Recent Results in Cancer Research
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Rare Hereditary Cancers

Diagnosis and Management

Indexed in PubMed/Medline

Springer

Recent Results in Cancer Research

Volume 205

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ISSN 0080-0015 ISSN 2197-6767 (electronic)
Recent Results in Cancer Research
ISBN 978-3-319-29996-9 ISBN 978-3-319-29998-3 (eBook)
DOI 10.1007/978-3-319-29998-3

Library of Congress Control Number: 2016931320

Printed on acid-free paper

This Springer imprint is published by SpringerNature
The registered company is Springer International Publishing AG Switzerland

Preface

Alongside rapid advances in scientific understanding about cancer genomics, there have been huge steps forward in genetic testing for pathogenic mutations in cancer predisposing genes, as well as the management of cancer risks associated with these mutations.

Until recently, families with a history of cancer suggesting high risk cancer predisposition genes as their cause have been counselled and managed within specialised genetic services. As the number of individuals eligible for cancer predisposition testing is rapidly increasing and more management options and treatments tailored to pathways disrupted by mutated cancer predisposition genes are developed, oncologists, surgeons and other healthcare specialists treating these patients have to become more involved in genetic testing and managing cancer risks in their patients.

Much has been written about the diagnosis and management of patients with common hereditary cancer such as breast/ovarian and colorectal cancer syndromes. However, there is limited information available to health professionals who diagnose and manage rare hereditary cancer syndromes, some of which present in childhood.

This book approaches the issue of the differential diagnosis and management of rare hereditary cancer syndromes from a practical angle, addressing the issues for each tumour type as seen by health professionals in their day-to-day practice.

The first chapter aims to update cancer specialists on the newest developments in genetic testing technology. It describes the strengths, limitations and caveats of these technologies to enable cancer specialists to use these tests safely and effectively for the benefit of their patients.

The subsequent chapters describe how patients with specific rare hereditary cancer syndromes may be identified through their personal and family history of cancer, which genes should be tested based on these criteria, the clinical picture of the respective cancer syndromes caused by mutations in these genes, as well as the appropriate management options.

The final chapter deals with the wider issues involved in genetic counselling and testing for cancer susceptibility for patients, families and health professionals.

In summary, this book has been written by leading specialists in the field to enable health professionals to correctly identify patients with these rare syndromes who will benefit from genetic counselling and testing and to provide them with the knowledge to manage patients and advise family members who may be at risk of an inherited cancer predisposition.

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Abbreviations

^{123}I-MIBG	Metaiodobenzyleguanindine
ACCs	Adrenocortical carcinomas
ACT	Adrenocortical tumours
AD	Autosomal dominant
AF	Aggressive fibromatosis
APC	Adenomatous polyposis coli
Array-CGH	Comparative genome hybridisation
ATM	Ataxia telangiectasia
BAP1	BRCA1 associated protein 1
BCC	Basal cell carcinoma
BHD	Birt–Hogg–Dubé syndrome
BRRS	Bannayan–Riley–Ruvalcaba syndrome
BWS	Beckwith–Wiedemann syndrome
(CAPS) consortium	Cancer of the pancreas screening
CCH	C-cell hyperplasia
ccRCC	Clear cell renal cell cancer
ccRCC	Renal cell carcinomas
CDKN1C	Cyclin-dependent kinase inhibitor 1C
CDKNIK1	Cyclin-dependent kinase inhibitor 1C
CEA	Carcinoembryonic antigen
CHRPE	Congenital hypertrophy of the retinal pigment epithelium
CLA	Cutaneous lichen amyloidosis
CNC	Carney complex
CRC	Colorectal cancer
CT	Calcitonin
CT	Computerised tomography
CTNNB1	Catenin-beta 1
DBC	Invasive ductal cancer
DGC	Diffuse gastric cancer
dHPLC	High performance liquid chromatography
DTC	Differentiated thyroid carcinoma
EB	Epidermolysis bullosa
EC	Endomentrial cancer
EGF	Epidermal growth factor
ENCODE	The Encyclopedia of DNA Elements

