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Application of genomics and proteomics to the prevention of colorectal cancer

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Synopsis

The completion of the Human Genome Project in 2003 led to significant developments in the field of genomics—the aim of which is to characterize all of the DNA coding elements that determine the phenotype of cells. Genomics can now be applied to issues involved in the hereditary transmission of diseases, as well as the molecular events that take place in the sequence of carcinogenesis.

The incidence of colorectal cancer and the mortality associated with it can be significantly reduced through the early detection and treatment of premalignant and malignant lesions. Colonoscopy is still the gold standard procedure, but there is growing interest in genomic approaches. Hereditary colorectal cancer includes syndromic familial diseases with autosomal-dominant Mendelian inheritance. It also includes nonsyndromic familial conditions in which multiple genetic variants interact with environmental factors. As emphasized by Burt and Neklason (2005), colorectal cancer occurs in three distinct situations:

- Syndromic familial cases (less than 5% of the total)
- Nonsyndromic familial clustering of cases (30% of the total)
- Sporadic cases with no increased familial clustering (65% of the total)

When patients have provided informed consent, gene testing of blood samples is now carried out routinely for syndromic familial colorectal cancer in order to detect individuals who are at risk in a family. Identifying the specific mutation requires DNA sequencing. Once the mutation has been characterized, a simpler site-specific analysis can be carried out in the other members of the family.

In the other two groups, using a systematic questionnaire to assess a proband's family antecedents helps distinguish between sporadic cancer and nonsyndromic familial cancer. The application of genomic techniques to tumor samples is now leading to more sophisticated analysis in both groups.

Identifying biomarkers for cancer depends on noninvasive tests that can identify alterations in the genes or proteins in the tumor. The reliability of these tests for early diagnosis and classification of hereditary and sporadic colorectal cancer is still a matter of debate.

Genomics and proteomics

The human genome

The DNA in the chromosomes is a double-helix structure consisting of two strands that run in opposite directions. Each strand has a phosphate at one end (5′) and a hydroxyl at the other end (3′). Each strand of DNA is a polymer of nucleotides consisting of a sugar, a phosphate, and a base, of which there are four types: adenine, guanine, cytosine, and thymine. Specific pairing between opposite bases in the two strands (A with T and G with C) results in a ladder structure in which the two chains are held together. Sequencing of the human genome (3.2 gigabases) was completed in 2003 (Lander et al. 2001, Collins et al. 2003), and the sequence is freely accessible from the Human Genome Project on the Internet. The genes coding for proteins represent less than 2% of DNA and they only number 30,000. Each gene has coding areas or exons and noncoding areas or introns; splicing signals are present in the introns.

DNA transcribes all the exons of the gene into the RNA; this is the open reading frame. The open reading frame in the RNA is assembled as a result of intron splicing: each triplet of nucleotides (or codons) codes for a specific amino acid. The protein corresponding to the gene is translated by messenger RNA (mRNA) in the microsomes of the cell. The molecules of RNA (which belongs to the transcriptome) are analyzed with the reverse transcriptase enzyme, which produces a DNA copy of each mRNA strand. This is known as the *complementary DNA* (cDNA), in contrast to *genomic* DNA. The reverse-transcribed mRNAs are collectively known as the cDNA library.

About 35% of genes have alternative splicing. In these cases, several isoforms of the protein with a distinct function are transcribed by the same gene; isoforms of proteins p53 and p63 are one example of this.

Polymorphism of the human genome

The genome is 99.9% identical in any two individuals with no family link; still, this small difference accounts for the 3 million genetic variants that determine each person's phenotype. The polymorphism of the genome results from mutations, most of which are harmless, although some result in diseases when the function of the gene is altered or silenced. Epigenetic alterations occurring after the constitution of the DNA chain, such as hypermethylation of the dinucleotide CpG islands, may also alter the expression of the gene.

Germ-line and somatic mutations. *Germ-line mutations* are inherited and present in all cells of the body; they help in the diagnosis of hereditary cancer. In *missense* mutations, the substitution of a single nucleotide results in the presence of one incorrect amino acid in the sequence of the translation product. The function of the resulting protein variant may be unchanged (no disease) or severely affected (disease). In *nonsense* mutations, the substitution of a single nucleotide results in a stop codon and the expression of a truncation product. Premature termination of translation can lead to disease.

