JECT. 2006;38:49–80
The Journal of The American Society of Extra-Corporeal Technology

Genetics and Outcome After Cardiac Surgery

Hilary P. Grocott, MD, FRCPC
Associate Professor of Anesthesiology and Critical Care Medicine, Duke University Medical Center, Durham, North Carolina USA

Well before the completion of the enormous task to characterize the human genome (1), it was long thought that one's genetic makeup had the ability to influence patterns of both health and disease. Although each of us contains roughly the identical 30,000 or so genes that were identified in the human genome project (surprisingly fewer genes than was initially predicted and also making us different from lesser species by only a few thousand genes), it is the subtle differences within these 30,000 genes that can dramatically influence both the susceptibility and response to various disease states. No better an example of these subtle differences and the ability of one to respond to injury is in the setting of cardiac surgery where already several genetic influences have been demonstrated to effect changes in both intermediate endpoints (such as measured cytokines of the inflammatory response) (2,3) as well as functional outcome [such as bleeding (4) and stroke (5)].

There are several types of studies that have been reported in cardiac settings that have added to our understanding of how genetics can influence outcome. The earliest were association studies focusing on the relationship between single gene variants or single nucleotide polymorphisms (SNPs) where individual nucleotides differ within specific genes (thus producing various alleles) (6). These studies were followed with more sophisticated ones (7,8) that focused on how particular SNPs influenced potentially important intermediate endpoints (such as the inflammatory response to bypass or physiologic parameters during bypass) (3,9–11).

The more recent and arguably more sophisticated types of studies, many of which are currently underway, focus on better linking the genetic influences of multiple genes to outcomes. Not only do these multi-gene studies look at how several genes can influence outcome, but they also examine critical gene-gene interactions. These more complex studies provide further information on gene associations and provide plausible biologic mechanisms explaining their associations to disease.

Of the earliest studies, Tardiff et al. in 1997 published preliminary results linking the APOE4 allele to adverse cognitive outcome after cardiac surgery (6). Although this study had several weaknesses (such as its relatively small size, $n = 65$), it heralded a new phase of investigation in cardiac surgery and spawned a series of further investigations trying both to corroborate this relationship and to understand the mechanisms surrounding this link. The presence of the APOE4 allele was the same specific allele to adverse cerebral outcome relationship that was found in studies linking both sporadic as well as late onset Alzheimer's disease with the APOE4 allele (12). A similar association of APOE4 worsened outcome has since been demonstrated after stroke, subarachnoid hemorrhage, closed-head injury, and several other cerebral injury syndromes (13–15). In short, it seemed plausible that this allele (present in approximately 25% of patients) that had already been associated with worse outcome in many other cerebral settings would also be associated with worse outcome after cardiac surgery.

To further elaborate on this APOE adverse cerebral outcome relationship, we undertook a number of investigations to better understand the potential mechanism by which the APOE4 allele affects the brain during cardiac surgery. In a study by Ti et al. APOE4 was found to have no effect on cerebral blood flow (CBF) during CPB negating any adverse effects on CBF during CPB as a potential influence on outcome. (16) A possible link between APOE4 and outcome was found by Mackensen et al. showing a relationship between worse atheroma burden and APOE4 in cardiac surgery patients (17). However, the paradox between APOE4 and atheroma was the fact that an association between worse atheroma and cognitive dysfunction has been difficult to demonstrate (18). Similarly, we examined whether APOE would have an influence on the inflammatory response to bypass thus trying to identify an APOE-specific effect on inflammation as a potential moderator of cerebral outcome after cardiac surgery. Indeed, we demonstrated that APOE4 was linked to an enhanced inflammatory response marked by APOE4 patients having lower levels of the anti-inflammatory cytokine IL-10 in effect representing a more pre-inflammatory state (3). These results were similar to the work performed by Grunenfelder et al. that also documented an APOE4-dependent effect on inflammation (2,19).

However, in the intervening time since this early APOE4 study was published, it has become increasingly likely that the relationship between adverse cognitive outcome and cardiac surgery and any genetic link is likely far more complex than a single isolated gene. Corroborating the APOE4 hypothesis (more than 10 years in the making) has been very difficult. Steed et al. (20) found no such relationship leading most investigators to question the significance of the impact of this one gene, or any one gene, on cognitive outcome. Gaynor et al. for example, was not only unable to demonstrate a similar APOE4 effect in pediatric cardiac surgery patients, but in fact demonstrated that it was the presence of APOE2 and not APOE4 that was associated with worse outcome (21). In summary, it seems unlikely that APOE, at least by itself alone, plays a significant role in cardiac surgery related cerebral injury.