EUS	Endoscopic ultrasound
FAMMM	Familial atypical multiple mole melanoma
FAP	Familial adenomatous polyposis
FDA	United States Food and Drug Administration
FDG-PET/CT	^{18}Fluorodeoxyglucose-positron emission tomography/computed tomography
FDR	First degree relative
FFPE	Formalin fixed paraffin embedded
FH	Fumarate hydratase
FIHP	Familial isolated hyperparathyroidism
FISH	Fluorescence in situ hybridisation
FLCN	Folliculin
FMTC	Familial medullary thyroid carcinoma
FNA	Fine needle aspiration
FNMTC	Familial non-medullary thyroid cancer
FPC	Familial pancreatic cancer
FPTC	Familial papillary thyroid cancer
FRCC	Familial non-syndromic renal cell cancer
FTC	Follicular thyroid cancer
GC	Gastric cancer
GEP	Gastroenteropancreatic
GIST	Gastrointestinal stromal tumours
GWAS	Genome-wide association studies
H&E	Haematoxylin and eosin
HBOC	Hereditary breast ovarian cancer
hCG	Beta-human chorionic gonadotropin
HDGC	Hereditary diffuse gastric cancer
HIF	Hypoxia inducible factor
HNPCC	Hereditary non-polyposis colorectal cancer
HP	Helicobacter pylori
HPT-JT	Hyperparathyroidism-jaw tumour syndrome
IC1	Imprinting centre 1
IC2	Imprinting centre 2
ICGC	International Cancer Genome Consortium
IGF2	Insulin growth factor-2
IM	Intestinal metaplasia
IPMK	Inositol polyphosphate multikinase
IPMN	Intraductal papillary mucinous neoplasm
JPS	Juvenile-polyposis syndrome
KCOT	Keratocystic odontogenic tumours
LAM	Lyphangioleiomyomatosis
LBC	Lobular breast cancer
LCCSCT	Large cell calcifying Sertoli cell tumours
LFLS	Li-Fraumeni-like-syndrome
LFS	Li Fraumeni syndrome

LOH	Loss of heterozygosity
LT4	Levothyroxine
MAP	MUTYH-associated polyposis
MCR1	Melanocortin receptor1
MDT	Multidisciplinary team
MEN1	Multiple endocrine neoplasia 1
MEN2/MENII	Multiple endocrine neoplasia 2
MEN2a/IIA	Multiple endocrine neoplasia type 2a
MEN2B/IIB	Multiple endocrine neoplasia type 2b
MEN4	Multiple endocrine neoplasia type 4
MET	Mesenchymal epithelial transition factor
MMR	Mismatch repair
MPLA	Multiplex ligation-dependent probe amplification
MPNST	Malignant transformation of peripheral nerve sheath tumours
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance Imaging
MS	Methylation-specific
MTC	Medullary thyroid carcinoma
mTOR	mammalian target of rapamycin
MTTs	Molecular targeted therapeutics
NANETS	North American Neuroendocrine Tumor Society
NBI	Narrow band imaging
NBS1	Nijmegen breakage syndrome
NCCN	National Comprehensive Cancer Network
NCR	Netherlands Cancer Registry
NET	Neuroendocrine tumour
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type II
NFPTR	National Familial Pancreatic Tumor Registry
NGF	Nerve growth factor
NGS	Next generation sequencing
NIH	National Institutes of Health
NMTC	Non-medullary thyroid cancer
OCA	Oculocutaneous albinism
OGD	Oesophago-gastroduodenoscopy
OPG	Orthopantomogram
OR	Odds ratio
PALLD	Palladin
PanIN	Pancreatic intraepithelial neoplasia
PARP	Poly ADP ribose polymerase
PAS	Periodic acid-Schiff
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PDT	Photodynamic therapy
PEComas	Perivascular epithelioid cell sarcomas

PG	Paraganglioma
PHTS	PTEN hamartoma tumour syndrome
PJS	Peutz–Jeghers syndrome
PKA	Protein kinase A
PPNAD	Primary pigmented nodular adrenocortical hyperplasia
PRKAR1A	Protein kinase regulatory subunit type 1 alpha gene
PTC	Papillary thyroid cancer
RB1	Retinoblastoma gene
RCC	Renal cell cancer
SCC	Squamous cell carcinomas
SDH	Succinate dehydrogenase
SDHAF2	Succinate dehydrogenase assembly factor 2
SDHB	SDH subunit B
SEER	Surveillance, Epidemiology and End Results Program
SI NET	Small intestinal NET
SMO	Smoothened
SNP	SNP single nucleotide polymorphism
SUFU	Suppressor of fused
TCA	Tricarboxylic acid
TCF	T-cell factor
TCO	Thyroid cancer with oxyphilia
Tg	Thyroglobulin
TGCA	The Cancer Genome Atlas Research
TSH	Thyroid stimulating hormone
TSC	Tuberous sclerosis complex
TYR	Tyrosinase gene
UDP	Uniparental disomy
USS	Ultrasound scan
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
VHL	Von Hippel–Lindau
VHL	Von Hippel–Lindau disease
VHL	Von Hippel–Lindau syndrome
VS	Vestibular schwannoma
VUS	Variant of uncertain significance
VUS/VUCS	Variant of unknown clinical significance
WBS	Whole body scan
WDTC	Well-differentiated thyroid cancer
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
XP	Xerodermapigmentosum