Somatic mutations are acquired; those occurring in tumor tissue as part of the carcinogenesis sequence do not help in the diagnosis of hereditary cancer.

Categories of mutations. *Single nucleotide polymorphism* (SNP) is the most frequent mutation. In an SNP, a single nucleotide is replaced by one or two

alternative nucleotides. When the SNP is located near a gene, the combination of two closely linked genetic loci is transmitted in the family more frequently than expected (linkage disequilibrium). This combination is known as a haplotype. When multiple SNPs are associated with the gene in a segment of the DNA strand, it represents a *haplotype block,* also subject to linkage disequilibrium. SNPs are useful for mapping the gene causing the disease and also affect the alternative splicing of the gene and the isoforms of proteins.

Microsatellites are small strings of mono-, di-, tri-, or tetra-, tandemly repeated DNA sequences, up to 100 nucleotides: for example, GTGTGTGTGTGT would be referred to as (GT)6. These are frequent, with uniform distribution in the genome, and are easily identified using polymerase chain reaction (PCR). Microsatellites are subject to extensive polymorphism, related to the length of the repetition with 8–12 alleles. Microsatellite polymorphism can modify the function of the gene.

Insertions and *deletions* of one or more nucleotides in the DNA sequence are relatively uncommon, and cause disease if they change the reading frame in the mRNA.

Methods in genomics and proteomics

Samples for study. Most noninvasive studies use *peripheral blood samples.* Immunohistochemistry is carried out on paraffin-embedded sections of *tumor samples* obtained during endoscopy or surgery, after fixation in neutral formalin. Tissue extracts and cell separation procedures are also used. Neoplastic lesions tend to shed cells in the lumen of the colon, and it has been suggested that *fecal samples* can be used as a source of tumor antigens.

Genetic biomarkers. Fecal DNA testing is a noninvasive method of diagnosis. DNA can be recovered and purified in fecal samples that contain tumor cells exfoliated into the lumen of the colon. One study has shown that the molecular weight of DNA fragments in the feces from patients with a colon tumor is higher than that isolated in control patients (Boynton et al. 2003). This suggests that cells shed from the tumor contain less degraded DNA. There is no proof yet that this test has any clinical relevance. Other studies have analyzed multiple biomarker genes (*APC*, *KRAS*, p53, and the microsatellite marker Bat26). Commercially available fecal tests include the PreGen-Plus assay (LabCorp, Burlington, North Carolina, USA), which includes a panel of 21 mutation tests for individuals at average risk of colorectal cancer, and the PreGen 26 assay for detecting microsatellite instability. As yet, there is no formal proof that the DNA fecal test is better than the simple guaiac fecal occult blood test; in any case, its price is 200 times higher, and colonoscopy is still required if the test is positive. Fecal DNA testing is not currently cost-effective for screening.

The potential usefulness of genetic biomarkers in the peripheral blood has also been investigated. The insulin-like growth factor-2 gene (*IGF2*), which encodes an important tumor growth factor, is silenced in healthy individuals by methylation (an epigenetic event). A recent study has shown that the loss of imprinting of *IGF2* could be a marker for a hereditary predisposition to nonsyndromic familial colorectal cancer (Cruz-Correa et al. 2004). This finding opens up new prospects for testing for the predisposition to common colorectal cancer.

Gene testing. In patients with a syndromic familial cancer, the strongly suggestive phenotype raises a suspicion that there is a specific germ-line mutation, and oncogenetic counseling is offered. If the mutation has not been previously identified in another member of the family, gene testing can be carried out if the patient and

family provide informed consent. DNA for gene testing is obtained from the white blood cells in a peripheral blood sample. The sample is sent to a laboratory with facilities for DNA sequencing. A world directory of gene testing laboratories is available (http://www.genetests.org.)

Initially, a short DNA fragment (150–300 bases long) suspected to contain the disease-associated mutation is selected and amplified using PCR. Various methods of selecting specific segments of DNA, based on recombination of the DNA strands after denaturation, have been developed. Denaturing gradient gel electrophoresis (DGGE) can detect 95% of the sequence changes.

