Personalized medicine in thrombosis: back to the future

Srikanth Nagalla and Paul F. Bray

The Cardeza Foundation for Hematologic Research and the Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA

Most physicians believe they practiced personalized medicine prior to the genomics era that followed the sequencing of the human genome. The focus of personalized medicine has been primarily genomic medicine, wherein it is hoped that the nucleotide dissimilarities among different individuals would provide clinicians with more precise understanding of physiology, more refined diagnoses, better disease risk assessment, earlier detection and monitoring, and tailored treatments to the individual patient. However, to date, the "genomic bench" has not worked itself to the clinical thrombosis bedside. In fact, traditional plasma-based hemostasis-thrombosis laboratory testing, by assessing functional pathways of coagulation, may better help manage venous thrombotic disease than a single DNA variant with a small effect size. There are some new and exciting discoveries in the genetics of platelet reactivity pertaining to atherothrombotic disease. Despite a plethora of genetic/genomic data on platelet reactivity, there are relatively little actionable pharmacogenetic data with antiplatelet agents. Nevertheless, it is crucial for genome-wide DNA/RNA sequencing to continue in research settings for causal gene discovery, pharmacogenetic purposes, and gene-gene and gene-environment interactions. The potential of genomics to advance medicine will require integration of personal data that are obtained in the patient history: environmental exposures, diet, social data, etc. Furthermore, without the ritual of obtaining this information, we will have depersonalized medicine, which lacks the precision needed for the research required to eventually incorporate genomics into routine, optimal, and value-added clinical care. (*Blood*. 2016;127(22):2665-2671)

> Variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions which we know as disease.
>
> Sir William Osler[1(p275)]

Personalized medicine is the wave of the present. It is an unusual modern medical center that has not developed a personalized medicine program. However, more than a century ago, the master clinician Dr Osler clearly appreciated the importance of the interindividual uniqueness of his patients, so what has changed? In short, the answer is the sequencing of the human genome in the early 2000s and the subsequent enthusiasm to apply this amazing information to patient management. However, most physicians would argue they practiced highly personalized medicine prior to the pre-2000 genomics era. Medical history-taking involves the most intimate details of someone's life and the physical examination involves probing an unclothed patient. Add to this, the trust of the patient in the doctor and the physician responsibility for the life of the patient, and very little in life is more personal, even in the pre-genomics era. This perspective will take a focused view of "personalized" with respect to thrombosis in the context of the pre-2000 approach to diagnosis and management and address whether the genomic revolution has had a substantive impact on how we diagnose and treat patients with thrombosis in 2016.

Evolution of personalized medicine

Approximately 30 years ago, the concept of evidence-based medicine entered the academic lexicon. The idea was straightforward and meant decisions about treatment should be based on well-designed randomized clinical trials (RCTs). Evidence-based medicine allowed physicians to make treatment decisions based on the average individual in the general population (or at least the population meeting study entry criteria). Of course, we never knew if our patient's unique biology put her/him at either extreme of the bell-shaped curve, but at least we had data for rational decisions. Thus, evidence-based medicine appears fine if your patient is average, but what about the outliers? Certainly William Osler would have agreed with Claude Bernard, father of modern experimental physiology, who said "Variation is absolute, and in physiology averages give nothing real."[2(pp72-73)] Who wants to be treated with a "one size fits all" approach?

The increased interest in personalized medicine began with the sequencing the human genome in the early 2000s. Early analyses comparing genomes of different individuals confirmed the remarkable similarities of sequence (>99% identical), but soon gave way to expectations that the millions of nucleotide differences among different individuals would enable clinicians to not only recognize each individual's biologic uniqueness, but to translate this knowledge into more precise understanding of physiology, more refined diagnoses, better disease risk assessment, earlier detection and monitoring, and tailored treatments to the individual patient; ie, personalized (or individualized or precision) medicine.

Personalized hemostasis-thrombosis laboratory testing

Laboratory testing in clinical medicine largely reflects constant advances in science and technology. The microscope in 1590

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was the computed tomography scan of 1972 and polymerase chain reaction of 1985. All are now commonplace in hospitals, and it is difficult to imagine practicing good medicine without them. Current clinical hemostasis-thrombosis laboratory testing reflects decades of basic science research that provided a detailed understanding of the molecular basis for coagulation, anticoagulation, and fibrinolysis. This molecular knowledge was translated into testing for disease diagnosis, management decisions, and treatment monitoring. Numerous clinical studies have provided evidence-based knowledge for the appropriate use of this testing.

