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Jumping on the Train of Personalized Medicine: A Primer for Non-Geneticist Clinicians: Part 3. Clinical Applications in the Personalized Medicine Area

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Abstract: The rapid decline of sequencing costs brings hope that personal genome sequencing will become a common feature of medical practice. This series of three reviews aim to help non-geneticist clinicians to jump into the fast-moving field of personalized genetic medicine. In the first two articles, we covered the fundamental concepts of molecular genetics and the methodologies used in genetic epidemiology. In this third article, we discuss the evolution of personalized medicine and illustrate the most recent success in the fields of Mendelian and complex human diseases. We also address the challenges that currently limit the use of personalized medicine to its full potential.

Keywords: Clinical utility, ethics, next generation sequencing, pharmacogenetics, prediction, personalized medicine.

INTRODUCTION

The observation of a familial clustering for human diseases was first reported by the Greek physician Hippocrates at the time of the 5th century BC [1]. He believed that hereditary material in all parts of the body affected health of next generation [1]. In 1865, Gregor Mendel published his seminal work on the laws of Mendelian inheritance from his experiments in peas [2]. In 1902, Archibald Garrod postulated that inborn errors of metabolism in humans might follow Mendel's laws and described how alkaptonuria, a rare human disorder, followed a pattern of recessive inheritance. This was the first report linking Mendel's laws and a human disease [3]. Garrod can be considered as the founder of human genetics, a field that has long been considered by most physicians as an esoteric academic specialty [4]. Times have changed with the development of clinical genetics and more recently with the emergence of the concept of personalized medicine.

Personalized medicine, also known as genomic medicine or precision medicine, originated with the idea of using an individual's unique genetic make-up to assess the risk of developing disease, predict the course and prognosis of disease, and tailor therapeutic interventions accordingly [4, 5]. It was this blueprint that inspired the United States National Research Council in 1990s to initiate the Human Genome Project [6, 7]. Completion of the Human Genome Project, the HapMap project and more recently the 1000 Genomes Project has resulted in an explosion of genetic discoveries related to human disorders [8-10]. Since then, there has been marked improvement in high-throughput technologies for both genotyping and sequencing, which along with advances in computational biotechnology, has fostered great promise in the potential of personalized medicine to revolutionize how we understand, diagnose, prevent and treat diseases.

Genetic screening is an important tool to use advances in genetics and genomics to improve public health [11]. However, in the first half of the 20th century, many scientifically unsound and socially harmful policies and laws based on "perceived genetic risks" had been adapted and implemented in many countries in the name of eugenics. Eugenics was coined by Sir Francis Galton in 1883 and he claimed that "a highly gifted race of men" could be generated by the process of selective breeding [12]. Among the most famous proponents of the eugenic idea, the United States (US) was the first country to take some actions. On one side, the US advocated "positive eugenics" to encourage reproduction among those who were presumed to hold superior gifted genes. On the other side, as many as 33 American states passed "negative eugenics" laws to promote compulsory sterilization surgeries to individuals who were mentally disabled or ill, morally undesirable (like the prisoners), or who belonged to socially disadvantaged groups living on the margins of society [13]. These laws were upheld by the US Supreme Court in 1927, but the "negative eugenics" movement led to more than 60,000 sterilizations across the US [13, 14]. German politicians and scientists endorsed the Nazi "racial hygiene" eugenic movement during 1933-1945. As a consequence of such motivation and actions, approximately 400,000 feeble patients were sterilized without consent and 275,000 of them were murdered by the Nazi "euthanasia" programs [15-18]. Some other countries also adapted such sterilisation programs, for example in Sweden, Canada and Japan [19-21]. In reaction

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to Nazi abuses, eugenics became almost universally reviled in many of the nations where it had once been popular. Scientists recognized the difficulty of predicting characteristics of offspring from their parents and demonstrated the inadequacy of simplistic theories of eugenics. The Universal Declaration of Human Rights was adopted by the United Nations in 1948 and affirmed, "Men and women of full age, without any limitation due to race, nationality or religion, have the right to marry and to found a family".

The modern concept of personalized medicine aims to use personal genetic information to predict or diagnose a disease (through prenatal diagnosis, neonatal screening, diagnosis of genetic disease in children, screening prospective parents for the carrier status of specific disorders, prediction for a serious late-onset disease), to minimize the exposure to environmental risks or to assess the differentiated response to a therapeutic drug [11, 22]. In this review, we will first discuss how to estimate the clinical utility of genetic testing; second, illustrate the current status of personalized medicine with examples; third, highlight the challenges on the way towards personalized medicine; and last, envision the future of personalized medicine.

HOW TO ASSESS THE CLINICAL UTILITY OF A GENETIC MARKER

Whereas some genetic variants have an obvious clinical utility in disease diagnosis (e.g. the mutation F508del in the *CFTR* gene and cystic fibrosis [23, 24]), others genetic variants despite being strongly associated with diseases do not necessarily imply a predictive value in clinical practice [25]. The measurements of genetic variant's effect sizes (odds ratio, relative risk, hazard ratio) commonly used in traditional epidemiology are not adequate to determine the potential value of a genetic marker for predicting individual risk. The efficiency of a new test is typically evaluated by discrimination using a receiver operating characteristic (ROC) curve [26], or an alternative *c* statistic in survival data [27]. The ROC curve is a plot of sensitivity or the true positive (the probability of a positive test among those with the disease) versus 1-specificity or the false positive (the possibility of a positive test among those without the disease). Each point on the ROC curve represents the decision criterion at a given threshold. Given a specific threshold, the predictor values above this are classified as positive (diseased category) and those lower than this are classified as negative (non-diseased category). The ROC curve also shows the trade-off between sensitivity and specificity. In other words, any increase in sensitivity will be accompanied by a decrease in specificity. The area under the curve (AUC) from the ROC analysis is used to assess how well the model can distinguish people who do have the disease from those who do not. By definition, an AUC of 0.5 indicates classification of cases and controls by chance and 1 designates a perfect classification. AUCs of 0.50-0.70 are considered as low, 0.70-0.90 are considered as moderate, and > 0.9 are considered as high [28]. For example, in a study of prediction of depression in dementia in Alzheimer's patients which was measured by the Cornell Scale based on signs and symptoms, an AUC of 0.91 meant that the probability was 91% that a randomly selected case had a higher Cornell Scale than a randomly selected non-case [29, 30]. This approach has been widely used to examine the clinical utility of common and rare genetic variants in predicting the risk of having common diseases [31-33]. These results for the most part have shown that the addition of genetic variants only slightly improve the performance of risk prediction compared with the models with standard clinical risk factors. This phenomenon may be explained by the small individual effect size (odds ratios<1.5) of genetic variants analyzed separately and by an insufficient knowledge of disease predisposing genetic variants. Notably, Pepe *et al*. have suggested that an odds ratio of 3.0 or smaller may be of clinical importance in characterizing population variations in risk but may have little impact on the ROC curve or *c* statistic [34]. In other words, a strong association between an outcome and a predictor does not imply that the ROC curve analysis or *c* statistic will give rise to a good estimate of discrimination. Additionally, the ROC curve and *c* statistic are insensitive to assessing the impact of adding new markers to an existing predictive model, especially when there is a correlation between them [30].

When it comes to risk factors, patients and physicians alike are interested in the likelihood of disease development and options for a better medical management afterwards, rather than the true positive rate and true negative rate if the patient has been diagnosed. This can be measured by calibration or reclassification, another measurement of clinical utility. If a model with novel predictive markers can more accurately classify individuals into higher or lower risk categories, it is better calibrated and will lead to a better clinical outcome. For instance, three independent studies performed reclassification analysis using genetic variants to predict the risks of cardiovascular diseases, type 2 diabetes and breast cancer [35-37]. These studies showed various risk reclassification improvements from 4 to 53% [35-37]. For example, in Wacholder and colleagues' study, after the addition of 10 common genetic variants associated and with breast cancer into the traditional risk model, the AUC increased from 58% to 61.8% which was modest; but 32.5% of patients were reclassified into a higher quintile, 20.4% into a lower quintile, and 47.2% remained in the same quintile [37]. Thus, different therapeutic options would be applied to different subgroups and improved outcomes would be expected. Furthermore, whether the reclassification is correct can be tested using the Hosmer-Lemeshow test [38]. Based on the reclassification table, a single measure named net reclassification index (NRI) was proposed by Pencina *et al*. [39]. It examines the proportions moving up or down categories among cases and controls separately and NRI = [Pr(up|case)-Pr(down|case)]-[Pr(up|control)-Pr(down|control)]. The most advantageous feature of NRI over ROC curve analysis and reclassification is that the categories of up and down can be defined according to clinically important risk estimates. As a result NRI can detect the prediction of clinically significant improvement due to genetic markers. Strictly speaking, NRI is a measure of discrimination rather than calibration. Therefore, when the clinical utility of genetic variants and other molecular signatures are investigated, careful selection of relevant statistical metrics, such as risk reclassification and NRI, is essential.

CURRENT PERSONALIZED MEDICINE APPLICATIONS

In the post-genomic era, the elucidation of genetic basis of human disorders is progressing with unprecedented rapidity. Genome-wide association studies (GWAS) have identified several thousand common and low-frequency single-nucleotide polymorphisms (SNPs) associated with human diseases. Whole-exome sequencing (WES) and whole-genome sequencing (WGS) have more recently led to the discovery of disease-causing rare variants. WES selectively sequences the coding regions and is useful to discover rare coding variants which usually have more severe functional consequences. WES has been successfully used to identify genetic determinants of both common and rare diseases [40-42]. WES is currently cheaper and more commonly used than WGS [43]. The applications of this new body of knowledge to state-of-the-art personalized medicine are described below.

