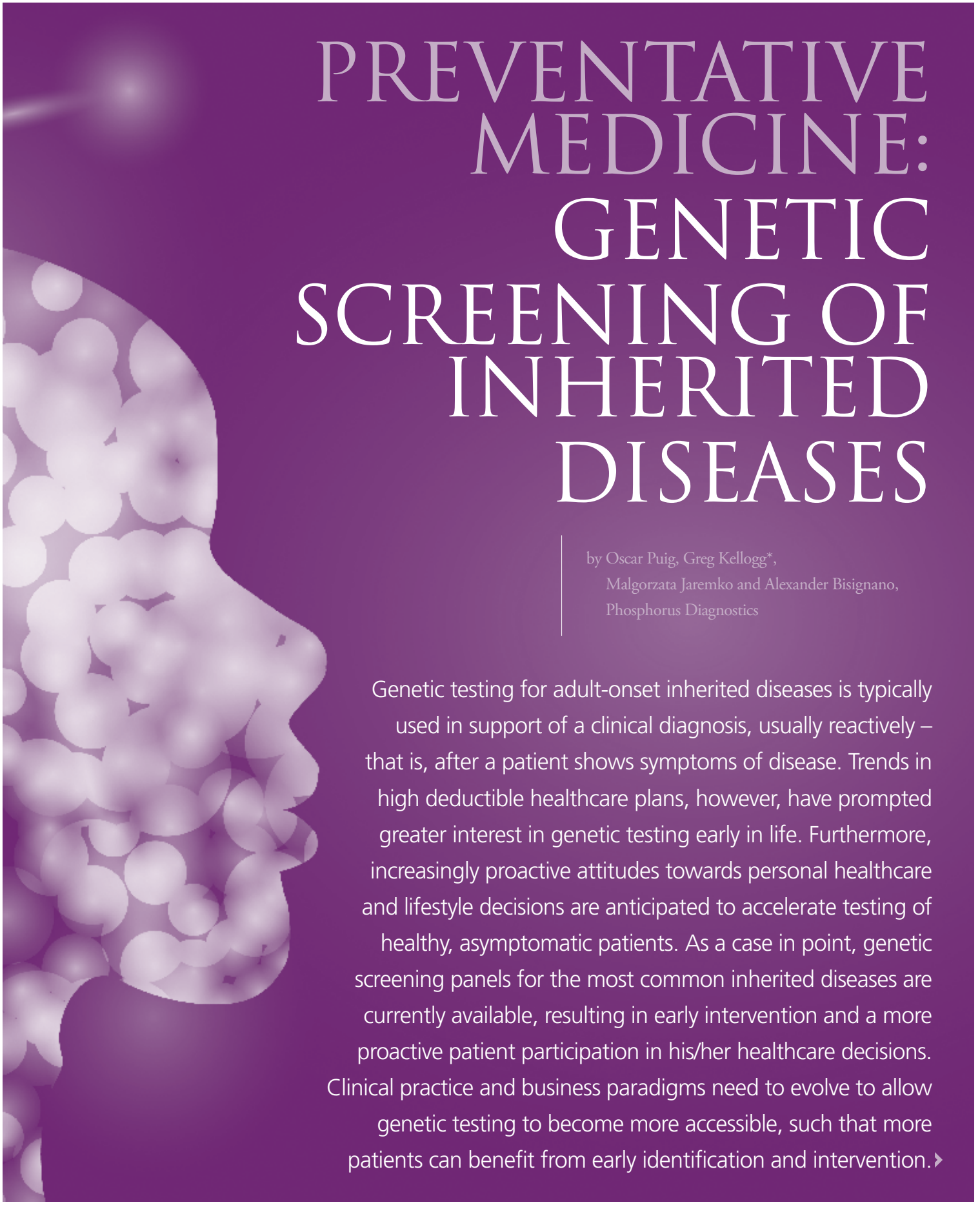

PREVENTATIVE MEDICINE: GENETIC SCREENING OF INHERITED DISEASES

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Genetic testing for adult-onset inherited diseases is typically used in support of a clinical diagnosis, usually reactively – that is, after a patient shows symptoms of disease. Trends in high deductible healthcare plans, however, have prompted greater interest in genetic testing early in life. Furthermore, increasingly proactive attitudes towards personal healthcare and lifestyle decisions are anticipated to accelerate testing of healthy, asymptomatic patients. As a case in point, genetic screening panels for the most common inherited diseases are currently available, resulting in early intervention and a more proactive patient participation in his/her healthcare decisions. Clinical practice and business paradigms need to evolve to allow genetic testing to become more accessible, such that more patients can benefit from early identification and intervention.

