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Cancer Genomics and Inherited Risk

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ABSTRACT

Next-generation sequencing (NGS) has enabled whole-exome and whole-genome sequencing of tumors for causative mutations, allowing for more accurate targeting of therapies. In the process of sequencing the tumor, comparisons to the germline genome may identify variants associated with susceptibility to cancer as well as other hereditary diseases. Already, the combination of massively parallel sequencing and selective capture approaches has facilitated efficient simultaneous genetic analysis (multiplex testing) of large numbers of candidate genes. As the field of oncology incorporates NGS approaches into tumor and germline analyses, it has become clear that the ability to achieve high-throughput genotyping surpasses our current ability to interpret and appropriately apply the vast amounts of data generated from such technologies. A review of the current state of knowledge of rare and common genetic variants associated with cancer risk or treatment outcome reveals significant progress, as well as a number of challenges associated with the clinical translation of these discoveries. The combined efforts of oncologists, genetic counselors, and cancer geneticists will be required to drive the paradigm shift toward personalized or precision medicine and to ensure the incorporation of NGS technologies into the practice of preventive oncology.

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INTRODUCTION

The last three decades have witnessed significant strides in our understanding of the genetic basis of cancer susceptibility. In the 1980s and 1990s, rare but highly penetrant cancer predisposition genes were identified by studying cancer-prone families showing Mendelian modes of inheritance. These investigations successfully implicated genes such as *BRCA1* and *BRCA2* in hereditary breast-ovarian cancer syndrome, DNA mismatch repair genes in Lynch syndrome, *p53* in Li-Fraumeni syndrome, and *APC* in familial adenomatous polyposis.[1] Identification of the genetic basis of such syndromes has had a powerful impact on the practice of preventive oncology. The incorporation of cancer genetic testing into oncology marked one of the first applications of personalized genomics in medicine, because it allowed tailored cancer screening, prevention, and, in some cases, therapeutic measures.[2-5]

Recently, the applications of next-generation sequencing (NGS) technology have led to multiplex gene-panel testing and genome-wide sequencing, posing broad new challenges to clinical oncologists (definitions of important terms involved in NGS are listed in Table 1). As genotyping costs continue to decrease, and computational abilities improve, there will be increasing demand for all patients with cancer to undergo tumor genome sequencing to guide targeted therapies.[6,7] In the process, patients' normal or germline DNA may also be scanned, thrusting oncologists into the position of providing genome-based risk assessment to patients and their families. This genomic information will include not only cancer-associated risk but also pharmacogenomic markers to guide treatment choices as well as noncancer disease risks. The task of integrating and translating this genomic information for patients with cancer may require consultation with genetic counselors and clinical cancer geneticists. As in the early days of *BRCA* testing, oncologists will again be called on to take on new challenges in the emerging field of genomic prevention and personalized medicine.

FROM RARE TO COMMON VARIANTS ASSOCIATED WITH RISK FOR CANCER

Although known cancer susceptibility syndromes now number > 100, mutations in high-penetrance genes explain only a fraction of the heritability of human cancers.[8] Largely on the basis of knowledge of tumor genomes and pathways, a candidate gene approach has also been applied to the study of cancer susceptibility. As an example, mutations in candidate genes in DNA damage response pathways (*ATM, CHEK2, BRIP1, PALB2*) were found to be associated with a modest increase in breast cancer risk and are now considered cancer susceptibility genes.[9-13]

Table 1. Definition of Terms

Term	Definition
NGS or MPS	High-throughput DNA sequencing technique that allows for parallel sequencing of thousands to millions of simultaneous sequences, producing vast amounts of data at a fraction of the cost of traditional Sanger sequencing
WGS	Sequencing of the complete DNA sequence of an individual
WES	Sequencing limited to protein-coding regions of the genome, constituting approximately 1% of the total human genome (approximately 30 Mb)
Multiplex gene-panel testing	Targeted analysis of multiple genes of interest simultaneously using NGS/MPS technologies
GWAS	Systematic hypothesis-free search for genetic variations, usually in the form of SNPs, across the genome to identify genetic association with disease or trait
VUS	Genetic sequence change whose association with disease risk is currently unknown
Clinical utility	Degree to which use of test informs clinical decision making and leads to improved health outcomes
Clinical validity	Accuracy with which genetic test can identify or predict presence or absence of a particular clinical condition, taking into account specificity, sensitivity, and penetrance of genetic variation
Incidentalome	Incidental and/or unexpected genomic findings that may result from genomic evaluation of an individual's DNA sequence
Pharmacogenomics	Identification of genetic factors associated with a specific response or side effect for a particular drug/treatment

Abbreviations: GWAS, genome-wide association study; MPS, massively parallel sequencing; NGS, next-generation sequencing; SNP, single-nucleotide polymorphism; VUS, variant of unknown significance; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Coinciding with the introduction of high-resolution genotyping arrays in approximately 2005, under the common disease–common variant hypothesis, wherein heritability is presumed to be determined by the joint action of multiple common genes, the genetic architecture of complex diseases, such as cancer, began to be dissected. Using these high-resolution genotyping platforms, genome-wide association studies (GWASs) were rapidly completed for nearly all common cancers.[14,15] Although hundreds of statistically robust risk variants, largely in the form of single-nucleotide polymorphisms (SNPs), were identified, each genetic variant was associated with only a modest increase in disease risk (relative risk, approximately 1.1 to 1.5). With > 90% of risk variants residing in noncoding introns, the causal variants in most implicated risk loci have remained elusive, and the biologic basis of most associations remains unclear, although recent mapping of genetic switches to noncoding regions promises greater insight into some variants.[16] Given the modest effect size for most risk variants identified, the clinical utility of genomic profiling for risk stratification based on GWAS data has been limited for most common cancers.[17-25] However, the clinical utility of common genetic variants in risk assessment continues to evolve. For example, as a result of large international consortia studies, 49 new loci were recently identified for breast cancer, 26 for prostate cancer, and eight for ovarian cancer.[26-33] With such additional discoveries, the incorporation of genetic susceptibility into models of risk stratification for public health programs and cancer screening may eventually be feasible.[34]

NGS TECHNOLOGY

Recently, a shift toward identifying rare genomic variants was made possible by the emergence of NGS using massively parallel sequencing (MPS) platforms that enable whole-exome sequencing (WES) and whole-genome sequencing (WGS) of tumors as well as normal tissue (Table 2; Fig 1 and Appendix Fig A1, online only). NGS technology may directly identify causative mutations, which can then be studied at a functional level. Although promising, NGS gives rise to significant computational and analytic hurdles.

As opposed to conventional Sanger-based capillary sequencing methods,[40] NGS allows for MPS through a series of repeating sequencing reactions, performed and detected automatically, with the production of thousands to millions of simultaneous sequences. MPS,

Table 2. Commercially Available MPS Machines Compared With Traditional Sanger Sequencing

Platform	Amplification Step	Sequencing Chemistry	Average Read Length (bases)	Run Time	Reads per Run	Bases per Run
Sanger 330XL*	Yes (PCR)	Chain termination by dideoxynucleotides	400 to 900	20 minutes to 3 hours	96	1,900 to 84,000
Pacific Biosciences/ PacBio RS†	No (single molecule)	Real-time single-molecule sequencing reactions	1,500 (C1 chemistry)	2 hours	45,000	100,000,000
454 GS FLX*	Yes (emulsion PCR)	Pyrosequencing detection of pyrophosphate release with incorporation of correct complementary base	700	24 hours	1,000,000	700,000,000
Ion Torrent (Personal Genome Machine, 318 Chip)†	Yes (emulsion PCR)	Detection of pH change by semiconductor technology with incorporation of correct complementary base	200	2 hours	5,000,000	1,000,000,000
SOLiD 4*	Yes (emulsion PCR)	Sequencing by ligation using four fluorescently labeled di-base probes	50 paired end	14 days	1,400,000,000	120,000,000,000
Illumina/HiSeq 2000*	Yes (bridge amplification PCR)	Sequencing by synthesis using base-specific fluorophores and cyclic reversible-chain termination	100 paired end	3 to approximately 10 days	3,000,000,000	600,000,000,000

Abbreviations: MPS, massively parallel sequencing; PCR, polymerase chain reaction.
*Data adapted.[35]
†Data adapted.[36]

