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Molecular Diagnosis for Hereditary Cancer Predisposing Syndromes: Genetic Testing and Clinical Impact

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MOLECULAR DIAGNOSIS FOR HEREDITARY CANCER PREDISPOSING SYNDROMES: GENETIC TESTING AND CLINICAL IMPACT

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No conflict of interests was declared by any of the authors or reviewers.

Molecular Diagnosis for Hereditary Cancer Predisposing Syndromes

Technology Name

Genetic tests for hereditary cancer predisposing syndromes

Disease/Condition

Individuals with hereditary cancer predisposing syndromes are at an increased risk of developing cancer. These syndromes can be diagnosed clinically, but in many instances, genetic tests must be conducted to make or refine the diagnosis.

Technology Description

Genetic tests, which can be used for diagnosis or screening, are required to detect a genetic alteration in an affected person. Diagnostic molecular genetic testing comprises the spectrum of modern molecular biological techniques.

The Issue

The overall effectiveness of using genetic tests to predict the likelihood of cancer is to be determined, given their limitations. The ability to detect a genetic alteration depends on many factors, including the gene, the nature of the mutation and the sensitivity and specificity of the test. The predictive power of genetic testing is also influenced by the complex interaction between genetic predisposition and environmental influences. There are unique social, ethical and legal issues related to genetic testing.

Assessment Objectives

- To determine the analytical and clinical validity, availability and cost of genetic tests for the screening and diagnosis of hereditary cancer predisposing syndromes.
- To document the impact of genetic testing on the clinical management of patients with hereditary cancer predisposing syndromes.

Methods

A systematic review was conducted using a literature search strategy. Based on predetermined selection criteria for included studies, two independent reviewers identified 457 relevant articles. The molecular genetic tests available and their impact on clinical management were investigated for 20 syndromes. A list was compiled of molecular techniques used to detect the most common hereditary cancer predisposing syndromes, their analytical and clinical sensitivity, their cost and their availability. Genetic testing services and laboratories for hereditary cancers in Canada were also listed.

Conclusions

- Genetic testing for many types of hereditary cancers is far from satisfactory because of the relatively high costs of genetic tests; their variable analytical and clinical validity; and their limited availability.
- Given the rapid development of new molecular techniques and the demand for access, implementing genetic tests into clinical management is justified for some disorders.

EXECUTIVE SUMMARY

The Issue

Hereditary cancers have a significant impact on public health, since up to 10% of the cancer burden is estimated to be the result of inherited genetic mutations. The defects associated with inherited cancer syndromes include germline mutations of oncogenes, tumour suppressor genes and DNA repair genes specific for different types of cancer. The molecular diagnosis of genetic diseases raises the possibility of genetic testing to diagnose patients affected with cancer and to predict the risk of cancer for unaffected relatives. This is accompanied, however, by unique social, ethical and legal issues. The size and complexity of some cancer-associated genes make mutation detection difficult, leading to technical challenges. The ability to predict the likelihood of cancer depends on the gene and the mutation identified. The interpretation and utilization of genetic test results can therefore present a problem. The complex interplay between genetic predisposition and environmental influences also limits the predictive power of genetic testing. The usefulness of molecular diagnosis and screening is based on the test's validity and its impact on clinical management. The rapid development of new molecular techniques for mutation detection, an increasing interest in genetic tests and the expanded use of testing will affect both population health and health care costs.

Objectives

Our objectives were to perform a systematic literature review of the evidence regarding the availability, cost and analytical and clinical validity of genetic tests for screening and diagnosis of hereditary cancer predisposing syndromes; and to document the impact of genetic testing on the clinical management of patients with specific hereditary cancer predisposing syndromes.

Results

Published and unpublished literature was obtained by searching electronic databases using a defined strategy, by hand searching bibliographies of selected papers and by contacting genetic test laboratories. Two reviewers independently selected 457 relevant articles.

A chart listing the 20 most common types of hereditary cancer predisposing syndromes; their prevalence, penetrance and the associated cancer risk; and the molecular techniques used to detect mutations was prepared. The molecular diagnosis and the impact of genetic testing on clinical management were described. A list of hereditary cancer genetics clinics in Canada was included.

Different hereditary cancer predisposing syndromes were grouped: those in which genetic testing may be considered part of the management for affected families; those in which clinical benefit of genetic testing has been demonstrated, but genetic testing is not considered part of standard clinical management; and those in which the clinical benefit of genetic testing is unclear.

Hereditary Cancer Predisposing Syndromes	Cancer Risk* (%)	GT Clinical Availability	Analytical Sensitivity (%)	Clinical Sensitivity (%)	Cost** (C$)
Genetic testing (GT) generally part of clinical management					
1. Cowden syndrome	50	Yes	n/a	81	1,950
2. Familial adenomatous polyposis	100	Yes	95 to 100	80 to 90	500
3. Hereditary breast and ovarian cancer syndrome 1 and 2	≤85	Yes	60 to 100	14.9 to 20.2	1,200
4. Multiple endocrine neoplasia 2	70	Yes	80 to 95	n/a	262
5. Retinoblastoma	90	Yes	n/a	10 to 70	3,700
6. von Hippel Lindau syndrome	45	Yes	40 to 100	n/a	390
Clinical benefit has been demonstrated but GT not part of standard clinical management					
1. Ataxia-telangiectasia	30 to 40	No	66	n/a	4,500
2. Basal cell nevus syndrome	90	Yes	85 to 99	n/a	2,400
3. Bloom syndrome	20	Yes	n/a	n/a	n/a
4. Fanconi's anemia	≤50	Yes	n/a	23.5 to 97	n/a
5. Hereditary multiple exostoses	≤2	No	80 to 95	70	n/a
6. Hereditary non-polyposis colon cancer	≤75	Yes	90 to 100	43 to 50	1,500
7. Multiple endocrine neoplasia 1	<10	Yes	85 to 99.5	n/a	1,050
8. Neurofibromatosis 1	≤5	Yes	60 to 97	67 to 68	n/a
9. Neurofibromatosis 2	Not increased	Yes	100	64 to 75	n/a
Clinical benefit of GT is unclear					
1. Familial melanoma	>90	Yes	100	50	900
2. Li-Fraumeni syndrome	90	Yes	80 to 98	70	n/a
3. Peutz-Jeghers syndrome	50	Yes	70	30 to 70	2,100
4. Wilms' tumour	100	No	90	n/a	1,250
5. Xeroderma pigmentosum	>90	No	n/a	n/a	n/a

*Cancer risk is the risk that a patient with a syndrome will develop cancer in his or her lifetime.
**The cost is a laboratory cost for new mutation detection. In the case of ataxia telangiectasia, the cost comes from a research setting.
n/a=information not available from literature search.

Conclusions

Genetic testing faces limitations due to the complexity of human disease with the interaction of many genetic and environmental factors, the gap between diagnosis and treatment, and the changing nature of genetic information. Concerns about molecular testing stem from its variable technical reliability, cost issues and its impact on clinical management. Technical issues depend on the techniques used. Laboratory techniques and their analytical validity vary. Full gene sequencing should have the highest analytical validity, but it is too cumbersome and expensive to use in routine clinical testing. The clinical impact of genetic testing depends on the therapeutic gap that can be bridged in terms of health outcomes and the psychological impact of testing. When a test has a poor ability to predict clinical outcomes and/or there is no effective treatment, testing is difficult to justify on either medical or social grounds.

Despite rapid development of new molecular techniques, the implementation of genetic testing in the standard clinical management of many disorders is unjustified. Additional data about the impact on health outcomes and more information on the costs of testing are needed to provide a basis for decision-making with respect to the integration of specific tests into clinical services and the funding of these tests.

TABLE OF CONTENTS

ABBREVIATIONS

ASEA   allele-specific expression assay
ASO   allele-specific oligonucleotide analysis
AT   ataxia-telangiectasia
BCNS   basal cell nevus syndrome
BRCA1   hereditary breast and ovarian cancer syndrome 1
BRCA2   hereditary breast and ovarian cancer syndrome 2
BS   Bloom syndrome
CMC   chemical mismatch cleavage
CS   Cowden syndrome
CSGE   conformation sensitive gel electrophoresis
DGGE   denaturing gradient gel electrophoresis
DHPLC   denaturing high-performance liquid chromatography
DNA   deoxyribonucleic acid
ES   exon scanning
FA   Fanconi's anemia
FAP   familial adenomatous polyposis
FGS   full gene sequencing
FISH   fluorescence in situ hybridization
FM   familial melanoma
HPLC   high performance liquid chromatography
LA   linkage analysis
LFS   Li-Fraumeni syndrome
HME   hereditary multiple exostoses
HNPCC   hereditary non-polyposis colon cancer
MEN1   multiple endocrine neoplasia type 1
MEN2   multiple endocrine neoplasia type 2
MHA   monochromosomal hybrid analysis
MSI   microsatellite instability
MSI-H   high frequency MSI
NF1   neurofibromatosis type 1
NF2   neurofibromatosis type 2
NPV   negative predictive value
PCR   polymerase chain reaction
PJS   Peutz-Jeghers syndrome
PPV   positive predictive value
PTT   protein truncation test
RB   retinoblastoma
RFLP   restriction fragment length polymorphism
SB   Southern blot analysis
SSCP   single strand conformation polymorphism
TDGS   two-dimensional gene scanning
TGGE   temperature gradient gel electrophoresis
VHL   von Hippel-Lindau syndrome
WT   Wilms' tumour
XP   xeroderma pigmentosum

GLOSSARY

Allele-specific oligonucleotide analysis (ASO): ASO is a method for detecting mutations by using short synthetic deoxyribonucleic acid (DNA) sequences that represent a specific mutant and hybridizing them to target sequences arrayed on nylon membranes.

Allele-specific expression assay (ASEA): ASEA is a method that identifies mutations as an imbalance in the representation of alleles in the RNA transcript.

Analytical validity: Analytical sensitivity refers to the ability of a genetic test to detect the mutations that it was designed to detect. Analytical specificity refers to the ability of the genetic test to identify correctly those without the mutation. The analytical validity of a test, which is high when sensitivity and specificity are high, depends on the method used for DNA analysis.

Autosomal dominant inheritance: An individual with a dominantly inherited genetic condition inherits a normal copy of the gene from one parent and an altered copy from the other parent. The presence of that single altered (mutated) copy is enough to cause the disease. Vertical transmission (an affected grandparent, parent and child) is consistent with a dominantly inherited condition. "Autosomal" refers to one of the numbered chromosomes (1 to 22) rather than one of the sex chromosomes (X or Y). Most hereditary cancer syndromes are dominantly inherited.

Autosomal recessive inheritance: Both copies of the gene must contain mutations for an individual to have a recevissively inherited genetic condition. An affected individual has inherited an altered copy of the gene from each parent. Individuals who have one altered copy and one normal copy of the gene are called carriers or heterozygotes. Heterozygotes do not usually have any symptoms or problems related to the disorder. Horizontal transmission (unaffected parents and two or more affected siblings) is consistent with a recessively inherited condition.

Cancer risk: Cancer risk means the risk that a patient diagnosed with a familial cancer predisposing syndrome might develop cancer in his or her lifetime.

Clinical validity: Clinical validity, which refers to the accuracy of a genetic test in diagnosing or predicting risk for a disorder (in this report, referring to a hereditary cancer predisposing syndrome and not a cancer), is measured by a test's clinical sensitivity, clinical specificity, positive predictive value and negative predictive value. Clinical sensitivity is the probability of a positive genetic test in a population with the disorder. Clinical specificity is the probability that the test will be negative in a population without the disorder. Positive predictive value is the probability that people with positive test results will get the disorder. Negative predictive value is the probability that individuals with negative results will not get the disorder. The clinical validity of a genetic test may vary depending on the health condition and the population to be tested.

Clinical utility: This involves identifying the test's clinical and psychological benefits and risks associated with positive and negative results. The clinical utility of a genetic test may vary depending on the health condition and the population to be tested. The clinical utility of a test has to be demonstrated before it is made available for clinical use.

Conformation sensitive gel electrophoresis (CSGE): This is a method of separating DNA fragments in a gel matrix. It is based on the intrinsic bending of DNA in solution and its changed conformation in the presence of an altered nucleotide sequence (e.g. mismatched bases). The conformational differences between homo- and hetero-duplexes in mildly denaturing solvents are enhanced. With electrophoresis, the hetero-duplexes show decreased mobility. A single base pair mismatch can be detected by CSGE.

Denaturing gradient gel electrophoresis (DGGE): This is a method of separating DNA fragments in a gel matrix containing a concentration gradient of a denaturing agent. This technique is based on the fact that the double stranded DNA denatures (separates into single strands) to different extents depending on the nucleotide sequence and the concentration of the denaturant. This changes the conformation of the DNA and hence its mobility through the gel matrix. This technique can detect differences in the denatured behavior of small DNA fragments (200 to 700 base pairs) that differ by as little as one base pair. Increasing temperature can also cause DNA denaturation. When the chemical denaturant gradient is replaced by a temperature gradient, the method is known as temperature gradient gel electrophoresis (TGGE).

Denaturing high-performance liquid chromatography (DHPLC): HPLC is a chromatographic method in which a sample is separated under high pressure, using a stationary and a mobile phase. DHPLC involves analysis using HPLC under partially denaturing temperature conditions. DHPLC is a sensitive and specific method with high throughput.

Electrophoresis: Electophoresis is the separation of molecules based on their mobility in an electric field.

Enzymatic mutation detection (EMD): EMD is a technique in which an enzyme, such as resolvase, is used to scan along a DNA fragment until it detects a structural distortion due to a point mutation, an insertion or a deletion.

Fluorescence in situ hybridization (FISH): FISH is a technique for determining the position of a gene, by using a fluorescent-labelled DNA probe and visualizing by fluorescence microscopy. It is a sensitive technique to detect specific nucleic sequences in specimens on fixed microscope slides. FISH detects large deletions or insertions and translocations.

Full gene sequencing (FGS): This involves determining the nucleotide sequence of a gene. In theory, it should be the most sensitive technique for detecting all possible mutations. With present technology, however, FGS is too cumbersome and expensive for routine clinical use and it can miss mutations that lie outside the coding region of a gene (e.g. in regions with introns, promoters or enhancers).

Gene: A gene is a sequence of nucleotides that occupies a specific locus on a chromosome. It represents a functional unit of inheritance.

Genotype: A genotype is a person's genetic makeup, as distinct from expressed features or phenotype.

Germline: A germline is a sequence of cells in the line of direct descent from zygote (fertilized ovum) to gamete (ovum or sperm), as opposed to somatic cells (all other body cells). Mutations in germline cells are transmitted to progeny (i.e. are hereditary), whereas mutations in somatic cells are not.

High-performance liquid chromatography (HPLC): HPLC is a form of liquid chromatography (which is a separation method based on the different interactions of the compounds with the mobile phase and with the stationary phase, as the speciment travels through a support medium) that gives high resolution, short analysis time and good reproducibility.

Linkage analysis (LA): This is used when the responsible gene is unknown or for disorders with too many mutations. The technique requires comparative testing of affected and unaffected siblings and parents; not every family will be accessible for this approach.

Microsatellite: Microsatellites are short sequences of dinucleotide or trinucleotide repeats of variable length distributed throughout the gene. Microsatellite instability (MSI) is defined as a change of any length due to insertion or deletion of repeating units in a microsatellite inside a tumour, when compared to normal tissue.

Mosaicism: In genetics, mosaicism is the presence in an individual of two or more cell lines that are karyotypically or genotypically distinct and that are derived from one zygote.

Mutation: A mutation is an error that has occurred in the DNA base sequence of a gene. It may impair the gene's ability to create a fully functional protein. A mutation can be a point mutation (single base pair substitution), a deletion or insertion with or without frame-shift (subtraction or addition of amino acid codons), an amplification or trinucleotide repeat (increase in the number of repeat sequences) or a translocation (interchromosomal exchange of large chromosome segments). A missense mutation is a mutation that changes a codon for a particular amino acid to one specifying a different amino acid. A nonsense mutation is a mutation that alters the coding sequence and prevents a protein from being synthesized.

Penetrance: Penetrance is the likelihood that a person carrying a particular mutant gene will have an altered phenotype.

Phenotype: A phenotype is the clinical presentation or expression of a specific gene or genes, environmental factors or both.

Polymerase chain reaction (PCR): PCR is the amplification technique that, through the judicious choice of primers, allows an assay to be designed to focus on the mutation of interest or on a "hot spot" in a gene containing several possible mutation sites, using minute amounts of starting material. It is especially valuable in detecting point mutations and micro-deletions that usually go unnoticed in a Southern blot.

Proband: A proband is an affected person ascertained independently of his or her relatives in a genetic study.

Protein truncation test (PTT): A PTT specifically detects mutations that cause termination of mRNA translation and subsequent protein truncation. It identifies nonsense mutations, frame-shift mutations and splice-site mutations, while overlooking missense mutations. The DNA fragment is usually amplified by PCR, then added to a transcription-translation system. The resultant protein and normal control products are run on a gel and compared for size.

Restriction fragment length polymorphism (RFLP): This technique involves cutting a gene into fragments with restriction enzymes, separating the DNA fragments by gel electrophoresis and identifying them by staining with ethidium bromide. Mutations in the gene abolish or create restriction enzyme cleavage sites, resulting in an altered pattern of DNA fragments.

RNase mismatch cleavage: This is a method in which cleavage by RNase of mismatched bases (which result from mutations in tumour samples) leads to fragments that are detected under ultraviolet light.

Single-strand conformation polymorphism (SSCP): This method, which is based on conformation differences, acts on single-strand species. It can detect point mutations in genes. Single base substitutions can alter the secondary structure of a DNA fragment, resulting in changed mobility when run on a gel.

Southern blot analysis (SB): SB is a technique for transferring electrophoretic DNA fragments from a gel to a membrane and subsequently detecting the fragment of interest by nucleic acid hybridization, using a labelled complementary DNA probe. SB detects large deletions by finding a loss or decrease in size of a target fragment.