Mendelian Diseases

Until the advent of high-throughput technology, positional cloning and candidate gene approach were the primary methodologies by which approximately 2,000 genes causing Mendelian diseases were identified [44, 45]. These genes represent the foundation on which the routine genetic tests that are widely used in clinical laboratories provide early diagnosis or early prediction. The relevance of mutations or structural variants responsible for Mendelian disorders is obvious in genetic tests as they have very clear effects on phenotype. The diagnosis of Mendelian disorders is more beneficial if efficient treatments are available. For example, permanent neonatal diabetes is caused by mutations in *KCNJ11* and *ABCC8* among other genes [46, 47]. The two genes encode Kir6.2 and sulfonylurea receptor 1 (SUR1), the two subunits of the ATP-sensitive potassium (K_{ATP}) channel, and trans-activating mutations in these genes result in a failure of the beta-cell K_{ATP} channel to close in response to increased intracellular ATP and impaired insulin secretion [48]. Ninety percent of patients carrying a mutation in *KCNJ11* or *ABCC8* genes reverse diabetes when they are shifted from insulin to oral sulfonylurea medication [47, 49]. However, the clinical diagnosis of permanent neonatal diabetes is based on Sanger sequencing of the PCR fragments from the *KCNJ11* and *ABCC8* genes. This molecular diagnosis is restricted to a limited number of the known mutations and other possible genetic loci elsewhere in the genome are not assessed. Recently, Bonnefond *et al*. performed WES for a permanent neonatal diabetes patient and identified a novel non-synonymous mutation (c.1455G>C/p.Q485H) in *ABCC8* gene which was missed by classical Sanger sequencing [50]. Using WES in the maturity-onset diabetes of the young (MODY) patients, the same research group found one mutation (p.Glu227Lys) in *KCNJ11*, indicating that such MODY patients can be ideally treated with oral sulfonylureas [51]. Although Sanger sequencing is the gold-standard DNA sequencing method, next generation sequencing (NGS) has its unique advantage at finding a novel disease-causing mutation in larger areas of the genome when the exact site of mutation is unknown.

When WES is performed, 20,000-30,000 genetic variants are typically identified in patients comparing to reference genomic sequences. A series of filtering strategies are then required to isolate the disease-causing variant(s) [52]. Since the first report of the targeted capture and massively parallel sequencing of the exomes of 12 humans in 2009 [43], WES has identified many novel disease mutations that contribute to both Mendelian and common diseases [52]. In 2010, Sarah Ng and colleagues used WES to sequence four patients who were affected with Miller syndrome (MIM#263750), an autosomal recessive inherited disorder. By simple filtering procedures using dbSNP and the HapMap databases to prioritize the candidate variants, they found Miller syndrome was caused by mutations in *DHODH* gene [53]. This was the first WES study that identified a causal gene for a Mendelian disorder. Targeted re-sequencing in another four affected individuals using Sanger approach found that all of them were compound heterozygotes for missense mutations in *DHODH*. Furthermore, each parent of the affected individual was a heterozygous carrier, none of the mutations appeared to be *de novo*, and none of the unaffected siblings were compound heterozygotes. All of these features supported the hypothesis that *DHODH* was the causal gene responsible of Miller disorder [53].

More recently, WES has not only led to the identification of a novel Mendelian mutation and the elucidation of a novel mechanism underlying inflammatory bowel disease (IBD), but also provided key information for the clinicians to find an effective treatment [54]. A boy started to present Crohn's disease-like symptoms when he was 15 months old. Comprehensive clinical evaluation and laboratory examinations (including genetic tests of defined forms of IBD) could not reach a conclusive diagnosis, thus his illness could not be controlled and was getting worse and life-threatening. When the patient was at age of 5 years and 8 month, a WES was conducted and a mutation in the X-linked inhibitor of apoptosis gene *XIAP* was identified. The affected boy was a hemizygote for a cysteine to tyrosine amino acid substitution, leading to a previously undefined form of IBD. This variant was confirmed and his mother was heterozygous carrier for the same mutation. XIAP protein has a central role in the pro-inflammatory response and bacterial sensing through the NOD signaling pathway [55, 56]. In *in vitro* tests with the patient's cells, the mutated protein had an increased susceptibility to activation-induced cell death and defective response to NOD2 ligands. After receiving an allogeneic hematopoietic progenitor cell transplant, the boy was able to eat and drink normally and there was no recurrence of gastrointestinal symptoms [54].

These studies clearly demonstrate that disease-causing variants for Mendelian disorders can be directly identified by WES in several unrelated individuals or in a single family. In addition to filtering variants based on a variety of reference databases, another strategy used to remove benign variants is bioinformatics-based prediction of the putative impact of point mutations on the structure and function of human proteins like the software PolyPhen-2 (Polymorphism Phenotyping v2) [57] which has been used in Bonnefond *et al*.'s study [50]. It should be known that such computational algorithms have at least 20% of false prediction [52].

In combination with other challenges encountered by WES during filtering and interpretation, current success rate of identifying causal mutations with WES is approximately 50% [52]. Theoretically, WES is expected to be more efficient when applied to recessive disorders because the likelihood to find homozygous or compound heterozygous carriers for rare non-synonymous variants is low.

Common Diseases

Unlike Mendelian diseases, the predictive value of common genetic variants with modest effects identified by GWAS is limited in the context of common diseases. Some common loci with unusual large effect sizes have been used for disease prediction in clinical settings, for example, *HLA* variants in autoimmune disease like type 1 diabetes and rheumatoid arthritis, *APOE* in Alzheimer's disease, and *BRCA1* and *BRCA2* in breast and ovarian cancers [58]. It is important to mention that these variants were identified by linkage studies or candidate gene approach before the GWAS advent. Among thousands of genetic variants identified by GWAS, except for a handful of variants having odds ratios greater than 3, most of them so far have small effects with a median odds ratio of 1.33 [59]. When the associated variants thus far are considered together they generally account for a small proportion of the heritability of a specific disease [60].

Is it too early to implement genomic information in the prediction of the risk of having a common disease? ROC analysis using genetic information from common variants identified by GWAS did not provide clinically relevant improvement in the prediction of type 2 diabetes or cardiovascular disease, even using more than 20 SNPs together [32, 61, 62]. Such failures are not surprising, as the variants selected in these studies are usually associated with the disease exceeding a stringent level of statistical significance ($P < 5 \times 10^{-8}$). Beyond these 'top hits', many genetic variants with true modest effects on the trait do not reach such a level of association because of statistical power issues. These variants are consequently excluded from the prediction analyses. Genome-wide association consortium initiative studies with very large samples and the use of new algorithms may enable a better prediction of the risk of common diseases.

Height is a polygenic trait with an estimated heritability of 80%. To date, a large-scale GWAS meta-analysis in close to 200,000 subjects identified hundreds of genetic variants in 180 loci conclusively associated with height that together explain 20% of the genetic variation of height [63]. Yang *et al*. chose a method of restricted maximum likelihood that simultaneously accounted for all the SNPs (N=294,831) genotyped in a DNA array and explained 45% of the genetic variation of height [64]. Stahl *et al*. developed a novel method based on Bayesian inference and evidenced that thousands of common SNPs were able to explain approximate of 50% of the heritability for both cardiovascular diseases and type 2 diabetes [65]. This suggests that many more SNPs contributing to the trait remain to be discovered and that GWAS from even larger studies and with better imputation methods (e.g. using the 1000 Genomes Project reference panel) will continue to be highly productive for the discovery of additional susceptibility loci for common diseases. In another study, Wei *et al*. used a sophisticated Support Vector Machine (SVM) algorithm to assess the risk of type 1 diabetes using whole-genome genotyping array data [66]. They demonstrated that SVM could accurately assess the risk of type 1 diabetes with an AUC of approximate 0.84 in two independent datasets. This study also reported that the higher the heritability is, the more accurate prediction SVM provides. These studies suggest that the current lack of clinical relevance of prediction models for common diseases may be related to incomplete knowledge of the disease-associated SNPs and to the use of suboptimal methodologies. The integration of common genetic variation information into efficient prediction models is definitely relevant in personalized medicine.

Psychiatric diseases are currently diagnosed by symptoms and psychopathological tests with criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM, 5th edition) [67]. These criteria are more categorical than quantitative, sometimes making the diagnosis ambiguous. Furthermore, it is common that different psychiatric disorders share biologic background and environmental exposures. Based on these, Bragazzi proposed to apply omics science and personalized medicine to the field of psychiatry to refine the disease classification and diagnosis and tailor the therapeutic regimen [68]. Recently, Professor Bernard Lerer, the director of the Biological Psychiatry Laboratory at Hadassah-Hebrew University Medical Center, Israel, won the Werner Kalow Responsible Innovation Prize in Global Omics and Personalized Medicine because of his achievements in the development of methodology and novel discoveries in the field of psychiatric pharmacogenetics [69]. This shows a strong international peer-recognition for the success and potentials of personalized medicine in psychiatric disorders.

Along with common variants, low-frequency SNPs and rare variants are also important in the elucidation of missing heritability and in prediction of the risk for common diseases [70, 71]. Many studies have provided clear evidence that rare variants contribute to chronic diseases [72-75]. By resequencing the exons and regulatory regions of 10 candidate genes, Nejentsev *et al*. identified that four rare variants in the exons and introns of *IFIH1* (encoding interferon induced with helicase C domain 1) gene were associated with type 1 diabetes, none of which was coupled with a known common SNP in the same gene, suggesting *IFIH1* gene is casual [72]. Large-scale exon re-sequencing of *MTNR1B* gene (encoding melatonin receptor 1B), which was initially found to be associated with type 2 diabetes by GWAS, revealed that 36 very rare variants with minor allele frequency less than 0.1% were associated with type 2 diabetes, and a pool of 13 of them having partial- or total-loss-of-function strongly increased the risk (odds ratio=5.67, 95% confidential interval: 2.17-14.82, $P=4.09 \times 10^{-4}$) [73]. Subsequent biological evaluation of these rare variants further confirmed the functional link between *MTNR1B* and type 2 diabetes. An extended haplotype association study in an enrichment population of Ashkenazi Jewish, in which the prevalence of Crohn's disease is several-fold higher compared with non-Jewish European ancestry, has found an ethnic-specific

missense rare mutation R642S in *HEATR3* to be associated with Crohn's disease [74]. An integrated simulation framework to mimic the empirical genetic data of common diseases suggested that rare variants played a significant causal role in explaining missing heritability, but it also excluded such an extreme hypothesis that rare variants are entirely responsible for disease [76]. Therefore, the combined effect of both common and rare genetic variants may significantly improve disease prediction [77].