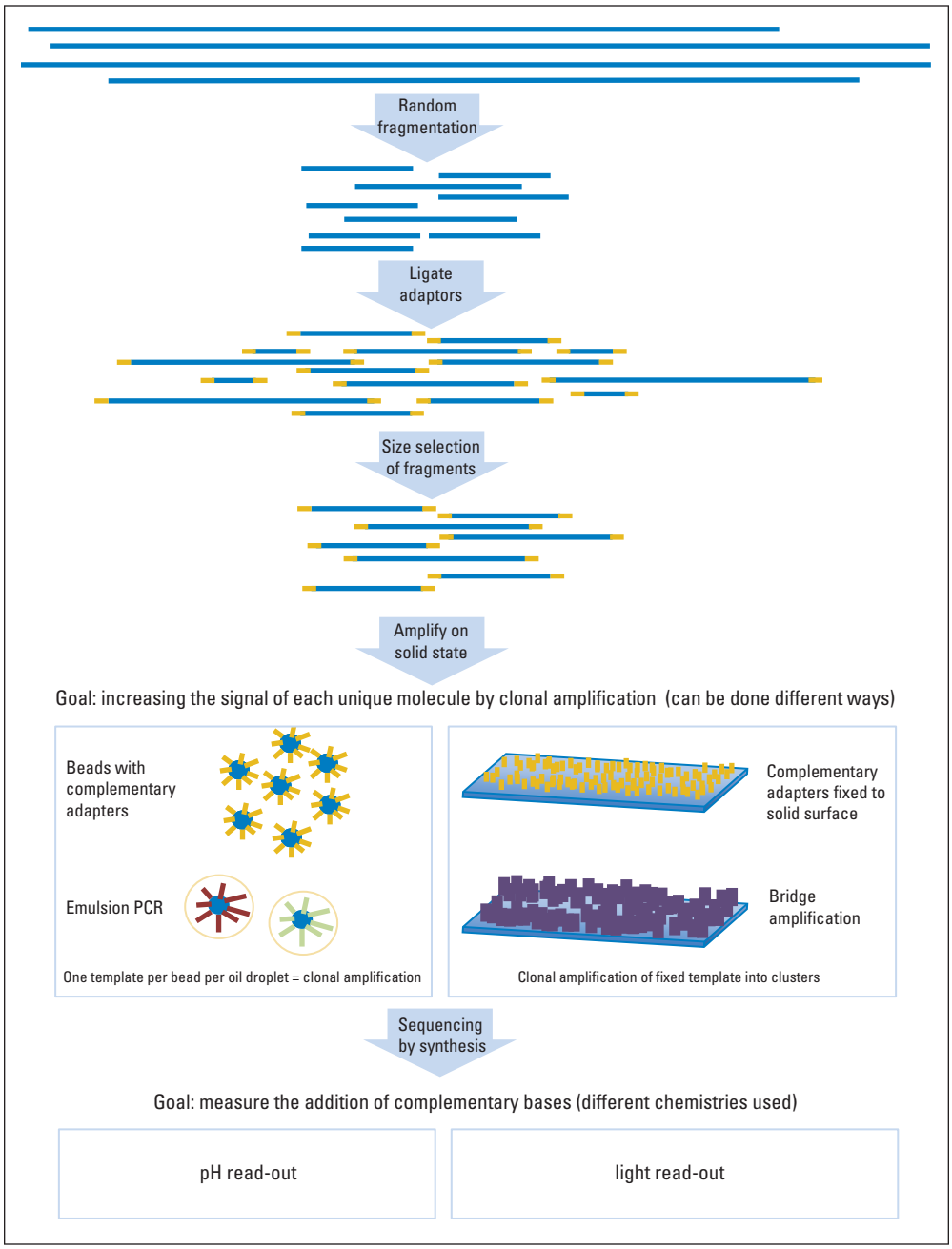

Fig 1. Principles of next-generation sequencing (NGS) technology. For NGS library preparation, DNA is randomly fragmented into desired size ranges. Adaptors containing the universal priming sites are ligated to the target ends of the fragments. After ligation, the template is immobilized to a solid support. Immobilization strategies for clonally amplified templates include either using emulsion polymerase chain reaction (emPCR)[37] or a solid-phase amplification.[38] In emPCR, an oil-aqueous emulsion reaction mixture is created to encapsulate bead-DNA complexes into single aqueous droplets. PCR is then performed within the droplets to create beads that contain several thousand copies of the same template sequence. The emPCR beads can then be attached to a glass slide or loaded into PicoTiterPlate (Roche Applied Science, Indianapolis, IN) wells. Solid-phase amplification relies on bridge PCR, where both forward and reverse PCR primers are tethered to a solid substrate by a flexible linker such that the clonally amplified clusters remain immobilized, thereby localizing to a single physical location on an array. At the conclusion of bridge PCR, each clonal cluster contains approximately 1,000 copies of a single member of the template library. Regardless of platform used, amplification is a necessary step because it allows the sequencing reactions to produce sufficient signal for detection by the imaging system of the instrument. Single-molecule as opposed to clonally amplified templates can also be accomplished using a number of possible approaches for immobilization. On the basis of whether clonally amplified or single-molecule templates are used, different sequencing and imaging strategies need to be applied. Although the DNA sequencing reactions vary among platforms, the most popular technologies, such as cyclic reversible termination, pyrosequencing, and the pH-based/semiconductor sequencing, perform sequencing by synthesis to sequence the template. The ability to move away from optically based detection systems to more scalable semiconductor technology has drastically reduced the costs associated with sequencing. Other sequencing technologies exist, such as the sequencing by ligation method, referred to as the SOLiD (support oligonucleotide) platform (Table 2).[39]

thereby, generates drastically more sequence reads per instrument run, at a significantly lower expense. Applications of NGS include WGS of tumor and germline DNA, as well as targeted sequencing of specific regions of interest, including WES or multigene (multiplex) gene-panel testing. In multiplex testing, the simultaneous interrogation of target genes of interest allows for an efficient and cost-effective method of screening panels of cancer genes concurrently, as opposed to screening on a gene-by-gene basis as occurs in Sanger sequencing.

The basic principles of NGS technology are shown in Figure 1; an in-depth review of NGS technologies can be found elsewhere.[41-44] Although each NGS technology is distinct (Table 2), all manufacturers aim to increase the amount of sequence output per run, increase the number of nucleotides per sequence read (or read lengths), lower cost, and improve base-calling accuracy. Although cost effective and highly efficient, disadvantages of NGS include higher error rates and shorter read lengths, enrichment of rare variants, and a large proportion of missing values. A comparison of the accuracy and completeness of variant calling for two commonly used sequencing platforms found that although both technologies achieved a relatively high concordance for unique single-nucleotide variants of 88%, for indels, concordance was only 27%.[45] Technologic advancements may improve base-calling accuracy; however, for now, all NGS-acquired data require analytic validation using alternate technologies. These technical limitations also constitute important caveats to regulators and clinicians seeking to certify or use NGS-based diagnostic panels for cancer predisposition or prognostic evaluation.

CANCER GENETICS THROUGH NGS TECHNOLOGIES

In the last 4 years, WES of unrelated individuals or families with multiple affected members with the same rare disorder has identified the genetic basis of diseases such as Freeman-Sheldon syndrome, Kabuki syndrome, Miller syndrome, and autosomal dominant spinocerebellar ataxias.[46-51] In 2010, the first report of WGS of a patient with Charcot-Marie-Tooth disease was published and was followed by a number of WGS studies of rare phenotypes.[52-54] The rapidly growing catalogue of NGS studies can be found in the NGS catalogue maintained by Vanderbilt University.[55,56] In cancer, the use of WES and WGS has enabled a dramatic improvement on the classical methods for gene discovery, such as linkage analysis.

Evaluation of Cancer Susceptibility Using WES and WGS Technologies

One of the first applications of NGS for cancer susceptibility was use of WES to identify a germline mutation in *PALB2*, a gene previously implicated in breast cancer risk, in an individual with familial pancreatic cancer.[57,58] WGS approaches also identified heterozygous variants in *ATM* in families with rare pancreatic cancer.[59] Homozygous mutations in *ATM* are known to cause ataxia telangiectasia,[60,61] whereas heterozygous mutations have previously been linked to breast cancer susceptibility.[62] However, these *PALB2*- and *ATM*-linked pancreatic cancer clusters have proven to be exceedingly rare.

NGS was also used to identify a novel gene involved in familial pheochromocytoma (PCC).[63] Although the *RET*, *VHL*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *NF1*, and *TMEM127* genes have already been linked to familial PCC,[64-67] approximately 10% of hereditary PCCs with autosomal-dominant pattern of inheritance are not explained by mutations within these genes.[68,69] WES in individuals with familial PCC identified mutations of *MAX*.[63] *MAX*, the MYC-associated factor X gene, is a transcription factor that regulates cell proliferation, differentiation, and apoptosis.[70] Similar to the sex-linked transmission of familial PCC cases associated with *SDHD* and *SHAF2* mutations, *MAX* mutations associated with familial PCC seem to have a preferential paternal transmission pattern. Taken in aggregate, these NGS discoveries set the stage for a panel of nearly a dozen genes that could be simultaneously tested in familial PCC.