Temperature gradient gel electrophoresis (TGGE): See DGGE.

1   INTRODUCTION

The identification of the sequence of the human genome and of human genes has changed our understanding of health and pathogenesis. We now recognize that virtually all diseases may have a genetic component. Thus, molecular genetics could become a powerful diagnostic and screening tool. The complex interplay between genetic predisposition and environmental influences, however, limits the predictive power of genetic testing.[1] Dissecting a patient's fundamental constitutional makeup also raises psychological, ethical and professional questions.[2-4]

Genetic susceptibility to cancer has been reviewed.[5-9] All cancers are disorders caused by mutations in the genes that control aspects of cellular biology such as proliferation and differentiation. These mutations occur most often in a somatic cell (i.e. sporadic cancer). Alterations in several oncogenes or tumour suppressor genes usually occur before a sporadic cancer develops. In some individuals, the mutation is inherited from a parent as the initial mutation occurs in a germline cell (i.e. hereditary cancer). The inherited mutation predisposes these individuals to cancer. It should be viewed as the initiating event in tumour development, which may in itself be insufficient to cause cancer. Hereditary syndromes in which individuals are at an increased risk of developing cancer are called hereditary cancer predisposing syndromes. This report focuses on the genetic testing of hereditary cancer predisposing syndromes.

In comparison with their sporadic counterparts, hereditary cancers have the following clinical features:[10]
- occurrence at an unusually young age compared with the usual presentation of that type of cancer
- multifocal development of cancer in a single organ or bilateral development of cancer in paired organs
- development of more than one primary tumour in a single individual
- family history of cancer of the same type or of several types associated with a cancer predisposing syndrome in close relative(s)
- high rate of cancer in a family
- occurrence of cancer in an individual or a family exhibiting congenital anomalies or birth defects.

Genes that can influence the initiation of cancer may be divided into tumour suppressor genes, oncogenes and DNA repair genes.[11] Mutations of these genes lead to uncontrolled cell growth, initiating tumour development. Tumour suppressor genes encode proteins that are responsible for suppressing cell growth; deletions or mutations result in a failure to produce proteins or the production of abnormal and inactive proteins that cannot perform their anti-oncogenic functions in the cell. In oncogenes, mutations can result in the production of proteins called oncoproteins, which become permanently activated to stimulate cell growth and proliferation. DNA repair genes are essential in protecting the integrity of the genes. Hereditary cancer causing mutations have been found most often in tumour suppressor genes, but they have also been found in a few oncogenes and DNA repair genes.

The increase in our understanding of the cancer predisposing process has arisen largely through the development of molecular genetic techniques. These techniques have allowed cancer predisposing genes to be identified, isolated, functionally characterized and mapped to regions of the human genome. The molecular diagnosis of genetic diseases, however, has been accompanied by complexities.

Diagnostic molecular genetics involves the spectrum of modern molecular biological techniques. These include single-strand conformation polymorphism (SSCP), denaturing high-performance liquid chromatography (DHPLC), denaturing gradient gel electrophoresis (DGGE), protein truncation test (PTT), Southern blotting (SB), polymerase chain reaction (PCR), PCR with allele-specific oligonucleotide (ASO) probes, fluorescence in situ hybridization (FISH) and DNA sequencing.

The choice of a technique to be used depends on whether the gene is known and its degree of heterogeneity.[12] All genetic disorders can be divided into two categories: those for which the causative gene has been identified and those for which it has not. The approach for those in the first category is direct gene mutation analysis; for those in the latter category, linkage analysis using polymorphic DNA markers nearby on the same chromosome, provided the disorder has been mapped to that chromosome. The degree of heterogeneity refers to the number of genes or the variety of mutations in one gene that can cause the same disease. The greater the heterogeneity, the more labour-intensive and costly the test becomes. With too much heterogeneity, direct mutation analysis may be unfeasible and linkage analysis must be used even though the causative gene has been cloned.

If a high proportion of the cancer-causing mutations in a gene are known and there are only a few mutations, then direct analysis for these mutations is simple and inexpensive. If the gene is large and the cancer-causing mutations are numerous and dispersed or occur in less accessible regions of the gene, then techniques that can screen large stretches of DNA must be used for mutation analysis. These tests, such as DGGE, SSCP, PTT, DHPLC and full gene sequencing, can be time-consuming and costly. Once a mutation is identified in an affected individual, subsequent testing of his or her family members is simpler, since it can be targeted to the abnormality. The mutation of interest can then be studied with the use of PCR. For those disorders with an unknown causative gene or with too many mutations, predictive diagnosis is possible in certain families using linkage analysis. The strategies for mutation detection are shown in Figure 1.

Genetic tests can be used for diagnostic or screening purposes. Genetic diagnostic tests are used to make or refine the diagnosis of a disorder or syndrome in a symptomatic individual or in a developing fetus. Genetic screening tests are used to identify the disorder in a healthy individual with or without a family history of the condition.

Figure 1: Strategies for mutation detection

Causative gene is known

↓

DIRECT MUTATIONAL ANALYSIS

To scan gene for mutations

To study precise mutations

Pre-screen
- DGGE
- SSCP
- PTT
- DHPLC
- Others

↓

Confirmation
- Sequence analysis

Direct confirmation
- FGS

Point mutation
- PCR and gel electrophoresis
- PCR and ASO
- Quantitative PCR

Large deletions
- SB
- FISH
- Quantitative PCR

The causative gene is unknown with chromosomal location known or disorder has too many mutations

↓

LINKAGE ANALYSIS

ASO=allele specific oligonucleotide testing
DGGE=denaturing gradient gel electrophoresis
DHPLC=denaturing high performance liquid chromatography
FGS=full gene sequencing
FISH=fluorescence in situ hybridization
PCR=polymerase chain reaction
PTT=protein truncation testing
SB=Southern blot analysis
SSCP=single strand conformational polymorphism

3

2 OBJECTIVES

1. To perform a systematic literature review of the evidence regarding availability and the cost analytical and clinical validity of genetic tests for screening and diagnosis of hereditary cancer predisposing syndromes
2. To document the impact of genetic testing on the clinical management of patients with specific hereditary cancer predisposing syndromes.

3 METHODS

3.1 Literature Search

Published and unpublished literature was obtained by searching several databases (for the detailed literature search strategy, see Appendix 1). On the DIALOG® system, MEDLINE®, EMBASE®, Cancerlit, BIOSIS Previews®, also PASCAL® were searched using MeSH headings, descriptors, gene names and text words in August 2002. The search strategy focused on the objectives of the report and a filter was used to restrict the large output to relevant studies. Retrieval was limited to "human" references. Regular database alerts and updates were established using the same headings and keywords as the full search to capture new references until April 2003. Additional databases searched included the Cochrane Library on CD-ROM, the National Center for Biotechnology Information's OMIM database (Online Mendelian Inheritance in Man) PubMed and GENETests databases. Clinical trial registries, health technology assessment (HTA) and related web sites were also searched. A Google™ search was performed to retrieve information regarding laboratories conducting the specific tests. These searches were supplemented by hand searching selected bibliographies and contacting genetics laboratories for additional information.

3.2 Selection Criteria and Method

There were two inclusion criteria for this review.

- The study must be relevant to the project's objectives. It must focus on genetic testing for the screening and diagnosis of familial cancers and on the technology, which must involve tests that are used to detect mutations of oncogenes, tumour suppressor genes and DNA repair genes.
- No language restrictions were placed on the selection of abstracts and the inclusion of articles.

Publication as a letter, editorial, or short note or a second publication of the same study presenting with the same results led to exclusion.

3.3 Data Extraction Strategy

Two reviewers (CH and SB) screened the articles according to the selection criteria. Information was captured on the:

- analytical and clinical validity of genetic tests for the detection of hereditary cancers
- technical issues and cost of genetic testing
- impact of genetic testing on the clinical management of hereditary cancer predisposing syndromes.

3.4 Limitations

The primary studies on the performance of genetic tests were non-randomized so that a critical analysis of the quality of the information could not be done. The variety of disorders covered, with the heterogeneity of the populations involved in the studies, also prevented a quantitative analysis of the data.

4   RESULTS

4.1   Quantity of Literature Available

The original electronic search strategy identified 1,786 abstracts. Of these, 637 potentially relevant articles were retrieved as full articles for a more detailed evaluation. Fifty articles from alerts and bibliographies were also retrieved as full articles; 230 articles did not meet the selection criteria and were excluded, leaving 457 relevant articles that were used in this report (for the flow of documents, see Appendix 2).

4.2   Summary of Hereditary Cancer Predisposing Syndromes and their Genetic Tests

The molecular techniques used to detect the most common hereditary cancer predisposing syndromes are listed in Table 1, with their analytical and clinical sensitivity, cost and availability. Prevalence of the disorders, with their cancer risk and penetrance, are included. More detailed information on molecular testing for each syndrome and its impact on clinical management is discussed in section 4.3. Genetic testing services and laboratories for hereditary cancers in Canada are listed in Appendix 3.

Table 1: Hereditary cancer predisposing syndromes and genetic testing

Disorder	Associated Tumour	Incidence or Prevalence of Disorder	Gene	Chromos-omal Location	Cancer Risk	Genetic Tests	Analytical Sensitivity	Clinical Sensi-tivity	Penetrance	Cost	Clinical Availa-bility
Tumour suppressor genes											
Basal cell nevus sydrome[13-18]	Skin cancer, brain tumour, ovarian and cardiac fibromas	Prevalence 1/40,000 to 1/164,000	PTCH	9q22.3	90%	FGS LA	85% 98% to 99%	n/a n/a	97%	US$1,600 (new mutation), US$350 (known mutation) (private laboratory)	Yes
Cowden syndrome[13,19-23]	Breast, endometrial, thyroid cancers and other carcinoma	Prevalence 1/200,000 to 1/250,000 in Dutch population	PTEN	10q23	50%	FGS	n/a	81%	May be 100%	US$1,300 (new mutation), US$350 (known mutation) (private laboratory)	Yes
Familial adenomatous polyposis[10,13, 24-34]	Colorectal, duodenal carcinoma	Incidence 1/6,000 to 1/13,000, prevalence 2.29 to 3.2/100,000, 15% of colorectal cancers are hereditary	APC	5q21-q22	100%	FGS PTT ASEA and PTT LA DHPLC SSCP	95% n/a >98% 100% n/a n/a	n/a 80% to 82% 80% to 90% n/a n/a n/a	Almost complete, 95% with FAP have polyps at age 35 years.	C$500 (new mutation) C$250 (known mutation) (Quebec)	Yes

Disorder	Associated Tumour	Incidence or Prevalence of Disorder	Gene	Chromos-omal Location	Cancer Risk	Genetic Tests	Analytical Sensitivity	Clinical Sensi-tivity	Penetrance	Cost	Clinical Availa-bility
Familial melanoma[10,1, 35-38]	Melanoma	Malignant melanoma 27,000 new cases in the US in 1988, 3% to 7% of melanoma cases are familial	CMM1 CMM2 (CDKN2, MST1, p16[INK4]) CDK4 (onco-gene)	1p36 9p21 12q14	>90%	DNA sequencing DHPLC SSCP	n/a 100% n/a	50% n/a n/a	Close to 100%	For TP16 gene mutation detection US$600 (new mutation), US$350 (known mutation) (private laboratory)	Yes
Hereditary breast and ovarian cancer syndrome 1 (BRCA1)[13 39-48]	Breast, ovarian carcinoma and cancer of fallopian tube, stomach, pancreas and colon	Prevalence 1/800 to 1/2,500 in general population (BRCA1 and BRCA2) • 7% of breast cancers are hereditary • 60% of hereditary breast cancers due to BRCA1	BRCA1	17q21	85% or less	FGS PTT SSCP DHPLC CSGE TDGS EMD IA	n/a 80% to 100% 65% to 96% 93% to 100% 60% 80% to 91% 93% n/a	n/a 20.2% 14.9% n/a n/a n/a n/a	0% (first 20 years), 49% (to age 50) 80% (lifetime risk), in high risk families 85% at 70 years	US$2,600 (private laboratory) C$1,200 (Ontario)	Yes
Hereditary breast and ovarian cancer syndrome 2 (BRCA2)[13,40, 42-44,47]	Breast, ovarian and pancreas carcinoma	Prevalence 1/800 to 1/2,500 in general population (BRC1 and BRC2) • 7% of breast cancers are hereditary • 20% of hereditary breast cancers due to BRCA2	BRCA 2	13q12	85% or less	FGS PTT IA	n/a n/a n/a	n/a n/a n/a	0% (first 20 years), 28% (to age 50), in high risk families 86% at 70 years	US$2,600 (private laboratory) C$1,200 (Ontario)	Yes

Disorder	Associated Tumour	Incidence or Prevalence of Disorder	Gene	Chromos-omal Location	Cancer Risk	Genetic Tests	Analytical Sensitivity	Clinical Sensi-tivity	Penetrance	Cost	Clinical Availa-bility
Hereditary multiple exostoses (HME)[49-53]	Osteo-chondroma or osteosarcoma	Prevalence 1/50,000, chondrosarcoma in general population 1/100,000 to 1/250,000, 5% of patients with a chondrosarcoma have HME	EXT1 EXT2	8q24 11p12 to p11	0.5% to 2%	SSCP DHPLC FGS CSGE	80% to 93% 95% n/a n/a	n/a n/a n/a 70%	96% at age 12 years, 5% at birth	n/a	No
Li-Fraumeni syndrome[13,54]	Sarcoma, breast carcinoma, leukemia, melanoma, cancer of pancreas, adrenal cortex and brain	Very rare, worldwide prevalence <300 families	TP53	17p13	90%	FGS Direct sequence-based DNA testing of exons 5 to 8 Chip-based DNA sequencing	98% 80% 90%	70% n/a n/a	90% n/a n/a	n/a	Yes
Multiple endocrine neoplasia, type 1 (MEN 1)[55-57]	Carcinoid tumours of pancreas, pituitary, adrenal and parathyroid glands	Prevalence 1/10,000 to 1/25,000	MEN 1	11q13	<10%	LA DNA sequencing SSCP	99.5% n/a 85%	n/a n/a n/a	90% at age 50 years	£420.25 (new mutation), £78.8 (known mutation) (UK)	Yes

Disorder	Associated Tumour	Incidence or Prevalence of Disorder	Gene	Chromos-omal Location	Cancer Risk	Genetic Tests	Analytical Sensitivity	Clinical Sensi-tivity	Penetrance	Cost	Clinical Availa-bility
Neuro-fibromatosis type 1[58-60 61-65]	Neurofibroma or sarcoma, pheochromo-cytoma	Incidence 1/3,000 to 4,000, prevalence 1/960 to 1/7,800	NF1	17q11	2% to 5%	PTT / DHPLC / SSCP / FGS	93% / 97% / 60% / n/a	67% / 68% / n/a / n/a	100%	n/a	Yes
Neuro-fibromatosis type2[13,61,63,66-69]	Neurofibroma, meningioma, schwannoma	Incidence 1/37,000	NF2	22q12	May not be increased	SSCP / RNase cleavage assay	n/a / 100%	64% / 75%	100%	n/a	Yes
Peutz-Jeghers syndrome[13,70-76]	Gastro-intestinal cancer, cancers of breast, uterus, ovary, lung, pancreas and testes	Incidence 1/120,000	STK11	19p13	50%	FGS for familial cases / SSCP for sporadic cases	70% / n/a	70% / 17%	100% / n/a	US$1,400 (new mutation) $350 (known mutation) (private laboratory)	Yes
Retinoblastoma [13,77-84]	Retino-blastoma, osteosarcoma	Prevalence 1/15,000 to 1/20,000 births; 60% of cases are unilateral, sporadic; 15% of cases are unilateral, hereditary; 25% of cases are bilateral, hereditary	RB1	13q14	90%	SB / DNA sequencing / HD-SSCP sequencing	n/a / n/a / n/a	10% / 70% / 72%	90%	C$3,700 (Ontario)	Yes
von Hippel-Lindau syndrome[13,85-87,87-91,91-96]	Hemangio-blastoma, cerebral, renal and retinal carcinomas, pheochromo-cytoma	Prevalence 1/36,000	VHL	3p25	45%	SB / TGGE / DHPLC / CSA / F-SSCP	40% / 100% / 95% / 100% / 100%	n/a / n/a / n/a / n/a / n/a	90%	US$260 (private laboratory)	Yes

Disorder	Associated Tumour	Incidence or Prevalence of Disorder	Gene	Chromos-omal Location	Cancer Risk	Genetic Tests	Analytical Sensitivity	Clinical Sensi-tivity	Penetrance	Cost	Clinical Availa-bility
Wilms' tumour[13,97-102]	Kidney tumour	Prevalence 1/10,000 children, 10% to 30% are hereditary (i.e. 1/30,000 to 1/100,000 children)	WT1 WT2 WT3	11p23 11p15.5 17q12-21	100%	FGS	90%	n/a	100%	£500 (FGS), £200 (exons 6 to 9 scanning) (Sheffield Children's Hospital, UK)	Yes
Oncogenes											
Multiple endocrine neoplasia, type 2 (MEN 2)[13,103-115]	Medullary thyroid carcinoma, pheochromo-cytoma, parathyroid hyperplasia, parathyroid-adenoma	Prevalence 1/30,000	RET	10q11	70% by age 70	PCR HD SSCP DGGE	80% to 95% n/a n/a n/a	n/a n/a n/a n/a	100%	£105 (two exons) (UK) US$300 (exon 16 scanning, private laboratory)	Yes
DNA repair genes											
(autosomal dominant)											
Hereditary non-polyposis colon cancer (HNPCC)[13,116,117]	Colorectal, uterine carcinoma	Prevalence 0.05% to 15% of all colorectal cancer, 1/100 to 1/3,000 of US population	MSH2 MLH1 PMS1 PMS2 MSH6	2p22 3p21 2q31 7p22 2p16	Colorectal cancer 74% in males 30% in females, uterine cancer 42%	MSI IHC FGS DGGE DHPLC	n/a 97% n/a 90% 100%	43% n/a n/a 50% n/a	As high as 75%	C$400 (MSI) (Ontario) C$205 (IHC) (Ontario) C$1,500 (MSH2 or MLH1 sequencing) (Ontario)	Yes