In addition to prediction based on GWAS data, the potential applications of WGS are being explored to predict the risk of common diseases. A report by Roberts *et al*. [78] constructed a mathematical model and used the information of incidence of 27 common diseases from large monozygotic twin studies to assess the capacity of WGS data in predicting who were at risk of these diseases. They concluded that the predictive value of this approach was small. This study raised much debate [79-81]. Begg and Golan criticized the analytic caveats in this study and proved that WGS could theoretically offer more optimistic risk prediction compared with what presented by Roberts *et al* [79, 80]. As pointed out by Topol, the predictive capacity of WGS is unlikely to be sufficiently powerful until the sequences of many individuals with the same well-defined trait and advanced analytic approaches are available [81]. He stated with optimism that his lab would sequence 14 million people at the end of 2014. Another study sequenced whole-genome for eight individuals, four at upper and four at lower deciles of risk for metabolic, cardiovascular, skeletal and mental health [82]. Approximately two-thirds of the genetic predictions were concordant with longitudinal clinical measurements.

Combining genomic information with regular monitoring of clinical status which measures other "omics" profiling with different high-throughput platforms will theoretically improve personalized medicine. Recently, Chen *et al*. first used "integrative personal omics profiling" (iPOP), which included genomics, transcriptomic, proteomic, metabolomics and autoantibody profiles, to evaluate healthy and diseased status [83]. They collected blood samples from a 54-year-old male volunteer at 20 time points during a 24-month study and captured snapshots of several molecular metrics at different conditions of health (i.e. healthy, during viral infection, recovery). The subject coincidentally developed type 2 diabetes during the monitored time frame. The results captured extensive and dynamic changes in diverse molecular features and biological pathways that occurred as the subject transitioned from healthy to diseased conditions. Using poly-omics dataset, Heather *et al*. recently developed a method called OmicKriging and showed substantially better performance in prediction of seven diseases than any single omics dataset in the study from the Wellcome Trust Case Control Consortium (WTCCC) [84]. With this strategy, collective databases with "omics" profiles from more individuals with different diseases may be valuable in the diagnosis and management of diseases, even if this approach may not be realistic in a clinical setting.

Pharmacogenetics

Traditionally, clinical trials classify patients into different groups on the basis of symptoms (e.g. mild/severe depression) or histological patterns (e.g. breast cancer stage I/II/III), assuming that the patients within the same subgroup will have similar responses to treatment. This current symptom-based treatment regimen leads to more than 2 million adverse drug reactions annually in US alone with a cost of $76 billion for drug-related morbidity and mortality [85]. Generally speaking, with a given medication, 25-60% of the patients gain therapeutic benefits and the rest either do not respond or suffer from drug toxicity [85]. Administrating a drug to non-responders also induces colossal loss of money for the public health system. For example, 30-40% of the psychiatric patients with major depression do not respond to treatment with fluoxetine [86]. These numbers highlight the fact that individuals vary greatly in their response to treatment, and part of this response may be inherited. If the patients are stratified using genetic markers (or genomic markers such as gene expression signatures in the broader context of pharmacogenomics), subgroups are expected to become more homogenous and display a more similar response to the same treatment.

Pharmacogenetics refers to genetic variations that affect individual responses to drugs, in terms of both clinical efficacy and adverse effects, thus predicting efficacy and toxicity and indicating dosage adjustments [87]. The genes harboring these genetic markers usually encode enzymes which are involved in the course of the pharmacokinetics and pharmacodynamics of the drug.

Cardiovascular medicine offers a good illustration of the impact of pharmacogenetics in clinical practice. Warfarin has been the most widely used oral anticoagulant for 60 years and it achieves therapeutic anticoagulation without excess risk of bleeding or thromboembolic events only within a narrow range of concentrations in the blood. The response to warfarin varies greatly from patient to patient and 10-20 fold differences in warfarin dosage have been reported to achieve the therapeutic effect [88]. As a result, warfarin use is associated with multiple dose adjustments, long periods of over- or under-anticoagulation for the patients, and inappropriate dosage of this drug is the leading cause of emergency department visits and hospitalizations due to an adverse drug reaction [88]. Finding new strategies for an effective and safe use of warfarin is therefore an ongoing and vital concern. Sequence variants in genes that encode cytochrome P450 2C9 (*CYP2C9*), a major enzyme that metabolizes warfarin, and vitamin K epoxide reductase (*VKORC1*), the molecular target of warfarin, have proved to contribute to more than 50% of dose variation among the patients [89, 90]. In 2009, the International Warfarin Pharmacogenetics Consortium established a dose algorithm based on these genetic variants and clinical relevant indicators [91]. The results showed that this algorithm was superior to predominant strategy, using clinical variables only, at directing the initial dosage to achieve desirable and stable therapeutic concentrations. It identified 49.4% of the patients that needed lower doses and 24.8% that required higher doses, in comparison to 33.3% and 7.2% from clinical algorithm, thus providing a better dose adjustment and improved treatment. This algorithm has been followed by evidence-based studies to evaluate its effectiveness. Initial warfarin dosage adjusted from the patient's genotype data could reduce the risk of hospitalization in outpatients by 31%

[92] and globally improve the clinical outcomes including significantly lower rate of serious hemorrhage [93]. Based on this evidence, Food and Drug Administration (FDA) modified the warfarin label, stating that *CYP2C9* and *VKORC1* genotypes may be useful in determining the optimal initial dose of warfarin [94, 95]. Most recently, two large randomized controlled trials tested the effect of the genotype-guided algorithm for warfarin dosing [96, 97]. The study by Kimmel *et al* recruited patients from different ethnic groups in US and showed that the percentage of time reaching the therapeutic range was almost identical in both genotype-guided and clinically guided groups (45.2% *vs*. 45.4%) and the rates of side effects did not differ either [96]. A significant interaction between dosing and race was observed. Controversially, Pirmohamed *et al.* reported significant improvement in the percentage of time reaching the therapeutic range (67.4% *vs*. 60.3%) and significant decrease in the rate of side effects in the genotype-guided *versus* clinically-guided groups of Europeans [97]. However, these two studies were underpowered to assess the more important end-point, the rate of bleeding and thrombotic complications, which was reported as the secondary outcome in both trials [98]. Therefore, meta-analysis of these outcomes or randomized controlled trials based on ethnic-specific algorithms may be necessary, indicating that the promise of genotype-based algorithm is proving to be more difficult than first predicted.

Another example of pharmacogenetics at work is statin, a cholesterol-lowering drug that effectively reduces the incidence of heart attack and stroke [99]. However, high doses of statin (e.g. 80mg/day) may induce myopathy [100]. A GWAS that selected 175 matched cases and controls from a 12,000-participant trial identified a non-coding SNP rs4149056 strongly associated with statin-induced myopathy [101]. This variant is located in the gene *SLCO1B1*, a well-known regulator of the hepatic uptake of statin. The homozygotes of the risk allele (CC) have 16.9 times higher risk of myopathy than non-risk allele homozygotes (TT). The screening of this genetic variant may help avoid serious side-effect of statin. However, the very low incidence of myopathy lowers the positive predictive value of this variant and reduces its cost-effectiveness, therefore, this pharma-cogenetic indication has not been pursued by FDA.

Cytochrome P450s (CYPs) consist of a large family of metabolizing enzymes which are active in the metabolism of clinically used drugs like warfarin discussed above. P450 genes are polymorphic and variations in *CYP2D6* and *CYP2C19*, alone or together, have also been shown to cause the ultra-rapid or delayed clearance of many psychiatric medications [102-104]. For example, citalopram is one of the widely prescribed antidepressant medications, but more than 50% of the patients do not have a complete remission of their symptoms [105]. Citalopram is a highly selective serotonin reuptake inhibitor metabolized by CYP2C19, CYP3A4 and CYP2D6 enzymes [106, 107]. Individuals who are homozygous for *CYP2C19**17/*17 genotype (ultra-rapid metabolizer) have 42% lower of serum concentration of citalopram compared with those with normal function alleles and increase the probability of therapeutic failure [108]. Therefore, increasing the starting dose is recommended. On the other hand, individuals with the *CYP2C19**2/*2, *2/*3, *3/*3 (poor metabolizer) genotypes have higher serum concentration and increased risk of side effects, thus using 61% of the standard dose has been suggested [109]. Although minimal downward dose adjustment has been suggested for poor CYP2D6 metabolizers, a potential interaction between *CYP2C19* and *CYP2D6* effect has been reported and labeled by FDA [104, 110].

The number of pharmacogenetic associations is increasing steadily [111] and the FDA has appended pharmacogenetic information to approximate 140 drug labels across a variety of diseases and 23 of them are psychiatric medications (http://www.fda.gov/Drugs/Science Research/ ResearchAreas/Pharmacogenetics/) [112]. Black box warnings on some drugs denote serious or life-threatening risk of adverse effects to patients related with specific genetic variants. Importantly, such pharmacogenetics-based genotype tests should be considered before initiating drug treatment to maximize the patients' benefits and minimize the drug side effects. When someday a clinical genetic program which integrates drug-gene interactions will be applied into patient electronic medical record system, a patient's tested genetic information will help the physicians to choose the optimal drug and its appropriate initial dosage [113]. In fact, patient electronic medical records are gradually being introduced into clinical practice and will keep updated with evidence from pharmacogenetic research [113].

Cancers

Cancer is a common disease that is standing on the frontier of personalized medicine. The importance of inherited cancer risk has long been realized and the American Society of Clinical Oncology (ASCO) released its first statement on genetic testing for cancer susceptibility in 1996 [114]. This statement has since been updated repeatedly to keep up with the rapid pace of new discoveries in genetics [115]. Some of the genetic variants identified from germline genetic testing are highly penetrant and confer substantial increases in cancer risk. *BRCA1* and *BRCA2* are such examples, where breast-cancer risk by the age of 80 years in carriers of the *BRCA1* and *BRCA2* pathogenic mutations are 90% and 40%, respectively, though their frequencies in the population are low [116]. Therefore, if the mutations in *BRCA1* and *BRCA2* are detected in a woman with multiple affected family members, clinical decisions of intensive screening with mammography or magnetic resonance image, and even preventive surgery would be prudent [115]. Most genetic variants identified from GWAS are low-penetrant and have limited clinical relevance in the context of the currently applied methodologies. Thus, they are not currently used as part of standard cancer diagnostics [115]. The challenge is how to parse the flood of data into simple and usable information. Recently, Massachusetts-based Foundation Medicine has developed software to interpret sequenced genomic data in tumor tissues and are now capable of sequencing up to 300 cancer related genes and extracting potentially actionable information for clinicians, and studies are ongoing to link the results to care recommendations [117].