Unlike most clinical laboratory testing, a relatively unique feature of traditional hemostasis-thrombosis testing is the ability to measure protein and platelet function. Almost all assays are performed using citrated or platelet-rich plasma. This not only allows assessment for qualitative differences in proteins, but often involves a read-out that "summarizes" physiologic pathways involving many gene products. This pathway assessment of a patient's intermediate phenotypes may be better predictors of disease than a single DNA variant with a small effect size or levels of single RNA species, metabolites, or proteins. Examples include the activated partial thromboplastin time for the intrinsic pathway of coagulation, the PFA-100 for platelet function in whole blood under shear stress, and the more refined assays like antithrombin activity for evaluating thrombosis risk. In 2016, there are still only 5 commonly considered genetic risk factors for venous thromboembolism (VTE; deficiencies of antithrombin, protein C, and protein S; factor V Leiden [FVL]; and the G20210A prothrombin gene variant). Discoveries of these 5 heritable thrombophilias were based on our understanding of coagulation and anticoagulation proteins, knowledge that derived from plasma-based hemostasis-thrombosis functional assays.

Like any assay, there are limitations to hemostasis-thrombosis and platelet reactivity assays, including reproducibility, certain preanalytic variables, interfering substances, and medications. Using platelet functional assays to identify platelet risk factors for thrombosis is particularly challenging because of numerous logistical hurdles. There is also a lack of consistent clinical thrombosis outcome data supporting the modification of antiplatelet therapy, especially purinergic 2 receptor 12 ($P2Y_{12}$) inhibitors, based on high platelet reactivity with these assays.[3] Nucleic acid–based assays can overcome some of the limitations of cell and plasma assays. To date, it cannot be argued that the genetic approaches offer a more personalized approach to thrombosis risk assessment and management. However, we believe that plasma-based and genetic assays can be complementary, and genetic testing has more potential than platelet function assays for assessing platelet risk factors for thrombosis.

Inheritance of venous and arterial thrombosis

Family and twin studies have established a heritable component to venous and arterial thrombosis.[4-10] For the vast majority of patients, thrombosis is a complex, multifactorial disease caused by a combination of numerous, often unknown, environmental and genetic factors. Notably, most common genetic risk factors are expected to have a rather small effect size.[11] Because of the strong heritability for both VTE and arterial thrombosis, and because 50% of patients with unprovoked VTEs do not have any of the 5 well-established venous thrombophilias, novel approaches are required to move knowledge forward regarding the genetic basis for thrombotic disease.

Value of genomics in personalized medicine

Diagnostic genetic testing is not new. The United States has a long history of performing newborn screening for >50 disorders, many of which are heritable.[12] In 1978, Kan and Dozy showed a *Hpa*I restriction fragment length polymorphism (RFLP) in DNA was associated with the hemoglobin β^s allele, and this RFLP was then used in prenatal diagnosis.[13] In 1994, Dahlbäck et al[14] discovered resistance to activated protein C (APCR) as a new cause for idiopathic VTE. One year later, Bertina et al[15] reported the genetic basis of APCR as a FVL variant, at which point DNA testing entered the field of thrombosis diagnostics. Rapid nucleic acid diagnostics is now state of the art for hemoglobinopathies and an expanding number of diagnostic uncertainties, including infectious agents not easily cultured in vitro, noninvasive prenatal diagnosis, heart transplant rejection surveillance, and predicting coronary obstruction in selected patients.[16-18]

The field of pharmacogenetics is rapidly expanding into many clinical disciplines, including hematology. Pharmacogenetics is based on the notion that genetic variations influence the clinical outcomes of drug therapies; ie, gene-drug interactions. An excellent review by Roden et al[19] nicely illustrated the potential limitation of the "one size fits all" strategy to medication use. Hypertensive patients randomized to hydrochlorothiazide had mean reduction in diastolic blood pressure of 6.9 mm Hg, but ~25% of patients had no change or an increase in their blood pressure. Similarly, clopidogrel is highly effective at lowering patient's adenosine diphosphate–induced platelet aggregation (mean reduction of 33%), but a fraction of subjects have no response. There is substantial individual variation in the response to warfarin, and for years, the effect of the *CYP2C9* and *VCORC1* gene variants on warfarin activity was the poster child for the utility of pharmacogenetics.[20-22]