Disorder	Associated Tumour	Incidence or Prevalence of Disorder	Gene	Chromos-omal Location	Cancer Risk	Genetic Tests	Analytical Sensitivity	Clinical Sensi-tivity	Penetrance	Cost	Clinical Availa-bility
(autosomal recessive)											
Ataxia telangiectasia [118,118-122]	Lymphoma, leukemia, breast cancer	Prevalence 1/30,000 to 1/100,000 gene carrier frequency 1% to 3% of general population	ATM	11q22.3	30% to 40%	PTT SSCP FGS	66% n/a n/a	n/a n/a n/a	100% in homozygotes	US$2,000 to US$4,000 (FGS) (research laboratory) $100 to $150 (PTT and SSCP) (research laboratory)	No
Bloom syndrome [13,123-126]	Lymphoma; leukemia; cancers of tongue, larynx and stomach	Gene carrier frequency 1/200 among Ashkenazi Jews	BLM	15q26.1	20%	FGS LA	n/a n/a	n/a n/a	100%		Yes
Fanconi's anemia [123,127-133]	Acute myelogenous leukemia	Prevalence 1/360,000, gene carrier frequency 1/300 to 1/600 in general population, 1/100 in Ashkenazi Jews	FANCA FANCB FANCC FANCD FANCE FANCF FANCG FANCH BRCA2	16q24.3 unmapped 9q22.3 3p25.3 6p21-p22 11p15 9p13 unmapped 13q12	As high as 50%	FGS SSCP PTT RT-PCR + CMC Fluorescent multiplex PCR	n/a n/a n/a n/a n/a	n/a 34% to 97% n/a 23.5% n/a	100%	n/a	Yes

Cancer risk is the risk that a patient with a syndrome will develop cancer associated with the disorder in his or her lifetime.
Clinical availability means that genetic tests for the disorder are available for patients for clinical purposes, as opposed to genetic tests that are done in a research setting for research purposes.
Penetrance is the likelihood that a person carrying a particular mutant gene will have altered phenotypes associated with the disorder.
n/a=information not available

ASEA=allele-specific expression assay
ASO=allele specific oligonucleotide testing
CMC=chemical mismatch cleavage
CSA=comparative sequence analysis
CSGE=confirmation sensitive gel electrophoresis
DGGE=denaturing gradient gel electrophoresis
DHPLC=denaturing high performance liquid chromatography
ES=exon scanning

FGS=full gene sequencing
F-SSCP=fluorescent single strand conformational polymorphism
HD=heteroduplex analysis
IA=immunoassay
IHC=immunohistochemical staining
LA=linkage analysis
MHA=monochromosomal hybrid analysis
MSI=microsatellite instability analysis

PCR=polymerase chain reaction
PTT=protein truncation testing
RFLP=restriction fragment length polymorphism
RT-PCR=reverse transcriptase PCR
SB=Southern blot analysis
SSCP=single strand conformational polymorphism
TGGE=temperature gradient gel electrophoresis
TDGS=two-dimensional gene scanning

4.3 Impact of Genetic Tests on Clinical Management

4.3.1 Ataxia-telangiectasia

Ataxia-telangiectasia (AT) is a rare, progressive neurological disorder that is inherited in an autosomal recessive pattern, with ATM as the responsible gene (11q22.3).[141-145] The ATM protein may contribute to the phenotype of AT,[146,147] and may play a role in repair and checkpoint pathways,[148] response to oxidative damage[149,150] and radiation resistance.[151,152] AT is characterized by cerebellar ataxia (i.e. loss of muscle coordination caused by disorder of the cerebellum), disturbed eye movements, slurred speech, endocrine dysfunction, immuno-deficiency, increased sensitivity to ionizing radiation, premature aging and telangiectasia (i.e. red marks due to dilation of small superficial blood vessels in sun-exposed skin),[120,153] with a predisposition to lymphomas, leukemias and other cancers.[121,154,155] Most affected individuals are wheelchair-bound by adolescence and rarely live past age 45. Because of the challenge of molecular diagnosis for AT, the diagnosis is clinical, combined with laboratory evidence regarding serum alphaprotein level and radioresistant DNA synthesis.[156]

a)   Molecular testing

Molecular testing of the ATM gene is available only on a research basis. Molecular testing for AT is technically challenging. The large size of the ATM gene, with the diversity of mutations (most mutations being unique and distributed uniformly along the length of the gene), limits the ability of direct mutation screening to act as a diagnostic tool.[157] ATM mutations have been identified using extended reverse transcriptase-PCR (RT-PCR) and restriction endonuclease fingerprinting[158] or SSCP.[159] A high-density oligonucleotide array-based assay has been used to screen for sequence variations in the ATM gene.[160] Screening for specific mutations may be feasible in subpopulations that carry a specific mutation. Given the high frequency (70%) of truncation mutations in the ATM gene, screening methods can be based on PTT, which can detect 66% of the mutations.[122] PTT and conformation sensitive gel electrophoresis (CSGE) have been used to detect truncated protein and other ATM mutations in selected ethnic groups.[122] Experts claim that a feasible approach to test carriers of ATM mutations involves identifying the most common mutations in selected ethnic populations and then developing rapid assays that use small amounts of genomic DNA and less costly methods.[122] The cost of molecular testing for AT in a private laboratory in the US is US$2,000 to $4,000 for FGS and $100 to $150 for PTT or SSCP.[122]

b)   Impact of molecular testing on clinical management

The clinical benefit of genetic testing for AT has been demonstrated, but it is not part of standard management. Individuals with AT are counselled to undergo periodic blood counts, careful skin examinations and upper gastrointestinal tract imaging, if symptoms are reported. Since studies show an increased incidence of breast cancer in ATM mutation carriers, surveillance programs are advised for heterozygous and homozygous women.[161] Since there is increased radio-sensitivity in carriers of the mutated ATM gene, concerns have been raised about whether x-rays, with their low rates of radiation, can be harmful.[152,162] ATM defects, however, are not the major cause of radiotherapy complications in women with breast cancer.[163] X-rays should only be used for diagnostic purposes rather than as a screening tool.[13] Aggressive prevention of infection is suggested for homozygous women.

4.3.2 Basal cell nevus syndrome

Basal cell nevus syndrome (BCNS) or Gorlin's syndrome is a rare autosomal dominant condition that is caused by mutation of the PTCH (9q22.3) gene.[13] The PTCH gene encodes a trans-membrane protein, the loss of which (due to the mutation) may lead to changed cell growth pattern and increased growth. Germline mutations include insertions, deletions and point mutations leading to premature stops or frame-shifts. The offspring of an affected individual has a 50% risk of being affected.[18] BCNS is characterized by the development of multiple jaw keratocysts, usually starting in the second decade of life; palmar and plantar pitting of the skin; or basal cell carcinomas, usually from the third decade. Ectopic calcification is present in over 90% of patients by age 20 years. Other than the high risk for skin cancer, individuals are at increased risk for a pediatric brain tumour, ovarian fibromas and cardiac fibromas.[18] About 5% of children with BCNS develop medulloblastoma, with a peak incidence at two years of age.[18] Cardiac and ovarian fibromas occur in 2% and 20% of individuals respectively.[18] Molecular diagnostic testing for the identification of a pathogenic mutation is used to confirm a clinical diagnosis of BCNS.

a)   Molecular testing

Molecular testing of PTCH gene is clinically available. Sequence analysis of the PTCH coding region, which can detect up to 85% of the mutations, is used to detect mutations in patients with the typical clinical findings of BCNS.[18] If the sequence analysis result is negative in these patients, SB may be performed to detect large deletions. Linkage analysis (LA), which has great accuracy in families with more than one affected family member, can detect up to 99% of the mutations.[18] Prenatal testing of fetuses at 50% risk for BCNS is clinically available if the mutation has been identified in an affected family member or if linkage testing has been informative in the family.

The cost for mutation detection by exon screening and sequencing in a new patient at a private laboratory is US$1,600. DNA sequencing of relatives for a known mutation costs US$350. Prenatal diagnosis using two samples (chorionic villus sampling and cultured amniocytes) is US$700. The turnaround time for testing new patients is about eight weeks, but it is two weeks when the mutation in the family is known.[14]

BCNS cannot be excluded on the basis of molecular genetic testing, since not all patients with clinical presentations consistent with BCNS will have detectable PTCH mutations. Missense mutations are common and may be difficult to interpret in an individual who has no family history of BCNS and who fails to fulfil diagnostic criteria.[18]

b)   Impact of molecular testing on clinical management

The clinical benefit of genetic testing for BSNS has been demonstrated, but it is not part of standard management. Early reports have not found a genotype-phenotype correlation in individuals with BCNS.[164] Due to the requirement for surveillance of BCNS complications, especially medulloblastoma, in childhood, clarification of the genetic status of at-risk children is considered appropriate by some experts.[18] Either gene sequencing or linkage analysis may be considered depending on whether a pathogenic mutation has been identified in an affected family member or if the family is informative for linked markers.

4.3.3 Bloom syndrome

Bloom syndrome (BS) is a rare autosomal recessive disorder characterized by severe growth deficiency, sun-sensitive facial erythema or telangiectasia, severe immune defects, infertility and elevated risk of malignancy, with rare survival to adulthood.[165-167] About 20% of individuals with BS develop cancer, with half of the cases occurring before age 20.[123,168] Malignancies include non-Hodgkin's lymphoma; acute leukemia; and carcinomas of the tongue, larynx and stomach. The mutated gene in BS is BLM (15q26.1), which encodes a DNA helicase that is involved in the surveillance of base abnormalities in DNA.[169] The mutated BLM gene is more common in Ashkenazi Jews.[126] The diagnosis of BS is usually made on clinical grounds with confirmatory laboratory testing for an increased rate of sister chromatid exchanges, which lead to excess breakage and possible loss of heterozygosity.

a)   Molecular testing

Clinical molecular testing for the BLM gene is available. About 1/231 of Ashkenazi Jews carries the identical 6-bp deletion/7-bp insertion at position 2281 of BLM (blm Ash).[124] This mutation, which is responsible for most if not all instances of BS in the Ashkenazi Jewish population, can be identified by a method based on restriction enzyme digestion of a PCR product containing the mutation.[125] Carrier testing is offered to couples of eastern European (Ashkenazi) Jewish ancestry as part of a prenatal screening profile.[13]

b)   Impact of molecular testing on clinical management

The clinical benefit of genetic testing for BS has been demonstrated, but it is not part of standard management. Heterozygotes for the BLM mutated gene are advised to have routine physical examinations and blood counts starting in childhood.[13] Individuals with BS (homozygotes for the mutated gene) should undergo surveillance for skin and gastrointestinal tract cancers. Affected females should also be monitored for cervical and breast cancers. Affected individuals are advised to avoid direct sunlight.[13]

4.3.4 Cowden syndrome

Cowden syndrome (CS) or multiple hamartoma syndrome is a rare disorder associated with germline mutations in the PTEN tumour suppressor gene (10q23).[170,171] PTEN encodes PTEN phosphatase, which acts by mediating cell cycle arrest and apoptosis (programmed cell death). Mutations include nonsense and missense mutations. Genetic studies have confirmed an autosomal dominant inheritance of CS with a high penetrance in both sexes and moderate interfamilial and intrafamilial differences in the expressivity of symptoms.[172] Although CS does occur in males, it occurs more often (6:1) in females.[172,173] While CS is characterized most commonly by non-cancerous mucocutaneous lesions and gastrointestinal hamartomas, it is also associated with a 25% to 50% lifetime risk of breast cancer, 10% risk of thyroid cancer and a risk of other cancers including endometrial cancer, renal cell carcinoma, melanoma and glioblastoma.[13,21,174] Dermatologic lesions, including multiple facial trichilemmomas, precede the development of malignancy and can be used to identify women with a high risk of developing breast or thyroid cancer.[175]

a)   *Molecular testing*

Methods for detecting mutations in the PTEN gene include direct sequencing, which is generally available; and other methods (SB, PCR-SSCP, PCR-DGGE), which are available on a research basis.[21,176-179] PCR-SSCP with non-isotopic silver staining, is more sensitive than automated direct sequencing for detecting PTEN point mutations.[176] Sequence analysis of each of the nine PTEN exons gives a clinical sensitivity of 81%.[19,20] The amount of tumour DNA in the sample is required to be 10% for PCR-SSCP, but 30% to 70% for direct sequencing.

The cost for mutation detection by exon screening and sequencing at a private laboratory in a new patient is US$1,300. Testing of relatives for a known mutation in the family costs US$350.[20] The turnaround time is six to eight weeks for a new patient and two weeks for relatives when the mutation is known.

To date, 82 germline mutations in the PTEN gene responsible for the clinical phenotype of CS (and over 300 somatic mutations in the PTEN gene responsible for sporadic cancers) have been identified.[23,178] It is estimated that 80% of families with CS will have an identifiable mutation in the PTEN gene.[21,177,180] PTEN germline mutations are also identified in 50% to 60% of families with Bannayan-Zonana syndrome and Proteus-like syndrome.[19,21,181]

CS has variable expression and is often manifested as subtle skin signs, so this condition is difficult to recognize. The mapped PTEN gene mutation is the sine qua non for the diagnosis of CS.[182] The genotype-phenotype correlations between PTEN mutations and CS, however, are unclear. The observed association between the presence of a PTEN mutation and breast cancer is not always confirmed. The clinical features of some CS patients without a PTEN mutation do not differ from those of CS patients with a PTEN mutation.[22] Data show, however, that the presence and absence of a PTEN mutation are associated with the type of breast involvement (i.e., malignant versus benign).[19,175]

b)   *Impact of molecular testing on clinical management*

Genetic testing is part of standard clinical management for CS. A presumptive diagnosis of CS is based on clinical signs. By definition, the diagnosis of CS is only made when a PTEN mutation is identified. Thus, molecular testing for the PTEN gene mutation is critical in the diagnosis and management of CS.[183] Early recognition of the dermatologic features of CS, particularly the pathognomonic facial trichilemmomas, is important for the identification of women who would benefit from molecular testing for the PTEN mutation. There seems to be an association between mutations upstream and in the phosphatase core motif (which contains most of the missense mutations) and the development of multi-organ disease.[19] Women with diagnosed CS should undergo frequent breast examinations, periodic mammography starting at age 30 to 35, biopsy of suspected lesions and screening for thyroid cancer.[13]

4.3.5 Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is a hereditary colorectal cancer predisposing syndrome with an autosomal dominant inheritance pattern. The responsible gene, adenomatous polyposis coli (APC) gene (5q21-q22), encodes the APC protein that controls apoptosis.[184] A mutated APC gene leads to chromosomal instability and a truncated APC protein, resulting in uncontrolled cell

growth and tumour formation.[185] FAP is characterized by the early onset of >100 colorectal adenomatous polyps and extracolonic manifestations. Cancer is inevitable, as one or more of the colorectal polyps will become malignant, if prophylactic colectomy is not performed.[186] The cancer may arise at any age from late childhood through the seventh decade, with a median age at clinical diagnosis of 40 years. FAP is a disorder of high penetrance (95% of those with FAP have polyps at 35 years).[28] Its diagnosis is based on clinical findings. Molecular genetic testing is most often used in the early diagnosis of FAP for at-risk family members and in the confirmation of FAP for patients with equivocal findings. [187,188]

a)   Molecular testing

Molecular diagnostic testing of FAP is clinically available. The Canadian Task Force on Preventive Health Care recommended in 2001 to include genetic testing or sigmoidoscopy in the periodic health examination of people in kindreds with FAP.[189] The analysis of the APC gene is difficult because of its large size (15 exons) and the number of known mutations scattered along the length of the gene. Full gene sequencing (FGS), a combination of allele-specific expression assay (ASEA) and PTT, PTT alone and LA are among the techniques used to identify the mutation on the APC gene.[190] An improved PTT technique increases the test clinical sensitivity.[191] DHPLC has been found to detect 100% of mutations (i.e. 100% analytical sensitivity).[24] A modified SSCP method, cold SSCP, could be a reliable and less expensive way to detect known mutations in the APC gene.[25] The PTT assay for APC gene testing for FAP gives about 20% false-negative results, i.e. the patient has a mutation but tests negative.[192] Since colorectal cancer develops in virtually all patients with FAP, false-negative results could lead to devastating consequences. Prenatal testing of fetuses at 50% risk for FAP is clinically available if the mutation has been identified in an affected family member or if linkage testing has been informative in the family.