Beyond genetic information, gene expression markers which measure the levels of messenger RNA (mRNA) are

extremely useful in all aspects of cancer management, from disease classification, response to chemotherapy, development of new therapeutics, and prognosis [118]. In some tumors, like breast cancers and glioblastmas [119], molecular markers have been implemented as disease classification criteria. Breast cancer has been classified into four molecular categories on the basis of histological patterns and gene-expression markers [120, 121]: basal-like cancers (estrogen-receptor (ER)-negative, progesterone-receptor (PR)-negative, and human epidermal growth factor receptor 2 (HER2)-negative), luminal-A cancers (ER-positive and histological low-grade), luminal-B cancers (ER-positive and histological high-grade), and HER2-positive cancers. This classification is still evolving as more data from microarray profiling, which measures thousands of mRNA transcripts simultaneously, increase the number of categories and classifications under each type of cancer, providing more precise targeted and efficient therapy. Gene-expression signatures also provide a unique approach to identify certain primary tissue from which the metastatic tissue develops, because expression pattern of the origin tissue are often retain in the cancer [118].

Another two categories of biomarkers, epigenetic changes and microRNA, are increasingly thought to drive the development of cancers [122-125]. Epigenetic changes are heritable and cause the changes of gene expression without alteration of DNA sequence [126]. DNA methylation is the currently most studied epigenetic mechanism which has been linked to both normal development and human diseases [126]. In cancer, epigenetic mechanisms act in term of silencing tumor suppressor genes and DNA repair genes and activating oncogenes [122]. For examples, methylation of tumor suppressor gene *BRCA1* is associated with breast cancer, and inactivated DNA repair gene *MGMT* is associated with glioblastomas [127, 128]. Recently, the genome-wide methylation technologies enable the comparison of DNA methylation patterns in normal and cancer cells [129]. Distinct patterns of DNA methylation have been reported to be associated with several cancers and their progression [130]. MicroRNAs are endogenous small (about 18-24 nucleotides) non-coding RNA molecules and are thought to play a key role in the regulation of translation and degradation of mRNA in physiological and pathological processes, including cancer [131, 132]. MicroRNA expression profiling using microarrays has been linked to a wide range of human cancers such as prostate and colorectal cancers [133]. Importantly, abnormal DNA methylation and microRNA expression levels in the plasma or serum are non-invasive and are consistent with the methylation and microRNA status in the primary tumor. Because both epigenetic changes and microRNA expression are involved at every step of cancer development and are potentially reversible by methylation inhibitors or antisense microRNAs, they hold promise in diagnosis, prognosis and specific tailored cancer therapies. But the clinical benefits are uncertain and lack scientific rigor at this early stage of evidence [125, 134].

Targeted therapy in cancer may also be directed by gene-expression based classification. Among breast cancer patients, 25-30% of them overexpress *HER2* gene which encodes a trans-membrane glycoprotein receptor and stimulates cell proliferation [135]. Meanwhile, the overexpressed *HER2* is highly associated with relapse within a short time and low survival rate. Trastuzumab, a recombinant monoclonal antibody, specifically targets *HER2*-postive breast cancer and improves the survival of patients [136, 137]. Similarly, Gefitinib targets the tyrosine kinase domain of the epidermal growth factor receptor, which is overexpressed in 40-80% of non-small-cell lung cancers and other epithelial cancers. However, only 10% of non-small-cell lung cancer patients harbor specific somatic mutations in the tyrosine domain and response quickly and well [138]. In the patients with the mutations, the response rate is 71% compared with 1% for those without [139].

Gene expression signatures including several dozens of genes have been applied to predict clinical outcomes, thus avoiding the hazards of unnecessary or ineffective chemotherapy and expensive costs. Before the prognostic gene signature for breast cancer, the clinical guidelines based on histological and clinical characteristics recommended chemotherapy for 85-90% of lymph-node-negative patients, even though about 60-70% of them would survive without it. A 70-gene signature (MammaPrint) derived from primary tumors has been used to predict distal metastasis and select patients for adjuvant systemic treatment [140]. The results showed that 52% of patients with "poor prognosis" needed chemotherapy, rather than 82% and 92% suggested by St Gallen and the National Institute of Health (NIH) guidelines, respectively. This predictive signature was later attested in an evidence-based study and approved by FDA [141, 142]. This signature provides a powerful tool to allow the clinicians to avoid adjuvant systemic therapy to a specific group of patients with low metastatic scores. Another 76-gene-expression profiling from an independent study was reported to present similar results [143]. In parallel, many other gene expression profiles have been developed to optimize the use of therapeutics, identify the novel targets for drugs, and design clinical trials [118, 144].

In spite of unprecedented development of genomic application in cancers and their promising potentials in personalized medicine, most of them do not have sufficient evidence to move to clinical application yet. Currently, there are only a few diseases and molecular subgroups in which the prognostic and therapeutic strategies are proved or recommended by FDA, ASCO or the Evaluation of Genomic Applications in Practice and Prevention Initiative (EGAPP) working group.

CHALLENGES AND CONCERNS

Technology and Computational Analysis Development

Massively paralleled technology has made the cost of DNA sequencing plummet. Nevertheless, WGS remains too expensive to study most common diseases as well-powered studies typically require several thousand individuals. WES is a cost-effective alternative to WGS, but it does not include copy number variants and non-coding variants which may also be critical to the development of diseases [145]. Because NGS technology which is currently used in WGS and WES can only read short lengths per run, identifying the copy number variants from WGS can be an arduous task.

However, many NGS companies have been making significant improvement in read length and algorithms are being developed to capture these variants with WGS data [146, 147].

Another challenge is how to store and interpret the massive amount data of WGS from a group of participants. Even in the context of affordable WES/WGS strategies, other costs including storage of the data, analysis, validation and implementation may be still too expensive to extend their application in common diseases [148]. There is also an urgent need to develop software to figure out the "actionable" components which can be used in a more straightforward way to make a diagnosis, guide the change of the patients' lifestyle, or provide specific targets for pharmaceutics [117].

Accuracy of Prediction

GWAS have identified numerous genetic variants associated with common diseases, pharmacogenetic studies have discovered many variants associated with the efficacy or hazards of a drug in a specific group of individuals, and plenty of gene-expression signatures have been reported to predict the outcomes of treatment; however, only a small portion of them have been approved for clinical use. There are three reasons for this. First, a genome-wide or an array-wide test may lead to many abnormal genomic findings which are unrelated to the primary reason, which is a phenomenon called "incidentalome" [149, 150]. As the number of tests (SNPs or gene expression) increases, the chance of a false-positive association increases as well. Second, researchers who discover novel genetic tests usually do not have the resources to conduct the evidence-based studies to examine their clinical utility. Third, there is insufficient clinical validation [151]. Three clinical trials testing the prediction of gene signatures on the outcomes of chemotherapy in non-small-cell lung cancer and breast cancer were suspended in 2011 because of the faults in the original data processing and analysis, and non-reproducibility [152].

Recently, some genomic companies (23andMe, deCODEme, GeneticHealth and Navigenic) have started to provide genetic and genomic tests on demand [153]. The relevance of this direct-to-consumer (DTC) medical service on disease risk estimation is controversial. The advocates may consider that DTC will improve the screening practices and motivate the buyers to switch to a healthier lifestyle; the opponents may ponder its safety, privacy and effectiveness [154]. The DTC results are not consistent when the same individual is assessed using different platforms offered by different companies, which may leave consumers confused or cause unnecessary anxiety from an unreliable diagnosis [155, 156]. The risk predictions, especially for some serious diseases, are somewhat contradictory. Ng *et al* ordered DTC tests for five individuals from two firms and they found that less than 50% of the risk estimations were consistent across them for seven diseases [155]. These discrepancies may be the consequences of different genetic markers used in different platforms. The genetic markers included in each platform are chosen from GWAS, but different companies may have their own criteria and more than 40% of the genomic variants used in commercial tests have not been replicated in meta-analyses [157]. The algorithms they use to calculate the risk only include genes that explain small portion of heritability and rely on preliminary clinical relevance [158]. Moreover, some companies may update the markers with the ongoing discoveries in research, and some may not. This exemplifies the lack of validation and oversight and the insufficient medical input in the DTC business.

Training Physicians and Medical Students

Today's physicians are facing the challenge of a transition from traditional to genomic medicine. Considering the growing number of approved genetic tests, a survey of American Medical Association members reported that only 10% respondents were confident enough to apply them in their practice [159]. Although the usefulness of epidermal growth factor receptor genetic testing in directing chemotherapy in lung cancer patients has been incorporated into the guidelines, one third of all physicians have yet to adapt it [160]. The emergence of DTC genomic service raises another challenge for traditional physicians. DTC has broken the established physician-patient relationship in which the clinical tests are ordered by physicians. Now thousands of people order their own genomic tests through DTC and bring the genomic profiles to their physicians. Many doctors are not familiar with the concepts of genomics and genomic medicine and are hard pressed to explain the estimated risks from such data [161, 162]. Some physicians may take the uncertainty of the genetic test results as an excuse to reject them. On one hand, many patients believe that the doctors have an obligation to help them interpret and use the genetic results [163]; on the other hand, 83% of Americans do not believe their doctors are sufficiently trained in this capacity [161]. These facts highlight the urgent need to integrate the education about the principles of genomic, targeted therapy, biomarker development, and biomarker-based clinical trials into the training curriculum and teaching program in the medical schools. Johns Hopkins University is leading this evolution by changing the teaching plans and opening new programs in the school of medicine [159]. The impetus came from the belief that every case is unique. A study introduced the 21-gene recurrence score assay to oncologists over standard tools to quantify the risk of distant recurrence and predict the extent of chemotherapy benefit in tamoxifen-treated patients with lymph node-negative, ER-positive breast cancer [164]. Before and after obtaining the score assay, the recommendation from the oncologists changed in 28 out of 89 cases. Among them, chemotherapy was removed from the treatment regimen in 20 cases. Meanwhile, the oncologists were more confident in their decision-making with the evidence from the score assay. Though this was a small study, it reflected the impact of genomic knowledge on the doctors' decision-making [159].