Advances in Genetic Testing for Hereditary Cancer Syndromes

Ellen Thomas and Shehla Mohammed

Abstract

The ability to identify genetic mutations causing an increased risk of cancer represents the first widespread example of personalised medicine, in which genetic information is used to inform patients of their cancer risks and direct an appropriate strategy to minimise those risks. Increasingly, an understanding of the genetic basis of many cancers also facilitates selection of the most effective therapeutic options. The technology underlying genetic testing has been revolutionised in the years since the completion of the Human Genome Project in 2001. This has advanced knowledge of the genetic factors underlying familial cancer risk, and has also improved genetic testing capacity allowing a larger number of patients to be tested for a constitutional cancer predisposition. To use these tests safely and effectively, they must be assessed for their ability to provide accurate and useful results, and be requested and interpreted by health professionals with an understanding of their strengths and limitations. Genetic testing is increasing in its scope and ambition with each year that passes, requiring a greater proportion of the healthcare workforce to acquire a working knowledge of genetics and genetic testing to manage their patients safely and sensitively.

Keywords

Genetic testing · Molecular diagnosis of inherited cancer · Diagnostic use of next-generation sequencing

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G. Pichert and C. Jacobs (eds.), *Rare Hereditary Cancers*,
Recent Results in Cancer Research 205, DOI 10.1007/978-3-319-29998-3_1

Contents

1 Introduction

The genetics of cancer has been the focus of a huge research effort for several decades. This can be divided into two main areas: firstly, the study of how genetic changes within a particular organ arise and accumulate, causing the development of an individual tumour; and secondly, the search for inherited genetic changes which increase a person's chance of developing cancer. The first category, known as 'somatic' genetic changes, occurs only in tumour cells and the tissue they developed from, while the second category, known as 'germline' or 'constitutional' genetic variants, is present in every cell in the body, including the germ cells (eggs and sperm) which pass on DNA to the next generation.

Cancers are initiated and driven by changes in a cell's DNA which cause it to divide uncontrollably, and to this extent, all cancers are genetic diseases. However, the majority of cancers are caused by a combination of lifestyle, environmental and stochastic (chance) influences with only a minor contribution from constitutional inherited genetic variation.

A significant minority of cancers (a variable proportion depending on the cancer type) are caused more directly by a rare single mutation, which is usually inherited in an autosomal dominant way. Diagnostic genetic testing can identify such mutations in individuals with a personal and family history of cancer. These tests must examine the entire sequence of the relevant gene(s) looking for the single mutation which could be causing the family's cancers. In some cases, even when there is a high suspicion of an inherited predisposition to cancer, no genetic cause is found, and the reasons for this will be discussed later in this chapter. Diagnostic tests can be carried out in individuals with a family history but no personal history of cancer. However, a negative test result in this situation is **uninformative and of limited value**, as it is not possible to tell whether there is a mutation in a known cancer gene in the family which has not been inherited by the individual tested, or whether there is no mutation in a known gene and the tested individual could still be at risk from an unidentified gene mutation.

Once a cancer-predisposing mutation has been identified in a patient with cancer, their relatives can be offered predictive testing to find out whether they have inherited the mutation and may be at increased risk of developing cancer in the future. This is a highly accurate test, because only the single genetic variant identified in the family needs to be tested. In general, predictive tests are cheaper and quicker than diagnostic tests, although their health implications are significant and appropriate counselling is always required. Individuals in these families who have inherited the mutation may have a very high risk of developing cancer—up to 100 % in some cases such as classical familial adenomatous polyposis. In addition, the cancers are likely to occur at a younger age than sporadic non-familial cancers, and may be of particular histological subtypes. A test showing that an individual has not inherited the familial mutation removes any increased risk for that individual related to their family history, unless they have a family history of cancers which cannot be accounted for by the familial mutation, for example if relatives of their unaffected parent have also had significant cancers. These individuals can be reassured, and additional surveillance for that cancer is not required following this test result.