Second generation studies have gone further and have described not only the genomic influences, but also the corresponding protein changes associated with various SNPs. Polymorphisms to several genes have been examined, including interleukin-6 (IL6), C-reactive protein (CRP) and, tumor necrosis factor α (TNFα), as to whether they influence the inflammatory response to bypass. What makes these particular studies stand out from the preceding studies was the fact that not only did they link a genetic polymorphism with an enhanced inflammatory response, but further linked that enhanced inflammatory response with increases in adverse outcome after cardiac surgery (22). The field of cardiac transplantation has also added to our understanding of genetics and outcome (23–27). With an increasing number of studies finding single effects on either intermediate endpoints (various proteins) or outcome, it is apparent that perhaps multiple genes could be working in concert to impact outcome.

With the advent of more economic and rapid technologies that now allow hundreds of genes to be queried for variants simultaneously, it is now evident that looking at single genes in isolation of others, although convenient (and previously "cutting edge"), likely represents a naïve ideal of how genes can impact outcome. Another point that has become very apparent in genetic studies is the large numbers of patients who will need to be genotyped before valid associations and mechanisms can be discovered. One of the largest hurdles in carrying out these studies is the need for enormous bioinformatic resources to handle the large amounts of data and constantly changing statistical methodology.

Most recently we initiated a large genomics initiative involving many thousands of patients examining more than 200 genes, and already several fascinating relationships between genetic constituents and outcome have been discovered. Although most of these represent associations, they represent the next step in understanding genetics and disease because they consider the importance of multiple single genes and gene-to-gene interactions. In addition, they represent attempts to describe distinct plausible biologic mechanisms.

Stafford-Smith et al. have established an association of a certain interaction between various SNPs and adverse renal outcome after cardiac surgery (28). In this study of 1671 patients undergoing CABG surgery, 12 SNPs on 7 different genes suspected (based on a priori hypothesis developed after surveying the literature for renally relevant genes) were studied. Several interesting findings were described. Firstly, a significant race effect was discovered with widely different gene relationships to outcome in Caucasians compared to African Americans highlighting the need to take into account issues related population structure in genetic analyses. In the Caucasians it was the presence of the minor alleles of angiotensinogen (AGT) and interleukin 6 (IL-6) that were associated with significant renal dysfunction. In the African American population, it was endothelial nitric oxide synthetase (eNOS) and angiotensin covering enzyme (ACE). Another important discovery was the way in which these genes significantly enhanced the ability of various clinically-based models to predict those patients suffering renal dysfunction.

In an unrelated study (n = 877) from our group published by Welsby et al(4) the possible polymorphisms associated with bleeding after cardiac surgery were investigated. In an elegant mechanistic fashion, he described, after studying 19 SNPs present on 13 genes, that 7 different SNPS related to thrombosis and hemostasis had an effect on the chest tube output 12 hours after surgery. Interestingly, some SNPs identified increased bleeding while others decreased bleeding risk. The ACE insertion/deletion SNP, for example increases ACE levels possibly enhancing vasoconstriction that mechanistically may have decreased bleeding (by altering tissue blood flow.) Alternatively, variants of the platelet glycoprotein IaIIa protein lead to enhanced activation of platelets during bypass thereby reducing their effectiveness at reducing bleeding after bypass. This is similar to the work done by Donohue et al. and adds to the work by Faraday et al. although with an additional degree of sophistication inherent in studying multiple genes as opposed to just single genes (29,30).

One of the most striking findings from our genetics initiative was related to neurologic outcome (specifically, stroke). In a study of 2140 patients examining 26 different SNPs, we identified an association between genes related to the inflammatory response (CRP and IL6) and an increased risk of stroke. The presence of the minor alleles of CRP, IL-6 had a three-fold increase in the risk of stroke after cardiac surgery (5). Interestingly, there was no single (or combination) of prothrombotic genes associated with stroke suggesting that inflammatory mechanisms supersede thrombotic in post-op patients at risk of a stroke.

Even more recently we have revisited the issue genetics and of cognitive dysfunction after cardiac surgery (31). This time we examined more than 30 genes, establishing not only a relationship between gene combinations and adverse outcome but also biologically plausible mechanisms based on work related to platelet activation after cardiac surgery. This work is still ongoing but has yielded exciting results implicating the inflammatory response to bypass as a mechanism of worsened cognitive outcome.

