least 1 family member, although we noted that few of them knew what, if any, medical evaluation had been performed on these relatives and whether they had been confirmed to have the secondary finding disorder. Again, it is possible that a 4-month interval is too short, and we suggest that future studies should explore the cascade evaluations in family members over a longer time period. Another possibility is that they felt their obligation to communicate results was fulfilled and it was not their business to explore what their relatives had done with the information.

Our experience from the ClinSeq cohort was that some patients and providers were perplexed by secondary findings referrals and that follow-up was ineffective in those circumstances, as we did not provide specific referrals and follow-up recommendations (L.G.B. et al., data not shown). In this protocol, we identified local resources for follow up to ease this process both for the patient and for the providers. Although we were generally directive, specific, and unambiguous about the need for, and nature of, the indicated follow-up care, four participants reported not being evaluated by the recommended specialist. These findings contrast with what has been widely assumed, which is that patients will over-react, have psychologic distress, and undergo excessive health care utilization. These data may suggest that, in contrast to the fear of secondary findings, it may instead be the case that, like many

medical screening and health promotion activities, secondary findings may be ignored or have inadequate follow up. This would suggest that instead of fearing secondary findings, we may need to reorient the field toward more directive support and promotion of the necessary follow up.

An important component of this study is that we have described the responses of research participants to a negative secondary finding report. We surveyed 235 eligible individuals who received such a report. About half (107) responded to our survey regarding their perceptions of, and reactions to, these negative results. The survey evaluated the recipients' knowledge and attitudes toward sequencing and secondary findings, depression, anxiety, and asked open-ended questions on these topics. Participants demonstrated good understanding of benefits and limitations of genomic testing, although the sub-scale means observed in our sample were somewhat lower than those observed in a study of healthy individuals undergoing consent for sequencing.[17] In addition, participants rated the potential benefit of receiving secondary findings highly and did not show evidence of significant anxiety or depressive symptoms. While the sample is small, the oft-raised concerns of detrimental effects of a secondary finding were not supported by these data.

A majority of participants reported being reassured by not having received a secondary finding. Most frequently cited reasons for feelings of reassurance included having additional information about one's health and a sense of ease and/or relief at not having secondary findings. We did detect a surprising degree of confusion among these participants regarding the distinction of primary from secondary findings. Our questions around this issue were asked several different ways. One tenth to nearly one third of participants cited a better understanding of their current, primary condition (depending on how the question was asked) as a reason why receiving a secondary finding would have been valuable to them. This suggests that disappointment over negative secondary finding results is a consequence of two related issues—conflation of secondary with primary results and a lack of an understanding that a negative secondary finding result is, in most respects, the desired result. These findings further suggest that while a substantial majority clearly did understand the distinction of primary condition from secondary findings and that a negative result was a favorable outcome, there was a notable minority of individuals who were confused on this issue, demonstrating a need for enhanced patient communication interventions about the nature and meaning of secondary findings.

The debate regarding secondary findings in research is complex.[16,18,19] The President's Commission[1] has determined that there is no general obligation to return secondary genomic findings. We recognize this conclusion but also that some research studies include clinical activities and relationships that are indistinguishable from routine clinical care.[16] There is a wide spectrum of genomic research activities, ranging from pure research to substantial clinical activity. The NIH Clinical Center is a hospital solely focused on clinical research, yet in this environment many clinical tests are performed (clinical chemistry, microbiology, imaging, etc.) and in such instances clinical care guidelines are routinely and uniformly applied. These considerations have been recently analyzed and recommendations made for provision of secondary findings based on the nature of the study and the relationship of the participants and investigators.[16] These considerations include the nature of the relationship of the participant to the researcher (clinical versus non-clinical), the nature of the study, the attributes of the study population, and the timeliness of the sequencing in relation to the collection of the sample.

