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Strategy for reporting secondary findings for diagnostic exome sequencing (DES).

Background

- Diagnostic exome sequencing (DES) results in the identification of thousands of variants unrelated to the indication for testing, often referred to as "incidental" or "secondary findings" (SFs). While the vast majority of these are likely benign polymorphisms or within genes whose function or pathogenicity remains obscure, a small number are well-described disease-associated mutations that may have important clinical and medical significance for the patient. For example, nonbiased massively parallel sequencing-based carrier-screening assays show that every individual carries an average of 2.8 mutations among genes known to cause severe pediatric disease in the recessive form. Among 37 well-described cancer predisposition genes, ~1% of individuals manifest a likely pathogenic cancer predisposition (Bell *et al.,* 2011).
- The program director of the NHGRI's Ethical, Legal, and social Implications Research Program likened the topic to "arguably the most pressing issue in genetics today." (Couzin-Frankel *et al.*, 2011).
- Recently, the ACMG set forth recommendations for reporting secondary findings from a list of 57 previously defined genes for all patients undergoing exome sequencing (Figure 2)(Green *et al.*, 2013).
- Herein, we provide a strategy for reporting secondary findings results, based on recent ACMG recommendations, empirical data from the preferences of the first 200 families undergoing DES at Ambry Genetics, and recent literature regarding disclosure of secondary findings in both children and adults (including Wolf *et al., 2012*; Fabstitz *et al.*, 2010; Berg *et al.,* 2012) (Table 1).

Strategies Utilized

Reference	Conclusion
ACMG Points to consider in clinical application of genomic sequencing . (Policy Statement, 2012)	1. Recommend disclosing the SF policy to patients and giving patients the option of not receiving certain or SFs.
ACMG Recommendations for reporting SFs. (Green *et al.*, 2013)	1. Recommended list of 57 genes to report 2. Report SFs for all patients regardless of age or preference for findings.
Retrospective study of patient preferences for SF results (Shahmirzadi *et al*, in press).	1. Study concluded that majority of patients chose to receive all available SF results including carrier status, cancer predisposition, adult-onset, and early-onset disease. 2. Provide patients with option to receive an expanded SF report with list of genes beyond the ACMG minimum.
Ethical Considerations	1. Based on the basic ethical principles and the discussion of delivery of secondary findings in minors, all strategies were developed to allow patients to opt out of receiving secondary findings. 2. Medical ethics consultations with bioethicists , genetic counselors, and geneticists.

Table 1. Delivery of Secondary Findings in Minors

General points:	Reference
• Testing in minors has been discouraged when the benefits of the test will not accrue until adulthood such as with late-onset diseases or conditions without medical management guidelines and is only recommended when the current potential benefits outweigh the harms.	Abdul-Karin et al., 2013; Wolf *et al.*,I 2008
• A recent chronological review of the ethical arguments for predictive genetic testing in minors describes a gradual shift toward ethical arguments in favor of testing.	Mand *et al.*, 2012
• Cognitive impairment has been discussed among the limited circumstances in which predictive genetic testing in minors may be appropriate.	Ross *et al.*, 2013

Arguments against disclosure of predictive test results in minors (Abdul-Karin R, *et al.*, 2013; Mand C, et al. 2012)	**Arguments in favor of disclosure of predictive test results in minors** (Duncan RE, et al.,2005; Duncan RE, et al., 2008; Mand C, et al. 2012; Murphy J, et al., 2008; Ross LF, *et al.*, 2013)
• Hindrance of confidentiality • Respect for patient autonomy (in reference to the child's future choice as an adult)	• Avoidance of paternalism • Multiple studies cite that few patients have regretted receiving results as a child • Psychological benefits including reduction of anxiety • Increase patient autonomy (in reference to the child's current choice)

Results

- By default, all patients undergoing DES will receive a secondary report with analysis of the **ACMG minimum gene list**.
- **For the Clinical Diagnostic Exome (CDE) orders only, patients may consent to a** patient-driven secondary findings report which includes the ACMG minimum list of genes in addition to options from the following four disease categories:
  - A) Recessive disease carrier status
  - B) Cancer predisposition
  - C) Early-onset disease
  - D) Late-onset disease predisposition
- All patients have the **option to opt-out** of all secondary findings
- Only alterations classified as **"mutation"** are reported
- Report issued for **proband** only.

Figure 1. Secondary Findings Report Options

FIRST TIER EXOME OR FIRST-TIER WITH REFLEX TO (CDE):	CLINICAL DIAGNOSTIC EXOME (CDE):
__ ACMG MINIMUM LIST	__ ACMG MINIMUM LIST Ambry's Secondary Findings Categories: __ Recessive disease carrier status __ Cancer predisposition __ Adult-onset disease predisposition __ Early-onset disease
__ I DO NOT WANT ANY SECONDARY FINDINGS REPORTED	__ I DO NOT WANT ANY SECONDARY FINDINGS REPORTED

