EDITORIAL I

Genetics and patient outcome after cardiac surgery: unravelling translational findings

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In the current issue of the *British Journal of Anaesthesia*, Frey and colleagues[1] present a study entitled 'Genetic interactions in the β-adrenoceptor/G protein signal transduction pathway and survival after coronary artery bypass grafting: a pilot study'. This report is an extension of a previously published study[2] designed to translate genetics from the laboratory bench into better understanding of human clinical cardiovascular outcomes after cardiac surgery. The goal of this editorial is to walk the clinical anaesthetist through this manuscript in ways that illuminate understanding overall, and also highlight strengths and weakness of the study. To do so, I start with a brief review of recent human genetic breakthroughs since they provide context for the study.

The year 2000 will be remembered as the year of the human genome. Indeed, the DNA sequence of the first human genome was submitted by two competing public and private groups in 2000, then officially published simultaneously in *Science* and *Nature* in February 2001.[3–5] Genetic variation between individuals was immediately noticed, including missing or added sequences, and also the most frequent variation called single-nucleotide DNA base pair alterations or polymorphisms (SNPs). Over 1.42 million SNPs have been identified in ~25 000 human genes and intervening sequences in the human genome.[6] Furthermore, it was quickly noticed that genetic variants often travel together during naturally occurring crossovers between maternal and paternal DNA strands which make up the DNA double-helix in every human. Such 'chunks' of DNA travelling together are called haplotypes and the statistical probability that two SNPs will travel together is described as linkage (the more precise mathematical term for this concept is linkage disequilibrium). Owing to initial expense

of sequencing all DNA in a given individual, as soon as the first human genome sequence was officially completed, the haplotype mapping (HapMap) project began with analysis of 270 individuals across four geographic populations.[7] The goal of this project, completed in 2005, was to identify which human SNPs travel together and use this as a way to map fragments of DNA associated with human traits, diseases, or the ability to modify disease. The ability to deduce haplotypes has become so commonplace in the intervening years that many commercial and university computational computer programs, now widely available, are capable of analysing human DNA sequences and inferring haplotypes based on variation in a given data set of human DNA.

Initially, clinical genetic studies focused on individual SNPs as they could be easily identified. These studies often targeted SNPs in 'known' biological pathways and then 'associated' them with the presence of disease, altered drug metabolism, or other traits; hence these were described as association studies. Since biological processes contributing to diseases are incompletely understood, and only a few SNPs were chosen for analysis, these studies were often not reproducible across populations. Whether this was due to genetic variation across populations themselves, small study size, inconsistent tracking of co-morbid diseases/drugs, or lack of robust sensitivity and specificity of defined outcomes, was unknown. Genome scientists argued that such studies were 'biased' since they assumed that important pathways were known, so 'unbiased' studies were proposed (sometimes called fishing expeditions by more traditional hypothesis-driven researchers) examining thousands of RNAs or proteins present after specific perturbations such as heat, glucose deprivation, specific diseases or drug

therapies, etc. Indeed, such approaches have revealed novel pathways mechanistically linked to disease. However, classical researchers quickly chimed in that with these thousands of comparisons, many false positives were possible. This is a valid criticism of genetic studies in general and therefore in parallel, statistical methods for genetics were developed to specifically control for multiple comparisons. These include variations on multivariate analysis, propensity analysis, and more explicit false discovery rate calculations. In all such analyses, the effect of varying SNP frequency in the populations being studied must be taken into account. As a result of these checks and balances, large populations are required to make any firm clinical outcome predictions. In addition, once an association is suggested, it is not considered validated unless there is biological evidence supporting the finding. Some researchers have turned to transgenic or knockout murine (mouse) models to test their findings, while others use biomarkers present in isolated human tissues or unrelated cells. It is important to note that to make their findings clearer, researchers tend to use extremes of expected biological effect (or phenotype), comparing homozygous SNPs (two copies of the major or minor alleles of DNA variants). Human physiology is not so simple, however, with many patients expressing one copy of the 'good' and one copy of the 'bad' SNP variant. This complicates clinical genetics studies.

