

Cancer Genomics and Inherited Risk

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Published online ahead of print at www.jco.org on January 21, 2014.

Supported by the Robert and Kate Niehaus Clinical Cancer Genetics Initiative at Memorial Sloan-Kettering Cancer Center, The Romeo Milio Lynch Syndrome Foundation, the Breast Cancer Research Foundation, and the Sharon Levine Corzine Fund for Cancer Research. Z.K.S. is a Damon Runyon Cancer Research Foundation Clinical Investigator Award recipient.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/14/3207w-687w/\$20.00

DOI: 10.1200/JCO.2013.49.7271

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.

J Clin Oncol 32:687-698. © 2014 by American Society of Clinical Oncology

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 germ-

line 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*, *BRIPI*, *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

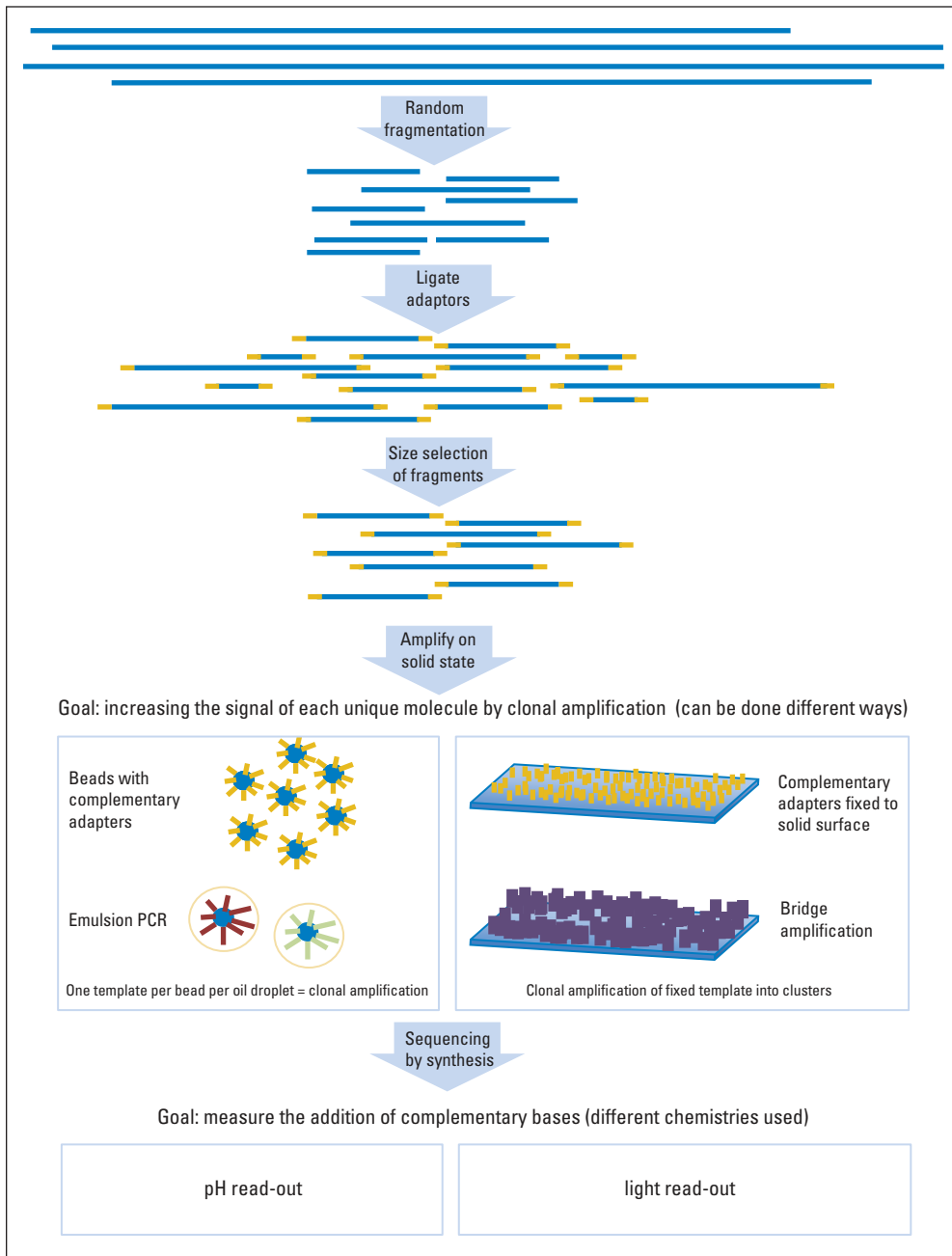


Fig 1. Principles of next-generation sequencing (NGS) technology. For NGS library preparation, DNA is randomly fragmented into desired size ranges. Adaptors containing the universal priming sites are ligated to the target ends of the fragments. After ligation, the template is immobilized to a solid support. 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*, *NFI*, 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 | <i>PALB2</i> | One affected with familial pancreatic cancer |
| Roberts et al ⁵⁹ | WGS and exome | <i>ATM</i> | WGS: 16 affecteds from six families; exome: 22 affecteds from 10 families |
| Familial pheochromocytoma | | | |
| Comino-Méndez et al ⁶³ | Exome | <i>MAX</i> | Three affecteds from three families |
| Hematologic malignancies | | | |
| AML with Emberger syndrome; Ostergaard et al ⁷³ | Exome | <i>GATA2</i> | Three unrelated affecteds (two with familial, one with sporadic) |
| Familial HL; Saarinen et al ⁷⁹ | Exome | <i>NPAT</i> | 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 | <i>PAX5</i> | Two families with exome sequencing of multiple affected and unaffected family members |
| Familial melanoma | | | |
| Yokoyama et al ⁸¹ | WGS | <i>MITF</i> | One affected with familial melanoma; also assessed in cases/controls |