In Quebec, Canada, the cost of the APC gene test using PTT followed by DNA sequencing (for a new patient) and PTT (for at-risk relatives from a mutation-known family) is estimated at C$500 per test and C$250 per test respectively.[26]

Since APC gene mutation testing is time-consuming and costly, the use of predictive genetic testing for FAP compared to conventional clinical screening (i.e. regular fecal occult blood testing and repeated sigmoidoscopies or colonoscopies) is an issue.[193-195] Since the probability of not detecting FAP decreases with age, the extent of cost savings depends on the age at which screening starts. A recent systematic review[26] shows that cost saving relative to clinical screening is achieved up to a starting age of 36 years. The average savings per person initially screened at age 12 are over C$900 but are reduced to C$200 by age 30. Another study comparing the cost of predictive genetic testing versus conventional clinical screening for FAP shows that predictive genetic testing, using heteroduplex analysis (HD) and PTT if necessary (C$4,975 per family), costs less than conventional clinical screening using flexible sigmoidoscopy (C$8,031), provided that the frequency of clinical surveillance is identical in both strategies.[196]

b)   Impact of molecular testing on clinical management

Genetic testing is part of standard clinical management for FAP. Molecular detection of an APC gene mutation in a member of a family with FAP allows differentiation of carriers and non-carriers of the mutant APC allele among relatives at risk. Since APC mutations occur early

during colorectal tumorigenesis,[197] molecular diagnosis testing for the APC gene mutations, if positive, may help in early detection and treatment of FAP. However, since traditional management strategies for at-risk family members involve regular sigmoidoscopic examination with prophylactic colectomy when polyps appear, most affected patients are still diagnosed endoscopically.[198] The molecular diagnosis of FAP is particularly valuable when it is truly negative (when it identifies non-carriers of an APC mutation), thus avoiding clinical and sigmoidoscopic surveillance for these individuals.[199]

Much effort has gone into discovering genotype-phenotype correlations that could become part of management decisions.[27,200] Profuse polyposis (an average of 5,000 polyps) has been reported with mutations in codons 1250 to 1464.[201] Mutations at codons 1309 and 1328 have been associated with a uniformly severe polyposis phenotype.[202] Mutations between codons 1444 to1581 are associated with a higher incidence of desmoid tumours.[203,204] Different mutations in the APC gene are associated with different expressions of FAP. Mutations in the 5' (5' to codon 158) and 3' (3' to codon 1596) are associated with a less severe phenotype of FAP (attenuated FAP).[205,206] The site of mutation in the APC gene influences the course of FAP, so molecular genetic testing could be used as a guide to surgical management.[207]

4.3.6 Familial melanoma

Familial melanoma (FM) or dysplastic nevus syndrome is a disorder in which malignant melanoma is inherited in an autosomal dominant pattern.[13,208-212] Individuals are considered to have FM if they have 10 to 100 dysplastic nevi (visible cutaneous lesions) or have two first degree relatives with melanoma.[213] It is estimated that 3% to 7% of melanoma patients are from genetically high risk families.[10,36] Three melanoma susceptibility genes have been identified: CMM1 (1p36); CMM2, also known as TP16 or MTS1 or CDKN2A (9p21); and CDK4 (12q14).[10,209] The TP16 gene encodes the TP16 protein, which mediates cell cycle regulation. Various mutations have been identified in the TP16 gene, including nonsense, splice donor site, missense, insertion and deletion.[10,38] The average age of a patient with FM and an initial melanoma diagnosis is 34, which is two decades earlier than the age at which the disease is typically seen in the general population. In individuals with FM, suspicious moles that change size or colour, unless excised early, have almost a 100% risk of becoming malignant. Astrocytomas and cancer of the pancreas have also been reported in people with FM.

a)   *Molecular testing*
Molecular testing for FM is clinically available. Methods for detecting mutations in the melanoma susceptible genes include DNA sequencing, DHPLC and SSCP, with DHPLC emerging as a fast, sensitive and cost-efficient method.[35,214] Although at-risk family members are easy to identify, uncovering the molecular basis of FM has been difficult. CDK4 is an oncogene, while CMM1 and TP16 are tumour suppressor genes. The TP16 germline mutation is the responsible factor in about 50% of patients with FM. Types of mutations include nonsense, missense, splice donor site and insertions, leading mostly to truncation of the protein p16.[13] Mutation of the CDK4 gene is rare; only a few families have germline mutations in this gene. In contrast with other cancer predisposing syndromes, the prevalence of germline mutations of CDKN2A and CDK4 is low (2%) in patients with early-onset melanoma.[35,215]

19

The cost for mutation detection by sequence analysis at a private laboratory is US$600 for the CDKN2A gene and US$150 for the CDK4 gene in an affected individual. DNA testing of relatives for a known mutation costs US$350. Turnaround time is eight weeks for a new patient and two weeks for a known mutation in relatives.[38]

b)   *Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for FM is unclear. The screening and control of FM are important, because of its high prevalence rate, the fact that it almost invariably arises from a visible cutaneous lesion and its grave prognosis when detected late in its course. The risks and benefits of cancer screening for this syndrome have not been established. Individuals with TP16 mutations have a melanoma risk that is increased by a factor of 75 and the pancreatic cancer risk is increased by a factor of 13. Individuals from FM kindreds without TP16 mutations have a risk of melanoma that is increased by a factor of 38.[209] For individuals with FM, surveillance involves monthly skin self-examination, twice yearly dermatologic evaluation with photography of the skin and early excision of suspicious lesions.[10] Individuals are advised to use sunscreen daily and to avoid sunburn.

4.3.7 Fanconi's anemia

Fanconi's anemia (FA) is a rare autosomal recessive disorder with diverse clinical symptoms including progressive bone marrow failure and increased cancer risk, mainly acute myeloid leukemia.[216] It is typically diagnosed around age six, because of anemia, bleeding or easy bruising, with a mean survival age of 16 years.[10,13,217,218] FA comprises at least eight subtypes, from FA(A) to FA(H), corresponding to eight responsible described genes for FA, from FANCA to FANCH genes,[129,130,219-223] with FANCA, FANCC and FANCE accounting for 66%, 13% and 13% of FA patients respectively.[224-226] Studies suggest that FA proteins of different groups cooperate in a common cellular pathway.[128,227,228] A recent study shows that mutations in BRCA2 gene are found in FA(B) and FA(D) patients,[127] suggesting that BRCA2 gene and FA proteins may cooperate in a common DNA damage response pathway. FA cells are hypersensitive to DNA crosslinking agents such as diepoxybutane and mitomycin C. Thus, FA is diagnosed by assessing the levels of chromosome breakage (that is invisible on a standard karyotype) and by using a mitomycin C chromosome stress test or a diepoxybutane (DEB) test.[229,230]

a)   *Molecular testing*

Molecular testing of FA is clinically available. The combination of immunoblot analysis and retroviral-mediated phenotype correction of FA cells is a rapid method of subtyping.[130] PCR- SSCP analysis has been used in determining sequence variation in FA genes of different subtypes; its clinical sensitivity varies between subtype populations, ranging from 34% to 97%.[131,231,232] Reverse transcriptase PCR and chemical mismatch cleavage (CMC) also have been used to determine the localization of a single splice mutation of the FANCC gene, which may account for most of the FA cases in Ashkenazi Jews.[132] In this population, PCR-CMC has a clinical sensitivity of 23.5%. Reverse transcriptase PCR and fluorescent CMC have been used to detect small mutations of the FANCA gene. They are combined with quantitative fluorescent multiplex PCR for gross deletions.[133] Splice, nonsense and missense mutations in the FANCG gene have been found using a combination of somatic cell fusion assays, retroviral complementation and immunoblotting methods.[233]

b)   Impact on clinical management

The clinical benefit of genetic testing for FA has been demonstrated, but it is not part of standard management. Genotype-phenotype correlations in FA have been found. The expression of a FAC-related protein, FRP-50, correlates with mild group FA(C). The endogenous PRP-50 levels in cells with delG322 is a measurable protective effect against the severe phenotypic features of FA.[234] The loss of one chromosome 7 (monosomy 7) has been associated with progression to leukemia and poor prognosis.[235] Monosomy 7 detection by fluorescence in-situ hybridization (FISH) can be adopted as standard practice to predict acute myeloid leukemia in patients with FA.[236] A functionally active retrovirus vector has been shown to be capable of transferring a functional FANCC gene to lymphoid cell lines established from FA(C) patients.[237] This suggests that gene therapy may be a potential strategy for FA patients.

4.3.8 Hereditary breast and ovarian cancer syndrome 1 and 2

Breast cancer is the second leading cause of cancer death in Canadian women. Seven per cent of breast cancers are hereditary, of which 60% are due to cancer predisposing mutations in the BRCA1 gene (17q21); and 20% are due to cancer predisposing mutations in the BRCA2 gene (13q12), both in an autosomal dominant manner.[43,238-245] These numbers suggest an overall carrier frequency of mutations in one of these two genes in the range of 1/800 to 1/2,500 across populations.[13,246-249] The BRCA1 gene mutation accounts for 30% to 45% of breast cancer cases in families with a high incidence of early onset breast cancer and nearly 90% of cases in families with a high incidence of breast and ovarian cancers.[41,242] BRCA2 gene mutation accounts for about 35% of families with early onset breast cancer.[243-245] BRCA1 mutations also predispose to ovarian cancer; prostate cancer; and cancer of the fallopian tube, stomach, pancreas and colon.[250,251] In Ontario, 11% of women diagnosed with fallopian tube cancer between 1990 and 1998 were positive for BRCA1 mutations and 5% were positive for BRCA2 mutations.[252] The most common detected mutations in the BRCA1 gene and BRCA2 gene have a frequency of 1.09% and 1.52% respectively in the general Ashkenazi Jewish population.[253-255]

a)   Molecular testing

BRCA1 and BRCA2 gene mutations can be detected using clinically available techniques. FGS is considered the gold standard with the highest sensitivity, but it is also the most costly and time-consuming. PTT and SSCP are the two most common screening techniques used. Other techniques include CSGE, exon scanning (ES), DGGE, enzymatic mutation detection (EMD) method, DHPLC, TDGS, immunoassay (IA),[39,47,256-259] ASO testing and allele specific PCR and recently automated PCR allele discrimination assay[260-262] techniques can be used in cases with known mutations.

Several studies look at the clinical validity and analytical validity of molecular techniques in the detection of BRCA1 and BRCA2 mutations.[45-48,263,264] In addition to its high validity, DHPLC is useful in the identification of known polymorphisms in the BRCA1 and BRCA2 genes, avoiding the need for sequencing to detect these benign alterations.[265] IA using antibodies can identify BRCA1 and BRCA2 mutations with better analytical sensitivity.[47] PTT with complementary 5' sequencing can identify 100% of truncating BRCA1 mutations.[39] The detection of germline mutations in the BRCA1 gene has been achieved by RNA-based sequencing.[263] Data from the main studies on test validity are summarized in Table 2.

Table 2: Validity of molecular diagnosis tests for BRCA1 and BRCA2 mutations

Reference	Year	Test Characteristics	Test						
			PTT	SSCP	DHPLC	CSGE	TDGS	EMD	IA
Andrulis et al.[34]		Analytical sensitivity (%)	80 to 100	67	93		80	93	
Geisler et al.[40]	2001	Clinical sensitivity (%)	20.2	14.9					
		PPV (%)	100	26.4					
		NPV (%)	99.1	99.8					
Eng et al.[41]	2001	Analytical sensitivity (%)		65	100	60	91		
Byrne et al.[42]	2000	Analytical sensitivity (%)							100
		Analytical specificity (%)							75
		PPV (%)							90
		NPV (%)							100
Gross et al.[43]	1999	Analytical sensitivity (%)		96	100				

IA=Immunoassay; PPV=positive predictive value; NPV=negative predictive value; PTT=protein truncation testing;
SSCP=single strand conformation polymorphism; DHPLC=denaturing high performance liquid chromatography
CSGE=conformation sensitive gel electrophoresis; TDGS=two-dimensional gel scanning; IA=immunoassay
EMD=enzyme mutation detection

Allele-specific gene expression is a new mutation detection strategy. This is based on detection of a "nonsense-mediated RNA decay". It shows a significant discrimination between the carrier and the non-carrier for BRCA1 and BRCA2 genes.[266] Improvement in the efficacy of BRCA1 mutation detection is shown in one study using the morphoclinical features of BRCA1-associated breast cancers as mutation indicators.[267] Estimation of BRCA gene mutation probability is important for pretest counselling. It may be determined by expert cancer risk counsellors or by using a computer model.[268-270] Mutation detection by risk counsellors and a computer model shows similar sensitivity in identifying BRCA gene mutation carriers, but specificity is better using the computer model.[271]

In Ontario, the cost for PTT sequencing is C$1,200 for an unknown mutation and C$1,500 if additional sequencing must be done. For a known mutation, it is C$250. For an ethnic-specific screen, it is C$325 (Nancy Carson, Children's Hospital of Eastern Ontario, Ottawa: personal communication, 2003 September 05). The cost for FGS at a private laboratory is about US$2,600. Results are reported in two to three weeks. Data from a decision model show that BRCA gene testing seems to be cost-effective in high risk women, with incremental costs ranging from US$3,500 to US$4,900 per quality adjusted life year.[272]The cost of BRCA gene testing, with its associated ethical and legal issues, requires careful patient selection.

The sensitivity and specificity of methods used to detect BRCA1 and BRCA2 mutations affect the accuracy of the molecular diagnostic data. Although cancer geneticists worldwide manage patients based on the probability of the gene mutations, the accuracy of these data is poorly defined. FGS is the most sensitive screening technique, but it is technically demanding, costly and time-consuming, especially for large genes such as BRCA1 and BRCA2 with their numerous

exon sequences. FGS also cannot identify large genomic deletions, smaller mutations involving primer-recognition sites and intron-exon splice sites or branch-point mutations that can lead to exon skipping.[273] FGS is impractical on a population-wide basis. PTT and SSCP are the two most common screening techniques, but their analytical validity is unsatisfactory. PTT detects only alterations that lead to prematurely truncated protein products. SSCP, HD, DGGE, CSGE cannot differentiate between causative mutations and polymorphisms.[48,274-277] A blinded comparison of the methods used by laboratories to estimate the prevalence of BRCA1 mutation shows significant false-negative results, with varying sensitivity and specificity, even in expert laboratories (sensitivity ranging from 60% to 100%).[46]

Mutation screening of the coding regions of BRCA1 and BRCA2 in families with a high incidence of breast cancer, supplemented with linkage analysis, cannot find the expected numbers of mutations in these two genes, suggesting the existence of at least one more breast cancer predisposing gene.[278,279] Negative test results for BRCA1 and BRCA2 can indicate that there is no inherited predisposition to breast cancer (the test is truly negative); that the mutation is not detected because large deletions or genomically complex alterations are missed; or that there is another unidentified susceptibility gene.[280] At least two additional genes, TP53 in Li-Fraumeni syndrome and PTEN in Cowden syndrome, are mutated in families with inheritable breast cancer.[42] Unless a specific mutation has been identified in other family member(s), a negative test result for BRCA1 and BRCA2 may not be truly negative and it is uninformative in most cases.[281-283] On the other hand, a positive test result yields a more informed risk assessment, but it does not provide an individual prediction regarding disease occurrence, because penetrance is still poorly characterized for BRCA1 and BRCA2.[240,284,285]

b) *Impact of molecular testing on clinical management*

Genetic testing is part of standard clinical management for familial breast cancer 1 and 2. Genetic counselling with an informed discussion regarding the ethical, legal and psychosocial implications is essential before genetic testing. Data from an interview study suggest that women are most interested in participating in genetic testing when a test with a high positive predictive value can be followed by effective, non-invasive preventive therapy, a situation that does not apply in hereditary breast and ovarian cancer.[286] Women with high breast cancer risk due to a familial predisposition can choose between regular surveillance and preventive therapies.[287] A prospective study on BRCA mutation carriers shows that genetic counselling and testing increase cancer surveillance by physical examinations and imaging studies.[288] Surveillance programs for women with high risk family histories (women carrying BRCA1 and BRCA2 mutations are not treated separately in the analysis) can detect 75% of tumours.[289,290] In the studies involving only women who carry BRCA1 or BRCA2 mutations, however, the efficacy of cancer surveillance or other measures to reduce breast cancer risk is inconclusive.[291] Studies show that breast MRI may be superior to mammography and ultrasound for the screening of women at high risk for hereditary breast cancer.[292] Surveillance can detect more cases of cancer in BRCA1 and BRCA2 carriers than in age-matched non-carrier women.[293] Early breast cancer and ovarian cancer screening are recommended for individuals with BRCA1 mutations. Early breast cancer screening is recommended for those with BRCA2 mutations.[291] However, a recent study shows that mammography leads to significantly more (p=0.01) false negative results in carriers than in controls (62% versus 29%) despite comparable tumour size and breast density.[294] The image of

the carriers presented significantly (p=0.01) less often as a spiculated mass (which is the usual recognition pattern of malignancy of a breast tumour), because the specific histologic features affect the margins of the tumour, yielding a better defined image. This finding suggests that the interpretation of mammography results must be regarded with caution in mutation carriers.

