Research Paper

Incidentalome from Genomic Sequencing: A Barrier to Personalized Medicine?

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A R T I C L E   I N F O

A B S T R A C T

Background: In Western cohorts, the prevalence of incidental findings (IFs) or incidentalome, referring to variants in genes that are unrelated to the patient's primary condition, is between 0.86% and 8.8%. However, data on prevalence and type of IFs in Asian population is lacking.
Methods: In 2 cohorts of individuals with genomic sequencing performed in Singapore (total n = 377), we extracted and annotated variants in the 56 ACMG-recommended genes and filtered these variants based on the level of pathogenicity. We then analyzed the precise distribution of IFs, class of genes, related medical conditions, and potential clinical impact.
Results: We found a total of 41,607 variants in the 56 genes in our cohort of 377 individuals. After filtering for rare and coding variants, we identified 14 potential variants. After reviewing primary literature, only 4 out of the 14 variants were classified to be pathogenic, while an additional two variants were classified as likely pathogenic. Overall, the cumulative prevalence of IFs (pathogenic and likely pathogenic variants) in our cohort was 1.6%.
Conclusion: The cumulative prevalence of IFs through genomic sequencing is low and the incidentalome may not be a significant barrier to implementation of genomics for personalized medicine.

1. Introduction

Incorporation of whole genome or exome sequencing (WGS/WES), hereafter referred to as genomic sequencing, in medical practice raises the disquieting issue of incidental findings (IFs), which has important and potentially far-reaching implications (Green et al., 2012; Knoppers et al., 2013; Wolf et al., 2012; Roche and Berg, 2015; Hegde et al., 2015; Ayuso et al., 2015; Krier and Green, 2015). IF, also called secondary findings and occasionally referred to as incidentalomes, are mutations in genes unrelated to the primary condition (phenotype) of the patient (Krier and Green, 2013). As genomic sequencing is a phenotype-agnostic test, it is not surprising that detection of IFs is of major concern and requires the decision of

whether and how return of these results to the individual should be practiced (Krier and Green, 2013). Another concern revolves around the additional burden this creates on the healthcare system. Individuals with medically actionable IFs will require long-term surveillance and anticipatory care, which is acceptable when it is appropriate, but may be hard to justify if there is only uncorroborated evidence for the pathogenicity of the mutation in question: e.g. even if the gene is a causal gene for the condition, the mutation could be a novel one and never reported before for the condition.

The American College of Medical Genetics and Genomics (ACMG) has recommended return of IFs for a minimum set of 56 actionable genes, where prevention and surveillance may significantly reduce mortality and morbidity (Green et al., 2013). While these 56 genes represent rare Mendelian disorders, backed by substantial years of prior research and clinical experience, mutations in these genes are indeed highly medically actionable and include well-publicized ones like *BRCA1* and *BRCA2*. By being classed as "medically actionable", these

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genes were prioritized to include disorders where preventative measures and/or treatments are available. For example, patients with mutations in cardiomyopathy-causing genes such as *MYH7* may have annual electrocardiogram (ECG) and echocardiography. Individuals with pathogenic mutations in these genes might be asymptomatic for long periods of time and therefore amenable to early intervention and prevention to reduce mortality and long-term morbidity. Patients with pathogenic *BRCA1* and *BRCA2* mutations have an 80% and 45% risk of developing breast cancer, respectively (Ford et al., 1998). Identification of a pathogenic variant in *BRCA1*/*BRCA2* significantly reduces the risk of developing breast cancer as close surveillance by an oncologist with clinical assessment, self-examination, mammogram and/or breast MRI allows for early detection, which, in turn leads to reduced morbidity and improved survival (Krier and Green, 2015; Krier and Green, 2013; Green et al., 2013; Ford et al., 1998; Warner et al., 2004). However about 50% of women harboring *BRCA1*/*BRCA2* mutation do not have a family history (Loman et al., 2001) and, hence, screening for breast cancer may not be recommended in this group of women. Indeed the absolute prevalence of breast cancer in this group of women is unclear. The incidental identification of individuals with these disease causing variants therefore allows the recommendation of follow-up screening offers a net benefit to individuals and society. Overall, the selection of these 56 genes may be conservative because many other genes are becoming medically important and actionable by the month.