Cost-Effectiveness of Genomic Tests

Cost-effectiveness, which assesses whether a new diagnostic tool or a new drug is worth of its investment, is a critical concern for a health agency in allocation of limited health resources. Therefore, beyond clinical validity, cost-

effectiveness presents another barrier to implement personalized genomic tests. In fact, genome-based diagnoses and therapies possess great potential to improve cost-effectiveness. Pharmacogenetic applications in cardio-vascular diseases will improve effectiveness and decrease adverse effects; and predictive magnitude of chemotherapy in cancers will prevent prescription of expensive drugs in the non-responders and avoid toxicity as well. The examples from rare diseases may even better demonstrate this. Without a definite diagnosis, the patient will seek a variety of examinations and treatments which are actually useless. A baby suffering from a cascade of infections caused by severe combined immunodeficiency (SCID) spent more than two months looking for many physicians before he got a conclusive diagnosis. At the end, he missed the treatment and died at 6 months and 15 days with a medical cost of $500,000. His younger sister who had the same disease was conclusively diagnosed by genotyping tests, received bone marrow transplantation at 16 days after birth, and survived with a lower bill than what her brother cost [165].

Currently, most of the research grants are invested in basic discovery research, diagnostic and therapeutic clinical trials. There is only a small portion of research evaluating candidate applications and developing evidence-based recommendations, even fewer studies investigating cost-effectiveness in genomic research. The genomic research is still being ever-improving, with test accuracy keeping improved over time and costs dropping even faster. Re-evaluation of the cost-effectiveness might be necessary. Someday when everyone has his own genome sequence available and the technologies are mature, cost-effectiveness may eventually not be a worry any more.

Gene Patenting and Prediction

A gene patent gives the owner of the gene exclusive rights for its application in research, diagnosis and therapeutics for 17 to 20 years and excludes anyone else from making, using or selling it. Up until 2010, approximately 20% of the human genes had been patented and more than 40,000 DNA-related patents have been generated since 1982, when gene patents were first allowed [166]. Although gene patents are incentive to innovation, they also impede other institutes and companies to contribute to important genetic discoveries and limit patient access to health services. Whether genes should be patentable was a hot topic in the last couple of years because of the lawsuit in 2009 involving Myriad Genetics, a biotechnology company, which had owned the patents of *BRCA1* and *BRCA2*. Since Myriad won these patents in 1998, all laboratories across US that were doing such tests stopped their practice, whereas Myriad started to monopolize the market with high price [167]. When a WES or a specific panel is able to sequence all exons and cancer-related genes in a single experiment, definitely including *BRCA1* and *BRCA2* and many other patent genes, doctors had to order them separately from other companies with authority or reported the results without the information of these genes if they did not buy licences. Furthermore, expensive cost for the patent genes adds another layer of complexity to cost-effectiveness analysis of genomic testing. In polygenic diseases, gene patents do stand in the middle to prevent scientists from doing better jobs towards personalized medicine. Fortunately and reasonably, on June 13, 2013, the US Supreme Court rejected Myriad's arguments and overturned the gene patents by saying that "genes are a product of nature and therefore not patentable by law, and Myriad did not create anything". As hoped by many scientists and doctors, including Francis Collins, the director of the National Institute of Health, *BRCA1*, *BRCA2* and many other patent genes are set free [167].

Ethical and Legal Issues

Many ethical and legal issues should be considered in the course of implementation of genetic and genomic testing [85]. People may reject genetic or genomic testing because they are afraid of genetic discrimination from insurance companies by denying coverage or from employers in employment decision. In 2008, the US Senate passed Genetic Information Nondiscrimination Act (GINA) to protect an individual's genetic information from insurance and employer discrimination [168]. This Act is also important to encourage Americans to make good use of genetic testing to prevent and prepare for potential diseases. Who else, except the patient, can the results be released to, and how to protect genetic privacy from the third party in the system of electronic medical record? There are still no answers for these questions. It is a challenging decision whether to inform children, adolescents or young adults when they have a diagnosis of a cancer due to the special age window. It is however admitted that their awareness of their disease should offer a psyco-social support, thus leading to better compliance and adherence to the treatment and better clinical outcomes [169]. There is always a consensus to conduct newborn screening for a panel of early-onset but treatable diseases; however, newborn screening for late-onset or no cure diseases is controversial [170]. Some may consider screening for late-onset or no cure diseases adds extra anxiety for the individuals and their families if there is no preventive and early treatment options or no immediate intervention needed [171]; others may think the testing can inform the individuals for their reproductive decision-making and the family for financial and psychological preparation. Some new concerns come with the advent of DTC. What are the proper procedures to obtain informed consents from DTC customers? Should only the results with sufficient clinical validity be reported to the patient or all of them? How to avoid the misleading or the uncertain results from DTC? Currently, there is no sufficient regulation on genetic and genomic testing. Some agencies like American Society of Clinical Oncology are calling for oversight from FDA and Center for Medicare and Medicaid Services to ensure highest standards for quality, accuracy, and reliability, but, on the flipping side, not hinder the scientific development or delivery of best available treatment and preventive care [115]. Fortunately, the FDA and other organizations have been active in addressing regulatory issues on personalized medicine. Very recently, the FDA has granted authorization for the first high-throughput genome sequencer, Illumina's MiSeqDx, for its clinical laboratory use because of its best performance in precision and reproducibility [172]. In February 2014, the FDA also withdrew the personal genome

service from 23andMe due to its potential risks of inaccurate results [158]. We believe that this decision is a step in the right direction, as the accuracy of genetic testing must be controlled by authorized agencies in the best interest of the patient. Some authorized organizations are making recommendations when personalized medicine is practiced [173, 174]. For example, the American College of Medical Genetics and Genomics (ACMG) published a policy statement on clinical sequencing that a minimal list of genes and variants (currently in 24 diseases) should be routinely evaluated and reported as the incidental or secondary findings to the clinician who orders the test [173].

THE FUTURE OF PERSONALIZED MEDICINE

Although many challenges and hurdles remain, for personalized medicine the future is bright. Recently, the term P4 medicine was coined by Leroy Hood [175]. It includes Predictive, Preventive, Personalized and Participatory aspects [175-177]. It is an approach beyond genomics and uses each person's system biology, in combination with bioinformatics, to generate "actionable" regimen and convert billions of data points into an intelligible synopsis that is accessible to physicians and care providers. System biology consists of unique genomic sequence data that is combined with dynamic molecular and cellular information, as well as elastic environmental and phenotypic measurements that are fundamental health determinants. Compared with genomic medicine using one-dimensional data, P4 medicine utilizes biological information in totality to detect the disease-disturbed components, providing deep insights into disease mechanisms and new targets for diagnosis and therapeutic drugs. By identifying the actionable information from a vast composite of information, P4 medicine is quasi-holistic in its aim not only to demystify diseases but also to improve wellness, which meets with the latest definition of health edited by the World Health Organization as a state of complete physical, mental and social well-being. This P4 model expands personalized medicine beyond genomic medicine. Furthermore, P5 medicine with an additional fifth P of Population science is proposed by Khoury, which is to be incorporated into each aspect of P4 [178]. Population science covers almost every aspects of health and uses ecologic model systems and mixed methods to input intelligence from multiple disciplines. It assesses the validity of evidence from P4 and is useful in guiding policy making [179]. From a population perspective, biological signatures from P4 models of uncertain clinical utility require strong evidence from randomized controlled trials before clinical use is recommended [178]. Among hundreds of reported predictive gene signatures of different cancers, only a handful of tests passed the FDA approval [152, 180]. Without sufficient clinical validation, the newly developed personalized medicine strategies from P4 medicine may be misleading and consequently may be a waste of resources and do more harm than good to the patients. Meanwhile, a different P5 model with the different fifth P of Psyco-cognitive aspect was proposed by Gorini and Pravettoni [181, 182]. Such a P5 medicine will not only inform the patients of their health status, but also empower them to be involved in their decision-making with doctors by their specific needs, values, behaviours, hopes and fears. Following this, a sixth P of Public was introduced by Bragazzi who was inspired by Salvatore Iaconesi's clinical story [183]. Salvatore Iaconesi is a skilled computer scientist and one day was diagnosed with a brain tumor. He posted his medical records on his website and desired to seek help from various sources and shared his experience with anyone who needed it [183]. In other words, P6 approach brings up the additional notion of e-health into personalized medicine. The sixth P is an interesting concept but it may lead to important ethical considerations such as confidentiality, discrimination and implications to family members, and therefore its applications are limited.

Hood and Flores also portray a stunning picture of future P4/P5 medicine and predict that it would likely become true within the next decade [184]. They assume that accurate assessments from genomic sequence to proteomics and their function, to conventional medical data, to enormous amounts of clinical diagnostic imaging and environmental measurements would be available, affordable and accessible for individuals. The leading edge biology and medicine in every field of "omics" will drive the development of new high-throughput technology and analytic tools to explore the multi-dimensional data from individuals, families, and across the population. P4/P5 medicine considers each person as unique, thus each has his own genome which would need to be sequenced only once, while measurements of other dynamic parameters, would require more regular assessments (e.g annually or biannually). By analyzing these data any transition from health to disease will be marked [185]. Genome and protein profiles will also be used to assess drug toxicities, avoiding unnecessary adverse effects. P4/P5 medicine model is characterized by stratifying health and disease based on different markers and extracting actionable components. Assuming that targeted drugs that are effective at different stages of disease progression are available in the future, tailored interventions will be engaged to correct a disease-perturbed network to restore an individual to wellness. All these information is linked to the individual's electronic medical records and the doctors will receive health messages in time such as health status change, drug choice and dosage, or progress/prognosis of a disease, achieving personalized prevention and treatment. More importantly, the P4/P5 medicine model postulates that individuals are active and networked rather than simple passive recipients of doctors' advice. Their participation will contribute to the advancement of medical and health knowledge and will eventually maximize their own wellness. They will be the most powerful drivers of the emergence of P4/P5 medicine. P4/P5 medicine also has the potential to drop the ever increasing costs of health care by active prevention, early diagnosis and specific treatments.