Despite the use of DNA diagnostic testing prior to 2000, it has been the exponential increase in our capacity to perform nucleotide sequencing that has been largely responsible for the relatively recent emphasis on personalized medicine. Completion of the HapMap project allowed for selection of genome wide single nucleotide variants (SNVs) that would tag common variants throughout the genome. This enabled genome-wide association studies (GWASs) for discovery of loci associated with clinical phenotypes. Advances in next-generation sequencing (NGS) have reduced the cost and time required for whole exome sequencing (WES) or whole genome sequencing (WGS), and we are continually improving our capacity for handling the storage, transfer, and analyses of huge amounts of sequence data. For these reasons, we are entering the window of time where WES and WGS may transition from a basic research tool to an accepted clinical assay.

These days, the term "actionable" is most often used in cancer genomics. Actionable refers to the ability of the laboratory information to enable management decisions. This issue becomes a major challenge for WES or WGS, where many thousands of DNA variants will be identified, most of which do not contribute to the disease being evaluated. Despite this challenge, there are a number of well-publicized examples of how WGS has led to effective treatments of diseases with poor prognoses by providing data on the responsible gene and allowing targeted therapy that would not have otherwise been used.[23,24] These cases illustrate the best possible outcome of actionable findings using WES or WGS.

However, beyond management decisions, we believe establishing a molecular etiology can also be a valuable actionable outcome of NGS (or any laboratory test). This may be especially true for rare complex pediatric cases of unknown etiology, which currently often involve

great financial and emotional expense for families. WGS has been successful for up to half of such patients[23] and is arguably cost-effective if instituted early in the workup. Some insurance companies are willing to pay the sequencing costs in these settings. Furthermore, establishing a diagnosis can affect a management plan, even if it does not lead to a specific drug therapy.

Despite these incredible advances, numerous complex issues need to be addressed before WES and WGS can and should become a common component of patient evaluation.[25-29]

Different genomics in thrombosis vs cancer and malignant hematology

A fundamental difference between the genomics of thrombosis and malignancy is that somatic cancerous cells acquire an altered genome with driver mutations that differs from the germline genome. Identification of these causative mutations in cancer cells may permit utilization of therapies targeting specific genes or gene products that have a large effect on the phenotype (cancer). Conversely, the genetic contribution to thrombosis (or other nonmalignant disorders) is housed in the germline genome. Unlike acquired driver mutations in malignancy, even the most penetrable thrombophilias (like antithrombin deficiency) are not so harmful as to prevent a healthy life until adulthood in subjects with haploinsufficiency. For adult complex multifactorial diseases like VTE and atherothrombosis, innumerable low effect genes are likely to explain only a fraction of the phenotypic variance.[11,30,31]

Current personalized medicine in thrombosis diagnosis and management

Venous thrombotic disorders

Genetic and acquired factors play a role in the pathogenesis of VTE. The current management of VTE is primarily determined by the presence or absence of a significant provoking and modifiable factor. Currently, the data support ≥3 months of anticoagulation therapy for patients with provoked VTE. However, common practice may use up to 6-month (or longer) anticoagulation where the treating physician personalizes treatment based on clinical factors she/he deems important, such as location/severity of the thrombosis and ongoing inflammation.

The situation with unprovoked VTE is different and typically involves long-term anticoagulation. When there is a question regarding the feasibility of long-term anticoagulation, patient characteristics (sex, age, and body mass index), nature of the initial VTE (distal deep vein thrombosis vs proximal deep vein thrombosis vs pulmonary embolus) and assays of global hemostasis like D-dimer are used for recurrence risk assessment and personalizing therapy. The abovementioned variables have been incorporated into recurrence risk assessment models like the Rodgers model (men continue and HER [hyperpigmentation, edema, redness] DOO2 [D-dimer high, obesity, old age]), Vienna model, and DASH score (D-dimer, age, sex, and hormone use) commonly used in practice.[32-34] These prediction models need comparison studies and assessment of performance across different patient groups. Because only about half of patients with an unprovoked VTE have a recurrence at 10 years after discontinuing anticoagulation,[35] there is an opportunity to further personalize treatment by identifying factors (including genetic factors) that identify those patients who are at low risk of recurrence after ceasing anticoagulation.

