



Published in final edited form as:

*Genet Med.* 2016 December ; 18(12): 1303–1307. doi:10.1038/gim.2016.47.

## Pharmacogenomic incidental findings in 308 families: The NIH Undiagnosed Disease Program experience

Elizabeth M.J. Lee, Karen Xu, Emma Mosbrook, Amanda Links, Jessica Guzman, David R. Adams, MD, PhD, Elise Flynn, Elise Valkanas, Camillo Toro, MD, Cynthia J. Tifft, MD, PhD, Cornelius F. Boerkoel, MD, PhD, William A. Gahl, MD, PhD, and Murat Sincan, MD

NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH; and National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA

### Abstract

**PURPOSE**—Using SNP chip and exome sequence data from individuals participating in the NIH Undiagnosed Diseases Program (UDP), we evaluated the number and therapeutic informativeness of incidental pharmacogenetic variants.

**METHODS**—Pharmacogenomics Knowledgebase (PharmGKB) annotated sequence variants were identified in 1,101 individuals. Medication records of participants were used to identify individuals prescribed medications for which a genetic variant might alter efficacy.

**RESULTS**—395 sequence variants, including 19 PharmGKB 1A and 1B variants, were identified in SNP chip sequence data and 388 variants, including 21 PharmGKB 1A and 1B variants, were identified in the exome sequence data. Nine participants had incidental pharmacogenetic variants associated with altered efficacy of a prescribed medication.

**CONCLUSIONS**—Despite the small size of the NIH UDP patient cohort, we identified pharmacogenetic incidental findings potentially useful for guiding therapy. Consequently, groups conducting clinical genomic studies might consider reporting of pharmacogenetic incidental findings.

### Keywords

Incidental findings; Secondary findings; SNP genotypes; exome sequencing; NIH Undiagnosed Diseases Program; personalized medicine; precision medicine

## INTRODUCTION

Incidental or secondary genetic findings are variants with medical or social implications discovered during genetic testing for an unrelated indication<sup>1</sup>. Recent discussions and the report by the ACMG Working Group on Incidental Findings in Clinical Exome and Genome Sequencing have focused on disease-associated genes and not genetic determinants of drug

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:[http://www.nature.com/authors/editorial\\_policies/license.html#terms](http://www.nature.com/authors/editorial_policies/license.html#terms)

**Correspondence:** Murat Sincan, M.D., Address: BG 10-CRC RM 5-2535 MSC 1470, 10 Center Drive, Bethesda, MD 20892, Phone: 301-827-1647, Fax: 301-480-0498, [sincanm@mail.nih.gov](mailto:sincanm@mail.nih.gov).

metabolism<sup>2</sup>. Given that genomic variation influences human responsiveness to many drugs and contributes to phenotypes ranging from life-threatening adverse drug reactions to lack of therapeutic efficacy<sup>3</sup>, the return of pharmacogenetic incidental findings has potentially significant medical benefit<sup>4</sup>.

The Clinical Pharmacogenetics Implementation Consortium (CPIC), which develops guidelines for incorporating pharmacogenomics findings into clinical practice, has identified variant-drug associations of high concern for clinicians, and provides drug-dosing guidelines based on patient genotype. We hypothesized, therefore, that designing and implementing a process to identify pharmacological incidental findings in the genomic data generated by the NIH Undiagnosed Diseases Program<sup>5</sup> (UDP) provides information to the medical community on the quantity and quality of pharmacogenetic incidental findings.

The NIH UDP routinely conducts SNP chip and exome sequencing analyses on probands and their family members. These data can be analyzed for pharmacogenetic incidental findings based on variant-drug associations listed in the Pharmacogenomics Knowledgebase (PharmGKB). The typical number of such pharmacogenetic incidental findings has not been widely studied, particularly when family members other than the proband are included in diagnostic studies. Consequently, more data are needed to assess the possible impact and need for resources.