The theme of transcription factor–associated cancer predisposition as revealed by WES also applies to acute myelogenous leukemia (AML). Inherited predisposition to myelodysplastic syndrome/AML had previously been linked to mutations within hematopoietic transcription factors *RUNX1* and *CEBPA*.[71,72] NGS studies linked mutations of the transcription factor *GATA2* to familial AML and, occasionally, Emberger syndrome, an autosomal-dominant primary lymphedema associated with widespread cutaneous warts, deafness, and predisposition to myelodysplastic syndrome.[73-75] Recently, our group demonstrated that inherited mutations of another transcription factor, *PAX5*, lead to childhood acute lymphoblastic leukemia.[76] The pedigrees in both *GATA2*- and *PAX5*-mutant kindreds demonstrate multiple mutation carriers who remain unaffected, illustrating the incomplete penetrance and variable expressivity associated with these NGS discoveries.

Genetic alterations in *KLHDC8B* have been associated with Hodgkin lymphoma risk,[77,78] and combining WES from one family member with genome-wide linkage data, a truncating germline mutation in nuclear protein ataxia telangiectasia locus (*NPAT*) was identified in familial nodular lymphocyte–predominant Hodgkin lymphoma.[79] With an important role in cell-cycle regulation and promotion of *ATM* activation, *NPAT* is a promising cancer susceptibility gene.[80]

WGS in an individual with familial melanoma (without identified *CDKN2A* or *CDK4* mutations) showed a single-nucleotide variant in the melanoma lineage–specific oncogene microphthalmia-associated transcription factor (*MITF*) to be segregating with melanoma in the family.[81] The variant had an odds ratio of 2.3 (95% CI, 1.2 to 4.7) in epidemiologic studies, and functional analysis demonstrated that MITF encoded by the E318K variant allele had impaired sumoylation and differentially regulated MITF targets. The E318K variant has since been implicated in susceptibility to both melanoma and renal cancers.[82] As in the case of the leukemia-associated transcription factors we have mentioned, this study illustrates a clinical conundrum common to many NGS-based gene discovery studies: The *MITF* risk variant did not segregate fully with disease status. The reduced penetrance and expressivity are more marked than those observed, for example, in *BRCA* mutation carriers, thereby complicating the genetic counseling process. Presumably, in these patients, cancer risk is affected by other genetic or environmental risk factors. Targeted resequencing of other melanoma families identified mutations in the promoter of telomerase reverse transcriptase (*TERT*), which create novel binding sites for Ets/TCF transcription factors.[83]

Despite extensive research, only approximately 30% of familial breast cancer risk is explained by known genetic factors,[14] and it was expected that NGS of unexplained kindreds would rapidly reveal the so-called low-hanging fruit of novel candidate genes. Such fruit, however, have not always ripened. WES in selected affected family members from 13 breast cancer families identified two families with mutations in x-ray repair cross-complementing 2 (*XRCC2*), including a protein-truncating change and a probable deleterious missense mutation.[84] WES in families with early-onset breast cancer also revealed variants in the Fanconi pathway gene *FAN1*.[85] Unfortunately, subsequent large-scale studies did not confirm the *FAN1* or *XRCC2* variants as associated with breast cancer risk.[85,86] We implicated another Fanconi pathway gene, *SLX4*, in only one of > 700 *BRCA*-negative breast cancer kindreds, suggesting that additional such mutations may in rare cases contribute to breast cancer risk.[87] Most recently, a large pooled NGS study focusing on DNA repair pathways identified mutations in the p53-inducible protein phosphatase *PPM1D* as occurring

Table 3. Identification of Cancer Susceptibility Genes Using NGS

Cancer/Cancer Syndrome	NGS	Gene Implicated	Cases Used for Identification of Cancer Susceptibility Gene
Familial pancreatic cancer			
Jones et al[57]	Exome	*PALB2*	One affected with familial pancreatic cancer
Roberts et al[59]	WGS and exome	*ATM*	WGS: 16 affecteds from six families; exome: 22 affecteds from 10 families
Familial pheochromocytoma			
Comino-Méndez et al[63]	Exome	*MAX*	Three affecteds from three families
Hematologic malignancies			
AML with Emberger syndrome; Ostergaard et al[73]	Exome	*GATA2*	Three unrelated affecteds (two with familial, one with sporadic)
Familial HL; Saarinen et al[79]	Exome	*NPAT*	One affected with familial nodular lymphocyte predominant HL, combined with genome-wide linkage data from family
Familial pre–B-cell ALL; Shah et al[76]	Exome	*PAX5*	Two families with exome sequencing of multiple affected and unaffected family members
Familial melanoma			
Yokoyama et al[81]	WGS	*MITF*	One affected with familial melanoma; also assessed in cases/controls
Horn et al[83]	Targeted sequencing	*TERT*	Targeted sequencing of four affecteds and one unaffected in melanoma family
Familial mesothelioma, melanoma, and RCC			
Testa et al[95]	Exome	*BAP1*	Two families with mesothelioma and uveal melanoma
Wiesner et al[96]	Targeted sequencing		Two families with uveal and cutaneous melanocytic tumors
Popova et al[97]	Exome		Two affecteds from one RCC family
Familial colorectal cancer and polyposis			
HMPS; Jaeger et al[91]	Targeted sequencing	*GREM1*	Large Ashkenazi Jewish family with HMPS; additional HMPS families
Colorectal adenomas and colon cancer; Palles et al[90]	WGS	*POLE, POLD1*	20 affecteds (colorectal adenomas ± colorectal cancer) from 15 families
Familial breast cancer			
Park et al[84]	Exome	*XRCC2*	Five affecteds from two families; also assessed in case/controls
Park et al[85]	Exome	*FAN1**	Four early-onset multiple-case breast cancer families
Ruark et al[88]	Targeted sequencing	*PPM1D* (mosaic)	1,150 with breast cancer ± ovarian cancer; replication in large case/control
Ovarian cancer			
Rafnar et al[89]	WGS	*BRIP1*	WGS of 457 Icelanders; case/control of imputed SNPs
Walsh et al[99]	Targeted sequencing	Multiple genes†	360 women with ovarian cancer

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; HL, Hodgkin lymphoma; HMPS, hereditary mixed polyposis syndrome; NGS, next-generation sequencing; RCC, renal cell carcinoma; SNP, single-nucleotide polymorphism; WGS, whole-genome sequencing.
*Identified variants in *FAN1* not found to independently influence breast cancer risk.[85]
†Germline mutations found in *BRCA1*, *BRCA2*, *BARD1*, *BRIP1*, *CHEK2*, *MRE11A*, *MSH6*, *NBN*, *PALB2*, *RAD50*, *RAD51C*, and *TP53*.[99]

mosaically in individuals with predisposition to breast and ovarian cancers.[88] Using imputed-risk SNPs identified via WGS in an Icelandic population, a novel frameshift mutation was discovered in the *BRIP1* Fanconi pathway gene, with an odds ratio of 8.1 for ovarian cancer.[89] Such testing for genes associated with the homologous recombination DNA-repair pathways, in addition to preventive applications, has an emerging role in screening for targeted therapies, such as poly (ADP-ribose) polymerase inhibitors.

Predisposition to colon polyposis and colorectal cancer is only partially explained by known cancer susceptibility genes. WGS in families with multiple adenomas and/or colorectal cancer recently identified heterozygous *POLE* and *POLD1* germline variations.[90] These mutations, mapping to the exonuclease domains of DNA polymerases, are predicted to cause defective correction of mispaired bases and seem to be high-penetrance cancer susceptibility genes, with *POLD1* mutations also increasing endometrial cancer risk. Using a combination of linkage analysis as well as high-throughput sequencing, *GREM1* is the first gene to have been implicated in the genetic etiology of hereditary mixed polyposis syndrome.[91] In prostate cancer, NGS of linkage regions on chromosome 17 helped to identify a particular mutation in the homeobox gene *HOXB13*, an important driver of prostate cancer risk.[92]

Somatic mutations in *BAP1*, a nuclear-localized, ubiquitin carboxy-terminal hydrolase that binds to the RING finger domain of *BRCA1*, were previously identified in mesothelioma and uveal and cutaneous melanomas.[93,94] More recently, the identification of germline mutations in *BAP1* in families with mesothelioma, melanoma, and renal cell cancer, in an autosomal-dominant manner, suggests a new *BAP1*-related cancer susceptibility syndrome.[95-98] The frequency, penetrance, and spectrum of malignancies associated with *BAP1*-related cancer susceptibility remain to be elucidated.

The handful of studies published to date (Table 3), with many more in progress, highlights the power of NGS technologies in elucidating the genetic basis of hereditary cancers. In addition to providing important insights into the molecular mechanisms underlying carcinogenesis, even where there is no preventive strategy evident (eg, leukemia- or lymphoma-associated risks), this testing can be used in reproductive planning.[100] In breast and colon cancers, these findings have already produced high-throughput screens for panels of genes.