Although great strides have been made in our understanding of how the genome can impact outcome after cardiovascular surgery, the genome represents only one layer of a many layered set of independent processes within the cell. These multilayer constituents include the genome (DNA), the transcriptome (messenger RNA), the proteome (which represents a collection of all of the proteins within the DNA), the physiome (which are the regulatory networks and signaling pathways with the cell), the metabolome (which is the whole set of metabolic entities and small pathways in cells, tissues, organs, and organisms), as well as what can broadly be considered the phenome (which is the quantitative description of the integrative functions of the living organism and how it interacts with this environment). It is only with this integrative systems approach that we will ultimately be able to quantitate and mechanistically describe the important and unifying signaling networks in cardiovascular medicine all of which will ultimately be focused upon impacting positively in both preventative as well as therapeutic approaches to the patient undergoing cardiac surgery (32). What we have accomplished thus far is only the tip of the iceberg and what lies below the surface is considerably vaster and exponentially less understood.

One of the ironies of understanding the influence of genetics on outcome after cardiac surgery is the fact that technology is moving faster than one can plausibly more forward with clinical trials. This requires not only a great deal of foresight in designing clinical trials (making them adaptable to newer techniques), but also emphasizes the need to develop expansive research consortia where large numbers of patients can be genotyped for many hundreds of genes. It is only in the large trials examining multiple relationships that meaningful associations and mechanisms will ever be elucidated. One thing is clearly emerging from these complex studies—they require an equally complex but none the less cohesive collaborative group with representatives with expertise in understanding the relevant clinical outcomes, access to large numbers of study subjects, clinical study design and conduct, genetics, bioinformatics, and statistics. Obviously no one person (nor likely research group) possesses all these components further emphasizing the need to form collaborative research consortia, either within or between institutions.

REFERENCES

1. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. Science. 2001;291:1304–51.
2. Drabe N, Zund G, Grunenfelder J, et al. Genetic predisposition in patients undergoing cardiopulmonary bypass surgery is associated with an increase of inflammatory cytokines. Eur J Cardiothorac Surg. 2001;20:609–13.