Individuals who have a positive predictive testing result will be offered a range of strategies to try to reduce their future cancer risks. Demand for genetic testing is therefore increasing, from patients and healthcare professionals, and advances in genetic testing technology described in this chapter have been introduced into clinical practice with the aim of making access to genetic testing broader and more equitable.

In between sporadic and inherited cancers are another loosely defined group where the patient has a family history which is likely to be relevant to their own cancer, but no mutation is detectable in a known gene. These families are likely to have one or several variants which are contributing to an increased cancer risk, but the level of risk is lower than with the inherited cancer gene faults. These families may be offered some additional surveillance, but genetic testing is usually not contributory or informative in this situation. However, this may change as our understanding of the whole spectrum of constitutional genetic predisposition to cancer improves with further large-scale genetic research projects.

2 Advances in Genetic Testing Technology

Traditionally, genetic testing for cancer predisposition genes has used capillary sequencing (also known as Sanger sequencing), which is a highly accurate but labour-intensive and expensive way of working through each individual exon of the gene of interest, requiring a large DNA specimen. Genetic testing has therefore been limited by cost and throughput to individuals with a clinical picture indicating a high likelihood of a cancer-predisposing gene mutation (Table 1).

Table 1 Comparison of tests used to make genetic diagnoses

Test	Use	Strengths	Limitations
Capillary (Sanger) sequencing	Sequencing of small genomic regions, e.g. individual exons	Highly accurate	Low throughput, labour intensive, expensive
Panel testing using next-generation sequencing	Simultaneous sequencing of genes causing a particular phenotype (up to several hundred genes)	Allows multipanel gene testing Useful in heterogeneous conditions	Needs adjusting when new genes are discovered, and coverage of each gene may not be as good as capillary sequencing
Array CGH	Detection of large structural chromosome rearrangements	Highly accurate, high throughput	
Exome sequencing	Simultaneous sequencing of all coding regions of the genome	Streamlines lab workflow and useful extension of the panel test	Coverage of some genes is inadequate, no information on structural rearrangements
Genome sequencing	Sequencing of the whole genome	More even coverage of all genes	Expensive, data storage and analysis costs are high, and non-coding regions hard to interpret

In the last 15 years, rapid advances have been made in genetic and genomic research and technology development, initially driven by the Human Genome Project which was completed in the year 2000 (Lander et al. 2001). The HapMap project then identified sites of common variation in different human populations (The International HapMap Consortium 2005), which led to the development of high-throughput accurate genotyping platforms.

This work laid the foundations for genome-wide association studies (GWAS), a large-scale population-based case-control study design exploiting linkage disequilibrium between ancient common variants to compare allele and haplotype frequencies in large cohorts of patient and control subjects. The GWAS design is based on the 'common disease–common variant' hypothesis that multiple small genetic effects combine to predispose individuals to complex diseases. Several thousand loci have now been reliably identified as contributing to a large range of common diseases and other phenotypes by this method, and this has provided insights into novel disease pathways and mechanisms (Hirschhorn and Gajdos 2011). However, in only a minority of cases has the precise gene or variant giving rise to the association signal been identified and its mechanism of action has been established, and the odds ratios for disease development associated with each individual variant identified on GWAS tend to be in the region of 1.1–1.5, indicating that their effect on disease risk in any individual person is small. Even when an individual's genotype at multiple risk single nucleotide polymorphisms (SNPs) is taken into account, these results only account for a small amount of the variation

in cancer risk between individuals. The GWAS effort has contributed to our understanding of the molecular processes and pathways underlying many diseases, and it is hoped that this will be translated into therapeutic advances. However, common SNP genotype tests have not been adopted as clinical tools due to their limited clinical utility, and therefore, the original hope that GWAS would lead to the use of SNP genotyping to stratify risk and deliver personalised medicine has not been realised.