Multiplex Analysis for Cancer Syndromes Using NGS

In addition to gene discovery, another potential application of NGS technology is comprehensive and simultaneous (multiplex) mutational analysis of known cancer susceptibility genes and candidate genes. In a research context, this approach was demonstrated by screening 360 ovarian carcinomas for germline mutations in 21

tumor-suppressor genes.[99] Of the 360 patient cases, 85 showed germline loss-of-function mutations in 12 genes: 40 (11.1%) in *BRCA1*, 23 (6.4%) in *BRCA2*, and 22 (6.1%) in 10 other genes, including *BARD1*, *BRIP1*, *CHEK2*, *MRE11A*, *MSH6*, *NBN*, *PALB2*, *RAD50*, *RAD51C*, and *TP53*. Multiplex testing using NGS also reidentified pathogenic mutations in 28 blinded specimens with known mutations in genes associated with Lynch and polyposis syndromes.[101] Similarly, some of the newly implicated cancer susceptibility genes were identified partially via NGS, which provided an efficient method for screening large numbers of candidate genes (Table 3).[83,88,91,95]

As opposed to the traditional stepwise one-gene-at-a-time approach to genetic testing, the dramatic decreases in the cost of DNA sequencing make multiplex genetic testing an efficient and economically advantageous approach (Table 4). At the same time, recent court decisions have eroded intellectual property barriers to diagnostic genetic testing, further stimulating the proliferation of NGS-based multiplex gene-panel testing for genetic predispositions.[103] However, multiplex testing will inevitably result in the identification of increased variants of uncertain significance. Although the technical ability for multiplex testing has arrived, mechanisms to provide meaningful counseling for multigene and possible variant results pose significant barriers to the responsible translation of these technologies.[102]

Table 4. Commercially Available Multiplex Gene Panels

Panel	Genes Included
Breast cancer	
BreastNext; Ambry Genetics, Aliso Viejo, CA	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11, TP53*
Colorectal cancer	
ColoNext; Ambry Genetics	*APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53*
ColoSeq; University of Washington Laboratory Medicine, Seattle, WA	*APC, BMPR1A, CDH1, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53*
Mayo Medical Laboratories; Rochester, MN	*APC, AXIN2, BMPR1A, CDH1, CHEK2, EPCAM, GREM1, MLH1, MLH3, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53*
Ovarian cancer	
OvaNext; Ambry Genetics	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, STK11, TP53*
Multicancer panels	
CancerNext; Ambry Genetics	*APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, SMAD4, STK11, TP53*
BROCA Cancer Risk Panel; University of Washington Laboratory Medicine	*APC, ATM, ATR, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK1, CHEK2, FAM175A, GALNT12, GEN1, GREM1, HOXB13, MLH1, MRE11A, MSH2 (+EPCAM), MSH6, MUTYH, NBN, PALB2, PMS2, PRSS1, PTEN, RAD50, RAD51, RAD51C, RAD51D, RET, SMAD4, STK11, TP53, TP53BP1, VHL, XRCC2*
Myriad myRisk; Myriad Genetics, Salt Lake City, UT*	*APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A (p16INKA and p14ARF), CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53*

NOTE. Listing of laboratory name here does not imply that testing meets regulatory criteria of relevant state health department. Data adapted.[102]
*Expected to be commercially available later in 2013; published with permission from Myriad Genetics.

NGS OF CANCER GENOMES: IMPLICATIONS FOR GERMLINE CANCER RISK ASSESSMENT

In cancer research, the most common application of NGS is in the identification of acquired (somatic) mutations in tumor genomes.[6] Genetic changes specific to tumor cells may result in the discovery of novel genes and important pathways involved in carcinogenesis. Although initial approaches focused on assessment of an individual patient's tumor(s),[104,105] rapid progress in deciphering the cancer genome is anticipated through ongoing international efforts, including the Cancer Genome Atlas and International Cancer Genome Consortium.[106,107] These collaborative efforts aim to sequence up to 500 clinically well-annotated tumors for selected cancer types, with generation of an enormous amount of genomic data. To date, the Cancer Genome Atlas has already published initial analyses of glioblastomas and ovarian, colorectal, and breast cancers, among other tumor types, demonstrating the feasibility of this large-scale project and identifying a number of deregulated genes that may set the stage for the development of targeted therapies.[108-111]

Importantly, the study of somatic mutations in tumors using NGS may purposefully or inadvertently also shed light on the presence of corresponding germline mutations (Figs 2 and 3 summarize two clinical case scenarios).[112]

Case Scenarios

Patient Case 1. A 41-year-old female never-smoker with incidental bilateral ground glass opacities identified on computed tomography (CT) scan underwent thoracoscopic surgery, with removal of three distinct adenocarcinomas of the lung. Pathology demonstrated morphologically distinct, synchronous primary lung adenocarcinomas. For treatment purposes, somatic evaluation of the patient's tumors was undertaken. In two tumors, the presence of the L858R mutation in exon 21 of the epidermal growth factor receptor (*EGFR*) gene was noted, whereas the third tumor exhibited deletion of exon 19 of the *EGFR* gene. Although both of these activating mutations in *EGFR* have been associated with an enhanced response to *EGFR* tyrosine kinase inhibitors (TKIs),[113-115] subsequent analysis demonstrated that all three tumors also exhibited the T790M *EGFR* mutation, associated with resistance to TKIs.[116,117] Family history at the time of diagnosis was unrevealing (Fig 2; pedigree case 1; noncritical clinical features have been changed to preserve confidentiality). The patient was referred to clinical genetics for counseling and genetic testing, in light of the fact that the *EGFR* T790M germline mutation has been linked to lung cancer susceptibility, and the identification of this somatic mutation in all of her tumors was suspicious for a potential germline etiology.[118] Germline analysis revealed the presence of the *EGFR* T790M mutation. Family testing revealed the *EGFR* T790M

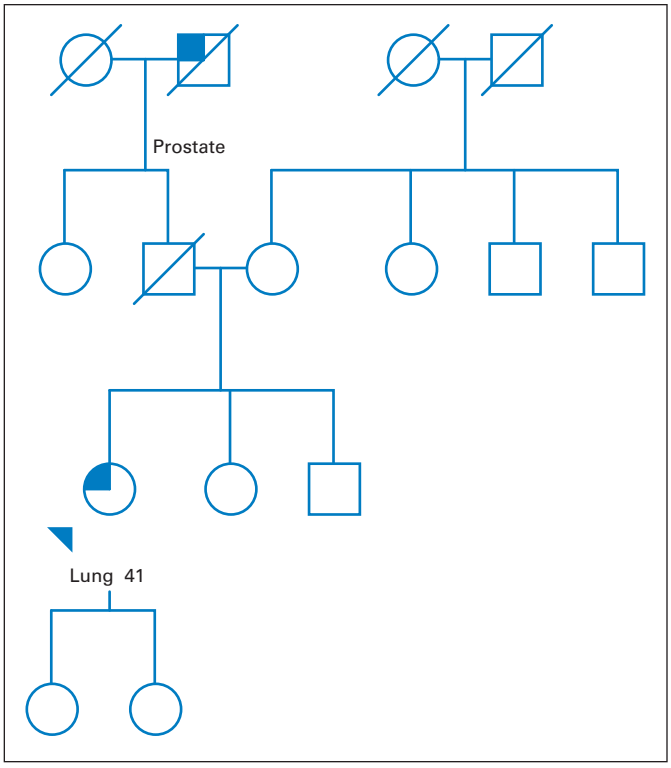

Fig 2. Pedigree case 1.

mutation in the patient's mother, in whom subsequent clinical workup confirmed a diagnosis of multifocal lung cancer. Currently, implications for unaffected germline *EGFR* mutation carriers remain unclear because of insufficient data regarding the risk of lung cancer (and/or other cancers) in such family members. Individualized counseling regarding the role of CT scan–based lung cancer screening and optional genetic testing for *EGFR* T790M were advised for relevant family members.

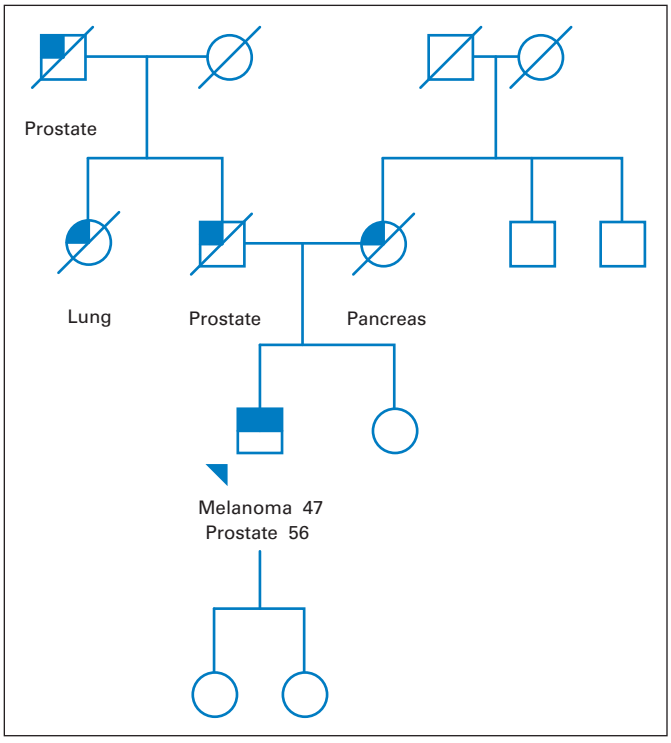

Fig 3. Pedigree case 2.