Screening and diagnostic paradigms for genetic testing

Screening for genetic disease has a long history, beginning in the early 1960s when Robert Guthrie developed a blood test for phenylketonuria (PKU), a metabolic disease that, untreated, leads to profound intellectual disability.[1] Identification of affected individuals allows for the restriction of dietary phenylalanine; patients treated early and effectively show almost normal development, demonstrating the dramatic effect that asymptomatic identification of a genetic disease can have.[2]

By 2006, screening of newborns for genetic disease was commonplace, with the American College of Medical Genetics recommending a core panel of 29 conditions tested.[3] The criteria for the inclusion of a disorder in newborn screening are largely informed by the Wilson and Jungner principles of screening for disease.[4] The most salient of these principles are 1) the need for an accepted treatment for patients with recognized disease; 2) adequate understanding of the natural history of the condition; 3) an agreed policy and criteria on whom to treat as patients; and 4) a test or examination that is acceptable to the population.[5] While screening for actionable genetic disorders with newborn onset has become commonplace, screening for actionable adult-onset disorders has not.

Newborn screening is primarily a biochemical test; analytes indicative of an underlying genetic disorder are assessed, and appropriate clinical follow-up is performed to determine if an individual is, in fact, affected. Many genetic disorders, however, do not have an identifying biochemical signature, and must therefore be diagnosed at a molecular level, by identifying deleterious changes in a patient's genes that lead to disease.

The techniques by which genes are analyzed, and the number of genes that can be assayed at a given time has grown dramatically,

from single gene tests for cystic fibrosis[6] and Duchenne muscular dystrophy[7] in the 1980s, to the ability to test the entire genome of more 20,000 protein coding genes at a given time.[8] The advent of accurate and cost-effective next-generation sequencing (NGS) has made molecular genetic testing an important component in the evaluation of inherited disease and is recommended in the work-up of many adult-onset inherited disorders, including hereditary cancers[9] and inherited cardiovascular disease.[10]

Identification of a disease causing mutation in an affected individual can clarify a clinical diagnosis, inform current and future management, and allow asymptomatic family members to be tested to determine if they are also at risk to develop disease.[11] The value of early identification and early intervention for at-risk relatives is well understood, and guidelines for the management of asymptomatic individuals with disease-causing mutations exist for many conditions.[12,13] Although the utility of identifying asymptomatic individuals is well characterized, recommendations for genetic testing are typically reserved for symptomatic individuals and their at-risk relatives, and not for the general population.

In 2013, the American College of Medical Genetics recommended that positive results for 56 disorders,[14] later updated to 59,[15] should be returned to patients if identified, regardless of the indication for testing, given that they are medically actionable. These recommendations support testing individuals for actionable genetic diseases even in the absence of a specific indication. Further, limiting genetic testing to only patients who meet current testing guidelines has been demonstrated to miss large numbers of patients.[16]

An illustrative example is familial hypercholesterolemia (FH), a genetic disorder caused by mutations in three genes (*APOB*, *LDLR*, *PCSK9*), which cause increased LDL-cholesterol levels in blood that lead to accumulation of plaque in arteries and a significant increase in the risk of coronary heart disease.[11] FH is an autosomal dominant condition with a prevalence of 1:250 in the US,[17] which translates to more than 1 million people affected. FH can be effectively treated by the administration of cholesterol lowering drugs,[18] and FH subjects who are treated with statins early live longer, healthier lives, compared with those who do not.[19] Furthermore, FH can be effectively diagnosed using a genetic test, which allows initiation of treatment at an age when it can be most effective. Yet, more than 90% of FH subjects in the US are undiagnosed,[20] leading to missed opportunities for intervention and consequent increases in medical costs to the system,[21] and increased morbidity and mortality. Genetic testing for FH is currently performed mainly on symptomatic individuals to aid in diagnosis and risk stratification;[9] using genetic testing for FH as a screening tool has the potential to identify individuals before they are symptomatic and allow them to be proactive about their healthcare.