3. Grocott HP, Newman MF, El-Moalem H, Bainbridge D, Butler A, Laskowitz DT. Apolipoprotein E genotype differentially influences the proinflammatory and anti-inflammatory response to cardiopulmonary bypass. J Thorac Cardiovasc Surg. 2001;122:622–3.
4. Welsby IJ, Podgoreanu MV, Phillips-Bute B, et al. Genetic factors contribute to bleeding after cardiac surgery. J Thromb Haemost. 2005;3:1206–12.
5. Grocott HP, White WD, Morris RW, et al. Genetic polymorphisms and the risk of stroke after cardiac surgery. Stroke. 2005;36:1854–8.
6. Tardiff BE, Newman MF, Saunders AM, et al. Preliminary report of a genetic basis for cognitive decline after cardiac operations. Ann Thorac Surg. 1997;64:715–20.
7. Lasocki S, Iglarz M, Seince PF, et al. Involvement of renin-angiotensin system in pressure-flow relationship: role of angiotensin-converting enzyme gene polymorphism. Anesthesiology. 2002;96:271–5.
8. Booth JV, Spahn DR, McRae RL, et al. Esmolol improves left ventricular function via enhanced beta-adrenergic receptor signaling in a canine model of coronary revascularization. Anesthesiology. 2002;97:162–9.
9. Gaudino M, Andreotti F, Zamparelli R, et al. The -174G/C interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation. Is atrial fibrillation an inflammatory complication? Circulation. 2003;108(Suppl 1):II195–9.
10. Tomasdottir H, Hjartarson H, Ricksten A, Wasslavik C, Bengtsson A, Ricksten SE. Tumor necrosis factor gene polymorphism is associated with enhanced systemic inflammatory response and increased cardiopulmonary morbidity after cardiac surgery. Anesth Analg. 2003;97:944–9.
11. Schroeder S, Borger N, Wrigge H, et al. A tumor necrosis factor gene polymorphism influences the inflammatory response after cardiac operation. Ann Thorac Surg. 2003;75:534–7.
12. Saunders A, Strittmatter W, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology. 1993;43:1467–72.
13. Coria F, Rubio I, Nunez E, et al. Apoplipoprotein E variants in ischemic stroke. Stroke. 1995;26:2375.
14. Alberts M, Graffagnino C, McClenny C, et al. ApoE genotype and survival from intracerebral haemorrhage (letter). Lancet. 1995;346:575.
15. Mayeux R, Ottman R, Maestre G, et al. Synergistic effects of traumatic head injury and apolipoprotein-ε4 in patients with Alzheimer's disease. Neurology. 1995;45:555–6.
16. Ti LK, Mathew JP, Mackensen GB, et al. Effect of apolipoprotein E genotype on cerebral autoregulation during cardiopulmonary bypass. Stroke. 2001;32:1514–9.
17. MacKensen GB, Swaminathan M, Ti LK, et al. Preliminary report on the interaction of apolipoprotein E polymorphism with aortic atherosclerosis and acute nephropathy after CABG. Ann Thorac Surg. 2004;78:520–6.
18. Bar-Yosef, S., M. Anders, G.B. Mackensen, L.K. Ti, J.P. Mathew, B. Phillips-Bute, et al. Aortic atheroma burden and cognitive dysfunction after coronary artery bypass graft surgery. Ann Thorac Surg 2004;78:1556-62; discussion 1562–3.
19. Grunenfelder J, Umbehr M, Plass A, et al. Genetic polymorphisms of apolipoprotein E4 and tumor necrosis factor beta as predisposing factors for increased inflammatory cytokines after cardiopulmonary bypass. J Thorac Cardiovasc Surg. 2004;128:92–7.
20. Steed L, Kong R, Stygall J, et al. The role of apolipoprotein E in cognitive decline after cardiac operation. Ann Thorac Surg. 2001;71:823–6.
21. Gaynor JW, Gerdes M, Zackai EH, et al. Apolipoprotein E genotype and neurodevelopmental sequelae of infant cardiac surgery. J Thorac Cardiovasc Surg. 2003;126:1736–45.
22. Gaudino M, Di Castelnuovo A, Zamparelli R, et al. Genetic control of postoperative systemic inflammatory reaction and pulmonary and renal complications after coronary artery surgery. J Thorac Cardiovasc Surg. 2003;126:1107–12.
23. Bijlsma FJ, Bruggink AH, Hartman M, et al. No association between IL-10 promoter gene polymorphism and heart failure or rejection following cardiac transplantation. Tissue Antigens. 2001;57:151–3.
24. Densem CG, Hutchinson IV, Yonan N, Brooks NH. Influence of tumor necrosis factor-alpha gene-308 polymorphism on the development of coronary vasculopathy after cardiac transplantation. J Heart Lung Transplant. 2001;20:1265–73.
25. Densem CG, Hutchinson IV, Yonan N, Brooks NH. Tumour necrosis factor alpha gene polymorphism: a predisposing factor to non-ischaemic myocardial dysfunction? Heart. 2002;87:153–5.
26. Morgun A, Shulzhenko N, Rampim GF, et al. Interleukin-2 gene polymorphism is associated with renal but not cardiac transplant outcome. Transplant Proc. 2003;35:1344–5.
27. Bedi M, Postava LA, Murali S, et al. Effect of the TNF-alpha-promoter polymorphism on cardiac allograft rejection. J Heart Lung Transplant. 2004;23:696–700.
28. Stafford-Smith M, Podgoreanu M, Swaminathan M, et al. Association of genetic polymorphisms with risk of renal injury after coronary bypass graft surgery. Am J Kidney Dis. 2005;45:519–30.
29. Donahue BS. The response to activated protein C after cardiopulmonary bypass: impact of factor V leiden. Anesth Analg. 2004;99:1598–603.
30. Faraday N, Martinez EA, Scharpf RB, et al. Platelet gene polymorphisms and cardiac risk assessment in vascular surgical patients. Anesthesiology. 2004;101:1291–7.
31. Mathew JP, Grocott HP, Podgoreanu MV, White WD, Newman MF. Inflammatory and prothrombotic genetic polymorphisms are associated with cognitive decline after CABG surgery. Anesthesiology. 2004;101:A274.
32. Podgoreanu MV, Schwinn DA. Genomics and the circulation. Br J Anaesth. 2004;93:140–8.

Development of a Novel Perfusion Technique to Allow Targeted Delivery of Gene Therapy–The V-Focus System

Arthur C. Preovolos, BAppSci; Mark T. Mennen, BSc; Adam Bilney, B.E(Hons); J. Mariani, MD; David M Kaye, MD; John M. Power, PhD
Baker Heart Research Institute, Melbourne, Australia

BACKGROUND

Current techniques for delivery of gene therapy, deliver the vector to the target organ and also to the systemic circulation. Targeted gene therapy aims at delivering the vector to specific and restricted cell populations, thus sparing all other cells of the