Patient Case 2. A 59-year-old man with a history of melanoma was being evaluated for oncologic treatment options for his newly diagnosed metastatic prostate cancer. In this setting, an outside laboratory performed targeted sequence analysis of > 200 cancer-related genes, using NGS technologies, on a sample of his prostate tumor. Germline DNA was not used for analysis. Results, after removal of known common germline genetic variants, revealed two genetic variations within the tumor, one in *p53* and the other in *BRCA1*. The patient was subsequently referred to the clinical genetics service for further evaluation of the two presumed somatic genetic events. In addition to his cancer diagnosis, family history was significant for a strong family history of prostate cancer (Fig 3; pedigree case 2; noncritical clinical features have been changed to preserve confidentiality). Although the *p53* nonsynonymous missense variation was noted to be a common somatic event in tumors, the identified, presumed somatic *BRCA1* mutation was also known to be a deleterious (pathogenic) germline mutation, diagnostic of hereditary breast and ovarian cancer (HBOC) syndrome. After genetic counseling, germline genetic testing revealed the presence of the *BRCA1* mutation within the germline, diagnostic of HBOC. Implications for family members, including the daughter, were explained. In this particular case, the presence of the *BRCA1* mutation may also have had an impact on future treatment for the patient, with clinical trials suggesting increased efficacy of poly (ADP-ribose) polymerase inhibitors in the treatment of *BRCA*-associated malignancies.[119,120]

Case Scenarios: Points to Consider

- Medical oncologists and other clinicians considering genomic analysis of tumors via NGS technologies (whole-exome and whole-genome sequencing, targeted cancer-panel testing) must understand that even without direct analysis of germline DNA, somatic (tumor) DNA analysis may reveal mutations in the constitutional (germline) genome that could have important implications for cancer (or other disease) risks for the affected individual as well as for at-risk family members.
- For this reason, there is a strong rationale for pretesting informed consent to include discussion of potential tumor findings, incidental germline findings, and plans for disclosure of results.
- Somatic genetic findings based on tumor analysis must be interpreted carefully within the context of the personal and family histories, preferably with the input of clinical cancer geneticists to identify situations in which interrogation of the germline is necessary.
- Some commercial laboratories performing somatic (tumor) analyses for targeted cancer panels report the results of somatic analyses without indicating if such genetic events resulted from germline mutations. Some laboratories may filter the germline entirely. Although such approaches may not be appropriate for prospective tumor genomic analysis, they may be justified in a retrospective research setting where specific consent may not be obtainable. In such a setting, one

alternative proposed by informaticians is simply to not subject the germline to variant calling algorithms.[121]

MAINTAINING PRIVACY IN THE ERA OF GENOMICS

Given the unique nature of an individual's DNA sequence, by definition, DNA data cannot truly be anonymized. A statistical approach was used to show that an individual's genomic data could be identified in large DNA data sets of aggregated SNP data.[122] This finding resulted in the National Institutes of Health establishing barriers for review and approval of open-access databases.[123] Recently, genomic and ancestry information was linked from online commercial databases to deduce a person's surname.[124] A conclusion thus emerges that research participants must be informed that although all efforts can be made to de-identify DNA samples, there still exists potential for breach of privacy. At present, certain federal grants require primary genomic sequences to be available to the research community, requiring institutional and/or participant consent for such data release. A controversial potential revision to the Common Rule 45 CFR, which has governed such research, would no longer allow DNA research to be deemed eligible for waivers resulting from sample anonymization (ie, permanent removal of personal identifiers).[125] Such a change would sharply limit the number of banked biospecimens eligible for federally funded research. Alternative solutions to this privacy concern would be regulatory deterrents to make it illegal to maliciously re-identify de-identified genomic data, as well as restrictions to data access ensuring that only credentialed researchers are able to use primary genomic data.

NGS DATA INTERPRETATION AND REPORTING OF RESULTS

With the profusion of WES and WGS data, a new challenge has been posed by the extent to which incidental or secondary genetic findings, termed incidentalomes,[126] should be clinically transmitted to patients. Unexpected results may include identification of genetic predispositions to non-cancer-related diseases for which medical interventions may be available, such as cardiac or neurologic disease risks.[127-129] Although there is emerging consensus that return of such actionable results should be offered to the patient,[130,131] perhaps even more problematic is the challenge to inform patients of the possibility of detecting variants of uncertain significance. In an exploratory study evaluating the recommendations of specialists in clinical genetics and/or molecular medicine, 100% concordance was found in favor of disclosing incidental pathogenic mutations to adults in 21 conditions and/or genes; however, substantial discordance existed with respect to disclosure of mutations without proven pathogenicity.[130]

Recognizing the potential for rapid integration of WES and WGS into the clinical practice of medicine, in 2012, the American College of Medical Genetics and Genomics (ACMG) issued a policy statement on genomic sequencing that emphasized the importance of incidental results in pretest patient counseling, clinical testing, and results reporting.[132] Subsequently, the ACMG specifically delineated a minimum list of genes in which germline mutations should be reported by the clinical laboratory, regardless of the indication for which the sequencing test was ordered.[133] Although a majority of these must-be-reported genes were associated with cancer risk, there were no oncologists on the panel that selected them, and the report generated a firestorm of controversy within the medical genetics community. The controversy stemmed from the dichotomy between those arguing for autonomous right of choice to learn results of incidental findings and the ACMG requirement to disclose all results regardless of patient choice, which was perceived as paternalistic.[134-136] However, this seems a false dichotomy, because the preferred outcome is for individuals to achieve a sufficient level of genomic knowledge to make an informed choice to specify which incidental findings they want to have transmitted. As evident to practitioners of cancer genetics, a substantial proportion of fully counseled patients do not wish, for example, to know inherited *p53* mutation results,[137] and it is possible to opt for effective cancer screening based on family history without knowing mutation results. As the earliest adopters of genomic scans in a clinical setting, oncologists will play an important role in mediating whether the personalized genome is perceived as a blanket liability to be imposed or an empowering choice for patients educated to understand precisely what health risks are at stake.[121]

ROLE OF THE ONCOLOGIST IN TRANSLATION OF NGS TO PREVENTIVE ONCOLOGY

Oncologists as well as other specialized health care providers may rightly ask how they may be expected to approach the process of informed consent for testing entire genomes, both tumor and normal, when the meaning of the vast majority of variants uncovered is currently not defined. Because of these concerns, one incontrovertible conclusion of the ACMG and other guidelines is that oncologists should provide counseling before NGS testing of tumor samples, including a discussion of the possibility of findings of inherited genomic variants with as-yet-unknown significance (Fig 4).

To address how oncologists can integrate the simultaneous findings of tumor and inherited genomes, in one model, pioneered at the University of Michigan, all patients undergoing NGS of their tumors had to meet with a genetic counselor before consenting to genomic analysis.[138] At most major cancer centers, including our own, an advisory body, genomics review board, or other expert entity is constituted to advise on the need to return germline findings that emerge in the context of tumor genome analysis. This body may be constituted separately from or alongside the committee that advises on the suitability of particular tumor mutations as targets for therapy. Regardless of the approach used, findings to be reported need to be analytically validated and returned in compliance with state regulations.

Once a particular genomic variant uncovered by NGS is analytically validated, it may range in its clinical utility and suitability for communication to the patient as a primary or incidental finding. Various models have been proposed to guide the communication of incidental findings, ranging from tiered approaches to binning to full return. We favor, and are testing in a trial, the binning system, where genomic findings are categorized depending on clinical utility and clinical validity.[139] One beneficial aspect of the binning process is that research participants would potentially have the ability to select upfront, at time of consent, which bin