In the fragment of DNA selected and amplified by PCR, sequencing determines the order of nucleotide bases in all codons of the gene. The mutation is characterized (type and location) by comparing the DNA sample with a DNA probe with a known sequence of nucleotides. The development of microarray chips (DNA arrays and cDNA arrays) has improved the method and speeded it up. The GeneChip (Affymetrix, Inc., Santa Clara, California, USA) is a highly ordered matrix of DNA oligomers fixed in a glass support. In the USA, gene testing is commercially available for hereditary nonpolyposis colorectal cancer (HNPCC), familial adenomatous polyposis (FAP), Cowden disease, Peutz–Jeghers syndrome, and juvenile polyposis. Once the mutation has been identified in an initial proband, site-specific mutation analysis, based on PCR with specific probes, is a simple method (with an accuracy of nearly 100%) of detecting its presence in other members of the family. In the USA, the cost of sequencing a gene is much higher (about $1500) than the cost of site-specific mutation analysis (about $200–300).

Proteomics. Protein antigens have been routinely used in histochemistry as biomarkers for neoplastic lesions; however, their predictive or prognostic value is very weak. Specific proteins have also been identified as tumor antigens in the peripheral blood; prostate-specific antigen (PSA) in prostate cancer is an example. In relation to gastrointestinal tract cancer, carcinoembryonic antigen (CEA), CA-19-9, 72-4 antigen, and also intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) show increased values in advanced cancer and are helpful for detecting recurrences. However, the predictive value of a single tumor antigen detected in the blood is often not satisfactory, and strategies are needed for identifying multiple biomarkers, which in combination could provide a better predictive value. The detection of tumor antigens in the blood is still not relevant for early diagnosis, even when multiple antigens are used.

Proteomic strategies (Krieg et al. 2002, Cutler 2003, Alfonso et al. 2005, Skandarajah et al. 2005) for identifying biomarkers of colorectal cancer include pattern profiling based on two-dimensional gel electrophoresis (2DE) and mass spectrometry. 2DE provides resolution of proteins based on their isoelectric point (first dimension) and molecular weight (second dimension), resulting in reproducible high-resolution two-dimensional maps that can be used to compare protein expression. Spots of interest in the map can be excised from the gel, and the identity of the proteins contained in these spots can be determined by mass spectrometry (MS) analysis. High-throughput protein/peptide profiling can also be achieved by MS-based methods such as surface-enhanced laser desorption/ionization (SELDI) and matrix-assisted laser desorption/ionization (MALDI). These strategies rely on sophisticated analytical tools for which commercial workstations have been developed. The clinical application of proteomics to the early diagnosis of cancer will be effective if distinct patterns can be individualized not only in the tumor, but also in peripheral blood samples. As yet, this has not been achieved.

Hereditary transmission of diseases

Methods of study

Mutations in the DNA sequence of the gene are characterized (by type and location) in comparison with DNA probes that have a known sequence of nucleotides.

Linkage analysis for Mendelian diseases uses marker loci to measure the cosegregation in a family: individuals exposed to the disease have the same distribution (disequilibrium linkage) for SNPs, microsatellites, and for the gene of the disease.

Association analysis explores the statistical correlation between a particular genetic variant and a disease, in cases and controls, when the gene has a small impact on the phenotype. Three steps are required: suggestion of a candidate gene for the disease; identification of genetic variants located near this suspect gene; and distinction of the phenotype "disease" from the phenotype "no disease."

Classification of genetic diseases

Chromosomic diseases, caused by deletion or addition of large segments of chromosomes, are often not compatible with normal development or induce abortion. Trisomy 21 is an example.

Mendelian diseases are caused by mutation on a single gene with high penetrance; there is a strict correspondence between the genotype and the phenotype. This applies to familial adenomatous polyposis. The transmission is dominant when the mutation on a single parental allele is expressed on the phenotype, and recessive when the mutation has to be present on both parental alleles. The mutation occurs in different loci of the incriminated gene; the same mutation is transmitted in one family. In another family, the locus on the gene is different.

Diseases with complex heredity depend on multiple genes with low penetrance, and the expression of the phenotype results from interaction between genetic and environmental factors. In diseases with complex heredity, some familial clustering occurs; this applies to nonsyndromic familial colorectal cancer.