In parallel with the technology used for large-scale SNP genotyping, similar protocols were developed to study larger changes in the genome known as structural variation (deletions—missing regions of the genome; duplications—extra copies of regions of the genome; and inversions—sections of the genome which have become rotated). It used to be thought that the overall structure of the healthy human genome was relatively invariant, because large genome rearrangements visible down the microscope were nearly all associated with significant medical and developmental difficulties (in a constitutional form) or were found as somatic changes in tumour cell genomes. However, once microarray techniques such as comparative genome hybridisation (known as array CGH) were developed to study copy number variation in more detail, it was discovered that smaller scale structural changes are often well tolerated and may not lead to any detectable phenotype. Array-CGH results have also led to the understanding that a significant minority of monogenic disease is caused by a structural variant affecting an important gene, and some families whose condition remained unexplained by DNA sequencing have a whole gene deletion; for example, deletions of the *APC* gene cause classic familial adenomatous polyposis.

The most recent major advance in genetic technology has been the exponential increase in sequencing capacity brought about in the last decade by the high-throughput platforms developed by Illumina (Bentley et al. 2008), Roche 454 (Margulies et al. 2005), ABI SOLiD (McKernan et al. 2009), and Complete Genomics (Peters et al. 2012). This has been made possible by the use of massively parallel sequencing, which uses simultaneous amplification of hundreds of millions of individual DNA fragments, which are imaged after each sequencing cycle to determine the order of nucleotides in each separate fragment simultaneously. Having taken several decades to generate the first draft of the human genome sequence in the years leading up to the millennium, in 2015 an entire individual human genome takes around a week to sequence, at a basic test cost not greatly exceeding $1000.

High-throughput sequencing generates huge volumes of data which require specialist computer hardware, software and informatics expertise to analyse. Bioinformaticians have developed many informatic techniques to map millions of sequence reads varying in length from 35 base pairs to around 700 base pairs to the genome, and to identify SNPs and structural rearrangements from the aligned reads (known as variant calling). Extensive testing of these algorithms has established the best parameters to maximise sensitivity of variant detection and minimise false positive variant calls. More recently, the increased body of experience in analysis of

high-throughput sequence data has allowed these analysis pipelines to become more standardised and automatable.

Following the use of high-throughput sequencing for whole-genome sequencing, technologies for sequencing selected parts of the genome have been developed. These include automated multiplex polymerase chain reaction (PCR) systems, where multiple individual targets are amplified using the traditional PCR technique but at much higher throughput. The more widely adopted mechanism uses target enrichment either of selected custom DNA targets such as a panel of genes known to cause a particular condition, or generic targets such as the entire coding sequence of the genome, known as the exome. This works by shearing DNA from the whole genome into small pieces, then capturing the fragments covering the genome regions of interest, before washing off the unwanted fragments, and sequencing the enriched library of targeted sequences.

Using a targeted approach known as a 'panel test', sequence data can be generated in one test for anything from a handful of genes up to several hundred genes. For example, the Lynch syndrome genes can be tested all together in a clinically available panel of nine bowel cancer genes, which is quicker and cheaper than sequencing each gene individually one after the other, and may avoid the need for immunohistochemistry to direct where to start with single gene testing. Panel testing has been introduced fairly widely to clinical practice, particularly in the diagnosis of heterogeneous conditions, where mutations in a number of different genes cause the same phenotype.

Exome sequencing has been used with great success to identify the genes responsible for dozens of monogenic disorders since the first publication (Ng et al. 2009). More recently, exome sequencing has also been used as an extension of panel testing in the clinical diagnostic context. The challenge with panel testing is that new genes causing each heterogeneous phenotype are discovered each year, and adding new genes to an existing panel test involves a lengthy and expensive process of adaptation and revalidation of the test. As sequencing costs have fallen, it has been suggested that it is more cost-effective to carry out exome sequencing by a standardised protocol on every sample, and then select the relevant genes for analysis. This provides a very flexible approach, where 'virtual panels' for analysis can be changed swiftly in response to new gene discoveries, and data can be revisited retrospectively without repeating the laboratory element of the assay.

The large volume of sequence data which has been generated in the last decade has highlighted the extent of variation found in every individual genome. Every exome or genome sequence identifies many thousands of variants, the majority of which have no relevance to the phenotype in question, and the process of prioritising and filtering these variants is one of the greatest challenges currently facing geneticists. Individual variants are commonly categorised using a five-point system, as described in Box 2.

Assessing variant pathogenicity to place each variant into the categories given above is a highly technical and time-consuming process which needs to be done by experienced molecular geneticists, and which is not yet amenable to a high degree of automation. This therefore represents the most significant bottleneck in the

high-throughput molecular diagnostic context at this time. A number of factors and techniques are commonly used to assess the pathogenicity of a variant; these are discussed in Box 3.