Preventive therapies include surgical or chemical prophylactic treatment.[295] There are no recommendations for or against prophylactic surgery (e.g. mastectomy, oophorectomy, hysterectomy); these surgeries are an option for mutation carriers.[296,297] One study shows that of the unaffected women with an identified mutation, 51% opt for bilateral mastectomy and 64% for oophorectomy.[298] In Ontario, 16 women with BRCA 1 or BRCA2 mutations underwent prophylactic oophorectomy between 1992 and 1998.[299] Recent data suggest that bilateral prophylactic mastectomy and oophorectomy reduce the risk of breast and ovarian cancer in women with BRCA1 and BRCA2 mutations.[300-304] Data suggest that the age at diagnosis of the first BRCA1 breast cancer should be taken into account when prophylactic mastectomy is considered.[305,306] The number of cases of fallopian tube cancer reported in studies of BRCA1 and BRCA2 mutation carriers may lead to a recommendation for hysterectomy in addition to other surgical prophylactic treatment.[252,307] According to one group that compares prophylactic surgical strategies in BRCA1 mutation carriers using a computed model, prophylactic mastectomy and prophylactic oophorectomy are the most effective in increasing life expectancy.[308] According to the model, having prophylactic mastectomy with bilateral oophorectomy at age 30 years may extend survival by 4.9 years over surveillance alone in women carriers of BRCA1 and BRCA2 mutations.[309,310] Data from different groups, however, support breast conserving treatment and show no evidence of radiotherapy adverse effects in women with BRCA1 and BRCA2 mutations.[311,312] A decision analysis model in one study on an Ashkenazi Jewish population looking at the effects of genetic screening for three mutations on survival and cost-effectiveness[313] shows that genetic screening may significantly increase average survival (95% probability interval). The study also shows that genetic screening followed by any of the three prophylactic surgical strategies (oophorectomy, mastectomy, oophorectomy and mastectomy) for women who test positive improves cost-effectiveness compared with surveillance (genetic test, physical examination, gynecologic examination, ultrasound, CA 125 measurement, mammogram, Pap test). Genetic screening is potentially cost-effective compared to different surveillance strategies only if all women who test positive undergo prophylactic surgery.[313]

Chemopreventive strategies, such as tamoxifen, have been considered for women carriers of BRCA1 and BRCA2 mutations.[314-317] A small randomized, double-blind trial shows that tamoxifen, as expected with its antiestrogen property, significantly reduces breast cancer incidence among healthy estrogen receptor (ER)-positive BRCA2 carriers, but not among women with ER-negative BRCA1 mutations.[318] A case-control study also shows that tamoxifen may have a protective effect on risk of contralateral breast cancer.[319] A systematic review of trials of tamoxifen given for at least three years estimates its likely prophylactic effect, with a 13% reduction in risk of breast cancer in BRCA1 mutation carriers and a 27% reduction in BRCA2 mutation carriers.[320] The studies, however, do not address how long BRCA1 and BRCA2 carriers are expected to take tamoxifen as a cancer preventive agent. This raises the ethical question of whether to expose healthy women to the potentially harmful side effects of

chemotherapy. Decision model analysis in one study shows that for 30-year-old women with BRCA1 or BRCA2 associated breast cancer, tamoxifen increases life expectancy from 0.5 years (low-penetrance mutations) to 1.3 years (high-penetrance mutations).[321] The effect of oral contraceptive use in reducing the risk of ovarian cancer in women with BRCA1 and BRCA2 genes mutations is unclear.[322,323] Women who are carriers of the BRCA1 and BRCA2 mutations and who have full-term pregnancies are more likely to develop breast cancer by age 40 than nulliparous carriers.[44]

Breast cancer patients with the same stage of disease can have different treatment responses and overall outcomes. Known predictors for metastases such as lymph node status and histological grade often fail to predict outcomes. Low BRCA2 mRNA levels may predict a good response to docetaxel treatment in breast cancer patients.[324] Efforts have been made to find a genotype-phenotype correlation or a gene expression profile of women with familial breast cancer that can be used as a predictor for clinical outcome. Ten-year follow-up data show there is a trend toward a worse prognosis for BRCA1 carriers whose tumours also overexpress the tumour suppressor gene p53.[325] Studies identify a gene expression profile that is strongly predictive of a short interval to distant metastases ("poor prognosis" signature). A signature that identifies tumours of BRCA1 carriers has been established.[326] BRCA1 tumours tend to be invasive, high grade, estrogen receptor negative (poor prognostic features), with a significant risk of contralateral breast cancer, but data on their clinical aggressivity are controversial.[327-330] Germline BRCA1 and BRCA2 mutations can independently predict outcomes after breast cancer.[331-334] In young women carriers with BRCA1 or BRCA2 mutations who develop breast cancer and who undergo breast-conserving surgery (lumpectomy) followed by radiotherapy, there are higher rates of ipsilateral and contralateral breast cancer at 12 years of follow-up compared with women with sporadic breast cancer. The management of healthy women carriers of BRCA1 and BRCA2 mutations should take into account the fact that the gene expression profile and genotype-phenotype correlations can provide a strategy to select patients who would benefit from preventive therapies. Individualized psychological support may be needed.

4.3.9 Hereditary multiple exostoses

Hereditary multiple exostoses (HME) is a rare disorder in which the clinical hallmark is the growth of bony protuberances (exostoses) from long bones resulting from dysplasia of the growth plate. Exostoses can be associated with a reduction in skeletal growth, bone deformation, restricted motion of joints, short stature and premature osteoarthrosis.[335] The most serious potential problem is malignant degeneration of exostoses. The disorder is transmitted in families as an autosomal dominant trait. Penetrance is about 95%. The proportion of individuals with HME who have clinical findings increases from about 5% at birth to 96% at age 12 years.[52] The median age of diagnosis is three years; nearly all patients are diagnosed by 12 years of age. The risk for malignant degeneration to osteochondrosarcoma increases with age, although the lifetime risk of malignant degeneration is around 1%.[336,337]

a) *Molecular testing*
Molecular testing for HME is available only on a research basis. Three loci on chromosomes 8q24.11-q24.13 (EXT1), 11p12-p11 (EXT2) and 19q (EXT3) have been identified through linkage analysis as being involved in the development of HME. EXT3 has not yet been cloned,

but EXT1 and EXT2 have been cloned and sequenced. At least 49 mutations have been described in EXT1 and 25 in EXT2 genes.[49,338] Most mutations lead to a truncated or non-functional EXT1 protein. Mutation screening of the EXT1 and EXT2 genes in families with HME done by CSGE found mutations in 70% of families.[53] Similar mutation detection rates have been achieved using fluorescent SSCP (F-SSCP) and DHPLC (80% to 93% and 95% respectively).[50] Of multiple exostoses families, 60% have shown linkage to the 8q24 region.[339]

Mutation detection data vary between studies. A study using F-SSCP and DHPLC techniques shows that 76% of families with the mutation carry it in the EXT1 gene and 24% of them carry it in the EXT2 mutation gene.[50] This is in contrast to reported EXT1/EXT2 mutation frequencies in two other mutation detection studies, 58% and 42%; and 50% and 50% using CSGE[53] and SSCP[51] respectively.

b)   *Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for HME has been demonstrated, but it is not part of standard management. The variable manifestations of HME may result from the different effects of different mutated genes. A recent review suggests that the chromosomal linkages of HME may predict the location, percentage of involvement and type of osteochondromas.[340] The HME patients whose responsible mutation is on chromosome 8 have radiographic and clinical features that are distinct from those in patients with non-chromosome 8 mutations. The percentage of sessile bony lesions (which cause more damaging effects than pedunculated lesions) and the severity of the angular deformity are statistically different between the two groups ($p<0.00001$) in favour of the non-chromosome 8 group. This genotypic-phenotypic correlation would help in the use of molecular diagnostic testing for optimum management and a better prognosis for HME.

4.3.10 Hereditary non-polyposis colon cancer

Hereditary non-polyposis colorectal cancer (HNPCC) is a common syndrome characterized by an autosomal, dominantly inherited predisposition to early onset colorectal cancer (mean age 44 years), an increased risk of uterine endometrial cancer and an increase in incidence of other cancers. It is reported to be the most common form of inherited colorectal cancer.[341,342] HNPCC is diagnosed on the basis of the pattern of cancer in the family according to the Amsterdam or Bethesda criteria,[13,343] the fulfilment of which might indicate a high frequency of MSH2 and MLH1 mutations.[344-346]

HNPCC is caused by germline mutations in five DNA repair genes: MSH2, MLH1, PMS1, PMS2 and MSH6. Mutations range from truncation to splice site loss, missense mutations and small deletions.[116,347] Mutations in MSH2 and MLH1 account for the majority of HNPCC[348] in 28% of patients diagnosed with colorectal cancer at <30 years of age.[349] Mutations in MSH2 and MLH1 genes have been identified with a higher rate in early onset colorectal cancer patients with high risk family history than in those without.[350] MSH2 and MLH1 protein expression analysis by immunohistochemical methods (IHC) shows that MSH2 and MLH1 protein loss is an early event in endometrial carcinogenesis.[351]

a)   *Molecular testing*

MLH1 and MSH2 molecular testing for HNPCC is clinically available. Screening for mutations of the MSH2 and MLH1 genes in HNPCC patients and their relatives has been done using methods that include DGGE, RT-PCR, PTT, SSCP, DHPLC and microsatellite instability (MSI) analysis.[352-358] DGGE can detect small base sequence alterations with >90% sensitivity; it is suited to detect splice site, missense, nonsense and frame-shift mutations. RNA-based methods, such as RT-PCR and PTT, are suited to detect large deletions, which are rare in HNPCC.[359] DHPLC analysis for MSH2 and MLH1 mutations initially found by sequence analysis shows that it is a highly sensitive (analytical sensitivity approaching 100%) and cost-effective technique.[360] Most programs in Ontario have acquired a DHPLC machine, so this may improve the turnaround time for testing (i.e., one month for most mutations).

Immunohistochemistry (IHC) and microsatellite instability (MSI) are two pre-screen methods used on tumour tissue. Immunohistochemistry (IHC) for MLH1 and MSH2 expression can be a rapid method for pre-screening tumours for mutations in NHPCC.[361,362] More than 90% of colorectal cancers of patients with MSH2 and MLH1 mutations show change-of-length mutations in multiple nucleotide repeat sequences. This is known as high-frequency microsatellite instability (MSI-H).[363,364] MSI-H has been identified in 43% of families enrolled in the high-risk colorectal cancer registry. Germline MSH2 and MLH1 testing using DGGE reconfirmed mutation in 50% of the MSI-H carriers.[365] One strategy is to first perform a MSI assay on the affected family members with colorectal tumour. If the tumour exhibits MSI, then germline testing may be considered for MSH2 and MLH1 mutations, for which commercial molecular testing is available.[366]

In Ontario, the cost for sequencing MSH2 or MLH1 genes is C$1,500 each for an unknown mutation and C$250 for a known mutation (Nancy Carson: personal communication, 2003 September 5).

b)   *Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for HNPCC has been demonstrated, but it is not part of standard management. The identification of mutations in families fulfilling clinical criteria makes genetic testing an important option in the management of HNPCC.[347] Morbidity and mortality can be reduced in individuals at the risk for HNPCC by using early and intensive screening.[367] In patients who are carriers of mutations associated with HNPCC, prophylactic colectomy is the most effective method of reducing the risk of colorectal cancer. A decision analysis model shows that the benefits of intervention range from 13.5 years of gains in life expectancy for endoscopic surveillance to 15.6 years for prophylactic colectomy compared with no intervention.[368]

4.3.11 Li-Fraumeni syndrome

Li-Fraumeni syndrome (LFS) is a rare (less than 300 families worldwide) cancer predisposition syndrome associated with soft tissue sarcoma; breast cancer; leukemia; osteosarcoma; melanoma; and cancers of the colon, pancreas, adrenal cortex and brain.[54] It involves early onset cancer. Malignancies can occur in both children and adults.[369,370] The lifetime cancer risk is about 85% to 90%; it is higher for women than men because of the high breast cancer risk.[13,54]

LFS is an autosomal dominant disorder. About 70% of patients diagnosed clinically have an identifiable mutation of the TP53 (also known as p53) gene (17p13). TP53 encodes the TP53 protein, which plays a crucial role in cell repair, apoptosis and genomic stability.

a) Molecular testing

Germline mutations of p53 can be detected by direct sequence-based DNA testing that is clinically available in a limited number of laboratories and is often confined to exons five to eight. A chip-based DNA sequencing assay is also available through research testing only. The mutations are mostly point mutations involving exons five through eight and nonsense and splice site mutations. The analytical sensitivity of FGS of p53 gene is 98%, of exons five to eight scanning 80% and of chip-based sequencing assay 90%.[54] The clinical sensitivity of current technologies is 70%. Information regarding the cost of LFS is unavailable.

Most p53 mutations occur in exons five through eight, but germline mutations can be found through the entire gene, including coding and non-coding exons in LFS families. The presence of mutations is verified by sequencing the complementary strand and by restriction digest analysis.[371] As a result, analysis of the entire gene is essential when screening LFS families.

Direct sequencing of all exons of the p53 gene cannot find germline mutations in around 30% of 21 LFS families.[372] This raises the question whether there are phenotypic differences between families with and those without identifiable p53 mutations.

b) Impact of molecular testing on clinical management

The clinical benefit of genetic testing for LFS is unclear. In a study on p53 gene mutation carriers among LFS families, more than half of the cancers and nearly a third of the breast cancers were diagnosed before 30 years of age.[373] The young age at onset of breast cancers in carriers of p53 germline mutations leads to a need to develop screening procedures for breast cancer (annual mammography and clinical examination of the breast every six months)[54] in asymptomatic carriers of germline p53 mutations by age 25 to 30. The use of mammography, however, is controversial given an increased sensitivity to x-rays in those with germline p53 mutations.[374] In addition, experts suggest that affected individuals should have CT scans of the head and abdomen and annual blood cell counts with a manual review of peripheral blood smears for leukemia.[10]

4.3.12 Multiple endocrine neoplasia type 1

Multiple endocrine neoplasia type 1 (MEN1) is a familial syndrome inherited in an autosomal dominant pattern.[375] The predisposing genetic defect in MEN1 has been located by linkage analysis.[57] The MEN1 gene (11q13) has been cloned, sequenced and identified as a gene that may be involved in the development of the familial cancer syndrome of MEN1.[376-378] MEN1 gene encodes menin, a protein that binds to JUND, which is a transcription factor. Individuals in affected families are predisposed to a varying combination of neoplastic lesions of the endocrine glands: parathyroid (95% of affected individuals), pancreas (73%), pituitary (44%) and adrenals (16%).[55,56,379] The penetrance of MEN1 is high, with about 99% of carriers of a mutant gene

affected by the fifth decade of life.[56] Symptoms largely depend on the substance secreted by the neoplastic tissue, but 90% of patients present with hypercalcemia, peptic ulcer, hypoglycemia and complaints referable to a pituitary mass.

a)   Molecular testing

Genetic testing for MEN1 is clinically available. Methods used to detect mutations in the MEN1 gene include DNA sequencing, LA and SSCP, with SSCP being able to detect 85% of mutations and LA 99.5% (analytical sensitivity).[56,57] A combination of SSCP and HD has been shown to achieve 100% analytical sensitivity.[380] Frameshift, nonsense, missense and in frame deletion mutations have been reported. Genetic mapping studies that investigated MEN1-associated tumours show a loss of heterozygosity in 70% of tumour tissue samples studied.

A diversity of germline mutations in the coding region of the MEN1 gene has been found in familial MEN1 and in sporadic MEN1.[377,381] Correlations between the MEN1 mutations and the clinical manifestations of the disorder seem to be absent.[382] Between 5% to 20% of MEN1 patients may not have mutations in the coding region of the MEN1 gene.[56,377,382,383] Mutation analysis for diagnostic purposes is time-consuming and expensive, because the spectrum of germline mutations has an apparent lack of genotype-phenotype correlations.

In the UK, the cost for testing MEN1 is £420 for an unknown mutation (C$1,050) and £78.8 (C$197) for a known mutation with a turnaround time of one to eight weeks.[384]

b)   Impact of molecular testing on clinical management

The clinical benefit of genetic testing for MEN1 has been demonstrated, but it is not part of standard clinical management. Genetic screening, which includes phenotypic screening (medical history, clinical examination, x-ray of the sella turcica for the pituitary, blood biochemical profile) and DNA analysis, has confirmed linkage with the MEN1 gene in affected members, while excluding others.[385,386] Thus, genetic screening is useful in drawing attention to susceptible subjects.

Their family history (onset of affected phenotype, age-related penetrance of the syndrome) reveals that most affected subjects would develop one or several endocrine tumours before the age of 30 years. The combined use of genetic and specific organ screening has reduced the age at which clinical organ involvement is detected in MEN1 to the late teens rather than the fourth decade.[387]

4.3.13 Multiple endocrine neoplasia type 2

Multiple endocrine neoplasia type 2 (MEN2) is a rare genetic syndrome transmitted as an autosomal dominant trait. It includes two subtypes: MEN2A and MEN2B.[105-107] MEN2A is characterized by a combination of medullary thyroid carcinoma, pheochromocytoma and benign parathyroid tumours. The age of onset of clinical symptoms of MEN2A occurs from childhood to late adulthood. MEN2B is characterized by medullary thyroid carcinoma; pheochromocytoma; multiple mucosal neuromas of the tongue, lips and eyelids; ganglioneuromas of the gastrointestinal tract; and musculoskeletal and ocular abnormalities. The age of onset of MEN2B

is earlier than that of MEN2A with a generally faster rate of progression and a more complicated course. The predisposing gene for MEN2 is the oncogene RET (10q11.2), a tyrosine kinase receptor, the mutation of which results in constitutive activation of the tyrosine kinase (MEN2A) or alteration of substrate specificity (MEN2B).[107,109] In addition to MEN2A and MEN2B, mutations in the RET oncogene are responsible for familial medullary thyroid cancer (FMTC)[388] and Hirschsprung's disease.[389,390]

a)   Molecular testing

Molecular testing for MEN2 is clinically available. Predictive testing for the inheritance of mutant alleles in individuals at risk for MEN2 was first performed with the application of linked DNA markers[391] and later improved using flanking dinucleotide repeats and restriction fragment length polymorphisms (RFLP).[392] Methods used to detect mutations in RET include DNA sequencing, PCR with restriction enzyme digestion of PCR products,[110,393] heteroduplex analysis,[111] SSCP[113,394] and PCR-based DGGE.[115,395] These tests have a sensitivity of >80% in detecting RET mutations.

In the UK, the cost for sequencing exons 10 and 11 (for MEN2A) and exons 15 and 16 (for MEN2B) is £105 (C$262) for a new mutation and £78.8 (C$197) for a known mutation.The turnaround time is from one to eight weeks.[384] The cost for scanning exon 16 of the RET gene for mutation detection in MEN2B at a private laboratory using PCR followed by DNA sequencing is US$300. Prenatal diagnosis using two samples (chorionic villus sampling, fresh amniocytes or cultured amniocytes) is US$700. The turnaround time is about three to four weeks for a new patient and two weeks for prenatal diagnosis for a known mutation.[104]

b)   Impact of molecular testing on clinical management

Genetic testing is part of standard clinical management for MEN2. Mutation analysis is useful to identify at-risk individuals in mutation-positive families; to find a mutation in members of a family with clinically apparent MEN2; to determine if an apparently sporadic case of medullary thyroid carcinoma or pheochromocytoma is the presenting lesion in a patient with MEN2; and to find a genotype-phenotype correlation that could help in managing the disorder.