Does this sound like a scientific fiction story? Are they castles in the sky? Because we have witnessed the unprecedented success of human genomic, this ambitious vision should not be rejected. However, in the first decade after deciphering the human genome, only a handful of genetic discoveries have been applied into routine medical practice and the clinical benefits are still far from enhancing the wellness and treating diseases for most individuals [25, 186]. In addition to genomics, integration of other types

of personal "omics" profiles including transcriptomics, proteomics, metabolomics, epigenomics, metagenomics will theoretically enable us to understand the onset, progression and prognosis of common diseases, thus broadening the capability of personalized medicine [187]. The laboratory experiments have shown that the levels of these "omics" vary greatly across time, within individuals, and between individuals, and this massive variation has made clear interpretations difficult. Meanwhile, many of these analyses are currently prohibitively expensive. Importantly, P4/P5 medicine is built on stringent assumptions that all these "omics" are accurately measured. Therefore, it is too optimistic to build up such a system with integration of huge data that are not yet fully-understood.

Will this become a reality in 10 years? P4/P5 medicine will use multi-level data within individuals and across a population to generate lots of information which can be used to improve health. Obviously, this complicated system in P4/P5 medicine model cannot be mimicked in the experiment settings. Therefore, one critical prerequisite to practice P4/P5 medicine is that all the elements in system biology should be clinically valid before they are used for final outcome syntheses. Over the past few years, numerous evidence-based studies were undertaken to assess the clinical validity and utility of emerging genetic testing. The Evaluation of Genomic Applications in Practice and Prevention Initiative (EGAPP) Working Group, established in 2005, reviews evidence reports from randomized controlled trials and/or observational studies and assesses the analytic validity and clinical validity, providing recommendations on the appropriate use of genetic tests in specific clinical scenarios. Currently, EGAPP have released 11 recommendations, in which only 3 have sufficient evidence. The lack of information on the clinical validity for most genetic and molecular tests is a major practical barrier to the implementation of P4/P5 medicine [188]. Another concern is that it takes an average of 17 years to translate a new scientific discovery to clinical practice, with a success rate of less than 15% [160]. Furthermore, this P4/P5 medicine revolution will not happen without a new generation of experts who are able to create algorithms to integrate and interpret all the diverse sources of information from genetics, molecular biology, clinical knowledge, statistics and bioinformatics, and eventually synthesize the actionable messages for the clinicians and patients. A shift in the organization of conventional health infrastructures is also mandatory. A new model of personalized medicine reference centers decoding the complex information for specific diseases from the information of electronic medical records and using revolutionary decision algorithms to translate this knowledge into medical actions is needed. We believe that P4/P5 medicine can progress with exponential acceleration as genomic science does, but it will be a long journey to reach the full potential of personalized medicine.

CONCLUSIONS

Because an individual's DNA sequence is static unless exceptional circumstances occur (eg. tumor, exposure to mutagenesis compounds), it is considered to be an easier and more reliable tool to predict long-term risk [189]. This review illustrates some of the successes of using personal genomic data in Mendelian and polygenic diseases. Personalized medicine is in its infancy and is also moving steadily forward, but many challenges remain. We describe the hopes and hypes of personalized P4/P5 medicine which is driven by advances in technologies such as omics platforms, computation, information integration, and analyses. We hope this review will encourage clinicians to be active contributors in this medical revolution.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Adams FL. The genuine works of Hippocrates. New York: William Wood 1886.
[2] Strachan T, Read A. Human molecular genetics. 7th ed: Garland Science 2011.
[3] Harris H. Garrod's inborn errors of metabolism. London: Oxford University; 1963.
[4] Guttmacher AE, Collins FS. Genomic medicine--a primer. N Engl J Med 2002; 347: 1512-20.
[5] Goodman DM, Lynm C, Livingston EH. JAMA patient page. Genomic medicine. JAMA 2013; 309: 1544.
[6] Lander ES, Linton LM, Birren B, *et al*. Initial sequencing and analysis of the human genome. Nature 2001; 409: 860-921.
[7] Venter JC, Adams MD, Myers EW, *et al*. The sequence of the human genome. Science 2001; 291: 1304-51.
[8] International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature 2004; 431: 931-45.
[9] Altshuler DM, Gibbs RA, Peltonen L, *et al*. Integrating common and rare genetic variation in diverse human populations. Nature 2010; 467: 52-8.
[10] Abecasis GR, Auton A, Brooks LD, *et al*. An integrated map of genetic variation from 1,092 human genomes. Nature 2012; 491: 56-65.
[11] Khoury MJ, McCabe LL, McCabe ER. Population screening in the age of genomic medicine. N Engl J Med 2003; 348: 50-8.
[12] Gillham NW. Sir Francis Galton and the birth of eugenics. Annu Rev Genet 2001; 35: 83-101.
[13] Kevles D. In the Name of Eugenics: Genetics and the Uses of Human Heredity. New York: Knopf; 1985.
[14] Carey A. Gender and compulsory sterilization programs in America: 1907–1950. J Historical Sociol 1998; 11: 74-105.
[15] Cohen MM Jr. Genetic drift. Overview of German, Nazi, and Holocaust medicine. Am J Med Genet A 2010; 152A: 687-707.
[16] Hughes JT. Neuropathology in Germany during World War II: Julius Hallervorden (1882-1965) and the Nazi programme of 'euthanasia'. J Med Biogr 2007; 15: 116-22.
[17] Zeidman LA. Neuroscience in Nazi Europe part I: Eugenics, human experimentation, and mass murder. Can J Neurol Sci 2011; 38: 696-703.
[18] Zeidman LA. Neuroscience in Nazi Europe part II: Resistance against the third reich. Can J Neurol Sci 2011; 38: 826-38.
[19] Adams M. The wellborn science: Eugenics in Germany, France, Brazil , and Russia. New York: Oxford University Press 1990.
[20] Caulfield T, Robertson J. Genetic policies in Alberta: From the systematic to the systemic. Alberta Law Review 1996; 35: 59-80.
[21] Tsuchiya T. Eugenic sterilizations in Japan and recent demands for an apology: A report. Ethics Intellect Disabil 1997; 3: 1-4.

[22] Andermann A, Blancquaert I. Genetic screening: A primer for primary care. Can Fam Physician 2010; 56: 333-9.
[23] McIntosh I, Cutting GR. Cystic fibrosis transmembrane conductance regulator and the etiology and pathogenesis of cystic fibrosis. FASEB J 1992; 6: 2775-82.
[24] Castellani C, Cuppens H, Macek M Jr., *et al*. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008; 7: 179-96.
[25] Collins F. Has the revolution arrived? Nature 2010; 464: 674-5.
[26] Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982; 143: 29-36.
[27] Harrell FE Jr., Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. JAMA 1982; 247: 2543-6.
[28] Fischer JE, Bachmann LM, Jaeschke R. A reader's guide to the interpretation of diagnostic test properties: Clinical example of sepsis. Intensive Care Med 2003; 29: 1043-51.
[29] Vida S, Des Rosiers P, Carrier L, Gauthier S. Depression in Alzheimer's disease: Receiver operating characteristic analysis of the Cornell Scale for Depression in Dementia and the Hamilton Depression Scale. J Geriatr Psychiatry Neurol 1994; 7: 159-62.
[30] Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007; 115: 928-35.
[31] Morandi A, Meyre D, Lobbens S, *et al*. Estimation of newborn risk for child or adolescent obesity: Lessons from longitudinal birth cohorts. PLoS One 2012; 7: e49919.
[32] Anand SS, Meyre D, Pare G, *et al*. Genetic information and the prediction of incident type 2 diabetes in a high-risk multiethnic population: The EpiDREAM genetic study. Diabetes Care 2013; 36: 2836-42.
[33] Simard J, Dumont M, Moisan AM, *et al*. Evaluation of BRCA1 and BRCA2 mutation prevalence, risk prediction models and a multistep testing approach in French-Canadian families with high risk of breast and ovarian cancer. J Med Genet 2007; 44: 107-21.
[34] Pepe MS, Janes H, Longton G, Leisenring W, Newcomb P. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am J Epidemiol 2004; 159: 882-90.
[35] Kathiresan S, Melander O, Anevski D, *et al*. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008; 358: 1240-9.
[36] Meigs JB, Shrader P, Sullivan LM, *et al*. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008; 359: 2208-19.
[37] Wacholder S, Hartge P, Prentice R, *et al*. Performance of common genetic variants in breast-cancer risk models. N Engl J Med 2010; 362: 986-93.
[38] Cook NR. Comments on 'Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond' by M. J. Pencina *et al*., Statistics in Medicine (DOI: 10.1002/sim.2929). Stat Med 2008; 27: 191-5.
[39] Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond. Stat Med 2008; 27: 157-72; discussion 207-12.
[40] Kiezun A, Garimella K, Do R, *et al*. Exome sequencing and the genetic basis of complex traits. Nat Genet 2012; 44: 623-30.
[41] Sanders SJ, Murtha MT, Gupta AR, *et al*. *De novo* mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 2012; 485: 237-41.
[42] Neale BM, Kosu Y, Liu L, *et al*. Patterns and rates of exonic *de novo* mutations in autism spectrum disorders. Nature 2012; 485: 242-5.
[43] Ng SB, Turner EH, Robertson PD, *et al*. Targeted capture and massively parallel sequencing of 12 human exomes. Nature 2009; 461: 272-6.
[44] Botstein D, Risch N. Discovering genotypes underlying human phenotypes: Past successes for mendelian disease, future approaches for complex disease. Nat Genet 2003; 33 Suppl: 228-37.
[45] Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2005; 33: D514-7.
[46] Gloyn AL, Pearson ER, Antcliff JF, *et al*. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 2004; 350: 1838-49.
[47] Babenko AP, Polak M, Cave H, *et al*. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. N Engl J Med 2006; 355: 456-66.
[48] Ashcroft FM. ATP-sensitive potassium channelopathies: Focus on insulin secretion. J Clin Invest 2005; 115: 2047-58.
[49] Pearson ER, Flechtner I, Njolstad PR, *et al*. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med 2006; 355: 467-77.
[50] Bonnefond A, Durand E, Sand O, *et al*. Molecular diagnosis of neonatal diabetes mellitus using next-generation sequencing of the whole exome. PLoS One 2010; 5: e13630.
[51] Bonnefond A, Philippe J, Durand E, *et al*. Whole-exome sequencing and high throughput genotyping identified KCNJ11 as the thirteenth MODY gene. PLoS One 2012; 7: e37423.
[52] Robinson PN, Krawitz P, Mundlos S. Strategies for exome and genome sequence data analysis in disease-gene discovery projects. Clin Genet 2011; 80: 127-32.
[53] Ng SB, Buckingham KJ, Lee C, *et al*. Exome sequencing identifies the cause of a mendelian disorder. Nat Genet 2010; 42: 30-5.
[54] Worthey EA, Mayer AN, Syverson GD, *et al*. Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 2011; 13: 255-62.
[55] Huang Y, Park YC, Rich RL, Segal D, Myszka DG, Wu H. Structural basis of caspase inhibition by XIAP: Differential roles of the linker versus the BIR domain. Cell 2001; 104: 781-90.
[56] Krieg A, Correa RG, Garrison JB, *et al*. XIAP mediates NOD signaling *via* interaction with RIP2. Proc Natl Acad Sci U S A 2009; 106: 14524-9.
[57] Adzhubei IA, Schmidt S, Peshkin L, *et al*. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7: 248-9.
[58] Jostins L, Barrett JC. Genetic risk prediction in complex disease. Hum Mol Genet 2011; 20: R182-8.
[59] Hindorff LA, Sethupathy P, Junkins HA, *et al*. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A 2009; 106: 9362-7.
[60] Goldstein DB. Common genetic variation and human traits. N Engl J Med 2009; 360: 1696-8.
[61] Paynter NP, Chasman DI, Buring JE, Shiffman D, Cook NR, Ridker PM. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. Ann Intern Med 2009; 150: 65-72.
[62] Lango H, Palmer CN, Morris AD, *et al*. Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. Diabetes 2008; 57: 3129-35.
[63] Lango Allen H, Estrada K, Lettre G, *et al*. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature 2010; 467: 832-8.
[64] Yang J, Benyamin B, McEvoy BP, *et al*. Common SNPs explain a large proportion of the heritability for human height. Nat Genet 2010; 42: 565-9.
[65] Stahl EA, Wegmann D, Trynka G, *et al*. Bayesian inference analyses of the polygenic architecture of rheumatoid arthritis. Nat Genet 2012; 44: 483-9.
[66] Wei Z, Wang K, Qu HQ, *et al*. From disease association to risk assessment: An optimistic view from genome-wide association studies on type 1 diabetes. PLoS Genet 2009; 5: e1000678.
[67] American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association; 2013.
[68] Bragazzi NL. Rethinking psychiatry with OMICS science in the age of personalized P5 medicine: Ready for psychiatome? Philos Ethics Humanit Med 2013; 8: 4.
[69] Ozdemir V, Endrenyi L, Aynacioglu S, *et al*. Bernard Lerer: Recipient of the 2014 inaugural Werner Kalow Responsible Innovation Prize in Global Omics and Personalized Medicine (Pacific Rim Association for Clinical Pharmacogenetics). OMICS 2014; 18: 211-21.
[70] Manolio TA, Collins FS, Cox NJ, *et al*. Finding the missing heritability of complex diseases. Nature 2009; 461: 747-53.
[71] Gibson G. Rare and common variants: Twenty arguments. Nat Rev Genet 2011; 13: 135-45.