In some respects, it may be more compelling to return secondary findings to research participants, as compared to those undergoing clinical sequencing. Research participants have attributes that are compatible with return of secondary findings, such as being generally information seeking (as opposed to information avoiding), accepting of greater risks and uncertainties in the course of their care, and having high levels of altruism, in that secondary findings may benefit others in their families.[20,21] For these reasons, we suggest that there are compelling reasons to return secondary findings to clinical research participants who have a clinical care component to the studies in which they are enrolled.[16] Our approach of integrating a CLIA-compliant process for secondary findings into ESGS in the research setting offers evidence related to its effectiveness in addressing unknown health risks in a protocol that is acceptable to research participants.

There are a number of limitations of this study. The participants were more educated than average for the US population. The NIH Clinical Center is a research institution, not an academic medical center with a mix of clinical and research patients. However, with respect to both of these attributes, they are likely more representative of research participants in general (as only individuals engaged in a research study can be eligible to receive secondary findings from research sequencing), but may be less generalizable to non-research patients. The process and content of the informed consent procedures for the parent studies were not standardized and thus the potential role of specific components of the consent process in the outcomes we measured cannot be evaluated. The numbers of interviews for the positive findings was small and may not generalize to others who receive secondary findings. The probands for the primary research studies were a mix of children and adults and our sample size was inadequate to perform separate analyses, which could identify important and relevant issues of secondary findings in adult versus pediatric research. A final weakness of the study was that we did not measure costs. In spite of these limitations, these pilot data suggest a number of potentially interesting questions for further study including how to effectively communicate secondary

findings to ameliorate conflation of primary and secondary findings and false reassurance from negative reports, more effective interventions to communicate results to facilitate sharing of information, cascade testing, and clinical evaluations of secondary findings among at-risk relatives.

Overall, we have demonstrated that a hybrid research-clinical exome-sequencing process can be implemented in a way that provides clinical investigators with research-grade exome data and simultaneously provides participants with clinically validated secondary findings analysis. We have shown the feasibility of detecting these findings and returning them to participants by telephone and demonstrated predominantly appropriate follow up and little distress on the part of the recipients. We also show for that a substantial minority of individuals who receive negative secondary findings evaluations have some confusion of secondary and primary results and that further improvements in effective communication strategies are needed to maximize the benefits of return of secondary findings. We suggest that a secondary findings process similar to CCGO could be implemented at academic medical centers where individual research groups are performing exome or genome sequencing but may not have resources within their groups to support secondary findings. This process could be implemented through a research support facility such as a sequencing core within a Clinical and Translational Sciences Award (CTSA) support facility or other core facility that was connected to a clinical genetic consultation service. In this way, the subset of sequencing studies that warrant return of secondary findings could be properly supported.

Supplemental Data

Supplemental Data include five tables, cover letter, and genetic counseling outline and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.07.018.

Consortia

NISC Comparative Sequencing Group: Beatrice B. Barnabas, Gerard G. Bouffard, Shelise Y. Brooks, Holly Coleman, Lyudmila Dekhtyar, Xiaobin Guan, Joel Han, Shi-ling Ho, Richelle Legaspi, Quino L. Maduro, Catherine A. Masiello, Jennifer C. McDowell, Casandra Montemayor, Morgan Park, Nancy L. Riebow, Karen Schandler, Chanthra Scharer, Brian Schmidt, Christina Sison, Sirintorn Stantripop, James W. Thomas, Pamela J. Thomas, Meghana Vemulapalli, and Alice C. Young.

Acknowledgments

The CCGO sequencing was funded by the Deputy Director's Challenge Funding Program of the Intramural Research Program of the National Institutes of Health. J.C.S., J.J.J., K.D., A.R.H., K.L.U., E.T., B.B.B., and L.G.B. were funded by the Intramural Research Program of NHGRI including grants HG200359-09, HG200387-04, and HG200317-14. T.A.M. was supported by the Office of the Director of the National Human Genome Research Institute. These individuals supported CCGO by participating in one or more advisory committees: David Adams, Ivona Aksentijevich, Larry Brody, Kenneth Fischbeck, Suzanne Hart, Javed Khan, Paul Meltzer, Sergio Rosenzweig, Wendy Rubinstein, and Jeffrey Schloss. We are grateful to Ashlyn Gruber for assistance in sending out secondary findings reports and to Katie L. Lewis for returning one of the positive results.