Screening for genetic disease has been demonstrated to be effective in the context of newborn screening. Many adult-onset genetic disorders fulfill Wilson-Jungner principles of screening for disease, justifying the case for population screening of these disorders. Given that current testing guidelines preclude most patients from being offered genetic testing by their provider, how can interested patients access proactive genetic testing that can improve their health?

Direct to Consumer (DTC) genetic testing

23andMe was the first company to offer DNA testing for genetic ancestry, and soon, commercialized genetic tests to detect human diseases – tests that anyone could purchase directly from their web page. This approach was questioned by the medical community[22] and led to FDA action. On November 2013, the FDA sent a warning letter to 23andMe asking them to stop selling DTC genetic testing as "personal genome services".[23-25] For several months, 23andMe had marketed a microarray genetic test with "intended medical use" without providing regulators with data supporting the analytical and clinical performance required to make the medical claims. The FDA's warning letter was a necessary step to ensure patient safety, and additional studies have demonstrated problems with the analytical validity of some DTC genetic tests.[26] In response, 23andMe agreed to keep marketing their test for ancestry purposes, and not for medical use.

A few months after the FDA warning letter, 23andMe submitted a 510 (k) application to the FDA for a test to diagnose Bloom syndrome, which was soon followed by submissions for other disorders. Recently, 23andMe received FDA approval for DTC testing for the three BRCA1/2 founder mutations present in the Ashkenazi Jewish population.[27] Anyone can go to the 23andMe webpage, buy a saliva kit, send it to 23andMe, and receive a medical report with information on three pathogenic mutations in BRCA1/2, without physician supervision.

'SCREENING FOR GENETIC DISEASE HAS BEEN DEMONSTRATED TO BE EFFECTIVE IN THE CONTEXT OF NEWBORN SCREENING'

Importantly, the three pathogenic mutations detected by this test (c.6869delAG, BRCA1 c.5266dupC, and BRCA2 c.5946delT) occur with a frequency 2.67% in the Ashkenazi Jewish population but 0.028% in the general population.[28] Since BRCA1/2 variants are estimated to occur in 1 in 400 individuals in the general population, testing only for the three Ashkenazi Jewish founder mutations would miss more than 88% of carriers of pathogenic BRCA1/2 variants. Further, multiple studies have demonstrated that in patients undergoing comprehensive NGS panel testing for hereditary breast cancer, BRCA1/2 variants account for only ~50% of identified mutations.[29] Given that the FDA has approved this test to be ordered without a clinician, there exists the potential for harm to

individuals who interpret their negative result to mean they are not at increased risk for hereditary breast and ovarian cancer.

This case illustrates the importance of determining the clinical utility of a genetic test, and that FDA approval does not necessarily mean an improved outcome for patients. Comprehensive diagnostic testing that screens for *all* BRCA1/2 mutations are available from many qualified vendors, and have shown clinical utility.[29,30] These genetic tests are regulated under CLIA, which is overseen by CMS and not the FDA. CLIA and medical societies have strict guidelines[31,32] that laboratories must follow to ensure appropriate test performance. Most CLIA laboratories also have CAP accreditation and all of them are required to participate in routine proficiency testing (a system where CAP sends blinded samples to the labs and labs report results), which allows the detection and correction of possible deficiencies in diagnostic laboratory processes. All these processes are in place to ensure patient safety, and, with few unfortunate exceptions, have served the community over the years with rapid and accurate diagnostic tests.[33]

Despite these controversies, the commercial success of 23andMe has clearly demonstrated a demand from consumers for information about their genetic health risks, which raises the question; how can consumers obtain high quality genetic testing that is both clinically actionable and accessible?