The European Society of Human Genetics (ESHG), on the other hand, has recommended against using genomic sequencing in the clinic, and instead recommends the use of targeted genomic tests; clearly in an effort to avoid the scenario of unexpected IF (van El et al., 2013). However, as the cost of genomic sequencing continues to drop, genomic sequencing in practice is inevitable and IF will be clearly an issue that we cannot avoid.

Although with good intent, the first issue that arises from such recommendations includes lack of data on the frequency of IFs to determine the burden on the testing laboratory as well as the referring physician and even the healthcare system. A recent review of exome sequencing data from 1000 individuals (500 European and 500 African descents) recruited in the National Heart, Blood and Lung Initiative (NHBLI) Exome Sequencing Project (ESP) estimated the prevalence of IFs at 3.4% for European descent and 1.2% for African descent (Dorschner et al., 2013). A follow-up study expanded to include 6503 individuals (4300 European and 2203 African ancestry) estimated the frequency of IFs at 1.7% for individuals of European ancestry and 1.0% for African ancestry (Amendola et al., 2015). In unrelated cohorts, IFs were detected in 8.8% of the participants recruited through National Institute of Health Undiagnosed Disease Program (n = 543) (Lawrence et al., 2014), 0.86% in the Baylor-Hopkins Center for Mendelian Genomics (n = 232) (Jurgens et al., 2015), and 1.9% in the UK WGS500 cohort (n = 500) (Taylor et al., 2015). Within Asia, a review of 196 Korean exomes detected IFs in 7% of control subjects (n = 100) and 6% of patients with disease (n = 96) (Jang et al., 2015). Although Singapore is a nation gearing up for genomics (Manolio et al., 2015), such data is lacking for an Asian population and hence, there are no policies and recommendations regarding IFs in Singapore. In this study, we set out to estimate the prevalence as well as define the types of IFs found in genomic sequencing that was performed in 2 cohorts of the Asian population in Singapore. Singapore, as an island country in South East Asia, is uniquely dominated by immigrant ethnic groups, comprising Chinese, Malays, and Indians. The IF analysis from our study should hence be representative of the profile in South East Asia.

2. Methods

2.1. Patient Recruitment

Individuals were recruited through institutional ethics review board approved genomics projects. Informed consent was obtained from the eligible individual (or parent/legal guardian, when the individual is a minor). Sequencing data of these individuals was anonymized, de-identified and analyzed in a cumulative manner.

2.2. Genomic Sequencing

Blood samples were obtained from the consented individuals and DNA was extracted by established methods. Samples were sequenced on HiSeq 2000/HiSeq 2500 or Ion Proton using established protocols. Data generated from genomic sequencing were aligned to the human reference genome using established bioinformatic algorithms and software (e.g. BWA-MEM followed by SAMtools to generate SAM/BAM files) (Li and Durbin, 2009). BAM files were processed using GATK (DePristo et al., 2011) to generate variant calling format (VCF) files.

2.3. Gene List Development

The list of genes we chose to analyze was confined to the 56 actionable genes recommended by ACMG (Supplementary Table 1) (Green et al., 2013). These genes were selected on the basis that deleterious variants would lead to specific conditions of high disease penetrance, for which evidence-based medical recommendations are available, implementation of which would arguably help towards preventing significant morbidity and mortality.