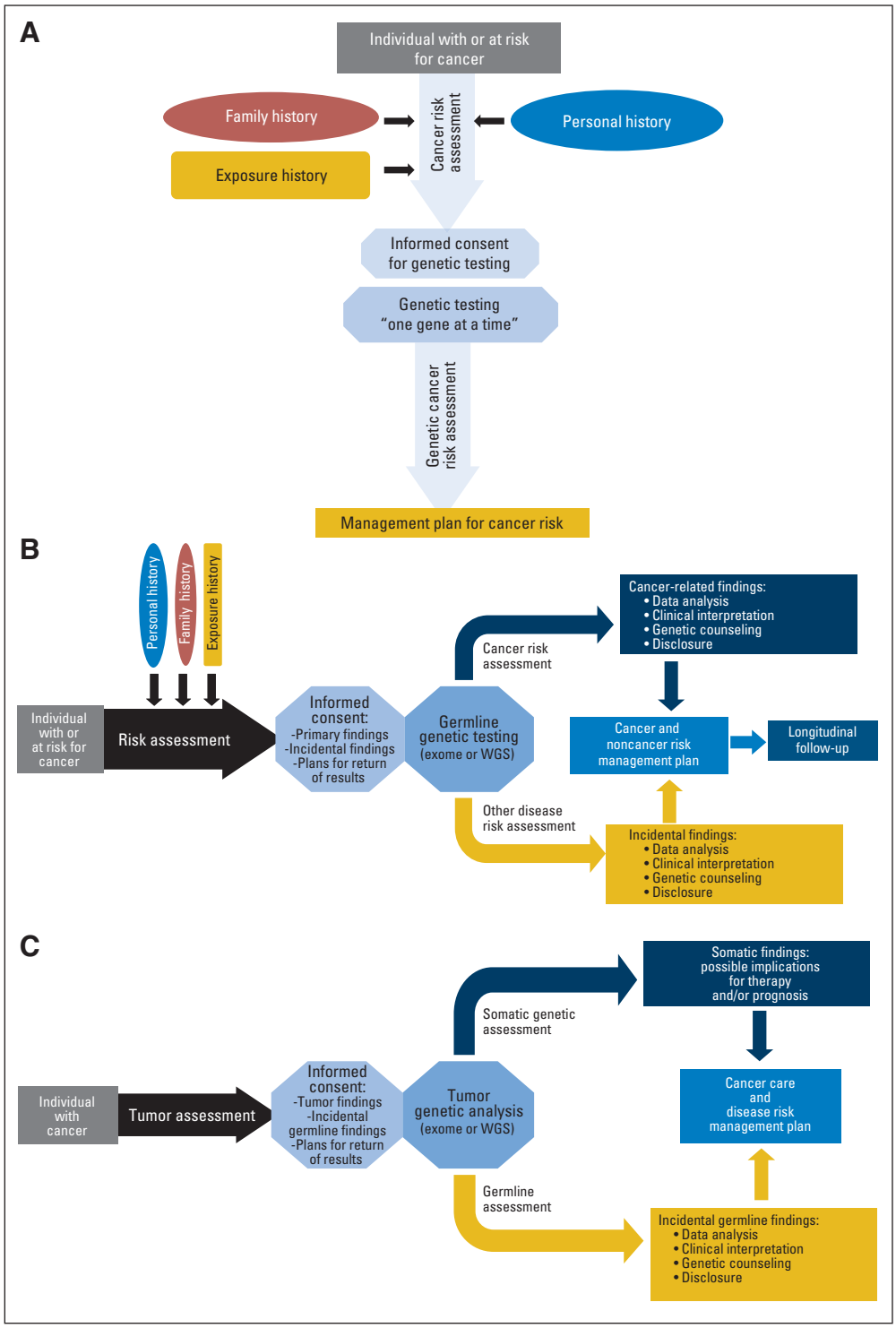

Fig 4. Challenging the traditional model of cancer genetic counseling. (A) Traditional model of clinical cancer genetics. (B) Incorporating next-generation sequencing (NGS) into genetic cancer risk assessment. (C) NGS of tumors with incorporation of incidental germline findings. WGS, whole-genome sequencing.

results they wish to receive at the results disclosure, allowing for a patient-directed approach. As the meaning of variants is defined over time, they can be deposited into bins for eventual transmission, depending on patient wishes. An important step forward in this regard is the creation by the National Human Genome Research Institute of the ClinVar database of genomic variation to provide a consensus of the biologic as well as clinical significance of genes and variants.[140]

Even if technically feasible, the premature translation of genomic information by oncologists also poses risks. These risks may be psychological, as a result of information overload. In addition, genetic information may create further health care disparities because of the high cost of these technologies or the medical interventions they motivate[141,142] and even pose health care risks if results are prematurely translated by commercial marketing directly to consumers outside of regulatory protection.[143]

DISCUSSION

Although common genomic risk variants identified to date have had limited clinical utility, ongoing WES and WGS approaches have already provided striking insights for some cancer-prone individuals. However, it is clear that sequencing technologies have outpaced our ability to interpret and apply the vast amounts of data generated. Pending these advances, we suggest a model incorporating a tiered approach to NGS studies of tumor and normal tissues. In addition to germline cancer risk assessment, such models would include flexibility for the provision of analytically valid and clinically useful risk assessment for other incidental noncancer disease risk findings.

Oncology is now ground zero for a tectonic shift in paradigms regarding personalized medicine.[144] Germline DNA profiles will be generated as references for DNA analysis of tumors to define therapeutic targets. As successfully accomplished during the prior era of cancer genetics, the practice of oncology will need to incorporate new concepts of genomic risk assessment. This will require new approaches to informed consent discussion with patients as well as decision algorithms to guide the communication of incidental germline findings that emerge from genome-wide tumor analyses.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Zsofia K. Stadler, Kenneth Offit
Collection and assembly of data: Zsofia K. Stadler, Kasmintan A. Schrader, Kenneth Offit
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Garber JE, Offit K: Hereditary cancer predisposition syndromes. J Clin Oncol 23:276-292, 2005
2. Robson M, Offit K: Clinical practice: Management of an inherited predisposition to breast cancer. N Engl J Med 357:154-162, 2007
3. Lindor NM, Petersen GM, Hadley DW, et al: Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: A systematic review. JAMA 296:1507-1517, 2006
4. Galiatsatos P, Foulkes WD: Familial adenomatous polyposis. Am J Gastroenterol 101:385-398, 2006
5. Villani A, Tabori U, Schiffman J, et al: Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: A prospective observational study. Lancet Oncol 12:559-567, 2011
6. Macconaill LE, Garraway LA: Clinical implications of the cancer genome. J Clin Oncol 28:5219-5228, 2010
7. Garraway LA: Genomics-driven oncology: Framework for an emerging paradigm. J Clin Oncol 31:1806-1814, 2013
8. Lichtenstein P, Holm NV, Verkasalo PK, et al: Environmental and heritable factors in the causation of cancer: Analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343:78-85, 2000
9. Athma P, Rappaport R, Swift M: Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. Cancer Genet Cytogenet 92:130-134, 1996
10. CHEK2 Breast Cancer Case-Control Consortium: CHEK2*1100delC and susceptibility to breast cancer: A collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 74:1175-1182, 2004
11. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al: Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 31:55-59, 2002
12. Seal S, Thompson D, Renwick A, et al: Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. Nat Genet 38:1239-1241, 2006
13. Rahman N, Seal S, Thompson D, et al: PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet 39:165-167, 2007
14. Stadler ZK, Thom P, Robson ME, et al: Genome-wide association studies of cancer. J Clin Oncol 28:4255-4267, 2010
15. Stadler ZK, Vijai J, Thom P, et al: Genome-wide association studies of cancer predisposition. Hematol Oncol Clin North Am 24:973-996, 2010
16. Bernstein BE, Birney E, Dunham I, et al: An integrated encyclopedia of DNA elements in the human genome. Nature 489:57-74, 2012
17. Wacholder S, Hartge P, Prentice R, et al: Performance of common genetic variants in breast-cancer risk models. N Engl J Med 362:986-993, 2010
18. Gail MH: Value of adding single-nucleotide polymorphism genotypes to a breast cancer risk model. J Natl Cancer Inst 101:959-963, 2009
19. Comen E, Balistreri L, Gönen M, et al: Discriminatory accuracy and potential clinical utility of genomic profiling for breast cancer risk in BRCA-negative women. Breast Cancer Res Treat 127:479-487, 2011
20. Antoniou AC, Beesley J, McGuffog L, et al: Common breast cancer susceptibility alleles and the risk of breast cancer for BRCA1 and BRCA2 mutation carriers: Implications for risk prediction. Cancer Res 70:9742-9754, 2010
21. Zheng SL, Sun J, Wiklund F, et al: Cumulative association of five genetic variants with prostate cancer. N Engl J Med 358:910-919, 2008
22. Salinas CA, Koopmeiners JS, Kwon EM, et al: Clinical utility of five genetic variants for predicting prostate cancer risk and mortality. Prostate 69:363-372, 2009
23. Johansson M, Holmström B, Hinchliffe SR, et al: Combining 33 genetic variants with prostate-specific antigen for prediction of prostate cancer: Longitudinal study. Int J Cancer 130:129-137, 2012
24. Dunlop MG, Tenesa A, Farrington SM, et al: Cumulative impact of common genetic variants and other risk factors on colorectal cancer risk in 42 103 individuals. Gut 62:871-881, 2013
25. Weitzel JN, Blazer KR, Macdonald DJ, et al: Genetics, genomics, and cancer risk assessment: State of the art and future directions in the era of personalized medicine. CA Cancer J Clin [epub ahead of print on August 19, 2011]
26. Michailidou K, Hall P, Gonzalez-Neira A, et al: Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 45:353-361, 2013
27. Garcia-Closas M, Couch FJ, Lindstrom S, et al: Genome-wide association studies identify four ER negative-specific breast cancer risk loci. Nat Genet 45:392-398, 2013
28. Bojesen SE, Pooley KA, Johnatty SE, et al: Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. Nat Genet 45:371-384, 2013
29. Pharoah PD, Tsai YY, Ramus SJ, et al: GWAS meta-analysis and replication identifies three new susceptibility loci for ovarian cancer. Nat Genet 45:362-370, 2013
30. Shen H, Fridley BL, Song H, et al: Epigenetic analysis leads to identification of HNF1B as a subtype-specific susceptibility gene for ovarian cancer. Nat Commun 4:1628, 2013
31. Permuth-Wey J, Lawrenson K, Shen HC, et al: Identification and molecular characterization of a new ovarian cancer susceptibility locus at 17q21.31. Nat Commun 4:1627, 2013
32. Eeles RA, Olama AA, Benlloch S, et al: Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. Nat Genet 45:385-391, 2013
33. Kote-Jarai Z, Saunders EJ, Leongamornlert DA, et al: Fine-mapping identifies multiple prostate cancer risk loci at 5p15, one of which associates with TERT expression. Hum Mol Genet 22:2520-2528, 2013
34. Burton H, Chowdhury S, Dent T, et al: Public health implications from COGS and potential for risk stratification and screening. Nat Genet 45:349-351, 2013
35. Liu L, Li Y, Li S, et al: Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012:251364, 2012
36. Quail MA, Smith M, Coupland P, et al: A tale of three next generation sequencing platforms: Comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genomics 13:341, 2012