Consumer-initiated genetic tests

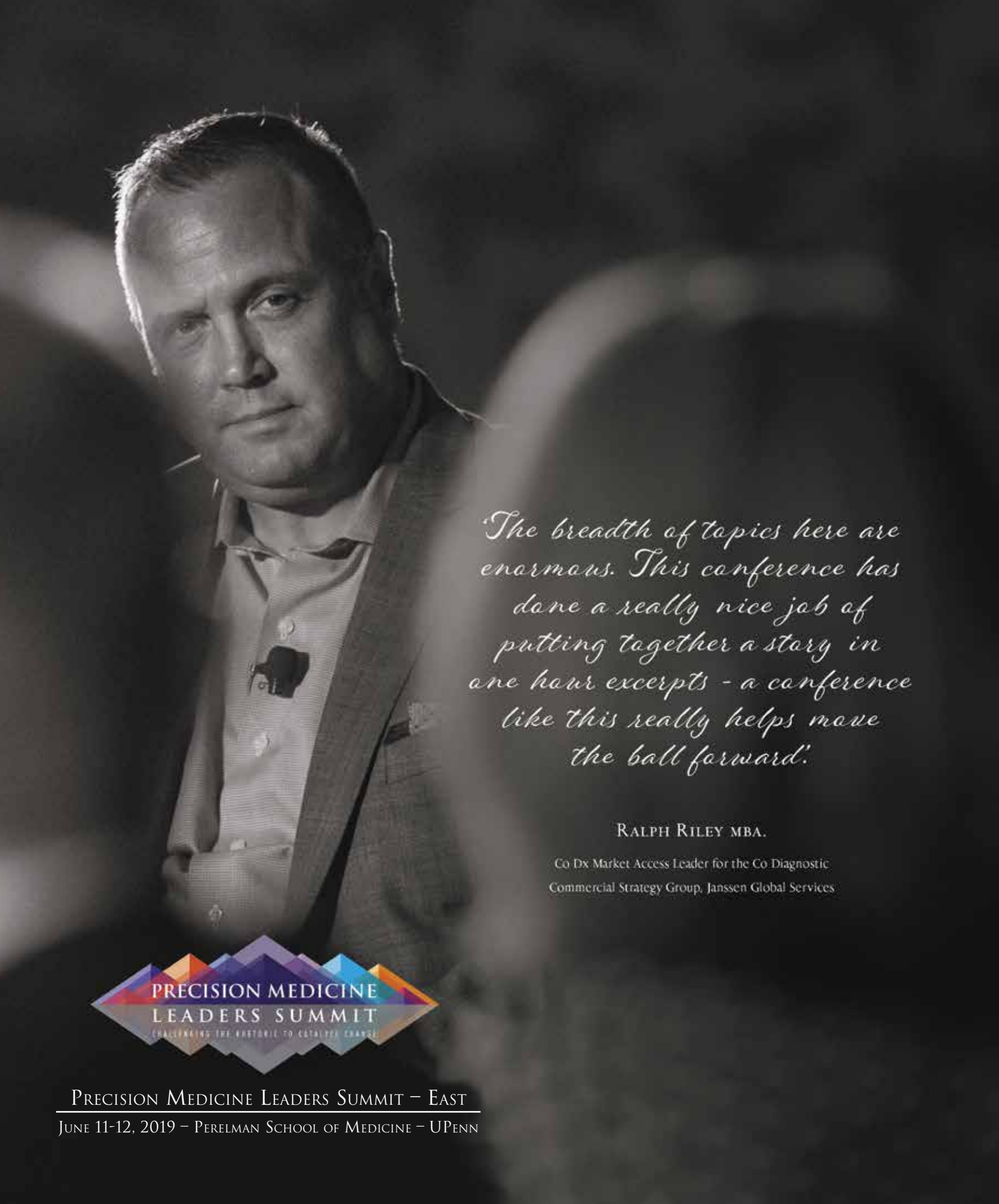
The above example has opened a debate about whether DTC genetic tests are safe when compared to physician-ordered tests. Is a test that provides potentially serious medical information safe when it is ordered without the oversight of a clinician to assist the patient in the interpretation and follow-up of that test? A third model, which combines the oversight of a physician ordered test with the accessibility of a DTC test, is the consumer-initiated genetic test.