Genetic testing for at-risk individuals is an established clinical procedure.[396] For all patients at risk or suspected of having MEN2, taking into consideration the limitations of biochemical screening,[397] molecular testing for RET mutations as early as possible is the first choice for a screening procedure.[114] For those who test positive, planning for prophylactic thyroidectomy should be considered and additional screening for pheochromocytoma and hyperparathyroidism is indicated. Prophylactic thyroidectomy is preferably done early, as medullary thyroid cancer in MEN 2B is aggressive with early metastatic spread.[112,398]

Molecular testing plays an important role in determining which of the apparently sporadic cases have heritable disease. For patients with apparently sporadic medullary thyroid carcinomas, retrospective data on MEN2A cases have shown that pheochromocytoma occurred two to 11 years subsequent to medullary thyroid carcinoma in more than 40% of these patients.[399] Thus, RET mutation analysis must be performed in patients with familial or sporadic medullary thyroid cancer.[108] The limited range of mutations makes this technically feasible in most cases. Where

there is no family history and no evidence of hyperplasia on the thyroidectomy specimen, a failure to find a mutation in exons 10, 11, 13, 14 and 16 of RET excludes MEN2A with >99% probability and if there is no abnormal phenotype, MEN2B too.[400]

Different mutations in the RET gene give rise to the different clinical types of MEN2, suggesting a genotype-phenotype relationship.[401] There is a statistically significant association (p<0.001) between the mutations at codon 634 and a phenotype that includes pheochromocytomas or hyperparathyroidism.[400] Mutations at codons 768 and 804 are only seen with familial medullary thyroid carcinoma, thus predicting that these patients would not develop pheochromocytoma or parathyroid disease. Codon 918 mutation is MEN2B-specific.[400] The association is too weak for prophylactic surgery to be offered to those with high risk mutations or to omit screening for those without the mutations.

4.3.14 Neurofibromatosis type 1

Neurofibromatosis type 1(NF1) (also known as von Recklinghausen disease) is one of the most common autosomal disorders (prevalence 1/960 to 1/7,800). The NF1gene (17q11.2) is responsible for NF1. The protein product of NF1 is neurofibromin, which down-regulates the proto-oncogene p21-RAS. The mutation of NF1 leads to a loss of neurofibromin, resulting in the activation of the p21-RAS oncogene and uncontrolled cell growth.[62] Of the mutations, 70% to 80% result in a truncated protein. NF1 is characterized by multiple neurofibromas, which may degenerate into malignancy (neurofibrosarcoma), café au lait spots, axillary and inguinal freckling, optic gliomas and Lisch nodules of the iris. The clinical manifestations are variable[402,403] with features that include short stature, macrocephaly, learning disabilities and seizures. NF1 can present at any age in any organ system. The penetrance of NF1 is almost 100% at the age of five years and new mutations represent half of the cases. The diagnosis of NF1 is made via clinical assessment.[64,404]

a)   *Molecular testing*

Molecular diagnostic testing of the NF1 mutation using PTT is clinically available. Several methods, including SSCP, DHPLC, DGGE and direct sequencing, have been used to identify mutations in the NF1 gene, with DHPLC having an analytical sensitivity of 97% and a clinical sensitivity of 68%.[58,59] With the PTT technique, RNA from white blood cells is reverse transcribed and converted into overlapping NF1 complementary DNA fragments that serve as templates for the synthesis of neurofibromin fragments in vitro. Each of the resulting neurofibromin fragments is separated by size on a denaturing polyacrylamide gel to assay for the presence of a truncated peptide. The PTT has a clinical sensitivity of 67% to detect pathogenic mutations in the NF1 gene and an analytical sensitivity of 93%.[65]

Approaches to identify disease-causing mutations in individuals affected with FN1 have been limited, owing to the large size of the NF1 gene (59 exons) and the diversity of mutations. Identification of mutations in individuals with NF1 is based on techniques that are time-consuming and expensive. In spite of the prevalence of NF1 and accumulated knowledge in molecular science, the understanding of the pathogenesis of the disorder remains limited. One perplexing feature of NF1 is its clinical heterogeneity, which a mutation of NF1 gene cannot explain. It is unlikely that diagnosis of NF1 could be excluded by using the molecular approach.[405]

b)   *Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for NF1 has been demonstrated, but it is not part of standard management. Even though 70% to 80% of NF1 gene mutations result in a truncated protein, about 10% of the mutations result in entire gene deletions, which are associated with mental retardation or learning disabilities, facial anomalies and a broad neck.[406] The sensitivity, specificity and PPV of the commercially available PTT have not been reported for a large patient group.[60] Prenatal diagnosis is often available for pregnancies in which one of the parents has NF1. Prenatal diagnosis for NF1 can be performed by direct analysis if a specific mutation has been identified in the family or by linkage analysis if enough family members are available for testing and the family provides enough genetic information for linked markers.[407] The inability to predict the severity of NF1 limits the usefulness of prenatal screening.

4.3.15 Neurofibromatosis type 2

Neurofibromatosis type 2 (NF2), or bilateral acoustic neurofibromatosis, is a hereditary disorder characterized by the development of multiple benign and malignant nervous system tumours - meningiomas, schwannomas, neurofibroma - in young adulthood and transmitted in families in an autosomal dominant pattern with complete penetrance by the age of 60.[61,408,13,63,409] The NF2 gene (22q12) encodes a protein, merlin, which is one of the cytoskeleton-associated proteins that help maintain the cell's shape and membrane stability.[410] Almost all affected individuals become hearing impaired due to the presence of bilateral schwannomas of the vestibular nerves by the age of 30. Malignant gliomas have been reported in people with NF2, but the cancer risk may not be increased. Studies suggest that there is a population incidence of one in 37,000, with 50% of cases being due to new mutations.[67]

a)   *Molecular testing*

Molecular testing for NF2 is clinically available. Molecular testing for NF2 comprises two DNA-based tests: mutation analysis of the NF2 gene and LA. Molecular detection methods for NF2 gene include SSCP, DGGE, direct sequencing and the RNA mismatch cleavage method.[66,69] Mutation analysis identifies germline mutations of the NF2 gene in 64% of patients by using exon scanning via single-strand conformation polymorphism (SSCP) followed by direct sequencing.[68] A new assay using an RNA mismatch cleavage method has shown an analytical sensitivity of 100% and a clinical sensitivity of 75% in detecting NF2 gene mutations.[69] Linkage studies are based on an accurate clinical diagnosis of NF2 in the affected family members and accurate understanding of genetic relationships in the family. Misleading linkage of NF2 families results may be obtained owing to the high rates of mosaicism in founders (i.e. the first person in the family to have NF2).[411]

Just like NF1, NF2 remains a clinical diagnosis. Mutation detection is time-consuming and expensive and may not reveal the causative mutation. Standard techniques such as SSCP can detect two-thirds of NF2 gene mutations. Confirmation of the NF2 gene mutation in individuals with positive results from an SSCP test requires direct DNA sequencing. Molecular testing of children at risk is problematic as it is unclear whether childhood protection will improve final outcomes.

32

b) *Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for NF2 has been demonstrated, but it is not part of standard management. Management strategies for NF2 are multidisciplinary.[412-414] Since it is unclear whether NF2 increases the risk of cancer development, no routine monitoring is recommended. A genotype-phenotype correlation would help in the prognosis and management of patients with NF2. Although there is no difference in disease course between males and females, there is a difference in natural history depending on whether the NF2 gene is inherited from the mother or father. Individuals with maternal inheritance of the NF2 gene have an earlier age of presentation (18 years versus 24 years in paternally inherited cases, p=0.027) and have a more severe course (the average age of death is 24 years versus 39 years in paternally inherited cases).[67] Correlations between germline mutations of the NF2 gene and NF2 severity and retinal abnormalities have been found. In one study, patients with nonsense or frameshift mutations were younger at onset and had a higher mean number of tumours (p≤0.05) than those with splice site mutations.[415] This supports the correlation between nonsense and frameshift muations and severe NF2.

4.3.16 Peutz-Jeghers syndrome

Peutz-Jeghers syndrome (PJS) is a rare disorder inherited in an autosomal dominant pattern. It is characterized by a combination of gastrointestinal polyposis and mucocutaneous pigmentation. Comparative genomic hybridization and target linkage analysis have linked PJS to mutation in the STK11 gene (19p13.3), which is one of the serine threonine kinase genes.[71,72,416] Mutations include intragenic rearrangements and deletions, nonsense mutations and splice site mutations. About 50% of patients have an affected parent and about 50% seem to be sporadic. Polyps can occur throughout the gastrointestinal tract (most commonly in the small intestine) and can turn malignant. Pigmented spots, which typically present in infancy and childhood, occur mainly on the lips and buccal mucosa. They are present in over 95% of cases. Individuals with PJS demonstrate a high risk for gastrointestinal cancers and cancers of other organs such as the breast, uterus, ovary, lung and testes.[13,70,73,417-419]

a) *Molecular testing*

Molecular testing for PJS is clinically available. Exon screening and sequence analysis of genomic DNA obtained from buccal swabs can detect STK11 gene mutations. The cost for mutation detection in a new patient at a private laboratory is US$1,400 and for relatives of a person with a known mutation, US$350.[74] Prenatal diagnosis using two samples (chorionic villus sampling, fresh amniocytes or cultured amniocytes) is US$700. For testing a new patient, the turnaround time is about six to eight weeks. For prenatal diagnosis, where the mutation in the family is known, the turnaround time is about two weeks.[74]

DNA sequencing has an analytical sensitivity of about 70% in families with linkage to the STK11 gene. End-to-end sequencing of STK11 has a clinical sensitivity that can detect 70% of probands with a family history of PJS.[75] For patients without a family history of PJS, CSGE followed by direct sequencing can detect 17% of the patients.[76] These data suggest the presence of genetic heterogeneity for PJS and the involvement of other loci.

b)   *Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for PJS is unclear. Linkage analysis can be used to identify non-carriers in large families that are linked to the STK11 gene. One source recommends that those with a mutated STK11 gene (and those with clear-cut PJS pigmentation) should be entered into screening programs for gastrointestinal polyps and cancers of the breast, ovary and uterus.[420]

4.3.17 Retinoblastoma

Retinoblastoma (RB) is a childhood cancer of the retina. It affects about 1/20,000 live births in developed countries: 60% of cases are unilateral and sporadic, 15% are unilateral and hereditary and 25% are bilateral and hereditary. The hereditary predisposition to RB results from germline mutations of the tumour suppressor gene RB1 (13q 14.1) transmitted as an autosomal dominant trait with high penetrance.[79-82] The most common presenting sign of RB is a white spot in the eye (leukocoria), followed by strabismus. Patients with deletion of the 13q chromosome and involvement of the 13q14 band have a distinct dysmorphic phenotype and neurological impairment.[421] Most affected children are diagnosed under the age of five years. Patients with germline mutations of RB1 gene are at an increased risk of developing tumours outside the eye, such as osteosarcoma, Ewing sarcoma, leukemia, lymphoma, melanoma, lung cancer and bladder cancer.

a)   *Molecular testing*

Molecular testing for RB is clinically available. Molecular testing for germline mutations in the lengthy (27 exons) RB1 gene has been challenging. Laboratories that perform a combination of techniques for mutation detection cannot provide 100% detection sensitivity. Hereditary RB diagnosis is still based on clinical criteria.[13]

The classical approach for the detection of a partial deletion in the RB1 gene is SB, which can detect the mutation in 10% of patients. For point mutation detection, screening methods based on multiplex fluorescent DNA fragment analysis, followed by direct sequencing, have a clinical sensitivity of 70%.[78] HD and non-isotopic SSCP detect 72% of the mutations.[84] A two-dimensional gene scanning method, combining PCR amplification and DGGE, provides an affordable alterna-tive to sequencing.[77] Deletions involving polymorphic loci can be detected by linkage analysis if the constellation of alleles in the parents is informative. Recently, a strategy that combines quantitative multiplex PCR, double-exon sequencing and promoter-targeted methylation-sensitive PCR has been shown to be a sensitive and efficient strategy to identify RB1 mutations.[422]

In Ontario, the cost for sequencing the RB1 gene is C$3,700. The cost comparison of molecular (DNA extraction, fragment analysis, sequencing, genetic counselling) versus conventional screening (regular complete retinal examinations) of relatives at risk for RB in 1994 shows a significant saving using the molecular route.[423] A report in 2003 shows that RB1 molecular testing accounts for a mean saving of C$6,591 per family in health care spending in a representative sample of 20 Ontario families.[422]

b)   *Impact of molecular testing on clinical management*

Genetic testing is part of the standard clinical management for RB. RB is a good example of a molecular diagnosis that saves health care dollars. Children without the mutation can avoid

invasive procedures and those with positive results can receive frequent ophthalmologic evaluations and be promptly treated. Since children with isolated unilateral RB might be the first evidence of a low penetrance RB1 mutation in the family,[424,425] molecular testing may be valuable for patients with unilateral RB. A recent study on families who underwent RB1 genetic testing suggests that genetic information and counselling are important and the long-term negative effects of genetic testing are rare.[83] The experimental application of gene therapy for RB shows that the transfer of the herpes simplex virus thymidine kinase gene into retinoblastoma cells increases the sensitivity of the cells to gancyclovir and acyclovir in vitro,[426] suggesting the possibility of gene therapy for RB.

4.3.18 von Hippel-Lindau syndrome

Von Hippel-Lindau syndrome (VHL) is an autosomal dominant inherited disorder predisposing patients to the development of cerebral, retinal and renal cell carcinomas; spinal hemangioblastomas; and pancreatic cysts. Cerebral hemangioblastomas are associated with headache, vomiting, gait disturbances or ataxia. Retinal hemangioblastomas may be the initial manifestation of the VHL syndrome and can cause vision loss. Renal cell carcinomas occur in 40% of patients and are the leading cause of mortality.[86] VHL syndrome is divided into two subtypes: VHL type 1 (affected individuals do not develop pheochromocytoma) and VHL type 2 (affected people develop pheochromocytomas).[13] VHL syndrome is often diagnosed when patients are in their 20s, although childhood symptoms, usually retinal hemangioblastomas, can occur. In one study, VHL occurs in 40% of individuals with hemangioblastoma.[427] VHL syndrome predisposing tumour suppressor gene VHL (3p25) encodes a protein that seems to inhibit transcription elongation. Loss of the protein may allow for unregulated cell growth. Mutations include deletions; insertions; and missense, nonsense and splice site mutations.[88,90]

a)   Molecular testing

Molecular genetic testing is clinically available for cases that meet the clinical criteria for VHL syndrome.[87] SB and quantitative SB are used to detect partial and complete gene deletions respectively and sequence analysis is used to detect point mutations. Deletions detected by quantitative SB are verified by FISH.[96] Parallel temperature gradient gel electrophoresis (TGGE) may provide a rapid mutation detection method for the screening of a large number of samples with high analytical sensitivity.[95] DHPLC has been introduced as a high throughput method to detect gene mutations. In one study, DHPLC and fluorescent SSCP have comparable high analytical sensitivity (95% to 100%) and specificity (100%) in detecting VHL mutations.[92] DHPLC using the Wave Nucleic Acid Fragment Analysis system was able to detect 93% of the VHL carriers in affected families for which specific mutations have been identified.[94] Direct sequencing can detect 100% of mutations, but it presents a financial burden in high throughput facilities. Comparative sequence analysis has shown its potential as a rapid and accurate method (analytical sensitivity and specificity 100%) to detect VHL mutations.[78]

The cost of molecular analysis of VHL syndrome using direct sequencing was US$260 in 1998. The cost of yearly screening (ophthalmologic examination, urinary catecholamine measurements) is US$650 (excluding time lost from work). Because the onset of symptoms occurs as early as five years and as late as 25 years, 20 years of regular follow-up is included in the calculation. The cost for 20 years of screening is $13,000. If the molecular testing is truly negative, the cost savings is over US$12,000 per person over 20 years.[89] In another study looking at the impact of molecular

genetic analysis of the VHL gene in patients with cerebral hemangioblastomas,[428] the cost of molecular diagnosis using SB and SSCP is 960 euros (C$1,500) or if sequencing is necessary, 1,070 euros (C$1,700). The cost of the clinical screening program in Germany, including magnetic resonance imaging (MRI) of the brain, the spinal canal and abdomen; ophthalmological examination; fluorescent angiography of the retina; and 24-hour urinary catecholamine excretion, is 2,570 euros (C$4,100) per year.[428]

b)   *Impact of molecular testing on clinical management*

Genetic testing is part of the standard clinical management for VHL. In addition to allowing accurate predictive testing, the characterization of germline VHL mutations may also provide information about the likely phenotype. This will help in the management of patients with VHL. Genotype-phenotype correlations in VHL syndrome have been described.[429-432] Germline mutations predicted to inactivate the VHL protein are associated with renal carcinoma and central nervous system hemangioblastomas without pheochromocytoma (VHL type 1). Germline mutations predicted to produce full-length VHL proteins are associated with pheochromocytoma in addition to the other manifestations of VHL (VHL type 2). Large deletions and mutations predicted to cause a truncated protein are associated with a lower risk of pheochromocytoma than missense mutations. Missense at codon 167 is associated with a high risk of pheochromocytoma (53% and 82% at 30 and 50 years respectively). As codon 167 mutations occur frequently, testing for this mutation in families with pheochromocytoma could be useful. Molecular testing for VHL, in addition to providing key information, enables screening for extra-neurological tumours of a patient and investigation of the patient's family. A consensus meeting in 1998 in the Netherlands, using an interactive voting system, reported that 56% of participants preferred molecular testing to be done before the age of five, 15% between the ages of five and 10 and 18% at an age where children can make their own choice.[433]