Over the last few years, targeting selected SNPs (by either biased or unbiased approaches) is falling out of favour overall. Instead, since the cost of DNA sequencing has plummeted (we are moving closer to the \$1000 genome), total exon (or exome) sequencing is becoming more commonplace. In these studies, DNA sequences encoding every exon in proteins are sequenced in highly characterized patient populations.[8][9] To save money and effort, such projects often use extremes of phenotypes (e.g. smaller numbers of patients with highest and lowest arterial pressures in response to a very specific stimulus), controlling for every other variable (e.g. gender, age, ethnicity, medications, medical conditions, etc.). While exome sequencing leaves out many regulatory sequences, it provides rapid and extensive DNA level sequence data across the genome, allowing for unexpected mechanisms to be revealed for specific diseases or drug responses. As can be imagined, robust computational, bioinformatics, and statistical genetics approaches are required to analyse such data. The final step in the road to personalized medicine will be to sequence the entire genome of every individual in a clinical study. This reality is very close, although appropriate data mining programmes designed to make analysis of such data simple enough for a 'regular' scientist have not yet caught up with sequencing technology.

Given this general background and context, we now return to the Frey manuscript and examine the methods utilized.[1] Over the last few years, Frey and colleagues have tested the overarching hypothesis that genetic variability in β-adrenoceptors (βARs) (β$_1$/β$_2$) *combined with key signal transduction system proteins* (in this case, the intermediary

stimulatory G protein, Gs) are important in patient outcome (death) after cardiac surgery. Key signalling proteins for βARs in the heart include G proteins (Gs for both β$_1$, β$_2$; Gi for both β$_2$, β$_3$) and the adenylyl cyclase moiety itself [which catalyses the formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate). The Frey study is an extension of a previously published study from 2009, which will be briefly summarized so we can discern the new information presented here. In the earlier publication in 2009,[2] the authors resequenced DNA in the *cis*-regulatory region and intron 1 of the gene encoding Gs protein (*GNAS*) from 10 unrelated Caucasians, six Chinese, and six Zimbabwe African individuals from blood transfusion studies and identified 11 novel SNPs. These SNPs formed three common haplotypes of differing promoter activity (*3 >*2 or *1 in terms of mRNA transcription in transfected cells grown in culture). Two key SNPs, G($-$1211)A and T2291C, were identified as defining the three main haplotypes; these two SNPs are therefore called 'haplotype-tagged SNPs'. In order to test whether *GNAS* haplotypes had any clinical effect in humans, differences between cAMP generation in cells by haplotype (homozygous *3 *vs* any other haplotype) were used to power a human study in coronary artery bypass grafting (CABG) patients. In total, 128 CABG patients on chronic βAR antagonists completed the study, and a subset of 58 patients had right atrium collected immediately upon cannulation for cardiopulmonary bypass, for functional testing. βAR signalling was examined along the signalling pathway using basal and stimulation at the level of the receptor (isoproterenol), G protein (NaF, GTP), and adenylyl cyclase moiety (forskolin). They concluded that *GNAS* haplotypes have functional consequences, with *3/*3 having the highest cAMP-producing activity, in spite of no difference in forskolin stimulation (so maximal cAMP stimulation possible is the same between patients). In addition, this was mirrored by *3/*3 having the highest post-anaesthesia induction cardiac index (perhaps related to statistically significant lowering of the systemic vascular resistance), lowest NYHA congestive heart failure (CHF) classification, and lower NT-proBNP (a serum marker for CHF) concentrations.

So what new data are presented in the current publication?[1] Essentially, the authors have taken the same patient population from the 2009 study (n=128), with 57 new patients for a total of n=185 enrollees, and followed up each patient to determine 1 yr clinical outcome. This is positive and important, since many anaesthesia studies never determine outcome beyond hospital stay or perhaps the first month after surgery, and long-term outcomes after surgery are important. As for *GNAS* basic science studies, since n=58 samples were obtained, presumably these data are identical to those published in the 2009 study showing that *3 *GNAS* haplotypes predict enhanced cellular and human atrial cAMP production, with stratification by risk alleles as a new spin. The authors do add further basic science characterization in cell culture models for these *GNAS* haplotypes, by examining reporter activity (an indirect method to determine whether precise regulatory sequences

may bind transcription factor enhancers or repressors) and electromobility shift assays (which determines whether interactions between nuclear DNA-binding proteins and their DNA recognition sequences occur), and erythrocyte western blotting (used to detect whether *GNAS* haplotype alters Gs protein expression in healthy blood donors—patients unrelated to the current CABG study). As mentioned above, these results support, and somewhat deepen, the conclusions of the 2009 study reporting that *GNAS* *3 haplotype has the highest activity by suggesting a possible mechanism.