Declaration of Interests

L.G.B. receives royalties from Genentech, honoraria from Wiley-Blackwell, and in-kind research support from ArQule Corp. and is an uncompensated advisor to the Illumina Corp.

Received: May 1, 2018
Accepted: July 24, 2018
Published: August 16, 2018

Web Resources

OMIM, http://www.omim.org/
Sequence Variant Interpretation, https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation

References

1. United States Presidential Commission for the Study of Bioethical Issues. (2013). Anticipate and communicate: ethical management of incidental and secondary findings in the clinical, research, and direct-to-consumer contexts. Presidential Commission for the Study of Bioethical Issues, Washington, D.C.
2. Jarvik, G.P., Amendola, L.M., Berg, J.S., Brothers, K., Clayton, E.W., Chung, W., Evans, B.J., Evans, J.P., Fullerton, S.M., Gallego, C.J., et al.; eMERGE Act-ROR Committee and CERC Committee; and CSER Act-ROR Working Group (2014). Return of genomic results to research participants: the floor, the ceiling, and the choices in between. Am. J. Hum. Genet. *94*, 818–826.
3. Wolf, S.M., Burke, W., and Koenig, B.A. (2015). Mapping the ethics of translational genomics: situating return of results and navigating the research-clinical divide. J. Law Med. Ethics *43*, 486–501.
4. Wynn, J., Martinez, J., Duong, J., Zhang, Y., Phelan, J., Fyer, A., Klitzman, R., Appelbaum, P.S., and Chung, W.K. (2015). Association of researcher characteristics with views on return of incidental findings from genomic research. J. Genet. Couns. *24*, 833–841.
5. Bollinger, J.M., Scott, J., Dvoskin, R., and Kaufman, D. (2012). Public preferences regarding the return of individual genetic research results: findings from a qualitative focus group study. Genet. Med. *14*, 451–457.
6. Klitzman, R., Appelbaum, P.S., and Chung, W. (2013). Return of secondary genomic findings vs patient autonomy: implications for medical care. JAMA *310*, 369–370.
7. Appelbaum, P.S., Waldman, C.R., Fyer, A., Klitzman, R., Parens, E., Martinez, J., Price, W.N., 2nd, and Chung, W.K. (2014). Informed consent for return of incidental findings in genomic research. Genet. Med. *16*, 367–373.
8. Green, R.C., Berg, J.S., Grody, W.W., Kalia, S.S., Korf, B.R., Martin, C.L., McGuire, A.L., Nussbaum, R.L., O'Daniel, J.M., Ormond, K.E., et al.; American College of Medical Genetics and Genomics (2013). ACMG recommendations for reporting of