37. Dressman D, Yan H, Traverso G, et al: Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proc Natl Acad Sci U S A 100: 8817-8822, 2003

38. Fedurco M, Romieu A, Williams S, et al: BTA, a novel reagent for DNA attachment on glass and efficient generation of solid-phase amplified DNA colonies. Nucleic Acids Res 34:e22, 2006

39. Valouev A, Ichikawa J, Tonthat T, et al: A high-resolution, nucleosome position map of C. elegans reveals a lack of universal sequence-dictated positioning. Genome Res 18:1051-1063, 2008

40. Sanger F, Air GM, Barrell BG, et al: Nucleotide sequence of bacteriophage phi X174 DNA. Nature 265:687-695, 1977

41. Mardis ER: New strategies and emerging technologies for massively parallel sequencing: Applications in medical research. Genome Med 1:40, 2009

42. Metzker ML: Sequencing technologies: The next generation. Nat Rev Genet 11:31-46, 2010

43. Koboldt DC, Ding L, Mardis ER, et al: Challenges of sequencing human genomes. Brief Bioinform 11:484-498, 2010

44. Shendure J, Ji H: Next-generation DNA sequencing. Nat Biotechnol 26:1135-1145, 2008

45. Lam HY, Clark MJ, Chen R, et al: Performance comparison of whole-genome sequencing platforms. Nat Biotechnol 30:78-82, 2012

46. Ng SB, Turner EH, Robertson PD, et al: Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461:272-276, 2009

47. Ng SB, Bigham AW, Buckingham KJ, et al: Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet 42:790-793, 2010

48. Hoischen A, van Bon BW, Gilissen C, et al: De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. Nat Genet 42:483-485, 2010

49. Ng SB, Buckingham KJ, Lee C, et al: Exome sequencing identifies the cause of a Mendelian disorder. Nat Genet 42:30-35, 2010

50. Wang JL, Yang X, Xia K, et al: TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing. Brain 133:3510-3518, 2010

51. Ku CS, Naidoo N, Pawitan Y: Revisiting Mendelian disorders through exome sequencing. Hum Genet 129:351-370, 2011

52. Lupski JR, Reid JG, Gonzaga-Jauregui C, et al: Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. N Engl J Med 362: 1181-1191, 2010

53. Sobreira NL, Cirulli ET, Avramopoulos D, et al: Whole-genome sequencing of a single proband together with linkage analysis identifies a Mendelian disease gene. PLoS Genet 6:e1000991, 2010

54. Rios J, Stein E, Shendure J, et al: Identification by whole-genome resequencing of gene defect responsible for severe hypercholesterolemia. Hum Mol Genet 19:4313-4318, 2010

55. Vanderbilt University: Bioinformatics and Systems Medicine Laboratory: NGS catalog. http://bioinfo.mc.vanderbilt.edu/NGS/index.html

56. Xia J, Wang Q, Jia P, et al: NGS catalog: A database of next generation sequencing studies in humans. Hum Mutat 33:E2341-E2355, 2012

57. Jones S, Hruban RH, Kamiyama M, et al: Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. Science 324:217, 2009

58. Tischkowitz M, Xia B: PALB2/FANCN: Recombining cancer and Fanconi anemia. Cancer Res 70:7353-7359, 2010

59. Roberts NJ, Jiao Y, Yu J, et al: ATM mutations in patients with hereditary pancreatic cancer. Cancer Discov 2:41-46, 2012

60. Gatti RA, Berkel I, Boder E, et al: Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. Nature 336:577-580, 1988

61. Li A, Swift M: Mutations at the ataxia-telangiectasia locus and clinical phenotypes of A-T patients. Am J Med Genet 92:170-177, 2000

62. Ahmed M, Rahman N: ATM and breast cancer susceptibility. Oncogene 25:5906-5911, 2006

63. Comino-Méndez I, Gracia-Aznárez FJ, Schiavi F, et al: Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet 43:663-667, 2011

64. Neumann HP, Bausch B, McWhinney SR, et al: Germ-line mutations in nonsyndromic pheochromocytoma. N Engl J Med 346:1459-1466, 2002

65. Hao HX, Khalimonchuk O, Schraders M, et al: SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 325:1139-1142, 2009

66. Yao L, Schiavi F, Cascon A, et al: Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. JAMA 304:2611-2619, 2010

67. Burnichon N, Brière JJ, Libé R, et al: SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet 19:3011-3020, 2010

68. Mannelli M, Castellano M, Schiavi F, et al: Clinically guided genetic screening in a large cohort of Italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab 94:1541-1547, 2009

69. Cascón A, Pita G, Burnichon N, et al: Genetics of pheochromocytoma and paraganglioma in Spanish patients. J Clin Endocrinol Metab 94:1701-1705, 2009

70. Atchley WR, Fitch WM: Myc and Max: Molecular evolution of a family of proto-oncogene products and their dimerization partner. Proc Natl Acad Sci U S A 92:10217-10221, 1995

71. Smith ML, Cavenagh JD, Lister TA, et al: Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med 351:2403-2407, 2004

72. Song WJ, Sullivan MG, Legare RD, et al: Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet 23:166-175, 1999

73. Ostergaard P, Simpson MA, Connell FC, et al: Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet 43:929-931, 2011

74. Mansour S, Connell F, Steward C, et al: Emberger syndrome-primary lymphedema with myelodysplasia: Report of seven new cases. Am J Med Genet A 152A:2287-2296, 2010

75. Hahn CN, Chong CE, Carmichael CL, et al: Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet 43:1012-1017, 2011

76. Shah S, Schrader KA, Waanders E, et al: A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. Nat Genet 45:1226-1231, 2013

77. Enciso-Mora V, Broderick P, Ma Y, et al: A genome-wide association study of Hodgkin's lymphoma identifies new susceptibility loci at 2p16.1 (REL), 8q24.21 and 10p14 (GATA3). Nat Genet 42: 1126-1130, 2010

78. Salipante SJ, Mealiffe ME, Wechsler J, et al: Mutations in a gene encoding a midbody kelch protein in familial and sporadic classical Hodgkin lymphoma lead to binucleated cells. Proc Natl Acad Sci U S A 106:14920-14925, 2009

79. Saarinen S, Aavikko M, Aittomäki K, et al: Exome sequencing reveals germline NPAT mutation as a candidate risk factor for Hodgkin lymphoma. Blood 118:493-498, 2011

80. Medina R, van der Deen M, Miele-Chamberland A, et al: The HiNF-P/p220NPAT cell cycle signaling pathway controls nonhistone target genes. Cancer Res 67:10334-10342, 2007

81. Yokoyama S, Woods SL, Boyle GM, et al: A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. Nature 480:99-103, 2011

82. Bertolotto C, Lesueur F, Giuliano S, et al: A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. Nature 480:94-98, 2011

83. Horn S, Figl A, Rachakonda PS, et al: TERT promoter mutations in familial and sporadic melanoma. Science 339:959-961, 2013

84. Park DJ, Lesueur F, Nguyen-Dumont T, et al: Rare mutations in XRCC2 increase the risk of breast cancer. Am J Hum Genet 90:734-739, 2012

85. Park DJ, Odefrey FA, Hammet F, et al: FAN1 variants identified in multiple-case early-onset breast cancer families via exome sequencing: No evidence for association with risk for breast cancer. Breast Cancer Res Treat 130:1043-1049, 2011

86. Hilbers FS, Wijnen JT, Hoogerbrugge N, et al: Rare variants in XRCC2 as breast cancer susceptibility alleles. J Med Genet 49:618-620, 2012

87. Shah S, Kim Y, Ostrovnaya I, et al: Assessment of SLX4 mutations in hereditary breast cancers. PLoS One 8:366961, 2013