2.4. Bioinformatic Filtering

Variants were quality filtered to exclude false positives according to standard thresholds (Quality scores >30, coverage >10×, and absence of clustered variants within a window size of 10 variants). From variants that passed this threshold, we extracted variants in each of the 56 genes in our gene list (Appendix 1) (Green et al., 2013). We then annotated the variants using our in-house bioinformatic pipeline to include information regarding the gene, chromosomal coordinate(s), genetic change, protein change, type of mutation (frameshift, nonsense, nonsynonymous, splicing, and synonymous); prediction of the variant from multiple algorithms (Polyphen-2, Adzhubei et al., 2013, SIFT, Ng and Henikoff, 2003, likelihood ratio test and MutationTaster2, Schwarz et al., 2014), allele frequencies in different databases (Exome Sequencing Project, dbSNP, 1000 Genomes, Complete Genomics, Exome Aggregation Consortium, and our in-house database of common variants (present in >5% of the population)), and annotation of variants in clinical mutation databases like Clinvar (http://www.ncbi.nlm.nih.gov/clinvar/), and Human Genetic Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) (Fig. 1). We then further analyzed the variant as per our filtering strategy illustrated in Fig. 1.
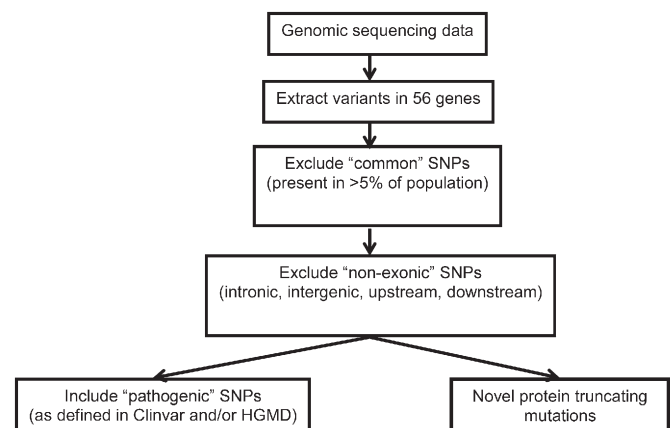

Fig. 1. Filtering strategy.

2.5. Criteria for Classification of Variants

Given that we are addressing IFs, we used stringent criteria for the classification of the variants to avoid false positives. Although certain mutations listed in the clinical mutation databases have been listed as pathogenic, the evidence for pathogenicity of some of these variants may be lacking (McLaughlin et al., 2014; Richards et al., 2015). Hence, we reviewed the primary literature regarding each of the filtered variants and reclassified them as per Table 1.

2.6. Quality Control

We bioinformatically reviewed the sequence aligned read of each of the samples to ensure that all exons and intron-exon junction of the 56 genes were adequately covered by the genomic sequencing. We excluded samples in which the genes were inadequately covered so as to reduce false negatives, which would underestimate the frequency of IFs.

2.7. Funding

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3. Results

3.1. Characteristics of the Participants

Individuals were recruited from 2 diverse cohorts of participants (Table 2). The first cohort (n = 245) comprised of individuals and their biological parents undergoing genomic sequencing to investigate the underlying genetic etiology of the individual's multiple congenital anomalies, developmental delay and/or intellectual disability. Genomic data from the proband as well as their family members was included in the analysis. However, a variant identified in related individuals was counted as one to avoid double counting of familial variants. The second

cohort (n = 138) comprised of adults who underwent genomic sequencing for population based studies and were reportedly healthy. Six of the samples did not fulfill QC criteria and were excluded from this analysis. Details of the second cohort have been published previously (Wong et al., 2013, 2014). Individuals in cohort one were given the option regarding return of IFs. Eighty-three percent opted for the return of such results.

3.2. Characteristics of Variants

In the first cohort, we identified 1381 variants in the 56 genes (Table 3). After excluding common variants, we found 6 variants that were reportedly pathogenic mutations according to Clinvar and/or HGMD. In the second cohort, we identified 40,226 variants in the 56 genes. As this cohort had undergone whole genome sequencing, the majority of these variants were in the non-coding region of the 56 genes. After filtering for coding and rare variants, we identified 5 variants that were reportedly pathogenic according to Clinvar and/or HGMD. As these mutation databases are biased against non-Caucasian population (Dorschner et al., 2013), we further analyzed for novel protein truncating mutations (stopgain, stoploss, frameshift, indels) in these 56 genes, and found three additional variants (one in cohort 1 and two in cohort 2). None of these variants were detected in more than one individual.