To delineate the impact of identifying pharmacogenetic incidental findings, we analyzed SNP chip data from 1,101 individuals derived from 308 families and research exome sequence data from 645 individuals derived from 158 families. For the 868 pharmacogenetic loci listed in the Pharmacogenomics Knowledgebase (PharmGKB), we identified 949 independent pharmacogenetic findings using the SNP chip and exome sequence data and found that each individual had at least one. For nine individuals, these constituted incidental findings relevant to a medication that they were taking. These data refine strategies for reporting of pharmacogenetic incidental findings.

## MATERIALS AND METHODS

Methods and Materials are available online

## RESULTS

The PharmGKB database includes 868 annotated SNPs with published pharmacological implications. PharmGKB has six levels of evidence based on published evidence and the drug-dosing guidelines of the Clinical Pharmacogenetics Implementation Consortium (CPIC)<sup>6</sup>. The top two levels (1A and 1B) have substantive evidence for clinical relevance and CPIC guidelines or known clinical implementation.

The SNP chips used in our study provided 46% coverage of the SNPs annotated in the PharmGKB database and 53% coverage of high priority SNPs (PharmGKB 1A and 1B categories). Variants identified through whole exome sequencing conducted on the patients in the UDP covered 45% of the SNPs in the PharmGKB database and 58% of PharmGKB 1A and 1B SNPs. Combining the SNPs from both the SNP chip genotyping and exome

sequencing covered 65% of the SNPs in the PharmGKB database and 81% of the PharmGKB 1A and 1B SNPs.

All UDP patients with SNP genotypes or exome sequencing data had potential pharmacogenetic incidental findings. Within the SNP chip data, there were 696 potential incidental findings per patient that were associated with 276 different drugs. These included 19 variants ranked within PharmGKB categories 1A and 1B, and these 19 variants were associated with the efficacy of 17 drugs. Within the exome sequencing data, there were 728 detectable variants per patient that were associated with 283 different drugs related to 388 PharmGKB documented SNPs. These included 21 variants within PharmGKB categories 1A and 1B that were associated with 14 different drugs. Combined SNP chip and exome data detected 949 variants per patient, and 29 were variants ranked in the PharmGKB 1A and 1B categories.

To determine if these variants constituted incidental findings, i.e., were of therapeutic relevance, we focused on the 359 individuals for whom we had medication records. None of these individuals had been prescribed a medication for which they carried a PharmGKB 1A or 1B category variant. We therefore tested whether any of the pharmacogenetic variants they carried might provide insight into their response to a prescribed medication. This identified five pharmacogenetically relevant variants or incidental findings among nine individuals (Table 1). Three of the variants were detectable by both the SNP chip and exome sequencing, and two were detectable only by exome sequencing.

The five variants were relevant to efficacy of four different medications (Table 1). These medications were escitalopram, carbamazepine, morphine, and acetylsalicylic acid. First, a female adult (Patient 1) prescribed escitalopram had a PharmGKB category 3 association: *rs6318* genotype CG, which has been associated with altered function of the X-linked serotonin 5-HT<sub>2C</sub> receptor and various phenotypes ranging from altered cortisol response to stress<sup>7</sup> to increased risk for cardiovascular disease mortality and morbidity<sup>8</sup>. Second, a child (Patient 2) who had seizures refractory to carbamazepine had a PharmGKB category 2B association: *rs1051740* genotype CT, which has been associated with a requirement for increased dosage of carbamazepine<sup>9</sup>. Third, a female adult (Patient 3), who had been prescribed morphine and reported that ibuprofen gave better pain control, had a PharmGKB category 3 association: *rs1799971* genotype AG, which has been associated with decreased efficacy of morphine in Caucasians<sup>10–12</sup>. Fourth, a male adult (Patient 8) prescribed acetylsalicylic acid and with a cardiomyopathy had a PharmGKB category 3 association: *rs1799983* genotype GT, which has been associated with acetylsalicylic acid promoting in-stent restenosis<sup>13</sup>. Fifth, a male adult (Patient 9) was prescribed acetylsalicylic acid, had a history of myocardial infarction, and had a PharmGKB category 3 association: *rs5985* genotype AC, which has been associated with an increased effectiveness of acetylsalicylic acid inhibition of Factor XIII activation, ultimately lowering an individual's risk of myocardial infarction and death<sup>14</sup>.