Personalized evaluation and management is used for selected unprovoked thromboses: (1) antiphospholipid antibody syndrome is generally treated with indefinite anticoagulation, and (2) paroxysmal nocturnal hemoglobinuria and myeloproliferative disorder therapies may include eculizumab or cytoreductive therapy, respectively.

Genetics of VTE

The 5 well-established inherited thrombophilias have an increased risk of an initial VTE.[35-40] Increased activity of many coagulation factors has also been associated with an increased risk of VTE, and there are known genetic variants altering these phenotypes. However, most studies have not demonstrated the value of thrombophilia testing in predicting recurrent VTE, perhaps because the assays only assess variables with a small or modest effect in the complex pathophysiology of VTE. Therefore, our practice is to not perform routine laboratory testing, genetic or nongenetic, for inherited thrombophilias except in a few circumstances: (1) occasional counseling and management of an asymptomatic woman with a strong family history of VTE in a hormonal milieu setting (pregnancy, oral contraceptives, or postmenopausal hormone replacement), (2) patients with VTE and family history of thrombophilia, (3) younger patients with recurrent unprovoked VTE, or (4) patient personal preference for a better understanding of their disease.

In 2007, Reitsma and Rosendaal[41] summarized the past and future of genetic research in VTE. This paper expertly laid out how the GWAS approach was limited to identifying only common variants in the population and had not substantively advanced our understanding of the genetic causes of VTE beyond what was previously known. Two relatively small platform and 2 large platform GWASs have since been performed for SNV associations with VTE.[42-45] These studies consistently identified associations with SNVs in *F5, ABO, F11, FGG*, and *F2*, genes for which there was prior evidence for a role in VTE etiology. Several other genes, including a few novel genes, have been identified by the GWAS approach (nicely reviewed by Morange et al[46]). To date, genotyping has not replaced plasma-based assays for diagnostic purposes with the exception of the prothrombin gene variant. Testing for APCR remains controversial, even with the second-generation plasma assays using factor V–deficient plasmas.[47] Some institutions simply do FVL DNA testing, whereas others use a less expensive plasma-based APCR assay and only do DNA testing for validation.

Reitsma and Rosendaal[41] finished their review with some optimism about large-scale sequencing. Unlike GWAS, deep sequencing permits the discovery of rare, family-specific mutations that contribute to the phenotype. In many instances, these variants and genes may have a large effect on the thrombosis risk. Even if the mutations are rare and have little impact on the population-attributable risk, they can identify novel genes whose product may become therapeutic targets. We are aware of only 1 NGS effort for VTE. Lotta et al[48,49] performed NGS on the coding regions of 186 candidate hemostasis/thrombosis genes from VTE patients and controls. Many novel rare and low frequency SNVs were identified. These represent potential variants that could affect protein function and risk for thrombosis, but much work is needed to establish causality or effect size of novel private variants.

Pharmacogenetics of anticoagulants

Numerous clinical studies have sought to address the benefit of *VCORC1* and *CYP2C9* genotype-based strategies for initiating

vitamin K antagonists (VKAs), including 3 moderate-sized RCTs.[50-52] Although a genotype-based algorithm may result in a greater percentage of time in therapeutic range[50,53] than fixed VKA dosing regimens, the benefit for initial VKA dosing seems marginal at best, considering expense and effort, and does not improve patient outcomes.[53,54]

A recently published study of 14 348 patients with nonvalvular atrial fibrillation showed that *CYP2C9* and *VKORC1* genotypes were able to identify patients more likely to bleed with warfarin and for whom edoxaban may have a better safety profile than warfarin.[55] The idea of genotype-guided warfarin management may lose relevance as the use of target specific oral anticoagulants becomes more widespread.

A GWAS performed on 2944 white patients in the Randomized Evaluation of Long-Term Anticoagulation Therapy trial identified a common *CES1* variant (rs2244613) associated with lower dabigatran levels and a reduced risk of bleeding on dabigatran compared with warfarin.[56] Genetic risk scores have been developed using multiple SNVs identified in GWASs associated with VTE with an ultimate goal of personalizing anticoagulation therapy for prevention of recurrent VTE.[57-59] Considerably more refinement and validation is needed before such genetic testing would be applied to treatment approaches.