[72] Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science 2009; 324: 387-9.
[73] Bonnefond A, Clement N, Fawcett K, *et al*. Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. Nat Genet 2012; 44: 297-301.
[74] Zhang W, Hui KY, Gusev A, *et al*. Extended haplotype association study in Crohn's disease identifies a novel, Ashkenazi Jewish-specific missense mutation in the NF-kappaB pathway gene, HEATR3. Genes Immun 2013; 14: 310-6.
[75] Ichimura A, Hirasawa A, Poulain-Godefroy O, *et al*. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature 2012; 483: 350-4.
[76] Agarwala V, Flannick J, Sunyaev S, Altshuler D. Evaluating empirical bounds on complex disease genetic architecture. Nat Genet 2013; 45: 1418-27.
[77] Janssens AC, Aulchenko YS, Elefante S, Borsboom GJ, Steyerberg EW, Van Duijn CM. Predictive testing for complex diseases using multiple genes: Fact or fiction? Genet Med 2006; 8: 395-400.
[78] Roberts NJ, Vogelstein JT, Parmigiani G, Kinzler KW, Vogelstein B, Velculescu VE. The predictive capacity of personal genome sequencing. Sci Transl Med 2012; 4: 133ra58.
[79] Begg CB, Pike MC. Comment on "the predictive capacity of personal genome sequencing". Sci Transl Med 2012; 4: 135le3.
[80] Golan D, Rosset S. Comment on "the predictive capacity of personal genome sequencing". Sci Transl Med 2012; 4: 135le4.
[81] Topol EJ. Comment on "the predictive capacity of personal genome sequencing". Sci Transl Med 2012; 4: 135le5; author reply lr3.
[82] Patel CJ, Sivadas A, Tabassum R, *et al*. Whole Genome Sequencing in support of Wellness and Health Maintenance. Genome Med 2013; 5: 58.
[83] Chen R, Mias GI, Li-Pook-Than J, *et al*. Personal omics profiling reveals dynamic molecular and medical phenotypes. Cell 2012; 148: 1293-307.
[84] Wheeler HE, Aquino-Michaels K, Gamazon ER, *et al*. Poly-Omic Prediction of Complex Traits: OmicKriging. Genet Epidemiol 2014.
[85] Pasic MD, Samaan S, Yousef GM. Genomic medicine: New frontiers and new challenges. Clin Chem 2013; 59: 158-67.
[86] Blazquez A, Mas S, Plana MT, Lafuente A, Lazaro L. Fluoxetine pharmacogenetics in child and adult populations. Eur Child Adolesc Psychiatry 2012; 21: 599-610.
[87] Klotz U. The role of pharmacogenetics in the metabolism of antiepileptic drugs: Pharmacokinetic and therapeutic implications. Clin Pharmacokinet 2007; 46: 271-9.
[88] Johnson JA. Warfarin pharmacogenetics: A rising tide for its clinical value. Circulation 2012; 125: ss1964-6.
[89] Sconce EA, Khan TI, Wynne HA, *et al*. The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: Proposal for a new dosing regimen. Blood 2005; 106: 2329-33.
[90] Rieder MJ, Reiner AP, Gage BF, *et al*. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005; 352: 2285-93.
[91] Klein TE, Altman RB, Eriksson N, *et al*. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009; 360: 753-64.
[92] Epstein RS, Moyer TP, Aubert RE, *et al*. Warfarin genotyping reduces hospitalization rates results from the MM-WES (Medco-Mayo Warfarin Effectiveness study). J Am Coll Cardiol 2010; 55: 2804-12.
[93] Anderson JL, Horne BD, Stevens SM, *et al*. A randomized and clinical effectiveness trial comparing two pharmacogenetic algorithms and standard care for individualizing warfarin dosing (CoumaGen-II). Circulation 2012; 125: 1997-2005.
[94] Gage BF, Eby C, Johnson JA, *et al*. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther 2008; 84: 326-31.
[95] Cavallari LH, Shin J, Perera MA. Role of pharmacogenomics in the management of traditional and novel oral anticoagulants. Pharmacotherapy 2011; 31: 1192-207.
[96] Kimmel SE, French B, Kasner SE, *et al*. A pharmacogenetic versus a clinical algorithm for warfarin dosing. N Engl J Med 2013; 369: 2283-93.
[97] Pirmohamed M, Burnside G, Eriksson N, *et al*. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 2013; 369: 2294-303.
[98] Furie B. Do pharmacogenetics have a role in the dosing of vitamin K antagonists? N Engl J Med 2013; 369: 2345-6.
[99] Baigent C, Keech A, Kearney PM, *et al*. Efficacy and safety of cholesterol-lowering treatment: Prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005; 366: 1267-78.
[100] Bowman L, Armitage J, Bulbulia R, Parish S, Collins R. Study of the effectiveness of additional reductions in cholesterol and homocysteine (SEARCH): Characteristics of a randomized trial among 12064 myocardial infarction survivors. Am Heart J 2007; 154: 815-23.
[101] Link E, Parish S, Armitage J, *et al*. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 2008; 359: 789-99.
[102] Hicks JK, Swen JJ, Thorn CF, *et al*. Clinical pharmacogenetics implementation consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. Clin Pharmacol Ther 2013; 93: 402-8.
[103] Sim SC, Nordin L, Andersson TM, *et al*. Association between CYP2C19 polymorphism and depressive symptoms. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 1160-6.
[104] Mrazek DA, Biernacka JM, O'Kane DJ, *et al*. CYP2C19 variation and citalopram response. Pharmacogenet Genomics 2011; 21: 1-9.
[105] Thase ME, Haight BR, Richard N, *et al*. Remission rates following antidepressant therapy with bupropion or selective serotonin reuptake inhibitors: A meta-analysis of original data from 7 randomized controlled trials. J Clin Psychiatry 2005; 66: 974-81.
[106] Olesen OV, Linnet K. Studies on the stereoselective metabolism of citalopram by human liver microsomes and cDNA-expressed cytochrome P450 enzymes. Pharmacology 1999; 59: 298-309.
[107] Von Moltke LL, Greenblatt DJ, Grassi JM, *et al*. Citalopram and desmethylcitalopram *in vitro*: Human cytochromes mediating transformation, and cytochrome inhibitory effects. Biol Psychiatry 1999; 46: 839-49.
[108] Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E. Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients. Clin Pharmacol Ther 2008; 83: 322-7.
[109] Sindrup SH, Brosen K, Hansen MG, Aaes-Jorgensen T, Overo KF, Gram LF. Pharmacokinetics of citalopram in relation to the sparteine and the mephenytoin oxidation polymorphisms. Ther Drug Monit 1993; 15: 11-7.
[110] Kirchheiner J, Nickchen K, Bauer M, *et al*. Pharmacogenetics of antidepressants and antipsychotics: The contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004; 9: 442-73.
[111] Feero WG, Guttmacher AE, Collins FS. Genomic medicine--an updated primer. N Engl J Med 2010; 362: 2001-11.
[112] Frueh FW, Amur S, Mummaneni P, *et al*. Pharmacogenomic biomarker information in drug labels approved by the United States food and drug administration: Prevalence of related drug use. Pharmacotherapy 2008; 28: 992-8.
[113] Chute CG, Kohane IS. Genomic medicine, health information technology, and patient care. JAMA 2013; 309: 1467-8.
[114] Statement of the American Society of Clinical Oncology: Genetic testing for cancer susceptibility, Adopted on February 20, 1996. J Clin Oncol 1996; 14: 1730-6; discussion 7-40.
[115] Robson ME, Storm CD, Weitzel J, Wollins DS, Offit K. American Society of Clinical Oncology policy statement update: Genetic and genomic testing for cancer susceptibility. J Clin Oncol 2010; 28: 893-901.
[116] Offit K. BRCA mutation frequency and penetrance: New data, old debate. J Natl Cancer Inst 2006; 98: 1675-7.
[117] Clark AE. Sequence thyself: Personalized medicine and therapies for the future: 2012 Yale Healthcare Conference. Yale J Biol Med 2012; 85: 421-4.
[118] McDermott U, Downing JR, Stratton MR. Genomics and the continuum of cancer care. N Engl J Med 2011; 364: 340-50.
[119] Mischel PS, Shai R, Shi T, *et al*. Identification of molecular subtypes of glioblastoma by gene expression profiling. Oncogene 2003; 22: 2361-73.