4.3.19 Wilms' tumour

Wilms' tumour (WT) or nephroblastoma is a childhood kidney tumour. WT, which occurs in one in 10,000 children, is the most common solid tumour in childhood. It is usually diagnosed at the age of five.[100] WT is rarely diagnosed in adult patients.[101] It is estimated that 1% of WT cases result from a gene mutation inherited from a parent. Hereditary WT is due to germline mutations of the tumour suppressor WT1 gene (11p13), WT2 gene (11p15.5) and WT3 gene (17q12-21), which are transmitted in an autosomal dominant pattern.[98,99] Loss of heterozygosity of 16q is a structural change seen in about 20% to 30% of WT cases.[434] The WT1 gene encodes a protein that is a transcription factor.[97] Certain point mutations in the WT1 gene produce severe genitourinary malformation, with pseudohermaphroditism and mesangial sclerosis of the kidney that may lead to renal failure in early childhood (Drash syndrome).[102] Unlike other tumour suppressor genes that do not manifest a loss of function in the heterozygous state, hemizygosity for WT1 causes developmental anomalies of the genitourinary tract, including cryptorchidism and hypospadias.

a)   *Molecular testing*

Genetic testing for WT is clinically available. WT1 mutations are screened by DNA sequencing including the intron-exon boundaries (Ann Dalton, Sheffield Children's hospital, Sheffield,UK: personal communication, 2002 July 11). The analytical sensitivity of the technique is claimed to be at least 90%. When a new change is identified, it has to be classified as a common polymorphism or

not, in the local population. When a change is close to the intron-exon boundary, a loss of splice site is also investigated. The cost for direct DNA sequencing is £500 (C$1,250) for full mutation screen of all exons (one to 10). The cost for screening exons six to nine (where most mutations occur) is £200 (C$500). The turnaround time varies and can be four to five months for the full screen. SB and MSI used for DNA analysis in sporadic WT cases show the involvement of two 1p regions in the etiology of Wilms' tumours.[435]

### b)	*Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for WT is unclear. There is a correlation between the loss of heterozygosity of 16q and the survival rate. Statistical analysis reveals significant differences in the occurrence of failure after treatment between the children with and without loss of heterozygoty 16q. Loss of heterozygosity 16q was present three times as often in tumours of dead compared with living children (p=0.0024).[434]

4.3.20 Xeroderma pigmentosum

Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder caused by an inability to repair DNA that is damaged by ultraviolet radiation. XP is characterized by extreme sensitivity to sunlight, resulting in pigmentary anomalies and a high incidence of skin cancer on sun-exposed areas.[135,436,437] The risk for basal and squamous cell carcinomas is thought to be 2,000-fold above that for the normal population. The average age for developing skin cancer is eight years (compared with age 60 in the general population). About 20% of affected children also demonstrate neurological abnormalities, including mental retardation, microcephaly and spasticity. Survival is reduced because of neoplasm, with only 70% of people with XP living to age 40.[135] Diagnosis is usually made on the basis of clinical features during childhood. There are a few laboratories that can confirm the defective DNA excision repair mechanism.

### a)	*Molecular testing*

Molecular testing for XP is available on a research basis only. There are seven mutated genes, from XPA through the alphabet to XPG, responsible for the seven complementation groups of XP.[134] The restriction site mutation procedure is a DNA-based method shown to be able to detect high mutation frequencies at different loci and used for genetic testing of repair-deficient cells in XP.[438] RT- PCR, PCR- RFLP analysis, SB and SSCP have been used to identify mutations in XP, including missense mutation, deletion leading to truncated protein, splicing mutation and nonsense mutation.[439-441] Prenatal diagnosis of XP can be done by fetal skin fibroblast or amniotic cell analysis of excision repair capacity.[442,443]

### b)	*Impact of molecular testing on clinical management*

The clinical benefit of genetic testing for XP is unclear. Individuals with gene mutations in group G (XPG) are more likely to have the most clinically diverse symptoms with associated neurological problems. Truncated XPG proteins have impaired nucleotide excision repair activity and account for the severe ultraviolet sensitivity of XP group G.[439] Mutations in the XPD gene are associated with a variety of clinical features in a range of severity with respect to features and cellular sensitivity to DNA damage.[444] Splice mutation in XP group C has been associated with autism and hypoglycinemia.[445] This genotypic-phenotypic correlation could be used in molecular diagnostic testing for optimum management and a better prognosis for XP. Retrovirus-mediated DNA repair gene transfer into XP cells from patients can recover normal ultraviolet survival, implying a possibility for gene therapy.[446]

5 DISCUSSION

The recent explosion of genetic knowledge, coupled with the development of new technologies, has increased our understanding of hereditable cancers. The discovery and identification of genes responsible for the development of hereditable cancers in high risk families raises hope for earlier diagnosis and management.

Genetic testing, with its complexities, is enjoying an increase in popular interest. In media reports,[447,448] experts lament the state of genetic testing in Canada. They suggest that tests for hereditary cancers, especially breast cancer and colorectal cancer, should be readily available to all Canadians through regional and provincial programs. A 2000 survey of Canadians found that most favour genetic testing for specific medical purposes, including determining the risk of disease transmission to children (91%) and determining the future risk of acquiring a medical condition (91%).[449] According to a 2001 report by Saskatchewan Health, the anticipated demand for genetic testing in the Saskatchewan health care system varies, with breast cancer and colorectal cancer ranked as being of moderate demand (breast cancer genetic testing is the most common referral for genetic testing in Saskatchewan).[450] A report with a description and an evaluation of tools to assist in the education of primary care practitioners and patients about genetic testing for late onset disorders was prepared for Health Canada.[451]

Parameters for evaluating genetic tests and criteria for implementing these tests have been suggested by several organizations. According to the American Society of Clinical Oncology,[452] cancer predisposing testing should be offered only when:

- the person has a strong family history of cancer or early age of onset of disease
- the test can be adequately interpreted
- the results will influence the medical management of the patient or family member.

For a genetic test to meet the US Blue Cross and Blue Shield Association Technology Evaluation Center criteria:[453]

- the test must have final approval from appropriate government bodies
- the scientific evidence must permit conclusions about the effect of the technology on health outcomes (i.e. analytical and clinical validity)
- the test must improve the net health outcome (i.e. clinical utility)
- the test must be as beneficial as established alternatives
- the improvement in health outcomes must be attainable outside investigational settings.

The basis of concerns about genetic testing stems from questions about the validity of the tests and the ethical challenges for health care practitioners. Guidelines for genetic testing issued by the Canadian Paediatric Society in 2003 are in accordance with the ethical principles for genetic services proposed by the World Health Organization.[454,455] Analytical validity, clinical validity, clinical utility and social consequences are criteria used to assess the benefits and risks of genetic tests.[456] As with any diagnostic test, the clinical utility of genetic testing depends on its ability to provide definitive results, whether positive or negative. The clinical utility of a genetic test

depends not only on its effect on the patient, but also on its effect on family members. Emotional relief in the family leading to undue decreased medical surveillance, anxiety and unjustified preventive measures can follow a test result. In families where sequence analysis yields a definitive positive result (i.e. a pathogenic mutation is identified), intensive surveillance and consideration of prophylactic surgery can be limited to those individuals showing the mutation. Family members without the mutation can undergo the same surveillance recommended for the general population.

If a germline mutation is identified in an affected person, then testing could be offered to at risk family members. If no mutation is found in the affected family member, this would not necessarily exclude an inherited susceptibility to cancer, but could indicate that the technology is too insensitive to detect the mutation. Alternatively, the family could have a mutation in a gene for which genetic testing is unavailable or could have a mutation in a yet unidentified gene that predisposes to cancer. Another explanation for a negative mutation test is that by chance, the tested individual has developed cancer as a sporadic case, while other members in the family have germline mutations. If no germline mutation is identified in an affected family member, testing should not be offered to at risk members. They would remain at increased risk of cancer by virtue of their family history and should continue with recommended screening for cancer.

The relevance and timeliness of implementing innovations in genetic testing in the health care system are associated with technical, clinical and service delivery issues.

Technical issues include issues of test performance. Laboratory techniques and analytical validity vary. The type of analysis depends on the gene in question and whether there is a known mutation in the family. Genetic testing for members of families with known mutations has higher sensitivity and specificity than testing for unknown mutations. Full gene sequencing should have the highest analytical validity, but it is cumbersome and expensive for routine clinical testing. Acceleration of the screening process is important since large studies are required to assess the susceptibility of distinct mutations to outbreak and the penetrance of familial cancers. Thus, there is a need for efficient techniques that can respond to high sample throughput.

Clinical issues or the clinical impact of genetic testing depend on the therapeutic gap that genetic tests can improve in terms of health outcomes and the psychological impact of testing. The genotype-phenotype correlations from genetic testing in familial cancers give a potential for better management and prognosis. When a test has a poor ability to predict clinical outcomes and there is no effective treatment, testing is difficult to justify on medical or social grounds. Appropriate counselling and follow-up are also critical.

Service delivery issues include laboratory quality assurance, quality of screening programs, costs of the tests, organization and coordination of service, manpower planning and training. The costs of molecular testing can range from hundreds to thousands of dollars. Health insurance plans may not consider genetic testing to be necessary.

The cost of meolecular testing is more expensive for detecting a new mutation. Once the mutation is identified, subsequent testing of family members for this known mutation is cheaper. This is a factor that decision-makers should consider.

The cost impact of a predictive genetic test depends on the technique, its application and the impact of the result on clinical management. According to a 2002 Ontario Ministry of Health and Long Term Care report,[457] the effect of genetic testing services on health care costs depends on the pattern by which the result classifies tested individuals as positive or negative and how individuals with these results change their health care consumption patterns. Coverage decisions for predictive genetic tests aim to provide access to those in need while avoiding inappropriate use that would generate large costs.

This report has limitations. Because of the observational nature of the primary studies on genetic test performance, there is no critical analysis of the quality of the available information. The variety of disorders covered by this document, with the heterogeneity of the populations involved, prevents a quantitative analysis of the data.

Additional data on the impact of genetic testing on health outcomes for patients with each disorder and more information on the costs of genetic testing are needed to provide a basis for decision-making with respect to the integration of specific tests into clinical services and to the funding of these tests.

6   CONCLUSION

This document reviews the molecular diagnosis of 20 hereditary cancer predisposing syndromes. These syndromes can be divided into three groups based on the medical benefits of genetic testing: those in which genetic testing may be considered part of the clinical management of affected families (either a positive or a negative result could change medical care); those in which the clinical benefit of genetic testing has been demonstrated but genetic testing is not considered part of standard management; and those in which the clinical benefit of genetic testing is unclear.

Hereditary Cancer Predisposing Syndromes	Cancer Risk* (%)	GT Clinical Availability	Analytical Sensitivity (%)	Clinical Sensitivity (%)	Cost** (C$)
Genetic testing (GT) is generally part of clinical management					
1. Cowden syndrome	50	Yes	n/a	81	1,950
2. Familial adenomatous polyposis	100	Yes	95 to 100	80 to 90	500
3. Hereditary breast and ovarian cancer syndrome 1 and 2	≤85	Yes	60 to 100	14.9 to 20.2	1,200
4. Multiple endocrine neoplasia 2	70	Yes	80 to 95	n/a	262
5. Retinoblastoma	90	Yes	n/a	10 to 70	3,700
6. von Hippel-Lindau syndrome	45	Yes	40 to 100	n/a	390
Clinical benefit has been demonstrated but GT is not part of standard clinical management					
1. Ataxia-telangiectasia	30 to 40	No	66	n/a	4,500
2. Basal cell nevus syndrome	90	Yes	85 to 99	n/a	2,400
3. Bloom syndrome	20	Yes	n/a	n/a	n/a
4. Fanconi's anemia	≤50	Yes	n/a	23.5 to 97	n/a
5. Hereditary multiple exostoses	≤2	No	80 to 95	70	n/a
6. Hereditary non-polyposis colon cancer	≤75	Yes	90 to 100	43 to 50	1,500
7. Multiple endocrine neoplasia 1	<10	Yes	85 to 99.5	n/a	1,050
8. Neurofibromatosis 1	≤5	Yes	60 to 97	67 to 68	n/a
9. Neurofibromatosis 2	Not increased	Yes	100	64 to 75	n/a
Clinical benefit of GT is unclear					
1. Familial melanoma	>90	Yes	100	50	900
2. Li-Fraumeni syndrome	90	Yes	80 to 98	70	n/a
3. Peutz-Jeghers syndrome	50	Yes	70	30 to 70	2,100
4. Wilms' tumour	100	No	90	n/a	1,250
5. Xeroderma pigmentosum	>90	No	n/a	n/a	n/a

* Cancer risk is the risk that a patient with a syndrome will develop cancer in his or her lifetime
** The cost is a laboratory cost for new mutation detection. In the case of ataxia telangiectasia, the cost is from a research setting.
n/a=information not available from our literature search

Despite the rapid development of new molecular techniques, the implementation of genetic testing for many disorders in standard clinical management has not been justified. With their high costs, their variable analytical and clinical validity and their limited availability, genetic tests for many types of hereditary cancers are far from satisfactory. Legal, social and ethical issues also affect the integration of genetic testing into the health care system.

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Appendix 1: Literature Search Strategy

Guide to Search Syntax (DIALOG®, Cochrane Library)

!   Explode the search term. Retrieves the search concept plus all narrower terms.
?   Truncation symbol, single character. Retrieves plural and variant endings of search terms.
*   Truncation symbol, any number of characters.
" "   Used to search phrases.
(w)   Proximity operator. Words must be adjacent.
()   Proximity operator. Words must be adjacent.
(n)   Proximity operator. Words must be near each other in any order.
ab   Search in article abstract.
de   Descriptor i.e., subject heading (a controlled, thesaurus term)
ME   Medical subject heading
ti   Search in titles
tw   Text word

DATABASES	DATES/ LIMITS	SUBJECT HEADINGS/KEYWORDS
DIALOG® OneSearch	Human	Neoplastic Syndromes, Hereditary!/de OR ((Hereditary or Familial) () (Cancer? OR Neoplasm?))/ti,ab OR Adenomatous Polyposis Coli/de OR Familial Adenomatous Polyposis/de OR Genes, APC/de OR FAP/ti,ab OR APC()Gene/ti, ab OR Sq21/ti,ab OR Familial()Polyposis(3N)Colon/ti,ab OR Polyposis()Adenomatous()Intestinal/ti,ab OR Gardner()Syndrome/ti,ab OR Adenomatous()Polyposis()Coli/ti,ab OR (Adenomatous Polyp/de AND Familial/ti,ab) OR Gardner Syndrome/de
Cancerlit	1975- 2002/Jun	
EMBASE®	1974- 2002/Aug W2	
BIOSIS®	1969- 2002/Aug W2	OR
MEDLINE®	1966- 2002/Aug W2	Basal Cell Nevus Syndrome/de OR Basal Cell Carcinoma/de OR Basal()Cell()Nevus()Syndrome/ti,ab OR Nevoid()Basal()Cell()Carcinoma/ti,ab OR Gorlin()Syndrome OR PTCH()Gene OR 9q22/ti,ab OR Gorlin()Gotz()Syndrome/ti,ab OR Basal()Cell()Carcinoma/ti,ab OR
PASCAL®	1973- 2002/Aug W2	Multiple()Basal()Cell()Nevi/ti,ab OR Gorlin(1N)Gotz()syndrome/ti,ab OR

DATABASES	DATES/ LIMITS	SUBJECT HEADINGS/KEYWORDS
		((Familial OR Hereditary) AND (Breast Neoplasms/de OR Breast Cancer/de OR Breast(2N)Cancer?/ti,ab OR Breast(2N)Neoplasm?/ti,ab)) OR Genes, BRCA1/de OR Genes, BRCA2/de OR BRCA1()Gene/ti,ab OR BRCA2()Gene/ti,ab OR Breast()Ovarian()Cancer?/ti,ab OR Breast?(2N)Neoplasm?/ti,ab)) OR Hereditary Breast Cancer/de OR Cowden()Syndrome/ti,ab OR Hamartoma Syndrome, Multiple/de OR Cowdens()Syndrome/ti,ab OR PTEN()gene OR 10q23/ti,ab OR Cowden Syndrome/de OR Cowden Disease/de OR Cowden()Disease/ti,ab OR Li-Fraumeni Syndrome/de OR Genes, p53/de OR Li()Fraumeni()Syndrome/ti,ab OR p53()Gene/ti,ab OR 17p13/ti,ab OR Li(1N)Fraumeni/ti,ab OR CDH1()Gene OR Cadherin()1/ti,ab OR 16q22/ti,ab OR ((Familial OR Hereditary) AND (Stomach Neoplasms/de OR Gastric Cancer/de OR Gastric Carcinoma/de OR Melanoma OR Gastic()Cancer/ti,ab OR Gastric()Carcinoma/ti,ab OR Stomach()Cancer?/ti,ab OR Stomach() Neoplasm?/ti,ab)) OR CDKN2()Gene/ti,ab OR CDK4()Gene/ti,ab OR p16/ti,ab OR 9p21/ti,ab OR 12q13/ti,ab OR Gorlin(1N)Gotz()Syndrome/ti,ab OR (Dysplastic()Nevus()Syndrome AND Hereditary) OR Multiple Endocrine Neoplasia Type 1/de OR Multiple Endocrine Neoplasia!/de OR Multiple Endocrine Neoplasia/de MEN1()GENE/ti,ab OR 11q13/ti,ab OR Endocrine()Adenomatosis()Multiple/ti,ab OR