Heredity and colorectal cancer

The number of cases of colorectal cancer arising in the setting of a penetrant syndrome with autosomal-dominant inheritance is small in comparison with the global burden. On the other hand, among common malignancies, colorectal cancer is one of the conditions with the greatest hereditary component (Burt and Neklason 2005, Doxey et al. 2005, Lazaridis and Juran 2005, Lazaridis and Petersen 2005, Strate and Syngal 2005): having a relative with colon cancer increases one's risk of developing malignancy. In a global analysis (Tables 1–3), colorectal cancer can be classified into three groups:

- *Syndromic familial colorectal cancer* arises in the setting of a penetrant autosomal-dominant inherited syndrome, and occurs in less than 5% of cases. Gene testing is applicable in this group. The two major diseases are familial adenomatous polyposis (FAP) and hereditary nonpolypoid colorectal cancer (HNPCC).

- *Nonsyndromic familial colorectal cancer,* with some familial clustering of cases, arises from a combination of hereditary and environmental factors. This situation, in which the criteria for syndromic familial cancer (HNPCC) are not met, represents one-third of the cases.
- *Sporadic colorectal cancer,* independent of hereditary factors and arising under the influence of environmental factors, occurs in two-thirds of the cases.

Syndromic familial colorectal cancer

Hereditary colorectal cancer with adenomatous polyposis

Familial adenomatous polyposis (FAP) is a genetic disease with autosomal-dominant transmission; its prevalence in the population is stable (2–3 per 100,000). One-third of new cases are caused by a de novo mutation. The phenotype appears early, and the risk for colorectal cancer is 100% at the age of 40. Colonic polyposis may be associated with duodenal periampullary adenomas, nonadenomatous gastric polyps, desmoid tumors, and osteomas (Gardner's syndrome), as well as tumors in the central nervous system (Turcot's syndrome).

Attenuated familial adenomatous polyposis (AFAP) is characterized by a smaller number of colonic adenomas (less than 100) and later appearance of the phenotype. The risk for colorectal cancer reaches 80% at age the of 50.

In FAP, the germ-line mutation is transmitted through a single parental allele. The alteration of the phenotype is early when a somatic acquired mutation occurs in the other parental allele. The mutation is on suppressor gene *APC* on chromosome 5 (locus 5q21.q22), which codes for Apc proteins that control the cell cycle (signal Wnt). The truncated Apc protein is unable to degrade β-catenin, a stimulant of cell proliferation; neoplastic lesions then develop with a sequence of mutations on K-*ras,* p53. More than 800 distinct germinal mutations of the *APC* gene have been described. Their type is identified by complete sequencing of the *APC* gene, consisting of 15 exons. Most identified mutations that result in truncation of the Apc protein are on exon 15, which encodes the open reading frame in the mRNA for the translation of the Apc protein. The phenotype is influenced by the site of the mutation: some tend to cause aggressive FAP with early onset, whereas others tend to cause osteomas, dental changes, and desmoid tumors. Patients who have a germ-line *APC* mutation in the first four exons, or in the alternatively spliced region of exon 9, usually develop AFAP with fewer tumors at an older age. When the first mutation has been characterized in one proband in a family, all other members can be tested, using a fast method—site-specific mutation analysis.

The *APC* mutation is absent in 5–10% of individuals with an FAP phenotype. This suggests mutation in another gene. A disease with autosomal-recessive inheritance with a mutation on the repair gene *MYH* on chromosome 1 (locus 1p34.3) may be present. The repair gene *MYH* codes for the Myh protein, affording protection against oxidative damage to DNA. The mutation is identified by complete DNA sequencing of this gene. Alternatively, it is possible to use a simple targeted analysis for the two most frequent mutations (85% of cases): Y165C and G382D.

To sum up, when an individual's phenotype meets the criteria for FAP, the clinician should request an oncogenetic consultation, followed by gene testing to identify the mutation. Endoscopic screening is recommended from the age of 10, only in individuals in a family that is found to be positive. In AFAP, endoscopic screening is recommended from the age of 18. If the *APC* mutation is not found and an *MYH* test

is negative, endoscopic screening is necessary in all members of the family. Timely colectomy can be carried out if colonic polyposis is confirmed. Ileoanal anastomosis is the procedure of choice; ileorectal anastomosis can be carried out in patients with AFAP.

