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 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 mechanism underlying inflammatory bowel disease (IBD), but also provided key information for the clinicians to find a novel gene which was responsible for Crohn’s disease-like symptoms. This variant was identified by WES and was confirmed by next generation sequencing [55].

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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 × 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 causal [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 × 10⁻⁸) [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
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%
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 pharmacogenetic 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 high 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 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/ScienceResearch/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 glioblastomas [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, increases 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 a 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-positive 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 cardiovascular 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 psycho-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 (e.g., 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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