incidental findings in clinical exome and genome sequencing. Genet. Med. *15*, 565–574.
9. Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., et al.; Exome Aggregation Consortium (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature *536*, 285–291.
10. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., et al.; ACMG Laboratory Quality Assurance Committee (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. *17*, 405–424.
11. Kaphingst, K.A., Facio, F.M., Cheng, M.R., Brooks, S., Eidem, H., Linn, A., Biesecker, B.B., and Biesecker, L.G. (2012). Effects of informed consent for individual genome sequencing on relevant knowledge. Clin. Genet. *82*, 408–415.
12. Weinfurt, K.P., Castel, L.D., Li, Y., Sulmasy, D.P., Balshem, A.M., Benson, A.B., 3rd, Burnett, C.B., Gaskin, D.J., Marshall, J.L., Slater, E.F., et al. (2003). The correlation between patient characteristics and expectations of benefit from Phase I clinical trials. Cancer *98*, 166–175.
13. Radloff, L.S. (1977). The CES-D scale a self-report depression scale for research in the general population. Appl. Psychol. Meas. *1*, 385–401.
14. Hays, R.D., Bjorner, J.B., Revicki, D.A., Spritzer, K.L., and Cella, D. (2009). Development of physical and mental health summary scores from the patient-reported outcomes measurement information system (PROMIS) global items. Qual. Life Res. *18*, 873–880.
15. Scheier, M.F., Carver, C.S., and Bridges, M.W. (1994). Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. J. Pers. Soc. Psychol. *67*, 1063–1078.
16. Darnell, A.J., Austin, H., Bluemke, D.A., Cannon, R.O., 3rd, Fischbeck, K., Gahl, W., Goldman, D., Grady, C., Greene, M.H., Holland, S.M., et al. (2016). A clinical service to support the return of secondary genomic findings in human research. Am. J. Hum. Genet. *98*, 435–441.
17. Sanderson, S.C., Suckiel, S.A., Zweig, M., Bottinger, E.P., Jabs, E.W., and Richardson, L.D. (2016). Development and preliminary evaluation of an online educational video about whole-genome sequencing for research participants, patients, and the general public. Genet. Med. *18*, 501–512.
18. Pyeritz, R.E. (2011). The coming explosion in genetic testing–is there a duty to recontact? N. Engl. J. Med. *365*, 1367–1369.
19. Kass, N.E., Faden, R.R., Goodman, S.N., Pronovost, P., Tunis, S., and Beauchamp, T.L. (2013). The research-treatment distinction: a problematic approach for determining which activities should have ethical oversight. Hastings Cent. Rep. (Spec No), S4–S15.
20. Loud, J.T., Bremer, R.C., Mai, P.L., Peters, J.A., Giri, N., Stewart, D.R., Greene, M.H., Alter, B.P., and Savage, S.A. (2016). Research participant interest in primary, secondary, and incidental genomic findings. Genet. Med. *18*, 1218–1225.
21. Facio, F.M., Brooks, S., Loewenstein, J., Green, S., Biesecker, L.G., and Biesecker, B.B. (2011). Motivators for participation in a whole-genome sequencing study: implications for translational genomics research. Eur. J. Hum. Genet. *19*, 1213–1217.

The American Journal of Human Genetics, Volume *103*

Supplemental Data

Evaluation of Recipients of Positive and Negative Secondary Findings Evaluations in a Hybrid CLIA-Research Sequencing Pilot

Julie C. Sapp, Jennifer J. Johnston, Kate Driscoll, Alexis R. Heidlebaugh, Ane Miren Sagardia, D. Nadine Dogbe, Kendall L. Umstead, Erin Turbitt, Ilias Alevizos, Jeffrey Baron, Carsten Bönnemann, Brian Brooks, Sandra Donkervoort, Youn Hee Jee, W. Marston Linehan, Francis J. McMahon, Joel Moss, James C. Mullikin, Deborah Nielsen, Eileen Pelayo, Alan T. Remaley, Richard Siegel, Helen Su, Carlos Zarate, NISC Comparative Sequencing Program, Teri A. Manolio, Barbara B. Biesecker, and Leslie G. Biesecker