Arterial thrombotic disorders

It is beyond the scope of this perspective to discuss the enormous literature relevant to the genetics and genomics of atherothrombotic disease. For adult hematologists, the relevance largely pertains to the central role of platelets in arterial thrombus formation and antiplatelet therapies,[60,61] which will be the focus of this section. Homocystinuria is a genetic disorder associated with arterial thrombosis typically diagnosed in childhood.

Platelet hyperreactivity plays a significant role in the pathogenesis of initial and recurrent arterial thrombosis.[62] Nevertheless, modifying antiplatelet therapy based on in vitro tests of platelet reactivity has not been shown to improve clinical outcomes in patients undergoing percutaneous coronary intervention (PCI) or coronary artery bypass graft.[63,64]

Genetics of platelet reactivity

A large component of the variability in platelet function is explained by putative genetic factors, with blacks having a higher heritability component in their platelet aggregation than whites.[65] Candidate gene studies and GWASs have identified numerous genes associated with platelet aggregation, platelet thrombus formation under shear stress, platelet number, and platelet volume.[66] Platelet transcriptomic approaches have also identified novel mRNAs and microRNAs associated with and regulating platelet reactivity.[67-69] Multi-omic approaches have shown white individuals have a high frequency of the common PAR4 gene (*F2RL3*) variant Ala120 (rs773902), whereas blacks have a high frequency of Thr120. The PAR4 Thr120 isoform induces greater signaling and is associated with greater PAR4-mediated platelet aggregation.[70]

Pharmacogenetics of antiplatelet therapy

Despite a plethora of genetic/genomic data on platelet reactivity, there is relatively little actionable pharmacogenetic data with antiplatelet agents. Clopidogrel, a $P2Y_{12}$ inhibitor, is activated by the cytochrome P450 system. Patients undergoing PCI who carry the *CYP2C19*2* allele metabolize clopidogrel poorly and are good candidates for alternative $P2Y_{12}$ inhibitors due to the higher risk of stent thrombosis. Although the use of newer $P2Y_{12}$ inhibitors like ticagrelor and prasugrel is increasing in PCI settings, clopidogrel is still the most widely used $P2Y_{12}$ inhibitor because of its low cost and effectiveness. The available data do not support a genomic-guided treatment approach for aspirin or the newer $P2Y_{12}$ inhibitors.

The platelet PAR1 antagonist, vorapaxar, was recently US Food and Drug Administration approved. The *F2RL3* rs773902 genotype does not affect vorapaxar inhibition of platelet PAR1 function, but a strong pharmacogenetic effect is observed with the PAR4-specific antagonist YD-3.[70]

A GWAS performed in the Women's Health Study found an effect of aspirin dependent on a rare Ile4399Met variant in Lp(a).[71] The authors reported that aspirin reduced the increased risk associated with Met4399 for a combined major cardiovascular event end point. However, the number of events was small, and there is no established mechanism linking aspirin with Lp(a).

History and physical examination initiates the genomic evaluation and provides value

Genetic testing will be of little value for most patients with acquired thrombosis. However, family history of VTE is a strong risk factor for VTE even after adjusting for major thrombophilic defects and common VTE-associated SNVs.[72] Thus, a personal and family medical history remains the cornerstone for evaluating thrombosis etiology. Unfortunately, obtaining an accurate family health history is often challenging given the time constraints on the medical personnel and limited resources. Online and patient facing family health history collection tools can be used to help obtain accurate family health histories.[73]

From the molecular genetic point of view, there is ample evidence supporting relationships between genomics/gene expression and simple demographic information obtained via a thorough medical history and physical examination. For example, selected genomic markers strongly correlate with self-identified race and ancient geographical ancestry.[74,75] Many coding and noncoding RNAs are significantly differentially expressed by age and sex.[76] Where a person lives accounts for nearly 25% of the variation in a substantial portion (one-third) of their cellular transcripts.[77] For the foreseeable future, patient diagnosis and management will be based on these traditional mechanisms of patient evaluation (history, physical, and laboratory), rather than the patient's genomic sequence. The former is more efficiently and cheaply obtained. Consider, for example, the value of the 4Ts score for the diagnosis of heparin-induced thrombocytopenia and thrombosis.[78]

Financing health care has begun transitioning away from fee-for-service to value-based medicine in the United States. Although many different algorithms have been implemented, the basic equation of value-based medicine is patient outcomes divided by the costs for delivering those outcomes. For patients, this means persistent effective care at reasonable cost. We believe a good medical history and physical examination can reduce unnecessary and expensive laboratory testing, imaging, and consultations and thereby enhance the value of care.