## DISCUSSION

By using the SNP chip data to analyze genotypes for 696 polymorphic variants and exome sequence data to define genotypes for 728 polymorphic variants, we were able to interrogate the genotype of 949 pharmacogenetically relevant variants for each individual enrolled in the NIH UDP. Focusing on the 359 individuals for whom we had medication information, we discovered five reportable variants among nine individuals from nine different families (eight probands and one unaffected family member). For five (56%) of these individuals, the pharmacogenetic incidental findings were potentially relevant to their medical management.

Although the sequencing conducted by the NIH UDP was not designed to screen pharmacologically relevant variants, 65% of the annotated variants in the PharmGKB databases were represented in the UDP sequencing data. All individuals in the study received SNP chip genotyping; exome sequencing was conducted on 59% of these individuals. Both SNP chip genotyping and exome sequencing were important in identifying the pharmacogenetically relevant variants. Although 50% of the variants that were discovered in the UDP dataset could be identified with either the SNP chip genotyping data or the exome sequencing data, 23% were found only within the SNP chip genotyping data and 27% were found only within the exome sequencing data.

Four additional issues arising during our analysis were 1) defining the level of relative risk warranting reporting of a potential variant, 2) determining how to weight variants identified in an ethnic group different from that of the subject, 3) the need for clinical correlation, and 4) obligations to family members. Relevant to the first issue, clinicians expressed a need for prioritization of the findings since there were such a large number of potential incidental findings per patient. To address this request, we used the clinical annotation levels of evidence of PharmGKB. Additionally, we reported the ethnicity within which the variant-drug associations were identified and highlighted any differences in ethnicity between the patient and that population and prioritized variant-drug associations accordingly.

Although filtering the incidental findings for PharmGKB 1A and 1B categories produces a manageable list of 21 medications, none of the individuals for whom we had medication data had a PharmGKB category 1A or 1B association. We therefore tested for associations ranked lower in PharmGKB and identified five PharmGKB category 2 or 3 associations. Five of these were potentially relevant to the care of the individuals, three patients and one family member (Table 1).

A one-sentence summary of the PharmGKB variant with the associated drug information was incorporated into the report entered into the NIH UDP database. Entry of the findings into the database also enabled sorting of the results and searching by individual, drug name and priority. Primary clinicians' discussions regarding whether to inform individuals enrolled under the NIH UDP protocol about the identified variants focused on the delineated and perceived obligations defined by the language of the consent document and the process by which the consent was explained. The choice of whether to report a given variant to a given study subject was deferred to the relevant clinical team. No clinical team elected to return a PharmGKB category 2 or 3 variant detected by this study for at least two reasons.

Firstly, the study consent reflected routine practice from the early days of the application of genome-scale sequencing to medical diagnostics. Only DNA variants that might contribute to the test indication were to be returned. Secondly, current ACMG guidelines do not include such variants among those recommended to be returned<sup>2</sup>. The UDP recognizes that these are areas of intense debate in the literature and elsewhere; the program is prepared to adjust its practice as the standard of care evolves.

Our analysis had some limitations. Some of the variant annotations in PharmGKB associate a variant to a class of drugs (e.g., bisphosphonates or antidepressants) instead of a specific drug. In contrast, UDP medication records list the specific drug name; therefore, our simple matching algorithm did not identify all associations. This problem can be readily addressed with drug ontology that would allow drug class matches. We also did not have access to the complete list of medications administered to our patients prior to their participation in our study. Despite these limitations, a small percentage of individuals with relevant medication prescriptions was identified in the cohort, indicating that there were likely an even larger percentage of medically relevant findings if the computational filtering mechanisms were refined.

