What sparked your initial interest in human platelet research? How has your work developed since you first entered the field?

My interest in this line of study resulted from the coalescence of several factors. First, during my haematology fellowship at the University of California, San Francisco, I became excited by the power of molecular biology and genetics to unravel the molecular basis for disease. Secondly, I was fortunate to identify very strong mentors: Dr Marc Shuman, whose expertise was in bleeding disorders, and Dr Y W Kan who focused on molecular genetics. Both were anxious to train MDs to conduct basic research. Shuman and I cared for a patient with the inherited platelet bleeding disorder Glanzmann thrombasthenia. Although it had been shown that platelets from these patients were deficient in two proteins required for normal platelet aggregation, the genes had not been cloned, which was a major limitation for understanding critical aspects of platelet physiology. My project was to fill this need.

Can you explain what platelets are and their importance to human health?

Platelets are one of the three types of cells that circulate in the bloodstream, and are produced in bone marrow. Although platelets function in numerous physiologies, their major role is to form plugs at sites of vessel injury in order to prevent excess blood loss. Thus, patient bleeding is a consequence of both an abnormally low number of platelets or platelet dysfunction. Equally important on a population basis is the role of platelets in heart attacks and strokes. Under these pathologic conditions, platelets do their job too well and form plugs or ‘clots’ in the vessels supplying blood to the heart or brain. The importance of platelets in these conditions is exemplified by the central role antiplatelet drugs like aspirin play in their management.

What are the major areas of investigation within your laboratory studies?

My laboratory focuses on the genetic basis of inter-individual variation in platelet function in order to better understand patient risks for bleeding and thrombosis. Had Charles Darwin a way to measure platelet function he would have been fascinated by the differences among otherwise healthy Homo sapiens. Our research uses state-of-the-art genetic approaches to characterise the molecular genetic causes of variation in this phenotype. The discovery system undertaken by the laboratory always involves human subjects, although we often test our human discoveries in animals or cell culture models. We also have a strong interest in how these novel gene variants alter the risk-benefit ratio of drugs.

Why are human platelets particularly useful when assessing the functional genomics of a cell?

Platelet function testing is rather unique in clinical medicine because we routinely remove these cells from the body and test their function ex vivo. Unlike other cells in the body, it is simple to obtain platelets in blood samples. In fact, a variety of platelet functions have been ‘hijacked’ for the study of other cells. For example, platelets and brain cells share many features: surface receptors that bind activating molecules, the transmission of signaling events within the cell, secretion of cargo-laden granules, etc. For decades, neuroscientists have used platelets as a surrogate for neurons. The platelet has also been a critical model for cell adhesion, an important physiologic process in healthy and cancerous cells.

Could you explain the link between RNA and disease? What are the primary limiting factors in achieving a greater understanding of the associations between disease and microRNA?

Genes in the DNA are transcribed into RNA, which is then translated into protein. Proteins are the primary regulators of cell function and physiology. RNA is one step closer to protein than DNA, and in this sense is one step closer to disease. To date, the primary limiting factor for establishing associations between microRNA and disease is that most studies of disease have not measured microRNAs, in part because of their relative recent discovery. Although there are increasing numbers of studies of microRNA levels obtained from whole blood, the most important source of microRNA is the tissue that is diseased – and few studies have ‘banked’ tissue for this purpose. With increased awareness of the importance of microRNAs, however, this limitation is being overcome.

Racial differences in blood clotting

Haematologist and experienced clinician-scientist Dr Paul F Bray discusses the basis of his long-running interest in the physiology of human platelets, as well as describing the problems they present in the formation of blood clots and inter-individual variation in platelet function.
Delving into genetic variations

Recent work conducted by a team of medical researchers at Thomas Jefferson University Hospital in Philadelphia, has uncovered the molecular basis of inter-individual differences in platelet function with significant implications for patients at risk of bleeding and thrombosis.

The question of whether or not people of different races have differing health needs can often be a thorny one. Although statistics show that people of certain ethnic backgrounds are more likely to suffer from some conditions than others, the fact is that unpicking the role of the genetic factors at work from the environmental and social factors so readily influenced by race is, in many cases, a very difficult task. It is also the subject of contention since, while most people can agree that on an individual level a subject’s genetic makeup has some influence on their health, there are many who see racial distinctions themselves as primarily social constructs with little bearing on genetics. This controversy aside, there is a mounting body of evidence suggesting that, in many fields of medicine, genetic variations across races do make a difference. Since the early 2000s, a number of studies have suggested that black people are more likely to suffer from heart disease than people of other ethnic backgrounds. In fact, the risk of suffering from heart disease for a black person is roughly double that of a white person, and even after accounting for other standard risk factors, outcomes are worse among black patients. Despite this striking discrepancy, investigating the causes of the phenomenon using medical data is difficult; black people typically represent less than two per cent of patients involved in clinical studies of heart disease.