In this model, the consumer approaches a genetic testing company directly, ordering and paying for the test, usually through a webpage. The company provides a saliva kit that the consumer ships directly to the testing lab. A third-party physician reviews the test order to determine if it is clinically appropriate, and, if so, becomes the ordering provider for that patient. When results are ready, they are first sent back to the physician, who determines if any follow-up, such as genetic counseling, is indicated. This model is considered proactive, or preventative, medicine, since there is no need for disease symptoms to be present for the consumer to get tested. Since the results are reviewed and relayed to the consumer by a physician, appropriate follow-up can be recommended.

Preventative medicine: the Geisinger MyCode initiative

Physician ordered tests, DTC tests, and customer initiated tests still only address a subset of the population that could benefit from screening for adult-onset genetic disorders. Population screening initiatives, spurred by trends towards high deductible healthcare plans,[34,35] and the need to reduce costs, have the potential to screen and identify large numbers of at-risk patients. One such program, the Geisinger Mycode genetic screening program, has screened over 100,000 patients in the Geisinger Health System, which serves Pennsylvania and parts of New Jersey.[36] The MyCode program combines whole exome NGS analysis of each MyCode participant, combined with longitudinal electronic health records (EMRs).

This initiative has shown impactful results. Genetic testing results are returned for 76 genes related to 27 diseases, and 1 in 76 individuals was found to have "actionable results", which means results leading to a direct change in their treatment. This prevalence ›



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projected into the population size covered by Geisinger implies that over 150,000 individuals will benefit from the program.[37] Considering conditions that were previously screened by the healthcare providers with alternative methods (i.e. mammograms and BRCA1/2 genetic testing for breast cancer, and colonoscopies for colon cancer), a significant number of individuals screening negative in these tests were identified by proactive, whole exome screening as being at increased genetic risk for these diseases.[37] Additional benefits from this program are correction of misdiagnosis produced by other methods, and early diagnosis of disease in those related to affected individuals through cascade screening.[37] Overall, by providing proactive genetic screening early in life, the healthcare system will reduce costs overall by reducing the eventual need for remedial palliative treatments. Other healthcare systems are following Geisinger's path.[37] This is a clear example of genetic screening in preventative medicine saving lives, and will likely reduce healthcare costs.

Issues with genetic screening

Genetic testing is not devoid of problems, and these are amplified when high throughput techniques, like exome sequencing, are used in screening of healthy people, mainly because of the extremely large number of hypotheses tested.

Test accuracy

No test is perfect, and genetic testing is not an exception. There is an associated number of false positives (results show a mutation that does not exist in the subject's DNA) and false negatives (subject carries a deleterious mutation, but testing missed it) with each test. Proficiency testing and CLIA regulations minimize the number of false positives and negatives in each test, but patients need to be properly informed of the risks that genetic testing carries. Different bioinformatics analyses (the ways data produced by DNA sequencers are processed), may produce very different results, depending on the software used, or even the version of each software package and the dependencies in it. Sometimes results have very low concordance.[38] Current medical society guidelines stress the importance of testing software performance as much as lab proficiency,[31,32] but this is a rapidly evolving field, with new developments happening at a fast pace, making it very time-consuming (although absolutely necessary) to version-control every step. Genetic screening of healthy individuals opens the possibility of unnecessary interventions, or of a false impression that disease has been avoided, so patient and physician education are critical to minimize undesired events.

'POSITIVE RESULTS IN A HEALTHY SUBJECT BRING A DILEMMA: TO TREAT OR NOT TO TREAT'

Variant interpretation

Often, genetic testing provides unequivocal determination of the presence of a variant in an individual's DNA; however, the effect of the variant on protein function is either unknown or not agreed upon. In cases where well-known, predefined mutations are sought and identified, results are usually interpretable. However, NGS queries entire genes, including not only regions where mutations clearly have a functional consequence on the protein coded by the gene (conserved protein domains, kinase active sites), but also those regions in which it is not clear how a mutation will affect gene function (non-conserved coding sequence, promoters, 5' and 3' UTRs). In the latter cases, pathogenicity (or the probability that the mutation causes the disease) of the genetic variant identified by the test may be unknown. Variants of Unknown Significance, or VUS, with unknown medical implications are common in genetic reports. An open debate exists in the field regarding which variants should be reported to patients, and what criteria should be used.[39]