In silico prediction tools such as Polyphen (Adzhubei et al. 2010), SIFT (Kumar et al. 2009) and Condel (González-Pérez and López-Bigas 2011) can be used as screening tools for large data sets. These use a combination of information on amino acid structures, known protein structures and evolutionary conservation to provide a quick and simple way of testing large batches of variants, but their sensitivity and specificity are low. In future, it is likely to become possible to prioritise non-coding variants using *in silico* tools as well, using data from projects such as ENCODE (the Encyclopedia of DNA Elements), which aims to catalogue functional and regulatory elements in the human genome (The ENCODE Project Consortium 2011). At present, these tools can be used to give a consensus suggestion about a particular variant, but they cannot be relied upon as a mainstay of clinical diagnostic variant interpretation.

Whole-genome sequencing is also being investigated now as a clinical diagnostic tool, chiefly within the 100,000 Genomes Project in the UK, which is generating whole-genome sequences in thousands of patients with rare disease or cancer in the NHS. Whole-genome sequencing is more expensive and generates volumes of data which are difficult to store, but has a number of potential advantages over exome sequencing. Firstly, an unknown proportion of disease-causing variants may lie outside coding regions, either in introns affecting gene splicing, or in promoter or enhancer regions affecting gene expression, and these variants will always be missed by exome sequencing. Secondly, exome sequencing requires a step in the DNA preparation where the coding regions of DNA are captured for sequencing. Some genomic regions do not pull down well or are hard to map back to the genome, due to repetitive DNA sequences or variations in the ratio of AT: GC nucleotides. Some genes are therefore consistently difficult to capture with exome sequencing, but genome sequencing does not involve this capture step and therefore covers these difficult regions more completely. Thirdly, genome sequence data allows structural variations (deletions, duplications, inversions) to be detected reliably as well as small sequence variation, so it is possible that a genome sequence will mean that array CGH will not be needed as a separate test.

3 Translation of Research Findings into Clinically Useful Genetic Tests

As described above, the technological aspects of genetic testing have improved rapidly over the last decade. These advances have been driven by the requirements of research, often by extensive multinational collaborations such as the Human Genome Project and the many international GWAS consortia. Following closely behind these developments have been efforts to translate the technological advances into clinical practice, to provide immediate clinical benefit for patients. However,

the requirements of genetic testing in the clinical context are different from the research context, as described in Table 2.

The ACCE framework (shown in Fig. 1) is a highly influential approach which has been designed to evaluate whether a test is appropriate to be used in clinical practice (Haddow and Palomaki 2003). This comprises a detailed assessment of the following:

Table 2 Comparison between research and clinical priorities

Test characteristic	Research priority	Clinical test priority
Accuracy	Global accuracy across the project is important	Individual accuracy for a clinical report for each patient is crucial
Throughput	Often needs to be very high	Healthcare system may not be able to afford high throughput
Cost	Moderate pressure to lower costs	High pressure to lower costs due to the requirement for cost-effectiveness evidence before implementation
Completeness	Some missing data will not significantly compromise the results	Missing data for an individual patient is a big problem
Time and labour required to perform test	High priority to minimise these, but no absolute deadline for results	Reliable turnaround time needed for clinical tests, including complex results

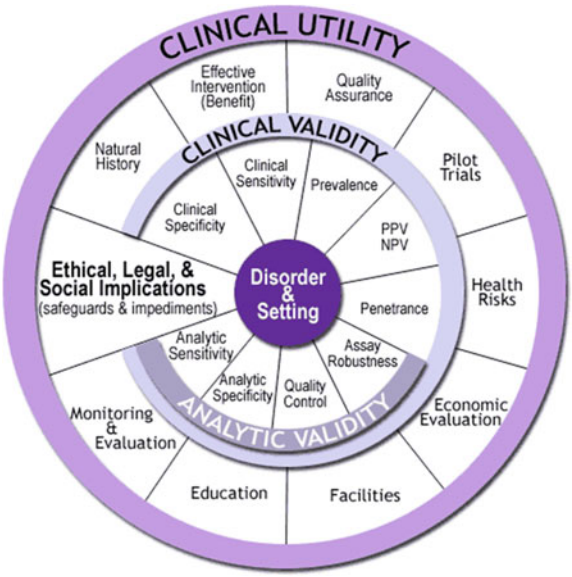

Fig. 1 The ACCE framework to assess the suitability of a genetic test for use in clinical practice, reproduced with permission from Haddow and Palomaki (2003)

- **A**nalytical validity,
- **C**linical validity,
- **C**linical utility and
- **E**thical, legal and social implications of the test.