| Horn et al ⁸³ | Targeted sequencing | <i>TERT</i> | Targeted sequencing of four affecteds and one unaffected in melanoma family |
| Familial mesothelioma, melanoma, and RCC | | | |
| Testa et al ⁹⁵ | Exome | <i>BAP1</i> | 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 | <i>GREM1</i> | Large Ashkenazi Jewish family with HMPS; additional HMPS families |
| Colorectal adenomas and colon cancer; Palles et al ⁹⁰ | WGS | <i>POLE, POLD1</i> | 20 affecteds (colorectal adenomas ± colorectal cancer) from 15 families |
| Familial breast cancer | | | |
| Park et al ⁸⁴ | Exome | <i>XRCC2</i> | Five affecteds from two families; also assessed in case/controls |
| Park et al ⁸⁵ | Exome | <i>FAN1*</i> | Four early-onset multiple-case breast cancer families |
| Ruark et al ⁸⁸ | Targeted sequencing | <i>PPM1D</i> (mosaic) | 1,150 with breast cancer ± ovarian cancer; replication in large case/control |
| Ovarian cancer | | | |
| Rafnar et al ⁸⁹ | WGS | <i>BRIP1</i> | 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

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

Table 4. Commercially Available Multiplex Gene Panels

| Panel | Genes Included |
|---|--|
| Breast cancer | |
| BreastNext; Ambray Genetics, Aliso Viejo, CA | <i>ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11, TP53</i> |
| Colorectal cancer | |
| ColoNext; Ambray Genetics | <i>APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53</i> |
| ColoSeq; University of Washington Laboratory Medicine, Seattle, WA | <i>APC, BMPR1A, CDH1, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53</i> |
| Mayo Medical Laboratories; Rochester, MN | <i>APC, AXIN2, BMPR1A, CDH1, CHEK2, EPCAM, GREM1, MLH1, MLH3, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53</i> |
| Ovarian cancer | |
| OvaNext; Ambray Genetics | <i>ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, STK11, TP53</i> |
| Multicancer panels | |
| CancerNext; Ambray Genetics | <i>APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, SMAD4, STK11, TP53</i> |
| BROCA Cancer Risk Panel; University of Washington Laboratory Medicine | <i>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</i> |
| Myriad myRisk; Myriad Genetics, Salt Lake City, UT* | <i>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</i> |

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.