Polygenic disease

Genetic counseling for monogenic diseases, or diseases caused by variants in a single gene, is usually straightforward. However, monogenic disease is relatively uncommon. Polygenic disease, caused by combined effects of multiple genes, is the most common source of inherited disease. It is often difficult to diagnose a polygenic disease, and although the treatment may be similar (i.e. statins in the case of polygenic hypercholesterolemia), it is often not clear the contributions of each gene, and the implications of a positive diagnosis derived from a polygenic score (or the combination of effects in multiple genes), as there may be unrecognized, counter-acting variations in other genes.[40] We anticipate that the integration of comprehensive genetic testing with longitudinal clinical follow-up (e.g., programs like the Geisinger MyCode initiative) will lead to a better understanding of how genetic changes of small effect impact patient health overall and in the long-term.

Patient support

Positive results in a healthy subject bring a dilemma: to treat or not to treat. Some diseases have incomplete penetrance, which means that subjects carrying known and well-studied pathogenic variants will never develop any symptoms.[41] In some cases, treatment involves major procedures (for example, the implantation of a defibrillator after testing positive for a variant associated with heart arrhythmia, or

undergoing mastectomy in BRCA1/2 positive subjects). Genetic testing, however, can also provide the impetus and justification for early screening, like mammography, colonoscopy, or echocardiography that can catch disease progression at an early stage. Appropriate patient counseling and support is therefore critical to inform correctly of the increased risk to disease, and the treatment options available to positive patients. To complicate matters, the size of the current genetic counseling workforce may not be large enough to meet the needs associated with a broad implementation of genomic screening programs in health care systems.[37]

Concluding remarks

Genetic screening for adult-onset disorders has the potential to change the way medicine is practiced by contributing to early diagnosis, treatment, and ultimately improved patient management. With increasing knowledge of the relationship between genes and health, improving technologies, and thoughtful regulation, genetic screening is providing a new paradigm of health care. ■