Hereditary colorectal cancer with nonadenomatous polyposis

Hyperplastic polyposis is not a hereditary disorder and there is no indication for genetic testing.

Juvenile polyposis (JP) is a disease with autosomal-dominant inheritance, with a prevalence of approximately one in 100,000 of the population; sporadic cases also occur in 2% of the pediatric population. The JP phenotype is characterized by multiple colorectal hamartomatous polyps, with a cystic structure and overgrowth of constituents of submucosa and muscular tissue. The polyps appear in the first decade of life. The lifetime risk for colon cancer linked to the occurrence of adenomatous foci is nearly 60%. Extraintestinal tumors also occur. Germ-line mutations can occur in three distinct genes:

- Mutation of the *SMAD4* or *MADH4* gene, on chromosome 1 (locus 18q21.1) is detected in only 15% of individuals with the JP phenotype. The *SMAD4* gene is involved in the signal pathway of transforming growth factor-β (TGF-β): Smad proteins mediate the transcription of nuclear factors inhibiting cell proliferation.
- A mutation is detected in the bone morphogenetic protein receptor, type IA gene (*BMPR1A*) in 25% of individuals with the JP phenotype; the mutation also involves the signal pathway of transforming growth factor-β (TGF-β).
- A mutation is detected on the *PTEN* gene in 5% of individuals with the JP phenotype.

In summary, gene testing with DNA sequencing can be offered to individuals with the JP phenotype. When the mutation has been identified, other members of the family can be tested using site-specific mutation analysis.

Endoscopic screening of the colon is recommended at 3-year intervals, no later than at the age of 20. Upper gastrointestinal endoscopy and small-bowel examinations are also recommended.

Peutz–Jeghers syndrome is a disease with autosomal-dominant inheritance, with a prevalence of approximately one in 200,000 of the population. The Peutz–Jeghers phenotype is characterized by mucocutaneous hyperpigmentation and hamartomatous polyps, located in the small bowel and developing in the second decade of life. The polyps have an arborizing pattern, with muscularis mucosae in branching fronds. Complications include bleeding, obstruction, and malignant transformation. The lifetime risk for colon cancer is nearly 40% and the cumulative risk of cancer in intestinal and extraintestinal sites (pancreas, breast, ovary, testicle, cervix) is as high as 90%. Patients with no familial antecedents have new mutations. A germ-line mutation in the *STK11* gene (previously known as *LKB1*) on chromosome 19p is detected by DNA sequencing in 60% of individuals with a family history.

In summary, gene testing with DNA sequencing can be carried out in individuals with the Peutz–Jeghers phenotype. When the mutation has been identified, other members of the family can be tested using simple targeted analysis. Endoscopic screening of the colon at 3-year intervals is recommended no later than the age of

20. Upper gastrointestinal endoscopy and small-bowel examinations are also recommended.

Cowden syndrome is a disease with autosomal-dominant inheritance with a prevalence of approximately one in 200,000 of the population, characterized by mucocutaneous lesions, macrocephaly, and frequent hamartomatous polyposis. The risk of cancer is much higher in the breast than in the large bowel. A germ-line mutation in the *PTEN* gene, a tumor-suppressor gene, on chromosome 10 (locus 10q23) can be detected in up to 80% of individuals who meet the criteria. The Pten protein is a lipid phosphatase that regulates the cell cycle. The somatic mutation may occur in sporadic colorectal cancer.

In summary, gene testing with DNA sequencing can be carried out in individuals with the phenotype. When the mutation has been identified, other members of the family can be tested using simple targeted analysis. Breast screening is recommended; systematic endoscopic screening of the colon is not necessary.

Hereditary nonpolyposis colorectal cancer (HNPCC)

HNPCC, or Lynch syndrome, a genetic disease with autosomal-dominant transmission, is the most common form of syndromic familial colorectal cancer (Aaltonen et al. 1998, Johansdottir et al. 1999, Lindor et al. 2005, Stormoken et al. 2005, Wheeler 2005). The phenotype is not characteristic, as the number of polyps is small and the lesions cannot be distinguished endoscopically from sporadic neoplastic lesions. A consensus group of experts therefore published a list of criteria (the Amsterdam I criteria) that suggest the presence of the HNPCC phenotype:

- There are at least three relatives with colorectal cancer in the family, one of whom is a first-degree relative of the other two.
- At least two successive generations are affected.
- Colorectal cancer has been detected before the age of 50 in one of the relatives.