DATABASES	LIMITS	SUBJECT HEADINGS/KEYWORDS
		OR Zollinger(1N)Ellison()syndrome/ti,ab OR Multiple Endocrine Neoplasia Type 2a/de OR Multiple()Endocrine()Neoplasia()Type()2/ti,ab OR Men()2()Gene/ti,ab OR Multiple()Endocrine()Neoplasia/ti,ab OR Medullary()Thyroid()Carcinoma/ti,ab OR PTC()syndrome/ti,ab OR Sipple()Syndrome/ti,ab OR Sipple Syndrome/de OR Exostoses, Multiple Hereditary/de OR Osteochondroma/ti,ab OR Osteosarcoma/ti,ab OR EXT1()Gene/ti,ab OR EXT2()Gene/ti,ab OR 8q24/ti,ab OR11p12-p11/ti,ab OR Multiple(1N)Exostoses/ti,ab OR Hereditary Multiple Exostosis/de OR Hereditary Multiple Exostoses/de OR Hereditary()Multiple()Exostos?s/ti,ab OR Neurofibromatoses/de OR Neurofibromatosis/ti,ab OR Neurofibroma/ti,ab OR Neurosarcoma/ti,ab OR NF1()gene/ti,ab OR Von()Recklinghausen()Syndrome/ti,ab OR Neurofibromatosis/de OR Neurofibromatosis()Type()2/ti,ab OR Genes, Neurofibromatosis Type 2/de OR Peutz-Jeghers Syndrome/de OR Peutz(1N)Jeghers()Syndrome/ti,ab OR Gastrointestinal()Tumo?r?/ti,ab OR STK11()Gene/ti,ab OR 19p13/ti,ab OR Polyposis()Hamartomatous()Intestinal/ti,ab OR Polyps(3N)Spots()Syndrome?/ti,ab OR Retinoblastoma/de OR Retinoblastoma/ti,ab OR RB1()gene/ti,ab OR 13q14/ti,ab OR

DATABASES	LIMITS	SUBJECT HEADINGS/KEYWORDS
		Hippel-Lindau Disease/de OR Von() Hippel(1N)Lindau ()Syndrome/ti,ab OR Hemangioblastoma/ti,ab OR VHL()Gene/ti,ab OR 3p25/ti,ab OR Von Hippel-Lindau Disease/de OR Von Hippel-Lindau Gene/de OR Wilms()Tumo?r?/ti,ab OR Nephroblastoma/de OR WT1()Gene/ti,ab OR 11p13/ti,ab OR WT3()Gene/ti,ab OR WT4()Gene/ti,ab OR ((Hereditary OR Papillary) AND (Carcinoma, Renal Cell/de OR Renal()Cell()Carcinoma)) OR Hereditary()Renal()Carcinoma/ti,ab OR Papillary()Renal()Carcinoma/ti,ab OR MET()Gene/ti,ab OR 7q31/ti,ab OR Hereditary()Papillary()Renal()Carcinoma/ti,ab OR Renal()Cell()Carcinoma()Papillary/ti,ab OR Hereditary Non-Polyposis Colorectal Cancer/de OR Hereditary Nonpolyposis Colorectal Cancer/de OR ((Colonic Neoplasms/de OR Colon()Cancer/ti,ab) AND (Hereditary()Non(1N)Polyposis)) OR Colorectal()Cancer/ti,ab OR Lynch()Cancer()Family()Syndrome/ti,ab OR Colorectal()Endometrial()Carcinoma/ti,ab OR MSH2()Gene/ti,ab OR MLH1()Gene/ti,ab OR PMS1()Gene/ti,ab OR PMS2()Gene/ti,ab OR MSH6()Gene/ti,ab OR Ataxia Telangiectasia/de OR Ataxia(1N)Telangiectasia/ti,ab OR ATM()Gene/ti,ab OR Louis(1N)Bar()Syndrome/ti,ab OR Bloom Syndrome/de OR Bloom()Syndrome/ti,ab OR BLM()Gene/ti,ab OR 15p26/ti,ab OR Bloom's Syndrome/de OR Bloom?s()Syndrome/ti,ab OR

DATABASES	LIMITS	SUBJECT HEADINGS/KEYWORDS
		Fanconi Anemia/de OR Fanconi's Anemia/de OR Fanconi Syndrome/de OR Fanconi?()An?emia/ti,ab OR FACA()Gene/ti,ab OR FA()gene/ti,ab OR FAA()gene/ti,ab OR Fanconi()Pancytopenia()type()1/ti,ab FANCD()GENE/ti,ab OR FANCE()Gene/ti,ab OR FANCF()Gene/ti,ab OR FANCA()Gene/ti,ab OR Xeroderma Pigmentosum/de OR Xeroderma()Pigmentosa/ti,ab OR Basa()Squamous()Skin()Carcinoma/ti,ab OR XPA(1N)XPE()Gene/ti,ab AND *(combining results from disease search with genetic tests' terms)* Genetic Techniques!/de OR Genetic Predisposition to Disease!/de OR Genetic Techniques/de OR Genetic Predisposition to Disease/de OR Genetic Screening/de OR Penetrance/de OR Mutation/de OR Gene Frequency/de OR Gene Privacy/de or Genetic Counselling/de OR Genetic Predisposition/de OR Mutational Analysis/de OR Genetic Testing/de OR Mutation Detection/de OR Genetic()Technique?/ti,ab OR Genetic()Predisposition?/ti,ab OR Genetic()Screening/ti,ab OR Mutation(3N)Detection?/ti,ab OR Genetic()Test?/ti,ab OR Molecular()Diagnos?s/ti,ab OR Gene()Privacy/ti,ab OR Genetic()Counselling/ti,ab OR Genetic()Analys?s?/ti,ab or Genetic Analysis/de OR Genetic()Susceptibilit?/ti,ab AND *(combining disease terms + genetic tests + terms to pick diagnostic studies relevant to the research questions)* Predictive Value of Tests/de OR Sensitivity and Specificity/de OR Analytical()Validit?/ti,ab OR Analytical()Sensitivit?/ti,ab OR Diagnostic Errors!/de OR Sensitivit?/ti,ab OR Specificit?/ti,ab OR Predictive()Value?/ti,ab

DATABASES	LIMITS	SUBJECT HEADINGS/KEYWORDS
		Total = 1786 unique hits *Medline = 1501 hits* *Cancerlit = 2 hits* *Pascal = 90 hits* *Biosis = 53 hits* *Performed 08/15/2002*
DIALOG® Alerts MEDLINE® EMBASE® BIOSIS Previews®		Weekly/bi-weekly alerts set up to capture new studies Same search strategy as the full search
Cochrane Collaboration & Update Software Ltd. The Cochrane Library, Issue 2 & 3, 2002; 1, 2003		MeSH headings and keywords as the original DIALOG® (excluding those that contain numbers as they are ignored by the software). Appropriate search syntax for Cochrane Library used.
National Center for Biotechnology Information OMIM Database (Online Mendelian Inheritance in Man)		MeSH headings and keywords
Children's Health System & University of Washington GENETests		Searched by keywords or gene
National Library of Medicine PubMed		MeSH headings and keywords to capture additional studies (pre-medline and other studies not covered by DIALOG®). Appropriate search syntax for PubMed was used.
Websites of HTA and related agencies; clinical trial registries; other databases		NICE;ECRI; National Research Register; University of York NHS Centre for Reviews and Dissemination – CRD databases

Appendix 2: Selection of Relevant Studies

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┌─────────────────────────────────┐
│   1,786 abstracts identified    │
└─────────────────────────────────┘
                │
                ▼
┌─────────────────────────────────┐
│   637 potentially relevant      │
│       articles retrieved        │
└─────────────────────────────────┘
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┌──────────────┐│          ┌──────────────────────────────────────────────────┐
│ 50 articles  ││          │ 230 articles excluded                            │
│ from alerts  │├─────────▶│  • studies on irrelevant technical issues (160)  │
│    and       ││          │  • reviews without relevant or additional  information (70) │
│bibliographies││          └──────────────────────────────────────────────────┘
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│  457 relevant articles included │
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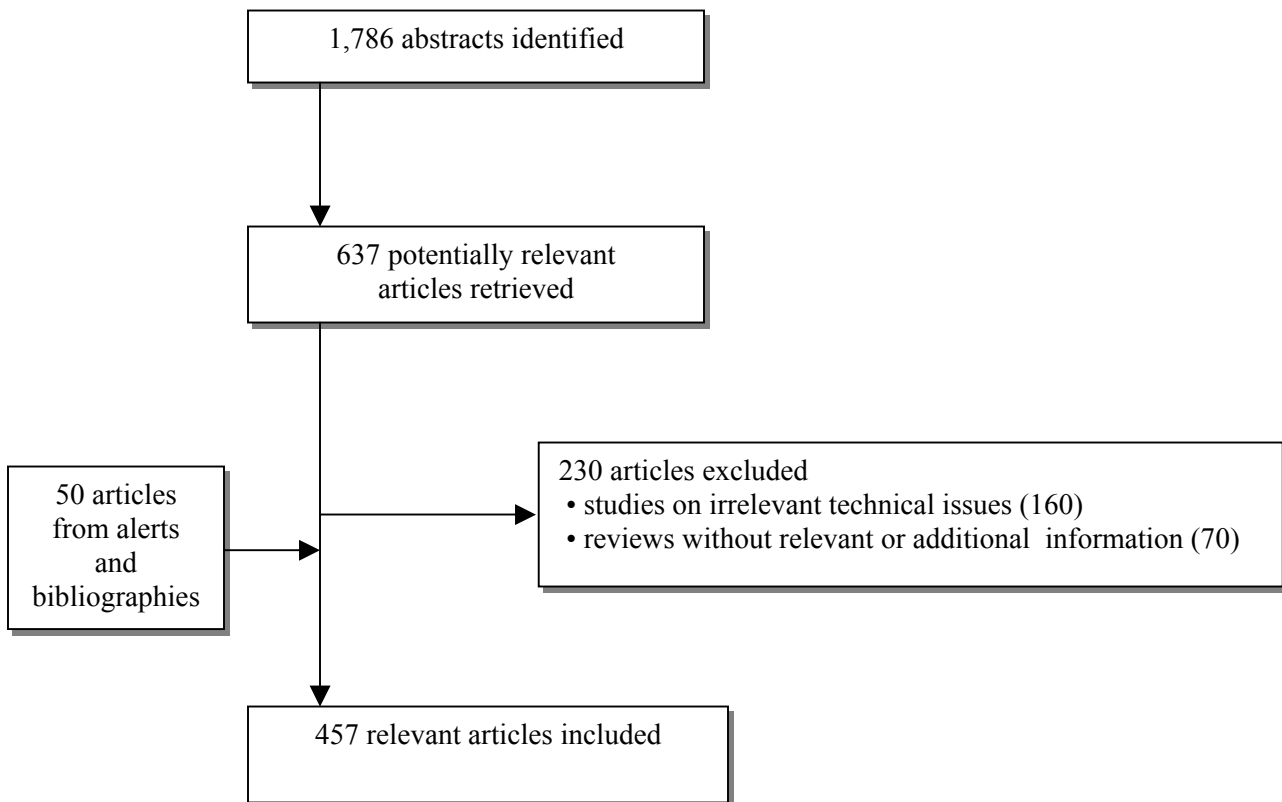
Appendix 3: Genetic Testing Services and Laboratories in Canada

Province	City	Clinic
Alberta	Calgary	Alberta Cancer Genetics Program Division of Epidemiology Rm. AE173B, Tom Baker Cancer Centre 1331-29 St. NW Calgary AB T2N 4N2 web site http://www.acgp.ca/Genetic_Counselling/
	Calgary	Cancer Genetics Research Clinic Tom Baker Cancer Centre Rm. CC110, 1331-29 St. NW Calgary AB T2N 4N2 Tel: (403) 670-2438 Fax: (403) 283-1651
	Edmonton	Cancer Genetics Clinic Rm. 8-53 Medical Sciences Bldg. University of Alberta Edmonton AB T6G 2B7 Tel: (780) 407-7333 Fax: (780) 407-6845
British Columbia	Vancouver	BC Cancer Agency 600 West 10th Ave. Vancouver BC V5Z 4E6 web site http://www.bccancer.bc.ca/
	Vancouver	Hereditary Cancer Program B.C. Cancer Agency PFC 600 West 10th Ave. Vancouver BC V5Z 4E6 Tel: (604) 877-6000 extension 2118 Fax: (604) 872-4596
	Victoria	Medical Genetics Victoria General Hospital 1 Hospital Way Victoria BC V8Z 6R5 Tel: (250) 727-4212 Fax: (250) 370-8750
Manitoba	Winnipeg	Hereditary Breast Cancer Clinic WHRA Breast Health Centre 100-400 Taché Ave. Winnipeg MB R2H 3C3 Tel: (204) 235-3674 Fax: (204) 231-3842

Province	City	Clinic
Newfoundland	St. John's	Medical Genetics Program Health Care Corporation of St. John's Health Science Centre 300 Prince Philip Dr. St. John's NF A1B 3V6 Tel: (709) 777-6223 or 777-4363 Fax: (709) 777 4190 or 777- 7317
Nova Scotia/New Brunswick/ PEI	Halifax	Maritime Medical Genetics Service IWK Health Centre 5850-5980 University Ave. P.O. Box 3070 Halifax NS B3J 3G9 Tel: (902) 428-8754 Fax: (902) 428-8709
Ontario	Hamilton	Cancer Risk Assessment Clinic Hamilton Regional Cancer Centre 699 Concession St. Hamilton ON L8V 5C2 Tel: (905) 387-9495 or 9711 extension 65920 Fax: (905) 575-6326
	Hamilton	McMaster University Medical Centre Hamilton Health Sciences Rm 3N20 Genetic Services 1200 Main St. W. Hamilton ON L8S 4J9 Tel: (905) 521-5085 Fax: (905) 521-2651
	Kingston	Familial Oncology Program Kingston Regional Cancer Centre 25 King St. W. Kingston ON K7P 2N7 Tel: (613) 544-2631 extension 4124 Fax: (613) 544-9708
	London	Cancer Genetics London Regional Cancer Centre 790 Commissioners Rd. London ON N6A 4L6 Tel: (519) 685-8727 Fax: (519) 685-8534
	Ottawa	Department of Genetics Children's Hospital of Eastern Ontario 401 Smyth Rd. Ottawa ON K1H 8L1 Tel: (613) 738-3979 Fax: (613) 738-4822

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	Ottawa	Hereditary Colon Cancer Programme Loeb Health Research Institute Ottawa Hospital Civic Site 725 Parkdale Ave. Ottawa ON K1Y 4E9 Tel: (613) 798-5555 extension 7805 Fax: (613) 761-5365
	Sudbury	Familial Cancer Familial Cancer Risk Northeastern Ontario Regional Cancer Centre 116, 41 Ramsey Lake Rd. Sudbury ON P3E 5J1 Tel: (705) 522-6237 extension 2060 Fax: (705) 523-7328
	Thunder Bay	Thunder Bay District Health Unit Northwestern Ontario Regional Cancer Centre 290 Munro St. Thunder Bay ON P7A 7T1 Tel: (807) 343-1610 Fax: (807) 345-2630
	Toronto	Ontario Cancer Genetics Network Division of Preventive Oncology Cancer Care Ontario 620 University Ave. Toronto ON M5G 2L7 web site http://www.cancercare.on.ca/prevention/ocgn.html
	Toronto	Genetics Department Credit Valley Hospital 1860, 2200 Eglinton Ave. W. Mississauga ON L5M 2N1 Tel: (905) 813-4104 Fax: (905) 813-4347
	Toronto	Familial Breast Cancer Clinic Mount Sinai Hospital 1286, 600 University Ave. Toronto ON M5G 1X5 Tel: (416) 586-3244 Fax: (416) 586-8659
	Toronto	Familial GI Cancer Registry Mount Sinai Hospital 1157, 600 University Ave. Toronto ON M5G 1X5 Tel: (416) 586-8334 Fax: (416) 586-8644 web site www.mtsinai.on.ca/familialgican

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	Toronto	Familial Ovarian Cancer Clinic Princess Margaret Hospital 610 University Ave. Rm M-700 Toronto ON M5G 2M9 Tel: (416) 946-2270 Fax: (416) 946-2288
	Toronto	Breast Cancer Clinic University Health Network Princess Margaret Hospital 8-502A, 610 University Ave. Toronto ON M5G 2M9 Tel: (416) 946-4409 Fax: (416) 946-4410
	Toronto	Genetics Programme North York General Hospital 391, 4001 Leslie St. North York ON M2K 1E1 Tel: (416) 756-6345 Fax: (416) 756-6727
	Toronto	Familial Breast Cancer Research Unit The Centre For Research in Women's Health Women's College Hospital 750A, 790 Bay St., 7th Floor Toronto ON M5G 1N8 Tel: (416) 351-3765 Fax: (416)-351-3767 web site http://www.utoronto.ca/crwh
	Toronto	Department of Preventive Oncology Toronto-Sunnybrook Regional Cancer Centre 2075 Bayview Ave. Toronto ON M4N 3M5 Tel: (416) 480-6835 Fax: (416) 480-6002 web site http://www.swchsc.on.ca/
	Windsor	Windsor Regional Cancer Centre 2220 Kildare Rd. Windsor ON N8W 2X3 Tel: (519) 253-5253 Fax: (519) 253-4204

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Quebec	Montreal	Division of Medical Genetics The Montreal General Hospital 1650 Cedar Ave., Rm. L10-120 Montreal QC H3G 1A4 Tel: (514) 937-6011 extension 4067 Fax: (514) 934-8273
	Montreal	Clinique des Cancers Familiaux de Montréal / CHUM Familial Cancer Clinic Pavillon Masson de l'Hôtel-Dieu 8-031 3850 St-Urbain Montréal QC H2W 1T8 Tel: 514-890-8104 Fax: 514-412-7131
	Quebec City	Département de génétique humaine Centre hospitalier de l'Université Laval 2705 boul. Laurier Québec QC G1V 4G2 Tel: (418) 654-2103 Fax: (418) 654-2748
Saskatchewan	Saskatoon	Saskatoon Cancer Centre 20 Campus Dr. Saskatoon SK S7N 4H4 Tel: (306) 655-6717 Fax: (306) 655-2639
	Saskatoon	Division of Medical Genetics University of Saskatchewan Royal University Hospital Saskatoon SK S7N 0X0 Tel: (306) 966-1692 Fax: (306) 966-1736