3.3. Variant Reclassification Based on Primary Literature

Out of the 14 pathogenic variants, two of the variants were in genes (*MUTYH* and *PCSK9*) where disease manifests in the presence of biallelic mutations and hence, detection of monoallelic variants in these genes was considered to be non-disease causing. Of the remaining 12 variants, we reviewed the primary literature for each of the variant and reclassified them as per our criteria (Table 4). Only four of the 12 variants were classified as pathogenic — two in *SCN5A* (associated with Brugada syndrome, OMIM: 601144), and one each in *BRCA2* (associated with hereditary breast and ovarian cancer, OMIM: 612555), and *TP53* (associated with Li-Fraumeni syndrome; OMIM: 151623) (Table 2); while an additional two variants were classified as likely pathogenic. Both were truncating mutations, one each in *TNNT2* (associated with cardiomyopathy, OMIM: 601494), and *COL3A1* (associated with Ehlers Danlos syndrome, vascular type, OMIM: 130050). The remaining six variants were classified as "variant of unknown significance" (VUS) (4 variants) and likely benign (2 variants) (Table 4). Overall, the cumulative prevalence of pathogenic IFs in our cohort of 377 individuals was 1.1%. After including likely pathogenic IFs, the estimated prevalence was 1.6%.

4. Discussion

Sequencing of the first human genome took more than a decade, completing in 2001, and costing more than US$2.7 million (Lander et al., 2001; Venter et al., 2001; NHGRI, 2010). In the subsequent decade, newer high-throughput genomic technologies, known as the Next-Generation sequencing technologies, have been able to sequence and detect genetic variation in humans with high levels of accuracy, at breakneck speeds and at a fraction of that cost: offering the promise of fundamentally changing medical practice and finally delivering personalized medicine (Manolio et al., 2013; Mayer et al., 2011). Next-generation clinical genomics has potential to transform healthcare by bringing us closer to delivering optimal treatment, prescribed based on an individual's genetic profile.

With improvement in genomic technologies, our ability to collect, analyze and aggregate data from large-scale sequencing continues to expand. The complexity of results generated from genomic sequencing thus presents unique challenges to clinicians, patients and their families in the areas of informed consent, genetic counseling and return of

Table 1
Variant classification criteria.

1. Pathogenic:
   1.1. Allele frequency of variant below the disease specific prevalence, AND
   1.2. Segregation in at least two unrelated families, OR
   1.3. Segregation in one family and identified in at least three unrelated affected individuals, OR
   1.4. Protein truncation where this event is known to cause disease, OR
   1.5. Functionally characterized variant
2. Likely pathogenic:
   2.1. Allele frequency of variant below the disease specific prevalence, AND
   2.2. Identified in at least three unrelated individuals, OR
   2.3. Segregation in one family, OR
   2.4. At least one *de novo* event in trio
3. Variant of uncertain significance:
   3.1. Allele frequency of variant below the disease specific prevalence, AND
   3.2. Identified in fewer than three unrelated affected individuals, OR
   3.3. No segregation studies, OR
   3.4. No *de novo* event in a trio
4. Likely benign
   4.1. Allele frequency of variant above the disease specific prevalence, AND/OR
   4.2. Seen in combination with a known pathogenic mutation

Table 2
Participant characteristics.

Study cohort	Type of genomic sequencing	Sample size	Sample size after QC	Ethnicity			
				Chinese	Malay	Indian	Others
Cohort 1	WES	245	245	182	10	25	28
Cohort 2	WGS	138	132	0	96	36	0
Total		383	377	182	106	61	28

WES: Whole exome sequencing, WGS: Whole genome sequencing.