The results of this study indicate that, by looking for pharmacogenetically relevant findings in SNP genotypes or exome sequencing data, incidental findings can be identified and potentially provide a valuable resource for patient care. The interpretation, use in medical practice, and returning of pharmacogenomics secondary findings are, however, different than the non-pharmacogenomic incidental findings listed in the original ACMG publication<sup>2</sup>. These pharmacogenomic variants different from non-pharmacogenomic incidental findings, in that, they are only relevant in the presence of an environmental factor, i.e., the drug that they have an effect on. This means that a pharmacogenomic variant may never be relevant in one person's lifetime but absolutely important for another person who is prescribed the drug in question. For example, in a recent study of 48 pharmacogenomically relevant variants among a 94 patient cohort<sup>15</sup>, the electronic medical record decision support system gave no alerts during these patients' hospital stay.

Our described methodology and findings, nonetheless, suggest that identifying and reporting pharmacogenetic incidental findings can improve patient care and personalized medicine. Additionally, if medically relevant findings can be found in the small UDP cohort with sequencing strategies designed for diagnostic purposes and not pharmacogenetic purposes, we hypothesize that such incidental findings occur in the sequencing data for other medical programs.

In summary, using SNP chip genotyping and exome sequencing, the NIH UDP identified pharmacogenetic incidental findings in its cohort. We established a framework for evaluating and selecting pharmacogenomic variants hypothetically useful for therapeutic management.

## Supplementary Material

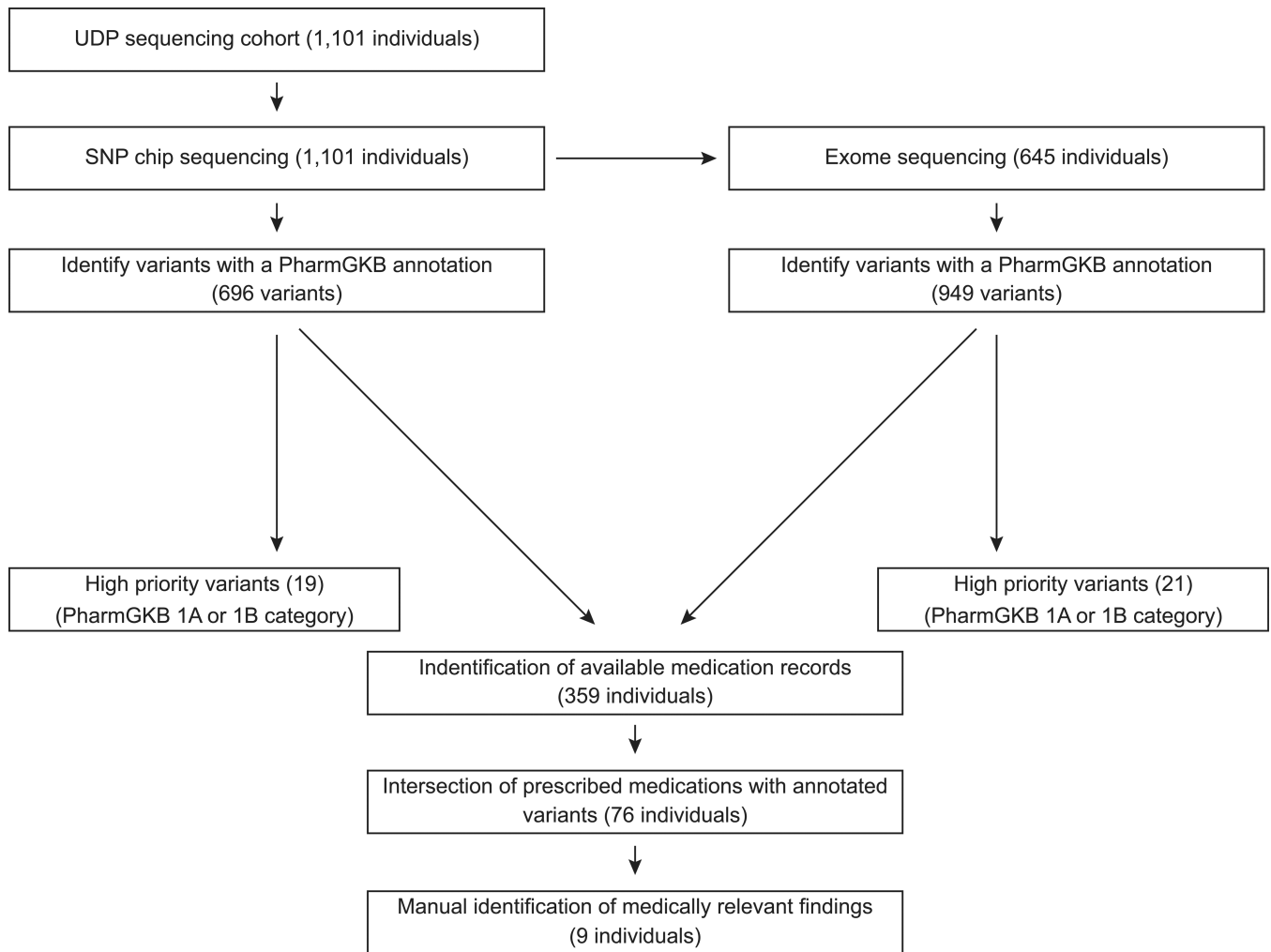
Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank the NHGRI Intramural Sequencing Center and Appistry, Inc. for their sequencing, alignment, genotyping and annotation services. This work was supported in part by the Common Fund, Office of the Director, and the Intramural Research Program of the National Human Genome Research Institute (NIH, Bethesda, Maryland)