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

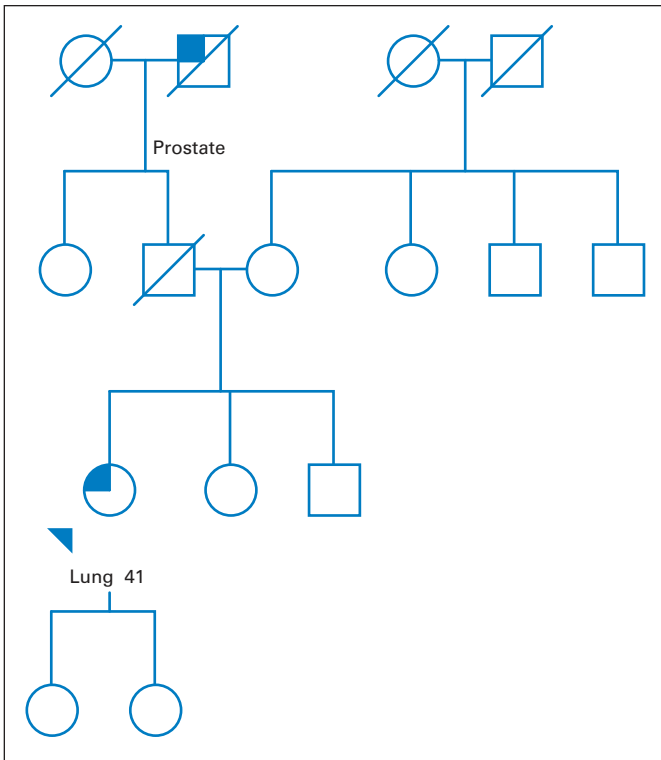


Fig 2. Pedigree case 1.

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

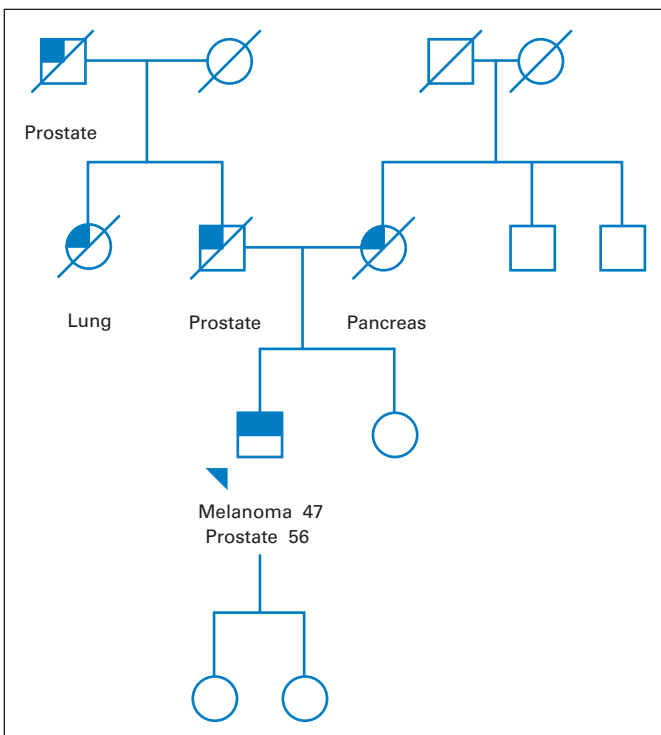


Fig 3. Pedigree case 2.

(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.