Analytical validity refers to the performance of the test in accurately identifying DNA sequence variation in the gene(s) of interest and measures the aspects of the test which occur in the clinical laboratory. Ensuring and demonstrating analytical validity for a new technology requires labour-intensive validation, improving the reliability and completeness of the test, testing samples with known mutations to compare the test with current gold standard tests and finally piloting the test on prospective clinical samples.

Clinical validity is a measure of the ability of the test to predict the disease or phenotype in question. For example, many of the SNPs identified in genome-wide association studies are readily measurable in the laboratory, but it would not be appropriate to measure these as clinical assays because the increased risk of cancer associated with each of these SNPs is so low that knowing an individual's genotype has no value in predicting their chance of developing cancer or tailoring their treatment accordingly. Establishing clinical validity requires epidemiological data on the clinical sensitivity and specificity of the test in a particular population, and on the penetrance of the mutation; these data need to have been generated in the research context before the test can be adopted for clinical use.

Clinical utility defines whether carrying out the test will lead to an improved outcome for the patient receiving the test. This will depend on an accurate prediction of the cancer risks caused by a particular mutation and the availability and effectiveness of surveillance and cancer risk reducing measures, and also on less tangible benefits such as the relief which some patients experience from understanding the cause of their personal and family history of cancer.

The ethical, legal and social implications of genetic tests also need to be considered. In addition, genetic tests can be expensive due to their complexity, and cost-effectiveness analyses are therefore required to determine which tests to use in which groups of patients to maximise the health benefit from these technologies.

4 Interpretation of Genetic Test Reports

4.1 Variants of Unknown Clinical Significance (VUS)

The advances in genetic testing described here are leading to ever-increasing numbers of patients receiving a genetic diagnosis confirming a constitutional predisposition to cancer running in their family. This enables patients and family members to appreciate their risk of developing cancer in the future, and helps clinicians to focus screening and prevention strategies on those at highest risk, who stand to benefit the most from available interventions. Test results in cancer

genetics must be accurate, robust and correctly interpreted to achieve these benefits. For example, if a variant is incorrectly designated as being the cause of a patient's cancer, relatives may undergo predictive testing which does not accurately reflect their future risk. This may lead to individuals being incorrectly informed that they are at high risk and using this information to access prophylactic surgery or inform reproductive decisions; it may also lead to inappropriate reassurance and removal of screening from individuals at high risk who go on to develop cancer.

In order to avoid these serious errors, the burden of proof required to designate a variant as pathogenic for diagnostic purposes is high. VUS results (see Box 2) are not used for diagnostic, predictive or reproductive purposes, and the family is managed as if no genetic diagnosis has been identified. As evidence accumulates, VUS can sometimes be reclassified as pathogenic or benign, and laboratories will revisit reports to assess this if requested.

4.2 Additional Unsought Genetic Findings

Traditional testing techniques only allowed one gene to be tested at a time, and therefore, genes were only tested in individuals with an associated phenotype predicted to have a high chance of being caused by a mutation in that gene. With the widening of testing to examine many genes simultaneously, a greater focus is needed on the relationship between mutations in a particular gene and the medical consequences of that mutation, in the context of an individual's lifestyle and environment (known as the phenotype). For example, a patient with bowel cancer who undergoes testing using the bowel cancer gene panel might be found to have a mutation in one of the Lynch syndrome genes, which would have a high likelihood of being pathogenic subject to the pathogenicity measures described above. However, if a mutation was found in the gene for Peutz-Jeghers syndrome (PJS), which is also on the panel because bowel cancer is part of this condition, the patient would need to be examined for the other clinical features of PJS, such as peri-oral pigmentation. If these features were found, the genotype and phenotype could be confirmed to match and the diagnosis would be clear.

If on the other hand a patient has a mutation in a gene for which they exhibit few or none of the classic clinical features, there are several possibilities which need to be distinguished:

1. The patient has a condition which is not the classic presentation of mutations in that particular gene, but the gene may be responsible for a more attenuated form of the phenotype and the result may therefore be relevant to the patient's presentation. This type of result occurs quite frequently, and our understanding of the spectrum of phenotypes which can be associated with mutations in each gene is increasing as a result.
2. The genetic variant is unrelated to the patient's presenting phenotype and is unlikely to be of medical relevance.