The Amsterdam II criteria also take into account the occurrence of other adenocarcinomas (endometrial, gastric, in the small bowel, ureteral, renal) in the family. At the age of 40–50, the cumulative risk of colorectal cancer is 80% in both sexes; for endometrial cancer in women, the risk is estimated at 40%.

An attenuated HNPCC phenotype has been recently described (Lindor et al. 2005) in individuals who meet the Amsterdam I criteria. In this situation, the risk of cancer is limited to the large bowel, the age of onset of cancer is later, and the familial risk is lower.

The germ-line mutation occurs in one of the mismatch repair genes. The function of the translated mismatch repair proteins is to repair small sequence errors occurring during replication of DNA. Mutations in both copies of one of the mismatch repair genes lead to the accumulation of DNA sequence errors in the segments with microsatellites. The replication errors in the microsatellites involve the deletion of one or several nucleotides.

Genetic testing in the peripheral blood. When the Amsterdam criteria are met, a mutation is detected in 50–70% of cases with gene testing of the peripheral blood. The proportion of positive findings increases when multiple mismatch repair (MMR) genes are tested. Most mutations (90%) occur in the *MLH1* gene (chromosome 3p22.3) or in the *MSH2* gene (chromosome 2p21). Some occur in the *MSH6* gene

(5–10%); mutations in the *PMS2* gene are very rare. The mutation that occurs in individuals with the attenuated HNPCC syndrome has not yet been identified.

Genetic testing in the tumor. Microsatellite instability (MSI) can be demonstrated using the replication error (RER) test carried out on five microsatellites. Testing for MSI is compared on two tissue samples: normal mucosa versus tumor (early or advanced colorectal cancer only). The malignant tumor is MSI-low if one of the five microsatellites tested is mutated, and MSI-high if two or more microsatellites are mutated. In HNPCC, the microsatellite instability test reveals a DNA deficiency in the MMR genes. In individuals with the attenuated HNPCC syndrome, the MSI test is negative. MSI also occurs in 15% of cases of sporadic colorectal cancer; in these cases, it is caused by aberrant methylation of the MMR genes (epigenetic alteration).

In practice, the MSI test can be used as a filter to select indications for gene testing in the peripheral blood; generalized use of the test in colorectal cancer would not be cost-effective. This is why the indications for MSI testing have been codified in the revised Bethesda criteria. The test should be carried out when one of the following criteria is met: when colorectal cancer is diagnosed under the age of 50; when there is synchronous or metachronous colon cancer or an extracolonic tumor; when the tumor detected in at least two first-degree relatives, regardless of age; and when the histopathology of the tumor shows infiltrating lymphocytes with mucinous differentiation in patients under the age of 60.

Immunohistochemistry in the tumor. The paraffin-embedded sections of samples from the colorectal tumor can be processed with immunohistochemistry (IHC) to test for the presence of MMR proteins translated from one of the four MMR genes. This test, which is lest costly than the RER test, is based on proteomics. A genetic dysfunction is confirmed if the test is positive, and gene testing in the peripheral blood is then carried out. However, false-positive and false-negative results are possible, and the IHC test is complementary to the RER test rather than a substitute for it.

In summary, genetic testing is very useful, as the HNPCC phenotype is not characteristic. DNA sequencing of the MMR genes in the peripheral blood is recommended from the age of 25 if the Amsterdam criteria are positive. When the mutation is not detected, MSI and IHC tests are carried out if one criterion from the revised Bethesda list is met. Colonoscopy screening in patients with confirmed HNPCC is recommended every 2 years from the age of 25. Prophylactic colectomy is not recommended. Total colectomy is recommended when a cancer has been detected. Screening for endometrial cancer in women (transvaginal ultrasonography) is recommended at 2-year intervals from the age of 25. There is debate regarding endoscopic screening for the stomach. In the attenuated HNPCC syndrome, colonoscopy is recommended at 3-year intervals.