## REFERENCES

1. Wolf SM, Crock BN, Van Ness B, et al. Managing incidental findings and research results in genomic research involving biobanks and archived data sets. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2012 Apr; 14(4):361–384. [PubMed: 22436882]
2. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2013 Jul; 15(7):565–574. [PubMed: 23788249]
3. Leeder JS. Developmental and pediatric pharmacogenomics. *Pharmacogenomics*. 2003 May; 4(3):331–341. [PubMed: 12718723]
4. Hawcutt DB, Thompson B, Smyth RL, Pirmohamed M. Paediatric pharmacogenomics: an overview. *Archives of disease in childhood*. 2013 Mar; 98(3):232–237.
5. Gahl WA, Tiftt CJ. The NIH Undiagnosed Diseases Program: lessons learned. *Jama*. 2011 May 11; 305(18):1904–1905. [PubMed: 21558523]
6. Relling MV, Klein TE. CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clinical pharmacology and therapeutics*. 2011 Mar; 89(3):464–467. [PubMed: 21270786]
7. Brummett BH, Babyak MA, Williams RB, et al. A putatively functional polymorphism in the HTR2C gene is associated with depressive symptoms in white females reporting significant life stress. *PloS one*. 2014; 9(12):e114451. [PubMed: 25514629]
8. Brummett BH, Babyak MA, Jiang R, et al. A functional polymorphism in the 5HTR2C gene associated with stress responses also predicts incident cardiovascular events. *PloS one*. 2013; 8(12):e82781. [PubMed: 24386118]
9. Makmor-Bakry M, Sills GJ, Hitiris N, Butler E, Wilson EA, Brodie MJ. Genetic variants in microsomal epoxide hydrolase influence carbamazepine dosing. *Clinical neuropharmacology*. 2009 Jul-Aug;32(4):205–212. [PubMed: 19620853]
10. Campa D, Gioia A, Tomei A, Poli P, Barale R. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. *Clinical pharmacology and therapeutics*. 2008 Apr; 83(4):559–566. [PubMed: 17898703]
11. Matic M, Simons SH, van Lingen RA, et al. Rescue morphine in mechanically ventilated newborns associated with combined OPRM1 and COMT genotype. *Pharmacogenomics*. 2014 Jul; 15(10):1287–1295. [PubMed: 25155931]
12. Reyes-Gibby CC, Shete S, Rakvag T, et al. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. *Pain*. 2007 Jul; 130(1–2):25–30. [PubMed: 17156920]
13. Shuvalova YA, Kaminniy AI, Meshkov AN, Shirokov RO, Samko AN. Association between polymorphisms of eNOS and GPx-1 genes, activity of free-radical processes and in-stent restenosis. *Molecular and cellular biochemistry*. 2012 Nov; 370(1–2):241–249. [PubMed: 22890915]
14. Undas A, Sydor WJ, Brummel K, Musial J, Mann KG, Szczeklik A. Aspirin alters the cardioprotective effects of the factor XIII Val34Leu polymorphism. *Circulation*. 2003 Jan 7; 107(1):17–20. [PubMed: 12515735]
15. Nishimura AA, Shirts BH, Dorschner MO, et al. Development of clinical decision support alerts for pharmacogenomic incidental findings from exome sequencing. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2015 Mar 5.