[120] Perou CM, Sorlie T, Eisen MB, *et al*. Molecular portraits of human breast tumours. Nature 2000; 406: 747-52.
[121] Sotiriou C, Neo SY, McShane LM, *et al*. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci USA 2003; 100: 10393-8.
[122] Esteller M. Epigenetics in cancer. N Engl J Med 2008; 358: 1148-59.
[123] Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature 2007; 447: 433-40.
[124] Calin GA, Sevignani C, Dumitru CD, *et al*. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 2004; 101: 2999-3004.
[125] Sethi S, Ali S, Sarkar FH. MicroRNAs in personalized cancer therapy. Clin Genet 2014; 86(1): 68-73.
[126] Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007; 128: 683-92.
[127] Esteller M, Silva JM, Dominguez G, *et al*. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 2000; 92: 564-9.
[128] Esteller M, Garcia-Foncillas J, Andion E, *et al.* Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 2000; 343: 1350-4.
[129] McCabe MT, Brandes JC, Vertino PM. Cancer DNA methylation: Molecular mechanisms and clinical implications. Clin Cancer Res 2009; 15: 3927-37.
[130] Fernandez AF, Assenov Y, Martin-Subero JI, *et al*. A DNA methylation fingerprint of 1628 human samples. Genome Res 2012; 22: 407-19.
[131] He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-31.
[132] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.
[133] Fabbri M. MicroRNAs and cancer: Towards a personalized medicine. Curr Mol Med 2013; 13: 751-6.
[134] Stefansson OA, Esteller M. Epigenetic modifications in breast cancer and their role in personalized medicine. Am J Pathol 2013; 183: 1052-63.
[135] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177-82.
[136] Vogel CL, Cobleigh MA, Tripathy D, *et al*. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002; 20: 719-26.
[137] Romond EH, Perez EA, Bryant J, *et al*. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353: 1673-84.
[138] Lynch TJ, Bell DW, Sordella R, *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129-39.
[139] Mok TS, Wu YL, Thongprasert S, *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009; 361: 947-57.
[140] Van 't Veer LJ, Dai H, Van de Vijver MJ, *et al*. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002; 415: 530-6.
[141] Van de Vijver MJ, He YD, Van't Veer LJ, *et al*. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 347: 1999-2009.
[142] Slodkowska EA, Ross JS. MammaPrint 70-gene signature: Another milestone in personalized medical care for breast cancer patients. Expert Rev Mol Diagn 2009; 9: 417-22.
[143] Wang Y, Klijn JG, Zhang Y, *et al*. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 2005; 365: 671-9.
[144] Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. N Engl J Med 2009; 360: 790-800.
[145] Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. Annu Rev Med 2010; 61: 437-55.
[146] Liu L, Li Y, Li S, *et al*. Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012; 2012: 251364.
[147] Medvedev P, Stanciu M, Brudno M. Computational methods for discovering structural variation with next-generation sequencing. Nat Methods 2009; 6: S13-20.
[148] Mardis ER. The $1,000 genome, the $100,000 analysis? Genome Med 2010; 2: 84.
[149] Kohane IS, Masys DR, Altman RB. The incidentalome: A threat to genomic medicine. JAMA 2006; 296: 212-5.
[150] Solomon BD. Incidentalomas in genomics and radiology. N Engl J Med 2014; 370: 988-90s.
[151] Ioannidis JP, Khoury MJ. Improving validation practices in "omics" research. Sciences 2011; 334: 1230-2.
[152] Goozner M. Duke scandal highlights need for genomics research criteria. J Natl Cancer Inst 2011; 103: 916-7.
[153] Bloss CS, Darst BF, Topol EJ, Schork NJ. Direct-to-consumer personalized genomic testing. Hum Mol Genet 2011; 20: R132-41.
[154] Bloss CS, Wineinger NE, Darst BF, Schork NJ, Topol EJ. Impact of direct-to-consumer genomic testing at long term follow-up. J Med Genet 2013; 50: 393-400.
[155] Ng PC, Murray SS, Levy S, Venter JC. An agenda for personalized medicine. Nature 2009; 461: 724-6.
[156] Fleming N. Rival genetic tests leaves buyers confused. Available from: http://www.timesonline.co.uk/tol/news/uk/science/article4692891.ece2008.
[157] Janssens AC, Gwinn M, Bradley LA, Oostra BA, Van Duijn CM, Khoury MJ. A critical appraisal of the scientific basis of commercial genomic profiles used to assess health risks and personalize health interventions. Am J Hum Genet 2008; 82: 593-9.
[158] Downing NS, Ross JS. Innovation, risk, and patient empowerment: The FDA-mandated withdrawal of 23andMe's Personal Genome Service. JAMA 2014; 311: 793-4.
[159] Marshall E. Human genome 10th anniversary. Waiting for the revolution. Science 2011; 331: 526-9.
[160] Meric-Bernstam F, Farhangfar C, Mendelsohn J, Mills GB. Building a personalized medicine infrastructure at a major cancer center. J Clin Oncol 2013; 31: 1849-57.
[161] Pandey A. A piece of my mind. Preparing for the 21st-century patient. JAMA 2013; 309: 1471-2.
[162] Najafzadeh M, Davis JC, Joshi P, Marra C. Barriers for integrating personalized medicine into clinical practice: A qualitative analysis. Am J Med Genet A 2013; 161: 758-63.
[163] McGuire AL, Diaz CM, Wang T, Hilsenbeck SG. Social networkers' attitudes toward direct-to-consumer personal genome testing. Am J Bioeth 2009; 9: 3-10.
[164] Lo SS, Mumby PB, Norton J, *et al*. Prospective multicenter study of the impact of the 21-gene recurrence score assay on medical oncologist and patient adjuvant breast cancer treatment selection. J Clin Oncol 2010; 28: 1671-6.
[165] Gura T. Rare diseases: Genomics, plain and simple. Nature 2012; 483: 20-2.
[166] Chuang CS, Lau DT. The pros and cons of gene patents. Publications 2010.
[167] Maxmen A. Personalized medicine enters a new era. 2013.
[168] Hudson KL, Holohan MK, Collins FS. Keeping pace with the times--the Genetic Information Nondiscrimination Act of 2008. N Engl J Med 2008; 358: 2661-3.
[169] Bragazzi NL. Children, adolescents, and young adults participatory medicine: Involving them in the health care process as a strategy for facing the infertility issue. Am J Bioeth 2013; 13: 43-4.
[170] Hiraki S, Green NS. Newborn screening for treatable genetic conditions: Past, present and future. Obstet Gynecol Clin North Am 2010; 37: 11-21.
[171] Goldenberg AJ, Sharp RR. The ethical hazards and programmatic challenges of genomic newborn screening. JAMA 2012; 307: 461-2.
[172] Collins FS, Hamburg MA. First FDA authorization for next-generation sequencer. N Engl J Med 2013; 369: 2369-71.
[173] Green RC, Berg JS, Grody WW, *et al*. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med 2013; 15: 565-74.
[174] Kocarnik JM, Fullerton SM. Returning pleiotropic results from genetic testing to patients and research participants. JAMA 2014; 311: 795-6.
[175] Hood L, Friend SH. Predictive, personalized, preventive, participatory (P4) cancer medicine. Nat Rev Clin Oncol 2011; 8: 184-7.

[176] Weston AD, Hood L. Systems biology, proteomics, and the future of health care: Toward predictive, preventative, and personalized medicine. J Proteome Res 2004; 3: 179-96.
[177] Tian Q, Price ND, Hood L. Systems cancer medicine: Towards realization of predictive, preventive, personalized and participatory (P4) medicine. J Intern Med 2012; 271: 111-21.
[178] Khoury MJ, Gwinn ML, Glasgow RE, Kramer BS. A population approach to precision medicine. Am J Prev Med 2012; 42: 639-45.
[179] Kindig D, Stoddart G. What is population health? Am J Public Health 2003; 93: 380-3.
[180] Subramanian J, Simon R. Gene expression-based prognostic signatures in lung cancer: Ready for clinical use? J Natl Cancer Inst 2010; 102: 464-74.
[181] Gorini A, Pravettoni G. P5 medicine: A plus for a personalized approach to oncology. Nat Rev Clin Oncol 2011; 8: 444.
[182] Pravettoni G, Gorini A. A P5 cancer medicine approach: Why personalized medicine cannot ignore psychology. J Eval Clin Pract 2011; 17: 594-6.
[183] Bragazzi NL. From P0 to P6 medicine, a model of highly participatory, narrative, interactive, and "augmented" medicine: some considerations on Salvatore Iaconesi's clinical story. Patient Prefer Adherence 2013; 7: 353-9.
[184] Hood L, Flores M. A personal view on systems medicine and the emergence of proactive P4 medicine: Predictive, preventive, personalized and participatory. N Biotechnol 2012; 29: 613-24.
[185] Qin S, Zhou Y, Lok AS, *et al*. SRM targeted proteomics in search for biomarkers of HCV-induced progression of fibrosis to cirrhosis in HALT-C patients. Proteomics 2012; 12: 1244-52.
[186] Varmus H. Ten years on--the human genome and medicine. N Engl J Med 2010; 362: 2028-9.
[187] Snyder M, Weissman S, Gerstein M. Personal phenotypes to go with personal genomes. Mol Syst Biol 2009; 5: 273.
[188] Phillips KA. Closing the evidence gap in the use of emerging testing technologies in clinical practice. JAMA 2008; 300: 2542-4.
[189] Lyssenko V, Jonsson A, Almgren P, *et al*. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008; 359: ss2220-32.

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