Table S3: Pathogenic or likely pathogenic variants not returned to participants as part of this sub-study either because they were monoallelic (*MUTYH*) or primary variants					
Variant Genomic Position GRCh37	**Gene Name & MIM**	**Variant cDNA**	**Predicted Protein Change**	**ACMG Scoring**	**Pathogenicity Assessment, Clinical Action**
chr1:g.45797228C>T	*MUTYH* 604933	NM_001128425.1:c.1187G>A	p.(Gly396Asp)	PS3, PS4, PM3, PP1, PP3, PP5	ACMG: Pathogenic Not Returned, monoallelic
chr1:g.45797230T>C	*MUTYH*	NM_001128425.1:c.1187-2A>G	p.?	PVS1, PM3, PS4_Supporting, PP5	ACMG: Pathogenic Not Returned, monoallelic
chr1:g.45798466C>T	*MUTYH*	NM_001128425.1:c.545G>A	p.(Arg182His)	PS3, PM3, PM5, PS4_Supporting, PM2_Supporting, PP3, PP5	ACMG: Pathogenic Not Returned, monoallelic
chr1:g.45798475T>C	*MUTYH*	NM_001128425.1:c.536A>G	p.(Tyr179Cys)	PS3, PS4, PM3, PP1, PP3, PP5	ACMG: Pathogenic Not Returned, monoallelic
chr7:g.6048649A>C	*PMS2* 600259	NM_000535.5:c.2T>G	p.(Met1?)	PVS1, PM2_Supporting, PP5	ACMG: Pathogenic Returned: Pathogenic Primary Finding
chr13:g.32953959_32953963del	*BRCA2* 600185	NM_000059.3:c.9026_9030del	p.(Tyr3009Serfs*7)	PVS1, PS4, PM2_Supporting, PP5	ACMG: Pathogenic Returned: Pathogenic Primary Finding
chr16:g.2138118G>A	*TSC2* 191092	NM_000548.3:c.5138G>A	p.(Arg1713His)	PS3_Moderate, PS4_Moderate PM2_Supporting, PP3, PP5	ACMG: Likely Pathogenic Returned: Likely Pathogenic Primary Finding
chr17:g.41276047_41276048del	*BRCA1* 113705	NM_007294.3:c.68_69del	p.(Glu23Valfs*17)	PVS1, PS4, PS3_Moderate, PP1, PP5	ACMG: Pathogenic Returned: Pathogenic Primary Finding
chr19:g.11200230del	*LDLR* 606945	NM_000527.4:c.6del	p.(Trp4Glyfs*202)	PVS1, PS4_Moderate, PM2	ACMG: Pathogenic Returned: Pathogenic Primary Finding

Table S3 (cont.): Pathogenic or likely pathogenic variants not returned to participants as part of this sub-study either because they were monoallelic (*MUTYH*) or primary variants

Variant Genomic Position GRCh37	Gene Name & MIM	Variant cDNA	Predicted Protein Change	ACMG Scoring	Pathogenicity Assessment, Clinical Action
chr19:g.11213445C>G	*LDLR*	NM_000527.4:c.296C>G	p.(Ser99*)	PVS1, PS4, PM2_Supporting	ACMG: Pathogenic Returned: Pathogenic Primary Finding
chr19:g.11216024T>C	*LDLR*	NM_000527.4:c.442T>C	p.(Cys148Arg)	PM2, PM5, PS4_Moderate, PP3	ACMG: Likely Pathogenic Returned: Upgraded, not included in results of study Primary Finding
chr19:g.11216096G>A	*LDLR*	NM_000527.4:c.514G>A	p.(Asp172Asn)	PS3_Moderate, PM5, PS4_Supporting, PM2_Supporting, PP5	ACMG: Likely Pathogenic Returned: Likely Pathogenic Primary Finding
chr19:g.11216171T>G	*LDLR*	NM_000527.2:c.589T>G	p.(Cys197Gly)	PM2, PM5, PS4_Supporting, PP3	ACMG: Likely Pathogenic Returned: Likely Pathogenic Primary Finding
chr19:g.11216236_11216238del	*LDLR*	NM_000527.4:c.655_657del	p.(Gly219del)	PS4, PS3_Moderate, PM4, PM2_Supporting, PP5	ACMG: Pathogenic Returned: Pathogenic Primary Finding
chr19:g.11218160G>A	*LDLR*	NM_000527.4:c.910G>A	p.(Asp304Asn)	PS3_Moderate, PM5, PS4_Supporting, PP3, PP5	ACMG: Likely Pathogenic Returned: Likely Pathogenic Primary Finding

[a.] ACMG refers to pathogenicity assessments per the ACMG/AMP criteria of Richards et al. The evidence codes are as specified in that manuscript, with the modification codes as per the ClinGen web site (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/)