Future opportunities for personalized medicine for thrombosis

Newer tests of global hemostasis, such as in vitro thrombin generation, may have a role in personalizing VTE management.

To capitalize on the power of NGS, large populations of patients will be required for novel thrombosis gene discovery and novel, actionable pharmacogenetic interactions. First-generation genetic risk scores and "thrombo-chips" may benefit from additional markers identified by NGS and will need further evaluation and confirmation before they can be used in clinical practice.

Because of the central role of platelets in atherothrombotic disease, there has been an increasing number of platelet RNA association studies, primarily in cardiovascular disease.[79-81] Transcriptome-wide association studies also provide an unbiased approach to novel functional gene discovery; eg, miRNAs associated with myocardial infarction.[82] Relevant to hemostasis/thrombosis, RNA is most easily available from platelets, which reflects the megakaryocyte transcriptome.[83] Serum and plasma can serve as a source for RNAs from platelets, liver, and endothelial cells, but it will be critical to consider confounding demographic variables and comorbid disease known to affect blood RNA levels.[76]

The potential of pharmacogenetics to impact on personalized medicine in thrombosis will require that clinical trials of antithrombotic agents include both genomic data and other measures of factors that affect the response to therapy, such as accurate clinical, environmental, social, and dietary information.[84] Gene-gene, gene-environment, and pharmacogenetic interactions are largely unexplored in thrombotic disease, and large sample sizes will be needed to tap this potential.

Because PAR4 is the only functioning receptor for thrombin in the presence of vorapaxar, it will be of interest to understand the pharmacogenetic effect of the PAR4 Ala120Thr variant in patients using any PAR1 or PAR4 blocker, and clinical trials are needed that consider the rs773902 genotype. Such pharmacogenetic data may provide a rational basis for decisions about benefit or harm of novel antiplatelet agents.

It is increasingly likely that hematologists will be faced with patients who have had their genomes sequenced and who ask about variants that may be associated with thrombosis or antithrombotic utility. The identification of rare, family-specific variants will require functional and clinical assessment for effect size on thrombosis risk. Because the number of such variants may be large, recommendations from expert subcommittees of international societies could promote personalized management.

The novel genetic markers discovered and validated in previously mentioned studies may have a higher impact in personalizing management as genome sequencing for all individuals becomes routine in the future due to rapidly reducing cost. Personalized medicine in thrombosis may someday include novel gene therapy approaches involving autologous transplantation of patient differentiated hematopoietic stem cells that have undergone gene editing with CRISPR/Cas9 (clustered regularly-spaced short palindromic repeats and CRISPR associated protein 9) or other techniques. Last, anti-microRNA therapy has recently been used to treat in vivo thrombosis,[85] and this novel therapeutic approach may benefit patients in whom dysregulated gene expression causes hypercoagulability.

Summary

Personalized medicine has been and will always be a valued and essential approach to patient management. Personalized medicine in the context of thrombosis in 2016 remains primarily grounded in a thorough patient history, physical examination, and hemostasis/thrombosis laboratory testing. However, for most patients with VTE (unprovoked or provoked, first or recurrent), extensive hemostasis/thrombosis testing is not warranted for management decisions. A genotype-guided approach compared with fixed-dose warfarin does not improve patient outcomes.

Genomics in thrombosis in 2016 is an important research tool for understanding disease mechanism. The genomic bench has not worked itself to the clinical thrombosis bedside. Genome-wide DNA/RNA sequencing should continue in research settings for causal gene discovery, pharmacogenetic purposes, and gene-gene and gene-environment interactions. The potential of genomics to advance medicine will require integration of personal data that are obtained in the patient history: exposures in home and work environment, diet, social data, etc. Furthermore, without this information, we will have depersonalized medicine, which lacks the precision needed to do the research required to eventually incorporate genomics into routine and optimal clinical care.

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Correspondence: Paul F. Bray, Thomas Jefferson University, The Cardeza Foundation for Hematologic Research and the Department of Medicine, Sidney Kimmel Medical College, Jefferson Alumni Hall, Room 394, 1020 Locust St, Philadelphia, PA 19107; e-mail: paul.bray@jefferson.edu.

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