References

1. Guthrie R, Susi A. A SIMPLE PHENYLALANINE METHOD FOR DETECTING PHENYLKETONURIA IN LARGE POPULATIONS OF NEWBORN INFANTS. Pediatrics. 1963;32:338-43.
2. Burgard P, Bremer HJ, Bührdel P, et al. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. Eur J Pediatr. 1999;158(1):46-54.
3. Newborn screening: toward a uniform screening panel and system. Genet Med. 2006;8 Suppl 1:1S-252S.
4. Wilson JM, Jungner YG. [Principles and practice of mass screening for disease]. Bol Oficina Sanit Panam. 1968;65(4):281-393.
5. Ross LF. Screening for conditions that do not meet the Wilson and Jungner criteria: the case of Duchenne muscular dystrophy. Am J Med Genet A. 2006;140(8):914-22.
6. Kerem B, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. Science. 1989;245(4922):1073-80.
7. Darras BT, Harper JF, Francke U. Prenatal diagnosis and detection of carriers with DNA probes in Duchenne's muscular dystrophy. N Engl J Med. 1987;316(16):985-92.
8. Choi M, Scholl UI, Ji W, et al. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci USA. 2009;106(45):19096-101.
9. Daly MB, Pilarski R, Berry M, et al. NCCN Guidelines Insights: Genetic/Familial High-Risk Assessment: Breast and Ovarian, Version 2.2017. J Natl Compr Canc Netw. 2017;15(1):9-20.
10. Ackerman MJ, Priori SG, Willems S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm. 2011;8(8):1308-39.
11. Sturm AC, Knowles JW, Gidding SS, et al. Clinical Genetic Testing for Familial Hypercholesterolemia: JACC Scientific Expert Panel. J Am Coll Cardiol. 2018;72(6):662-680.
12. NCCN. National Comprehensive Cancer Network guidelines for colorectal cancer screening. 2016
13. Daly MB, Pilarski R, Berry M, et al. NCCN Guidelines Insights: Genetic/Familial High-Risk Assessment: Breast and Ovarian, Version 2.2017. J Natl Compr Canc Netw. 2017;15(1):9-20.
14. 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(7):565-74.
15. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genetics in medicine : official journal of the American College of Medical Genetics. 2017; 19(2):249-255.
16. Beitsch PD, Whitworth PW, Hughes K, et al. Underdiagnosis of Hereditary Breast Cancer: Are Genetic Testing Guidelines a Tool or an Obstacle? Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018
17. Akioyamen LE, Genest J, Shan SD, et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ open. 2017; 7(9):e016461.
18. Versmissen J, Oosterveer DM, Yazdanpanah M, et al. Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. BMJ (Clinical research ed.). 2008; 337:a2423.
19. Neil A, Cooper J, Betteridge J, et al. Reductions in all-cause, cancer, and coronary mortality in statin-treated patients with heterozygous familial hypercholesterolaemia: a prospective registry study. European heart journal. 2008; 29(21):2625-33.
20. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J. 2013;34(45):3478-90a.
21. Lázaro P, Pérez de isla L, Watts GF, et al. Cost-effectiveness of a cascade screening program for the early detection of familial hypercholesterolemia. J Clin Lipidol. 2017;11(1):260-271.
22. Kaye J. The regulation of direct-to-consumer genetic tests. Hum Mol Genet. 2008;17(R2):R180-3.
23. Park A. FDA halts 23andMe genetic tests. Time. 2013;182(24):18.
24. Annas GJ, Elias S. 23andMe and the FDA. N Engl J Med. 2014;370(23):2248-9.
25. Yuji K, Tanimoto T, Oshima Y. 23andMe and the FDA. N Engl J Med. 2014;370(23):2248.
26. Tandy-Connor S, Guiltinan J, Krempely K, et al. False-positive results released by direct-to-consumer genetic tests highlight the importance of clinical confirmation testing for appropriate patient care. Genetics in medicine : official journal of the American College of Medical Genetics. 2018
27. Direct-to-Consumer Test for Mutations Authorized. Cancer discovery. 2018; 8(5):OF5.
28. PDQ Cancer Genetics Editorial Board. Genetics of Breast and Gynecologic Cancers (PDQ®): Health Professional Version. PDQ Cancer Inf. Summ. 2018.
29. Tung N, Lin NU, Kidd J, et al. Frequency of Germline Mutations in 25 Cancer Susceptibility Genes in a Sequential Series of Patients With Breast Cancer. J Clin Oncol. 2016;34(13):1460-8.
30. Dong L, Wu N, Wang S, et al. Detection of novel germline mutations in six breast cancer predisposition genes by targeted next-generation sequencing. Hum Mutat. 2018;39(10):1442-1455.
31. Rehm HL, Bale SJ, Bayrak-toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 2013;15(9):733-47.
32. Roy S, Coldren C, Karunamurthy A, et al. Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines: A Joint Recommendation of the Association for Molecular Pathology and the College of American Pathologists. J Mol Diagn. 2018;20(1):4-27.
33. Kim AS, Bartley AN, Bridge JA, et al. Comparison of Laboratory-Developed Tests and FDA-Approved Assays for BRAF, EGFR, and KRAS Testing. JAMA Oncol. 2018;4(6):838-841.
34. Lewey J, Gagne JJ, Franklin J, Lauffenburger JC, Brill G, Choudhry NK. Impact of High Deductible Health Plans on Cardiovascular Medication Adherence and Health Disparities. Circ Cardiovasc Qual Outcomes. 2018;11(11):e004632.
35. Abdus S, Keenan PS. Financial Burden of Employer-Sponsored High-Deductible Health Plans for Low-Income Adults With Chronic Health Conditions. JAMA Intern Med. 2018;178(12):1706-1708.
36. Carey DJ, Fetterolf SN, Davis FD, et al. The Geisinger MyCode community health initiative: an electronic health record-linked biobank for precision medicine research. Genet Med. 2016;18(9):906-13.
37. Addie S, Hackmann M, Wizemann T, Beachy S, Genomics R, Health P, et al. Implementing and Evaluating Genomic Screening Programs in Health Care Systems. National Academies Press; 2018.
38. Hwang S, Kim E, Lee I, Marcotte EM. Systematic comparison of variant calling pipelines using gold standard personal exome variants. Sci Rep. 2015;5:17875.
39. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.
40. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet. 2018;50(9):1219-1224.
41. Coll M, Pérez-serra A, Mates J, et al. Incomplete Penetrance and Variable Expressivity: Hallmarks in Channelopathies Associated with Sudden Cardiac Death. Biology (Basel). 2017;7(1)

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