Problematic Platelets

One possible cause of this inequity in heart disease may be as a result of inter-individual differences in platelet function. Under normal circumstances, this cell type responds to breaches in the blood vessel by adhering to cells around it and coagulating with other platelets – but if this happens when there is no breach at all, then problems can arise. A number of heart conditions are either caused or aggravated by the action of thrombi, blood clots formed when platelets bind to one another and move freely through the bloodstream. This is the root cause of many cases of myocardial infarction, or heart attack. Unfortunately, the reactivity of a patient’s platelets will usually not be assessed by their physician, even if they show risk factors for cardiovascular disease. The problem is that, because black people are underrepresented in clinical trials and platelet reactivity is not often tested, very little data exists to test the hypothesis that inter-individual differences in platelet function affect the rate of heart disease in this population. One team of clinician-researchers at Thomas Jefferson University Hospital in Philadelphia, however, has done exactly this. Dr Paul F Bray is Director of Hematology at the hospital, and has been investigating the physiology of platelets for more than 30 years. Recently, his team has utilised a new dataset to reveal a critical variant that accounts for more than half of racial differences in platelet function and the consequent disparities in the effectiveness of antiplatelet drugs.

Under PAR

In the human system, platelets are activated by the protein thrombin via two G protein-coupled receptors – PAR1 and PAR4. Once activated, platelets aggregate and bind together into thrombi. PAR1 is very sensitive to thrombin, and sends a strong, fast signal when activated; PAR4, on the other hand, sends a signal that is more sustained, but takes time to reach its peak. Until Bray began his investigations, it had been assumed that the two receptors functioned in a similar way, and the possibility that genetic differences between races could lead to variations in this signalling had not been examined. The Jefferson scientists were able to break ground in this area as a result of their pioneering dataset, as Bray explains: ”We were the first platelet researchers to develop a large, multi-ethnic, multi-’omic’ dataset with measures of platelet physiology, subject demographics, DNA variation and large and small RNA levels.”

The scientists therefore embarked on the Platelet RNA and Expression study (PRAX1), an investigation designed to identify the associations between platelet aggregation,
VARIATION IN PLATELET FUNCTION: THE GENETICS OF PLATELET GENE EXPRESSION

OBJECTIVE
To elucidate the role of platelets in cardiovascular disease and disorders of bleeding and excessive blood clotting.

KEY COLLABORATORS
Dr Leonard Edelstein; Dr Michael A Holinstat, Department of Medicine, Thomas Jefferson University, USA
Dr Chad Shaw, Department of Molecular and Human Genetics, Baylor College of Medicine, USA

PARTNER
Thomas Jefferson University

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CONTACT
Dr Paul F Bray
Director of Hematology
Department of Medicine
Sidney Kimmel Medical College at Thomas Jefferson University
Jefferson Alumni Hall
1020 Locust Street
Suite 394
Philadelphia
Pennsylvania 19107
USA
T +1 215 955 8544
E paul.bray@jefferson.edu
http://bit.ly/PaulBray
www.plateletomics.com

Patient genes and demographic variables. PRAX1 took 154 healthy subjects, 70 black and 84 white as determined by self-identified race and ethnicity, and collected and purified platelets from their blood. When the reactivity of these platelets was compared, the results were striking. The team discovered little difference between PAR1 signalling in black and white subjects, but thrombin-induced PAR4-mediated aggregation was 3.7 times faster in platelets from black subjects. What is more, numerous differences in RNA expression based on race and PAR4 reactivity were also identified.

PHARMACOGENETIC IMPLICATIONS
Bray and his colleagues subsequently pinned down the genetic variation to a single nucleotide polymorphism in the PAR4 gene that results in dimorphism. One variant of the gene is more common in white subjects, the other more so in black subjects – the form more prevalent in black subjects results in greater platelet aggregation following the activation of PAR4. Interestingly, PAR4 is not only expressed in platelets, but in tissues all over the body, and this dimorphism could therefore be at the root of other racial differences in health risks beyond the cardiovascular.

The immediate implications of this discovery for disease management, however, are even more intriguing. The fact is that many drugs – including vorapaxar, a treatment for myocardial infarction approved by the US Food and Drug Administration (FDA) only last year – target the PAR1 receptor alone. It may therefore be the case that many drugs provided as a standard for the management of heart conditions are simply not as effective for black patients as they are for white patients – a revelation that has huge implications for pharmacogenetics.

“My own bias is that the most practical benefits of personalised medicine pertain to pharmacogenetics,” Bray reflects, and in this sense, the advances he has achieved along with his collaborators constitute a big step in precision treatment.

CARE AND SHARE
“Too often public repositories become ‘data tombs’ where data go to die because of barriers for biologists and non-informaticians who are not able to write computer code,” Dr Paul F Bray laments. Consequently, the people who need previously collected raw genomic and transcriptomic data are frequently unable to access it.

Bray is determined that this will not be the fate of his team’s valuable data, however. In order to avoid such an eventuality, Bray at Jefferson and his collaborator Dr Chad Shaw at Baylor College of Medicine have created an accessible and user-friendly public web tool to enable anyone to question their 154-subject dataset.

Hosted at www.plateletomics.com, the site allows users to probe relationships between platelet aggregation, mRNA levels, miRNA levels, demographics, plasma fibrinogen and von Willebrand factor levels between patients. It also features links to external resources annotating genes and miRNA.

“This site extends the utility of our data as a resource for analyses by other investigators and presents a valuable model for how such data may be shared to increase discovery,” Bray enthuses.