As for clinical aspects of this study, this reviewer has concerns about the strength of the final conclusions. Basic haemodynamic parameters with the added 57 patients essentially replicate findings of the 2009 study, with *GNAS* *3 haplotype demonstrating less severe NYHA CHF class, but no effect on ejection fraction. The authors show the Kaplan–Meier curves that compare the time from CABG surgery with cardiac specific death or loss to follow-up; *GNAS* haplotypes show some mild changes depending on a specific haplotype. The authors then add β_2AR (*ADRB2*) SNPs (note: βAR SNPs and their role in perioperative outcomes and response to βAR blockade have recently been reviewed).[10] While the authors clearly state that β_2AR SNPs themselves are not associated with mortality in this study, the last Kaplan–Meier curve suggests otherwise. However, further examination reveals that these data are derived from three patient deaths in a group of only $n=6$ with the appropriate mix of β_2AR genotypes and *GNAS* haplotypes. It is important to remember that the 2009 study was originally powered by extremes of haplotype physiology (*3/*3 *vs* others) in cell culture in terms of cAMP generation,[2] with a goal of examining only *GNAS* haplotypes. The current study is simply not powered to derive any conclusions from multiple new *GNAS* and β_2AR SNP combinations. Although death is about as 'crisp' a phenotype as one can get, with only 11 cardiovascular deaths overall in 1 yr, there are also not enough clinical outcomes to draw any conclusions either. Hence, the labelling of this study as a pilot is appropriate. Having said this, the findings are potentially intriguing, but will likely take thousands of patients to sort out definitively.

In conclusion, genetic influences on human biology are important in patient care. The presence, or absence, of 'good'

or 'bad' genes affects life span, onset of disease, response to medical therapy, and overall quality of life. The hard work of sorting out which genetic variants are crucial to examine is rapidly coming to fruition, but will take large clinical trials before strong and reproducible conclusions can be reached.

Conflict of interest

None declared.

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References

1 Frey UH, Kottenberg E, Kamier M, *et al.* Genetic interactions in the β-adrenoceptor/G protein signal transduction pathway and survival after coronary artery bypass grafting: a pilot study. *Br J Anaesth* 2011; **107**: 869–78

2 Frey UH, Adamzik M, Kottenberg-Assenmacher E, *et al.* A novel functional haplotype in the human *GNAS* gene alters Gαs expression, responsiveness to β-adrenoceptor stimulation, and peri-operative cardiac performance. *Eur Heart J* 2009; **30**: 1402–10

3 Lander ES, Linton LM, Birren B, *et al.* Initial sequencing and analysis of the human genome. *Nature* 2001; **409**: 860–921

4 Venter JC, Adams MD, Myers EW, *et al.* The sequence of the human genome. *Science* 2001; **291**: 1304–51

5 International Human Genome Sequencing consortium. *Nature* 2004; **431**: 931–45

6 Sachidanandam R, Weissman D, Schmidt SC, *et al.* A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001; **409**: 928–33

7 McVean G, Spencer CCA, Chaix R. Perspectives on human genetic variation from the HapMap project. *PLoS Genet* 2005; **1**: 413–8

8 Ng PC, Levy S, Huang J, *et al.* Genetic variation in an individual human exome. *PLoS Genet* 2008; **4**: 1–15

9 Cooper GM, Shendure J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. *Nat Rev Genet* 2011; **12**: 628–40

10 von Homeyer P, Schwinn DA Pharmacogenomics of β-adrenergic receptor physiology and response to β-blockade. *Anesth Analg* Epub ahead of print: 29 September 2011