Table S4 – Selected quotations from interviewed/surveyed participants

Quotes from interviews[a] with recipients of secondary findings		
Participant Characteristics	**Quote**	**Code**
61-year-old female recipient of a secondary *BRCA2* result enrolled in an NIDCR study	*"It was totally a shock for me. One, not knowing how to communicate to my family, because we never knew -- I never knew we had that. And two, […] how do I cope with it now? That is the only downfall, I would say, that it is the surprise. We were not expecting anything. It was a total shock, a surprise. You know, it was not a pleasant surprise."*	Initial reactions
	"I think I'm more at ease now, and I […] really thank God for having to go through the other protocol that I was going [through] if it wasn't for that, I don't think I would have ever found out."	Feelings changing over time
61-year-old female recipient of a secondary *BRCA2* result enrolled in an NCI study	*"So, honestly all of this has been positive. I'm very glad that I did the study and that we found this [secondary finding] at the point where [the researchers] did find it. So, yes, it was a surprise, but it was okay I guess this is a good thing to know about."*	
61-year-old male recipient of a secondary *DSG2* result enrolled in an NICHD study	*"I think it's useful enough that I need to take action on it. […] bad things can happen to you and [you] go, "Oh my God, I didn't know that." But, in this case I have some warning that I do.* *[…] You never know when something like that's going to pop up and cause you a serious problem. Or it could even kill you, I guess. […] Well, it gives me a chance to do something about it."*	Reassured/grateful for knowledge

Example responses[b] from surveyed participants who received negative secondary findings reports			
Question text	**Participant Characteristics**	**Quote**	**Code**
"In what ways do you think learning a secondary finding may have been useful to you?"	22-year-old male recipient of a negative SF report enrolled in an NINDS study	*"I think it's very beneficial because at the end of the day I go to sleep knowing we are one step closer to a diagnosis."*	Better understanding of current/primary condition
	55-year-old female recipient of a negative SF report enrolled in an NIDCR study	*"…could have helped me understand where any pain, soreness, or lack of energy issues i have may have been coming from."*	

	70-year-old female recipient of a negative SF report enrolled in an NINDS study	*"Knowing how to manage or watch for particular signs or symptoms related to the findings"*	Seek follow-up care related to secondary finding
"If you had received a secondary finding, what would it have meant for your health and well-being?"	20-year-old female recipient of a negative SF report enrolled in an NIAMS study	*"If the second finding revealed that my lupus had gotten worse, I would seek out more help."*	Better understanding of current/primary condition
	41-year-old male recipient of a negative SF report enrolled in an NINDS study	*"…it means I can more properly and accurately deal with the issues I am having."*	
	25-year-old male recipient of a negative SF report enrolled in an NCI study	*"It would have meant that I would take precautions in my life to prevent it from developing if possible or make regular check ups to see if something develops from it."*	Seek followup care related to secondary finding
"How valuable do you believe this information may have been to you and your family?"	56-year-old male recipient of a negative SF report enrolled in in an NCI study	*"…this disease affects not just me, it affects the whole family support system. information gives us power and hope to proceed in the right direction."*	Valuable/useful
	64-year-old male recipient of a negative SF report enrolled in in an NCI study	*"Very valuable. If I can help my family live a healthier life it is definitely worth it."*	Valuable/useful

[a.] Quotes selected from various portions of interview

[b.] Question prompts from survey provided for each quote

Table S5: Protocol titles and NIH Institutes supported by CCGO

NIH Institute	Protocol title(s)
National Institute of Dental and Craniofacial Research (NIDCR)	Characterization of Diseases With Salivary Gland Involvement (NCT02327884)
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)	Genetic Causes of Growth Disorders (NCT02311322)
National Heart, Lung, and Blood Institute (NHLBI)	Characterization of the Pathogenesis of Primary and Secondary Lymphatic Disorders (NCT02156115)
National Institute of Neurological Disorders and Stroke (NINDS)	Genetic and Physical Study of Childhood Nerve and Muscle Disorders (NCT01568658)
National Heart, Lung, and Blood Institute (NHLBI)	Genes Involved in Lipid Disorders (NCT02311335)
National Institute of Allergy and Infectious Diseases (NIAID)	NIAID Clinical Center Genomics Opportunity Protocol (NCT02417766)
National Eye Institute (NEI)	Whole Exome and Whole Genome Sequencing for Genotyping of Inherited and Congenital Eye Conditions (NCT02077894)
National Institute of Mental Health (NIMH)	Evaluation of the Genetics of Bipolar Disorder (NCT00001174)
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)	Study of Systemic Lupus Erythematosus (NCT00001372) Natural History and Development of Spondyloarthritis (NCT01422694)
National Cancer Institute (NCI)	Collection of Serum and Tissue Samples From Patients With Biopsy-Proved or Suspected Malignant Disease (NCT00026884)
National Human Genome Research Institute (NHGRI)	Genetic, Brain Structure, and Environmental Effects on ADHD (NCT01721720)

