LETTER TO THE EDITOR

Response to "Predictable difficulty or difficulty to predict"

Using our automated bioinformatics tool, we recently classified 40 point mutations in ABC transporters as "neutral" or "disease-associated," followed by experimentally testing three of the predictions.¹ Scanning the scientific literature, Varadi *et al.* found support for nine of our predictions, whereas suggesting that 10 were incorrect. However, the interpretation of the literature by Varadi *et al.* is itself not unambiguous, as illustrated by at least 6 of the 10 examples of presumed misprediction.

The first five examples are likely pleiotropic mutations, with a different impact on a different function:

Mutation Q141K in the breast cancer resistance protein BCRP does not significantly influence the disposition of HIV drug lamivudine,² thus not supporting the unequivocal "disease-associated" classification by Varadi *et al.*

Mutation P269S in BCRP is associated with a 40% decrease in vesicular uptake of [(3)H]estrone-3-sulfate and [(3)H]methotrexate compared to the wild-type form,³ which is inconsistent with the unambiguous "neutral" annotation.

Mutation V444A in the bile salt export pump BSEP does not change taurocholate transport function *in vitro*;⁴ homozygous V444A mutations were prevalent in both progressive familial intrahepatic cholestasis patients and in noncholestatic patients;⁵ and this variant has also been observed in other healthy populations.⁶ Thus, a clearcut classification as "disease-associated" is not justified.

Mutant V1251I of ABCB1 showed decreased transport of BODIPY-FL-paclitaxel, but increased transport of calcein-AM in an assay using transfected HEK293T cells,⁷ arguing against an unequivocal "disease-associated" classification.

Mutation S1141T in ABCB1 showed either normal or increased transport in the yeast assay,⁸ and increased function in transfected HEK293T cells.⁷ Therefore, this mutation should not be classified simply as "disease-associated."

The final, sixth case is an example of the lack of evidence for the classification by Varadi *et al.*: Mutation A1291T in ABCC6 occurs simultaneously with a nonsense mutation (Y1069X) in the analyzed form of the transporter.⁹ Therefore, we do not yet know whether the nonsense mutation or the A1291T mutation (or both) is causative of the disease

state, contrary to a definitive "disease-associated" classification.

If one accepts the qualifications above, 5 of the 40 predictions by our automated tool are invalidated, including one by our experiments¹ and four by the scientific literature cited by Varadi *et al.*; and 11 mutations are confirmed, including two by our experiments¹ and nine by the literature cited by Varadi *et al.* The corresponding accuracy level (69%) is in fact comparable to that estimated by the random forest test (Fig. 4 in Ref. 1).

In conclusion, we agree with Varadi *et al.* that there are inherent difficulties in predicting the functional impact of point mutations. Moreover, as we illustrate above, there are also inherent difficulties in characterizing the functional impact of mutations by experiment, and in interpreting the corresponding scientific literature. Classification of mutations into only two classes (such as our "neutral" and "disease-associated" classes) is a serious limitation, given the complexities of protein localization, degradation, interactions with other small molecules and macromolecules, as well as function in general, resulting in the pleiotropy of some mutations.⁷ Nevertheless, the difficulty of the problem does not mean we should not address it as long as the progress is properly qualified, as we believe we did in our publication.

LIBUSHA KELLY
HISAYO FUKUSHIMA
RACHEL KARCHIN
JASON M. GOW
LESLIE W. CHINN
URSULA PIEPER
MARK R. SEGAL
DEANNA L. KROETZ
ANDREJ SALI*

*California Institute for Quantitative Biosciences at UCSF
Department of Pharmaceutical Chemistry University of
California at San Francisco UCSF MC 2552,
Byers Hall Room 503B, 1700 4th Street, San Francisco,
CA 94158-2330

(Received 3 November 2010)

References

1. Kelly L, Fukushima H, Karchin R, Gow JM, Chinn LW, Pieper U, Segal MR, Kroetz DL, Sali A (2010) Functional hot spots in human ATP-binding cassette transporter nucleotide binding domains. *Protein Sci.* PMID: 20799350.
2. Kim HS, Sunwoo YE, Ryu JY, Kang HJ, Jung HE, Song IS, Kim EY, Shim JC, Shon JH, Shin JG (2007) The effect of ABCG2 V12M, Q141K and Q126X, known functional variants *in vitro*, on the disposition of lamivudine. *Br J Clin Pharmacol* 64:645–654. PMID: 17509035.

*Correspondence to: Andrej Sali, E-mail: sali@salilab.org

DOI: 10.1002/pro.555

Published online 16 November 2010 proteinscience.org

3. Lee SS, Jeong HE, Yi JM, Jung HJ, Jang JE, Kim EY, Lee SJ, Shin JG (2007) Identification and functional assessment of BCRP polymorphisms in a Korean population. *Drug Metab Dispos* 35:623–632. PMID: 17237154.
4. Lang C, Meier Y, Stieger B, Beuers U, Lang T, Kerb R, Kullak-Ublick GA, Meier PJ, Pauli-Magnus C (2007) Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. *Pharmacogenet Genomics* 17:47–60. PMID: 17264802.
5. Chen HL, Liu YJ, Su YN, Wang NY, Wu SH, Ni YH, Hsu HY, Wu TC, Chang MH (2008) Diagnosis of BSEP/ABCB11 mutations in Asian patients with cholestasis using denaturing high performance liquid chromatography. *J Pediatr* 153:825–832. PMID: 18692205.
6. Lang T, Haberl M, Jung D, Drescher A, Schlagenhauser R, Keil A, Mornhinweg E, Stieger B, Kullak-Ublick GA, Kerb R (2006) Genetic variability, haplotype structures, and ethnic diversity of hepatic transporters MDR3 (ABCB4) and bile salt export pump (ABCB11). *Drug Metab Dispos* 34:1582–1599. PMID: 16763017.
7. Gow JM, Hodges LM, Chinn LW, Kroetz DL (2008) Substrate-dependent effects of human ABCB1 coding polymorphisms. *J Pharmacol Exp Ther* 325:435–442. PMID: 18287207.
8. Jeong H, Herskowitz I, Kroetz DL, Rine J (2007) Function-altering SNPs in the human multidrug transporter gene ABCB1 identified using a *Saccharomyces*-based assay. *PLoS Genet* 3: e39. PMID: 17352537.
9. Plomp AS, Florijn RJ, Ten Brink J, Castle B, Kingston H, Martin-Santiago A, Gorgels TG, de Jong PT, Bergen AA (2008) ABCC6 mutations in Pseudoxanthoma elasticum: an update including eight novel ones. *Mol Vis* 14: 118–124. PMID: 18253096.