results (Green et al., 2012; Wolf et al., 2012; Roche and Berg, 2015; Hegde et al., 2015; Krier and Green, 2015; Burke et al., 2013; Kleiderman et al., 2014; Yu et al., 2014a, 2014b). There are many aspects requiring clarity and attention. Patients will need to understand the implications of pathogenic mutations, and what it means for family members who may be carriers of the mutation. Referring clinicians will need to understand that not all mutations (more accurately called variants) are necessarily pathogenic although they may occur in disease causing genes. Genetic counseling will need to address issues of possible pathogenic variants, variants of unknown significance, di-genic or multi-genic inheritance, and disease penetrance. After clarifying the implications from results in genes related to the primary condition, we are then faced with the equally, if not more challenging issue of variants in other "medically important" genes, that may be unrelated to the patient's primary medical condition or that may be confounded by one's ethnicity. In our busy and time-constrained health care systems, explaining the complexities of the genomic sequencing process is a challenge and will need deliberate effort to address. We anticipate an increase in the use of genomics in clinics in the coming years, some of which may be driven by well-informed patients themselves or by commercial entities. There is undoubtedly an urgent need for recommendations and a build-up of reliable experience.

Detection of IFs has implications not only for the individual, but also his/her family members (McLaughlin et al., 2014). For example, WES on a patient with intellectual disability may detect pathogenic variants in *BRCA1*. This mutation could be inherited from the parents, which means that the affected parent (and his/her siblings) is at risk of developing cancer and would require surveillance and monitoring. This family may or may not be prepared to receive such information and this issue needs to be discussed during the informed consent process. While the Genetic Information Non-discrimination Act (GINA 2008) in USA offers protection to individuals against discrimination for health insurance and employment based on their genetic information, it is not comprehensive and excludes life insurance; and similar laws are lacking in other countries (Dorschner et al., 2013). Indeed individuals with a genetic diagnosis, especially those with incidental findings, may face discrimination at work or be denied of medical insurance without any avenue for legal redress.

Our study detected a combined prevalence of IFs at 1.6% among a representative South East Asian population, which is similar to the prevalence in other ethnic groups and is consistent with the rates of prevalence of these disorders. In addition, a majority of individuals (83%)

responded favorably to the return of IFs, which highlights the importance of including the patient in the decision making process. Four of the 6 variants are in cardiac-related genes (*SCN5A, TNNT2* and *COL3A1*). Mutations in *SCN5A* and *TNNT2* are associated with Brugada syndrome and cardiomyopathy, respectively, and can present with sudden death as their first presentation. Detection of carrier status for the mutations allows for anticipatory guidance in the form of regular electrocardiogram and echocardiography, and avoidance of triggers such as certain medications like macrolides. These measures can avert the usual catastrophic presentation of these disorders (Priori et al., 2013). In addition, cascade screening of family members can identify other at-risk individuals who can then be managed accordingly (Priori et al., 2013). Similarly, guidelines and recommendations exist for patients with mutations in *BRCA2, TP53*, and *COL3A1* which allows for anticipatory management and minimizes mortality and long-term morbidity (Warner et al., 2004; Ballinger et al., 2015; Lum et al., 2011). These results suggest that comprehensiveness of WES/WGS tests should be explained to the interested patients and treated as an opportunity to provide important health information.

While we studied the prevalence of variants in highly actionable genes, we did not include genes in which mutations could lead to conditions where no therapy exists to manage the consequences of the deleterious mutation (Berg et al., 2011) (e.g. Huntington disease, Prion disease or frontotemporal dementia), i.e. results that are "not-actionable". The list also does not include rare diseases where less knowledge is available about their level of penetrance (Berg et al., 2011) (e.g. *APOE4* allele and Alzheimer disease risk), or where individuals may in fact only be carriers of these recessive conditions (e.g. thalassemia, cystic fibrosis). There are also variants in pharmacogenetic-relevant genes which may not be disease causing, but could have clinically meaningful information for future management of the patient (e.g. *CYP2CP* and *VKORC1* single nucleotide polymorphisms and warfarin dosing) (Grossniklaus, 2010). As our understanding of each of these contexts improves, the list of "actionable" genes and their potential burden on the healthcare system may increase with time.