Figure 2. ACMG Minimum List of Genes

Ambry Genetics

GENE	DISORDER
BRCA1, BRCA2,	Hereditary Breast and Ovarian Cancer
TP53,	Li-Fraumeni Syndrome
STK11,	Peutz-Jeghers Syndrome
MLH1, MSH2, MSH6, PMS2,	Lynch Syndrome
APC,	Familial adenomatous polyposis
MUTYH	MYH-Associated Polyposis: Adenomas, multiple colorectal, FAP type 2; Colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas
VHL,	Von Hippel Lindau syndrome
MEN1,	Multiple Endocrine Neoplasia Type 1
RET,	Multiple Endocrine Neoplasia Type 2
RET, NTRK1,	Familial Medullary Thyroid Cancer (FMTC)
PTEN,	PTEN Hamartoma Tumor Syndrome
RB1,	Retinoblastoma
SDHD, SDHAF2, SDHC, SDHB	Hereditary Paraganglioma- Pheochromocytoma Syndrome
TSC1, TSC2,	Tuberous Sclerosis Complex
WT1,	WT1-related Wilms
NF2,	Neurofibromatosis type 2
COL2A1,	EDS - vascular type
FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYLK, MYH11,	Marfan Syndrome, Loeys-Dietz Syndromes, and Familial Thoracic Aortic Aneurysms and Dissections
MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA,	Hypertrophic cardiomyopathy, Dilated cardiomyopathy
RYR2	Catecholaminergic polymorphic ventricular tachycardia
PKP2, DSP, DSC2, TMEM43, DSG2	Arrhythmogenic right ventricular cardiomyopathy
KCNQ1, KCNH2, SCN5A,	Romano-Ward Long QT Syndromes Types 1, 2, and 3, Brugada Syndrome
LDLR, APOB, PCSK9,	Familial hypercholesterolemia
RYR1, CACNA1S	Malignant hyperthermia susceptibility

Figure 3. Patient-Driven Secondary Findings Report

Ambry Genetics — SAMPLE REPORT - 09/10/12

Ordered By
Physician: Sample Doctor
Ph: 888-999-1010 Fx: 949-900-5501
Client: Sample Client 2
12345 Wonderful Lane Orgggggg Address
Somewhere NY 99999-9999
Contact ID: 3965 Org ID: 00403

Patient Name: Sample, Report Exome
Accession #: 11-40998 Specimen#: RFID
Specimen: Blood
Birth Date: 01/01/80 Age: 31y 8m
Gender: F Collected: 09/02/11
MRN#: Received: 09/29/11
Family#: 52711 Authorized: 09/29/11
Ethnicity: Caucasian
Indication: Carrier Screen

CLINICAL DIAGNOSTIC EXOME SECONDARY FINDINGS REPORT

The following report of secondary findings is provided for carrier status of autosomal recessive disorders, cancer pre-disposition syndromes, and adult and childhood onset disorders. Alterations to be reported for secondary findings include previously described alterations reported within peer-reviewed publications or known deleterious mutations based upon ACMG guidelines in established disease causing genes (ACMG Recommendations for Standards for Interpretation and Reporting of Sequence Variations. Revision 2007. Genet Med 2008;10:294). Literature support is evaluated for evidence. Final interpretation of pathogenicity may differ from that presented by HGMD due to lack of evidence in the primary literature cited or subsequent conflicting evidence. Novel alterations, synonymous alterations, or variants of unknown significance in known disease causing genes are not provided. ACMG category mutations are not provided in this report for genes that are not yet known to be associated with disease.

The following alterations were flagged as disease causing based upon ACMG guidelines or citation in HGMD.

The following alterations were not confirmed by Sanger sequencing. Confirmation of these results by Sanger sequencing or another method of analysis is recommended prior to clinical utilization. To find a laboratory offering clinical testing for a specific gene or condition please visit: GeneTests (http://www.ncbi.nlm.nih.gov/sites/GeneTests/) or the Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/).

Genetic counseling is a recommended for all patients undergoing genetic testing.

RESULTS SUMMARY

Carrier Status (Recessive Disorders)

GENE	MUTATION	EXON	INCLUSION CRITERIA	HGMD/OMIM	INHERITANCE	PROBAND
ARSA	p.L428P	8	Gieselmann, V et al. 1994	CM940113	Autosomal Recessive	Heterozygous
ATM	p.L1420F	28	Fletcher, O et al. 2010	CM000653	Autosomal Recessive	Heterozygous
DGUOK	p.Q170R	4	Freisinger, P et al. 2006	CM063953	Autosomal Recessive	Heterozygous
MPL	c.1653delG	11	ACMG	No Record	Autosomal Recessive	Heterozygous
TCTN1	p.M1I	1	ACMG	No Record	Autosomal Recessive	Heterozygous

***ARSA* p.L428P (c.1283C>T)**
The *ARSA* gene encodes the arylsulfatase A enzyme which catalyzes the degradation of cerebroside-3-sulfate (sulfatide). Mutations in *ARSA* are associated with autosomal recessive metachromatic leukodystrophy (MLD) characterized by accumulation of intralysosomal cerebroside sulfate in the cells of the white matter within the central and peripheral nervous systems resulting in progressive demyelination and various neurological impairments (Eng, B. et al. *Hum Mutat* 2003;22:418-421, Grossi, S et al. *Hum Mutat* 2008;29:E220-E230).

***ATM* p.L1420F (c.4258C>T)**
The *ATM* gene encodes for the ataxia telangiectasia (A-T) mutated protein which is involved in the detection and repair response to DNA double-stranded breaks. Homozygous and compound heterozygous mutations in *ATM* gene are associated with autosomal

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Table 2. Reportable Mutations

Reportable Mutations
Mutations with: • HGMD/OMIM mutation record – or- • Classification as mutation based on ACMG-guidelines No variants of uncertain significance
Variants of uncertain significance (VUS) and benign alterations not reported
SFs report issued for proband only

Conclusions

- This strategy supports the recommendations set forth by ACMG, while preserving the autonomy of the patient.

References

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