Nonsyndromic familial colorectal cancer

Familial clustering in nonsyndromic familial colorectal cancer occurs in one-third of all new incident cases. In this situation, the Amsterdam criteria are not met, and inherited low-penetrance factors play a role. As in all diseases with complex heredity, the phenotype is expressed under the influence of environmental factors.

DNA fingerprinting has been attempted in tumors. Multiple genes associated with the common category of familial colon cancer are beginning to be identified. Various candidate genes have been suggested, either in the model of attenuated FAP or *MYH* syndrome, or polymorphism of methylene tetrahydrofolate (MTHF) reductase,

IGF2, or *TGFBR1* genes. It is hoped that gene testing will become available in the future for patients with a moderate inherited risk.

It has recently been suggested that an association of five criteria is predictive of the prognosis of sporadic colorectal cancer (Risques et al. 2003): DNA ploidy, microsatellite instability, location in proximal colon, p53 and K-*ras* mutation. For example, tumors of the proximal colon with microsatellite instability have a good prognosis, while aneuploid tumors of the distal colon with a p53 mutation have a poor prognosis.

In summary, in the absence of gene testing, screening recommendations for nonsyndromic familial colorectal cancer are not based on the fecal occult blood test but on primary colonoscopy. Screening should start at the age of 40 if a single relative over the age of 60 is affected with colorectal cancer, and colonoscopy should be repeated after 10 years. Colonoscopy should be repeated at 5-year intervals if two or more first-degree relatives have colorectal cancer.

Sporadic colorectal cancer

Syndromic heritable colorectal cancer accounts for less than 5% of cases. The other 95% of the cases occur either with or without some familial clustering. When there is no familial clustering, colorectal cancer is called sporadic; this accounts for 65% of all cases. In sporadic cancer, there is no role for gene testing in the peripheral blood. In colonic tumors, somatic mutations can be used as biomarkers of progression, but their predictive value for the outcome is a matter of debate. In the peripheral blood, the presence of tumor antigens (proteins or glycoproteins) has prognostic value and is useful for detecting recurrences, but is not helpful in the early diagnosis, even when multiple biomarkers are combined. In the future, there is some hope that new diagnostic tools will be developed using novel techniques in proteomics.

Appendix

Genome. The genome is the DNA material packed into the nucleus of the cells.

Genomics is the discipline that examines the structure, function, and interaction of all the genes. Interindividual variations (polymorphism) in the human genome are relatively limited, as the number of generations since the first ancestor is not larger than 5000. The genotype determines observable genetic characteristics modified by the environment: this is an individual's phenotype. Databases for genomics are available on PubMed services and in the Human Genome Browser (http://www.ncbi.nlm.nih.gov/ and http://www.genome.ucsc.edu/).

Transcriptome. The transcriptome is the complete collection of elements transcribed from DNA to RNA. It includes messenger RNA (mRNA), which carries the information to the ribosomes, where proteins are translated, and noncoding RNAs. Alterations in the structure or levels of expression of any one of these RNAs or their proteins can contribute to disease.

Transcriptomics is a discipline that examines the structure and function of RNA. It is easier to study RNA molecules after they have been transcribed into complementary DNA (cDNA) using the reverse transcriptase enzyme.

Proteome. The proteome is the complete collection of human proteins.

Proteomics is an emerging discipline that studies the structure and function of proteins in health and disease. Obviously, there is a close association between genomics, transcriptomics, and proteomics. In contrast to the genome, the proteome is a dynamic, constantly changing structure; some insights into its function have been published, but most will need further confirmation. Databases are available at the proteomics site (http://www.hupo.org/).

Genetic epidemiology investigates the transmission of susceptibility to diseases among families or populations, and the interaction of genetic factors with environmental factors.

Medical genomics aims to characterize the coding elements that determine the phenotype of cells and analyzes the molecular mechanisms involved in carcinogenesis. Diagnostic tests and even pharmacological treatment (chemoprevention) can be carried out if a mechanism is elucidated.

References

The texts of all of these references are available at www.esge.com

Aaltonen LA, Salovaara R, Kristo P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338:1481–7.