In conclusion, genomic sequencing is rapidly moving into medical practice. The scope of understanding and interpreting genomic sequencing is imperative in context of the patient's primary condition, but contemporaneously uncovered incidental mutations (or variants) in medically actionable genes not related to the primary condition will also need attention. The data presented here helps to improve our understanding of the nuances relating to implementation of clinical genomics as we pursue precision medicine on all fronts and is anticipated to benefit national policymakers as well as medical bodies as they discuss, debate and formulate recommendations for clinical genomic testing. Given the net benefit to the individual and society and relatively low burden of IFs, efforts to develop effective processes for separating and reporting only serious disease causing findings with necessary education should be treated as an opportunity and not as a burden.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.01.030.

Table 3
Filtering strategy and variants detected in the study cohorts.

Filter strategy	Number remaining after filtering		
	Cohort 1 (n = 245)	Cohort 2 (n = 132)	Total (n = 377)
Total number of variants in 56 genes	1381	40,226	41,607
Exclude "common" SNPs[a]	768	14,447	15,215
Exclude "non-exonic" SNPs[b]	523	435	958
Include "pathogenic" SNPs[c]	6	5	11

[a] Common SNPs defined as present in >5% of population.
[b] Intronic, intergenic, non-coding, upstream, downstream.
[c] Defined as pathogenic in Clinvar and/or HGMD.

Competing Interests

None.

Table 4
Classification of variants based on review of primary literature.

Gene name	Variant (hg19)	Primary associated condition (OMIM #)	Amino acid change	dbSNP ID	Ethnicity
Pathogenic					
SCN5A	chr3:38645439C>G;het	Long QT syndrome, Brugada syndrome (#601144)	p.Gly552Arg	rs3918389	Chinese
SCN5A	chr3:38592986C>T;het	Long QT syndrome, Brugada syndrome (#601144)	p.Arg1625Pro	rs199473283	Chinese
BRCA2	chr13:32915033G>T;het	Hereditary breast and ovarian cancer (#612555)	p.Gly2181*	rs371067421	Malay
TP53	chr17:7577094G>A;het	Li-Fraumeni syndrome (#151623)	p.Arg282Trp	rs28934574	Chinese
Likely pathogenic					
TNNT2	chr1:201332522G>A;het	Hypertrophic and/or dilated cardiomyopathy (#601494)	p.Arg158*	NA	Chinese
COL3A1	chr2:189861145C>T;het	Ehlers Danlos syndrome, vascular type (#130050)	p.Arg562*	rs375737772	Malay
Variants of uncertain significance					
SCN5A	chr3:38592386C>T;het	Long QT syndrome, Brugada syndrome (#601144)	p.Arg1826His	rs137854610	Chinese
SCN5A	chr3:38640472C>T;het	Long QT syndrome, Brugada syndrome (#601144)	p.Glu654Lys	rs199473138	Others
SCN5A	chr3:38622640A>G;het	Long QT syndrome, Brugada syndrome (#601144)	p.Cys1004Arg	rs199473183	Indian
FBN1	chr15:48888525G>C;het	Marfan syndrome (#154700)	p.Arg165Gly	rs113905529	Malay
Likely benign					
RET	chr10:43601830G>A;het	Multiple endocrine neoplasia type 2 (#171400)	p.Val292Met	rs34682185	Chinese
BRCA2	chr13:32972626A>T;het	Hereditary breast and ovarian cancer (#612555)	p.Lys3326*	rs11571833	Indian
Exclude					
MUTYH	chr1:45797760T>C;het	MYH associated polyposis (#132600)	c.934-2A>G	rs77542170	Malay
PCSK9	chr1:55527110C>T;het	Familial hypercholesterolemia (#603776)	p.Arg289*	rs373323910	Malay

Contributions

ZT, JYL, WKML, AJ, WKL, HYL, EST, AL, IN, YYT, BV, BR, and ECT participated in data acquisition. JLK and WLWT performed bioinformatic analyses. SSJ and MB reviewed variants and reclassified them based on primary literature. SSJ, MB, and RF wrote the manuscript. Every author has reviewed the manuscript, approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

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