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

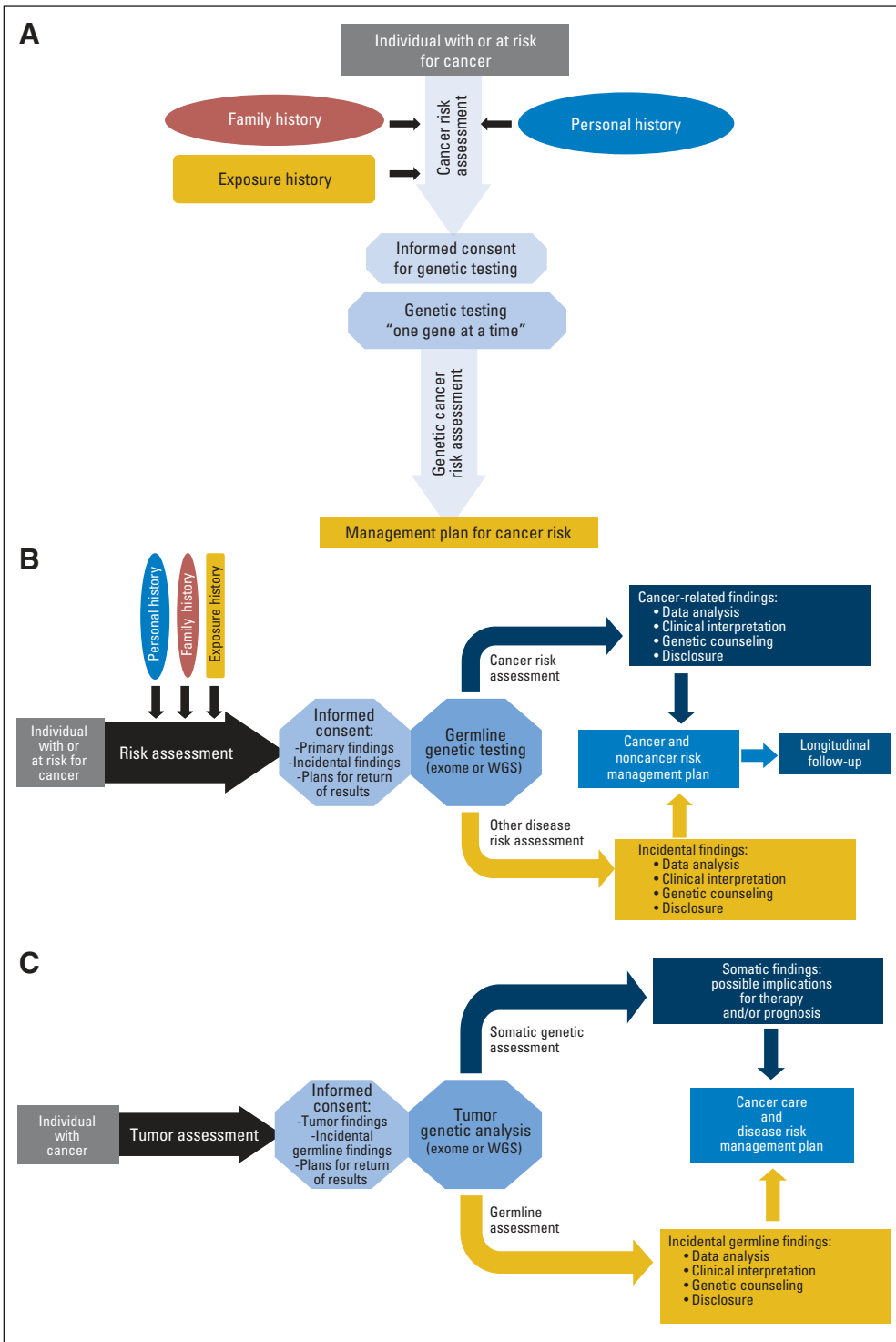


Fig 4. Challenging the traditional model of cancer genetic counseling. (A) Traditional model of clinical cancer genetics. (B) Incorporating next-generation sequencing (NGS) into genetic cancer risk assessment. (C) NGS of tumors with incorporation of incidental germline findings. WGS, whole-genome sequencing.

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

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Data analysis and interpretation: All authors

Manuscript writing: All authors

Final approval of manuscript: All authors

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Appendix

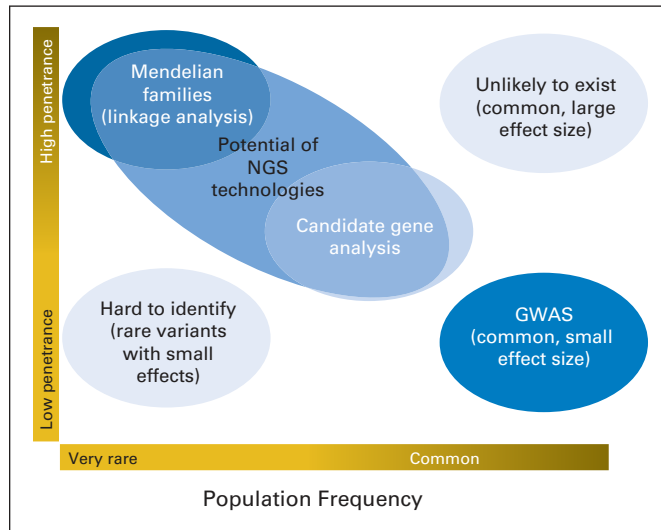


Fig A1. Gene discovery: the potential of next-generation sequencing (NGS) technology. GWAS, genome-wide association study.