**Figure 1.**

Flow chart summarizing the NIH Undiagnosed Diseases Program analysis of and observations for the pharmacogenetic variants listed in PharmGKB. The observations were derived from analysis of SNP chip and exome sequence data. The SNP chip data were derived from a cohort of 308 families consisting of 355 affected individuals, 278 unaffected siblings, 459 unaffected parents, and 9 other unaffected family members; this cohort included 564 females and 537 males. The exome sequence data were from a subset of this cohort, 158 families consisting of 182 affected individuals, 150 unaffected siblings, 313 unaffected parents, 326 females and 319 males.

Table 1

Medically relevant pharmacogenomics variants in the NIH Undiagnosed Diseases Program cohort

UDP Patient	SNP	Genotype	Age, Sex, Race	Phenotype	Drug	Pharm GKB Rank	Pharmacological Implication
1	rs6318	CG	46, Female, Caucasian	Fibromyalgia, Depression, Hypertension	escitalopram	3	CG associated with greater efficacy for treatment of nerve pain in males
2	rs1051740	CT	14, Male, Caucasian	Episodic Ataxia Type 1, Seizures	carbamazepine	2B	CT associated with increased metabolism of carbamazepine in people with Epilepsy
3	rs1799971	AG	27, Female, Caucasian	Facial and abdominal pain, lung nodules, facial masses, recurrent pericarditis and pleuritis	morphine	3	AG associated with increased metabolism and decreased efficacy for pain reduction
4	rs5985	AC	62, Female, Caucasian	Autonomic dysfunction, ataxia	acetylsalicylic acid	3	Associated with inhibition of FXIII activation
5	rs1799983	GT	57, Male, Caucasian	Neurological, no cardiovascular disease risks	acetylsalicylic acid	3	Associated with increased in-stent restenosis in men with Coronary Artery Disease
6	rs1799983, rs5985	GT, AC	52, Male, Caucasian	Fever, CSF Pleocytosis	acetylsalicylic acid	3	Associated with increased in-stent restenosis in men with Coronary Artery Disease and inhibition of FXIII activation
7	rs5985	AC	68, Male, Caucasian	Fever, pulmonary	acetylsalicylic acid	3	Associated with inhibition of FXIII activation
8	rs1799983	GT	53, Male, Caucasian	Cardiac, heart murmur, aortic valve replacement, myopathy	acetylsalicylic acid	3	Associated with increased in-stent restenosis in men with Coronary Artery Disease
9	rs5985	AC	60, Male, Caucasian	Myopathy, fatigue, Myocardial infarction, bicuspid aortic valve, pulmonary embolism	acetylsalicylic acid	3	Associated with inhibition of FXIII activation