S1. Cover letter sent with negative findings

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Social and Behavioral Research Branch
National Human Genome Research Institute
National Institutes of Health
31 Center Drive (31/B1B36) – MSC 2073
Bethesda, MD 20892-2030
(301) 443-0283

Date

Dear

As you know, you recently participated in a research study at the National Institutes of Health (NIH) whose goal was to learn the genetic cause of a health condition that affects you or a family member. This study uses extensive genetic testing technology that allows researchers to "read through" most of a person's genes. This technology is called "genome sequencing."

In addition to helping NIH investigators understand more about the genetics of a particular health condition, genome sequencing can tell us about other genetic differences that a person may have that are unrelated to the reason why they had the testing done. We call these differences "secondary variants." In the NIH study you participated in we routinely screen for secondary variants. If we identify one or more secondary variants that could be very important for a person's health we notify the participant.

We have about 20,000 genes and in this study we look carefully for secondary variants that could be important for a person's health in only 56 of them. The American College of Medical Genetics endorses these genes as medically actionable because we can interpret the risk to a person's health and we have experience in helping people manage the risk to reduce disease. Variants in these genes are rare (about 2-3%) so most people do not have one.

The attached report indicates that we **did not find** any highly significant secondary variants in in this limited set of genes. Stated another way, when looking at a very limited group of genes that, when altered, can cause significant and treatable health problems, we did not find any genetic variants that we think would be very important for you to know about. It is important to remember that genome sequencing technology is not perfect. Also, the attached report is relevant to only a very small fraction of your genes – it is very likely that there are other genetic variants present that are not covered by this report.

Through participation in this study, we would like to ask you to complete an online follow-up survey in three months. You will be contacted by the research team at that time with a web-link to complete the survey. Thank you very much for participating in our study.

Sincerely,

Name of Research Assistant
NIH/NHGRI/SBRB
CCGO 0017 Research Assistant

S2. Genetic counseling outline for positive disclosure sessions

1. Introduction
    a. Review of goal of primary research study
    b. Definition/review of primary and secondary genetic variants
2. Contracting
    a. Goal of session is to disclose a positive secondary variant
    b. Provide participant with information and support necessary so that they can initiate
        i. Specific healthcare actions
        ii. Familial conversations
        iii. Psychosocial adaptation
3. Disclosure of result
    a. Basic and brief genetics review
    b. Gene and mutation disclosure
        i. Evidence for pathogenicity
        ii. Who carries mutation (proband only or parent as well)
    c. Phenotype associated with mutation
    d. Inheritance pattern of associated disorder
    e. Overview of clinical recommendations specific to finding
4. Risk assessment
    a. Targeted medical history
    b. Targeted family history
5. Psychosocial assessment
    a. Psychological impact of variant receipt
    b. Limited psychosocial intervention
    c. Referral if needed
6. Plan
    a. Documentation of finding
        i. Logistics of report receipt (overnight FedEx)
        ii. CRIS Genetic Counseling Note
    b. Recommendations regarding additional validation if needed
    c. Referrals
        i. Local genetics services
        ii. Specific support group/disease information websites/resources
    d. Questions/concerns
        i. Understanding/clarification
        ii. Familial communication/testing
        iii. Barriers to referrals
    e. Communication with primary team
        i. Result disclosure summary
        ii. Referral back to primary team for questions regarding primary variant