Alfonso P, Nunez A, Madoz-Gurpide J, et al. Proteomic expression analysis of colorectal cancer by two-dimensional differential gel electrophoresis. Proteomics 2005;5:2602–11.

Boynton KA, Summerhayes IC, Ahlquist DA, Shuber AP. DNA integrity as a potential marker for stool-based detection of colorectal cancer. Clin Chem 2003;49:1058–65.

Burt R, Neklason DW. Genetic testing for inherited colon cancer. Gastroenterology 2005;128:1696–716.

Collins FS, Morgan M, Patrinos A. The Human Genome Project: lessons from large-scale biology. Science 2003;300:286–90

Cruz-Correa M, Cui H, Giardiello FM, et al. Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. Gastroenterology 2004;126:964–970.

Cutler P. Protein arrays: the current state-of-the-art. Proteomics 2003;3:3–18.

Doxey BW, Kuwada SK, Burt RW. Inherited polyposis syndromes: molecular mechanisms, clinicopathology, and genetic testing. Clin Gastroenterol Hepatol 2005;3:633–41.

Krieg RC, Paweletz CP, Liotta LA, Petricoin EF 3rd. Clinical proteomics for cancer biomarker discovery and therapeutic targeting. Technol Cancer Res Treat 2002;1:263–72.

Johannsdottir JT, Bergthorsson JT, Gretarsdottir S, et al. Replication error in colorectal carcinoma: association with loss of heterozygosity at mismatch repair loci and clinicopathological variables. Anticancer Res 1999;19:1821–6.

Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860–921.

Lazaridis KN, Juran BD. American Gastroenterological Association future trends committee report: the application of genomic and proteomic technologies to digestive disease diagnosis and treatment and their likely impact on gastroenterology clinical practice. Gastroenterology 2005;129:1720–52.

Lazaridis KN Petersen GM. Genomics, genetic epidemiology, and genomic medicine. Clin Gastroenterol Hepatol 2005;3:320–8.

Lindor NM, Rabe K, Petersen GM, et al. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. JAMA 2005;293:1979–85.

Risques RA, Moreno V, Ribas M, et al. Genetic pathways and genome-wide determinants of clinical outcome in colorectal cancer. Cancer Res 2003;63:7206–14.

Skandarajah AR, Moritz RL, Tjandra JJ, Simpson RJ. Proteomic analysis of colorectal cancer: discovering novel biomarkers. Expert Rev Proteomics 2005;2:681–92.

Stormoken AT, Bowitz-Lothe IM, Noren T, et al. Immunohistochemistry identifies carriers of mismatch repair gene defects causing hereditary nonpolyposis colorectal cancer. J Clin Oncol 2005;23:4705–12.

Strate LL, Syngal S. Hereditary colorectal cancer syndromes. Cancer Causes Control 2005;16:201–13.

Wheeler GM. Epigenetics, mismatch repair genes and colorectal cancer. Ann R Coll Surg Engl 2005;87:15–20.

Table **1** Proportion of inherited and sporadic colorectal cancers (Burt and Neklason 2005)

	Proportion of incident cases
Autosomal-dominant inheritance	
FAP	1.0%
HNPCC	2.5%
Hamartomatous polyps	0.1%
Sporadic, no hereditary transmission	
Sporadic colorectal cancer	Up to 65%
Sporadic, with complex heredity and some familial clustering	30%

FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer.

Table **2** Risk of colorectal cancer in individuals with inherited syndromes (Burt and Neklason 2005)

	Cumulative risk of cancer at age 40–50
FAP	100%
Attenuated FAP	80%
HNPCC	80%
Peutz–Jeghers syndrome	40%
Juvenile polyposis	10–70%
Cowden disease	10%

FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer.

Table **3** Lifetime risk of common colorectal cancer in the general population and in individuals with family antecedents (Burt and Neklason 2005)

	Lifetime risk of cancer
General population	5%
One first-degree relative with colon cancer	10–15%
Two first-degree relatives with colon cancer	15–20%
First-degree relative with colon cancer at age < 50	15–20%
One second- or third-degree relative with colon cancer	7.5%
Two second- or third-degree relatives with colon cancer	10–15%
One first-degree relative with adenomatous polyp	10%

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