88. Ruark E, Snape K, Humburg P, et al: Mosaic PPM1D mutations are associated with predisposition to breast and ovarian cancer. Nature 493:406-410, 2013

89. Rafnar T, Gudbjartsson DF, Sulem P, et al: Mutations in BRIP1 confer high risk of ovarian cancer. Nat Genet 43:1104-1107, 2011

90. Palles C, Cazier JB, Howarth KM, et al: Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet 45:136-144, 2013

91. Jaeger E, Leedham S, Lewis A, et al: Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. Nat Genet 44:699-703, 2012

92. Ewing CM, Ray AM, Lange EM, et al: Germline mutations in HOXB13 and prostate-cancer risk. N Engl J Med 366:141-149, 2012

93. Bott M, Brevet M, Taylor BS, et al: The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet 43:668-672, 2011

94. Harbour JW, Onken MD, Roberson ED, et al: Frequent mutation of BAP1 in metastasizing uveal melanomas. Science 330:1410-1413, 2010

95. Testa JR, Cheung M, Pei J, et al: Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43:1022-1025, 2011

96. Wiesner T, Obenauf AC, Murali R, et al: Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 43:1018-1021, 2011

97. Popova T, Hebert L, Jacquemin V, et al: Germline BAP1 mutations predispose to renal cell carcinomas. Am J Hum Genet [epub ahead of print on May 14, 2013]

98. Farley MN, Schmidt LS, Mester JL, et al: Germline BAP1 mutation predisposes to familial clear-cell renal cell carcinoma. Mol Cancer Res [epub ahead of print on May 24, 2013]

99. Walsh T, Casadei S, Lee MK, et al: Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A 108:18032-18037, 2011

100. Offit K, Sagi M, Hurley K: Preimplantation genetic diagnosis for cancer syndromes: A new challenge for preventive medicine. JAMA 296:2727-2730, 2006

101. Pritchard CC, Smith C, Salipante SJ, et al: ColoSeq provides comprehensive Lynch and polyposis syndrome mutational analysis using massively parallel sequencing. J Mol Diagn 14:357-366, 2012

102. Domchek S, Bradbury A, Garber J, et al: Multiplex genetic testing for cancer susceptibility: Out on the high wire without a net? J Clin Oncol 31:1267-1270, 2013

103. Offit K, Bradbury A, Storm C, et al: Gene patents and personalized cancer care: Impact of the myriad case on clinical oncology. J Clin Oncol 31: 2743-2748, 2013

104. Mardis ER, Ding L, Dooling DJ, et al: Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 361: 1058-1066, 2009

105. Ding L, Ellis MJ, Li S, et al: Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 464:999-1005, 2010

106. Cancer Genome Atlas. http://cancergenome.nih.gov

107. International Cancer Genome Consortium. https://www.icgc.org

108. Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061-1068, 2008

109. Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. Nature 474:609-615, 2011

110. Cancer Genome Atlas Network: Comprehensive molecular characterization of human colon and rectal cancer. Nature 487:330-337, 2012

111. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 490:61-70, 2012

112. Oxnard GR, Miller VA, Robson ME, et al: Screening for germline EGFR T790M mutations through lung cancer genotyping. J Thorac Oncol 7:1049-1052, 2012

113. 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 350:2129-2139, 2004

114. Paez JG, Jänne PA, Lee JC, et al: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497-1500, 2004

115. Pao W, Miller V, Zakowski M, et al: EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 101:13306-13311, 2004

116. Kobayashi S, Boggon TJ, Dayaram T, et al: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 352:786-792, 2005

117. Pao W, Miller VA, Politi KA, et al: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2:e73, 2005

118. Bell DW, Gore I, Okimoto RA, et al: Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. Nat Genet 37:1315-1316, 2005

119. Sandhu SK, Omlin A, Hylands L, et al: Poly (ADP-ribose) polymerase (PARP) inhibitors for the treatment of advanced germline BRCA2 mutant prostate cancer. Ann Oncol 24:1416-1418, 2013

120. Balma¯na J, Domchek SM, Tutt A, et al: Stumbling blocks on the path to personalized medicine in breast cancer: the case of PARP inhibitors for BRCA1/2-associated cancers. Cancer Discov 1:29-34, 2011

121. Bombard Y, Robson M, Offit K: Revealing the incidentalome when targeting the tumor genome. JAMA 310:795-796, 2013

122. Homer N, Szelinger S, Redman M, et al: Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays. PLoS Genet 4:e1000167, 2008

123. Zerhouni EA, Nabel EG: Protecting aggregate genomic data. Science 322:44, 2008

124. Gymrek M, McGuire AL, Golan D, et al: Identifying personal genomes by surname inference. Science 339:321-324, 2013

125. US Department of Health and Human Services: ANPRM for revision to Common Rule. http://www.hhs.gov/ohrp/humansubjects/anprm2011page.html

126. Kohane IS, Masys DR, Altman RB: The incidentalome: A threat to genomic medicine. JAMA 296:212-215, 2006

127. Ashley EA, Butte AJ, Wheeler MT, et al: Clinical assessment incorporating a personal genome. Lancet 375:1525-1535, 2010

128. Damani SB, Topol EJ: Emerging genomic applications in coronary artery disease. JACC Cardiovasc Interv 4:473-482, 2011

129. Biskup S, Gasser T: Genetic testing in neurological diseases. J Neurol 259:1249-1254, 2012

130. Green RC, Berg JS, Berry GT, et al: Exploring concordance and discordance for return of incidental findings from clinical sequencing. Genet Med 14: 405-410, 2012

131. Bombard Y, Offit K, Robson ME: Risks to relatives in genomic research: A duty to warn? Am J Bioeth 12:12-14, 2012

132. ACMG Board of Directors: Points to consider in the clinical application of genomic sequencing. Genet Med 14:759-761, 2012

133. Green RC, Berg JS, Grody WW, et al: ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med 15:565-574, 2013

134. Allyse M, Michie M: Not-so-incidental findings: The ACMG recommendations on the reporting of incidental findings in clinical whole genome and whole exome sequencing. Trends Biotechnol 31: 439-441, 2013

135. Wolf SM, Annas GJ, Elias S: Point-counterpoint: Patient autonomy and incidental findings in clinical genomics. Science 340:1049-1050, 2013

136. McGuire AL, Joffe S, Koenig BA, et al: Point-counterpoint: Ethics and genomic incidental findings. Science 340:1047-1048, 2013

137. Lammens CR, Aaronson NK, Wagner A, et al: Genetic testing in Li-Fraumeni syndrome: Uptake and psychosocial consequences. J Clin Oncol 28: 3008-3014, 2010

138. Roychowdhury S, Iyer MK, Robinson DR, et al: Personalized oncology through integrative high-throughput sequencing: A pilot study. Sci Transl Med 3:111ra121, 2011

139. Berg JS, Khoury MJ, Evans JP: Deploying whole genome sequencing in clinical practice and public health: Meeting the challenge one bin at a time. Genet Med 13:499-504, 2011

140. National Center for Biotechnology Information: ClinVar database. http://www.ncbi.nlm.nih.gov/clinvar/

141. McBride CM, Bowen D, Brody LC, et al: Future health applications of genomics: Priorities for communication, behavioral, and social sciences research. Am J Prev Med 38:556-565, 2010

142. Mardis ER: The $1,000 genome, the $100,000 analysis? Genome Med 2:84, 2010

143. Offit K: Genomic profiles for disease risk: Predictive or premature? JAMA 299:1353-1355, 2008

144. Offit K: Personalized medicine: New genomics, old lessons. Hum Genet 130:3-14, 2011

Appendix

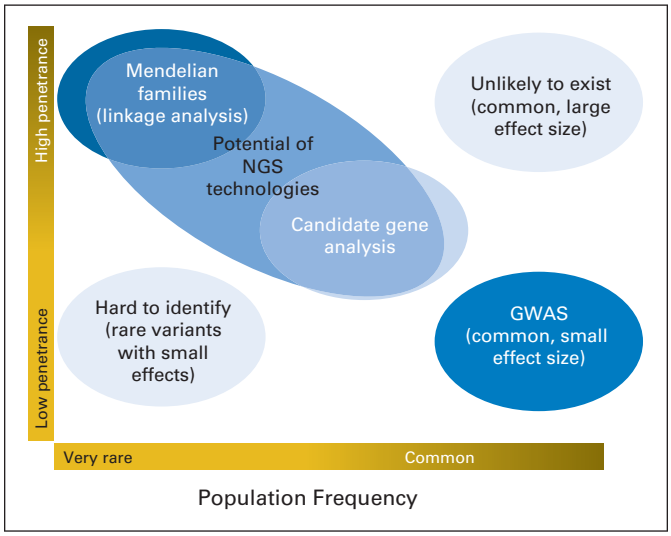

Fig A1. Gene discovery: the potential of next-generation sequencing (NGS) technology. GWAS, genome-wide association study.