Abstract. Hereditary cancer syndromes comprise approximately 5-10% of diagnosed carcinomas. They are caused by mutations in specific genes. Carriers of mutations in these genes are at an increased risk of developing cancer at a young age. When there is a suspicion of a hereditary cancer predisposition syndrome a detailed family tree of the patient requesting screening is constructed. DNA is isolated from all available members of the family. Mutation detection is carried out on DNA from an affected family member. If a mutation is found the remaining family is screened. The genetic basis of a large number of inherited cancer predisposition syndromes is known. In this paper the focus is on mutations in genes responsible for colorectal cancer, meaning adenomatous polyposis coli (APC), which is involved in familial adenomatous polyposis and homo sapiens mutL homolog 1 (hMLH1) and homo sapiens mutS homolog 2 (hMSH2), involved in hereditary non-polyposis colorectal cancer. In addition, the genes responsible for inherited breast and/or ovarian cancer, breast cancer genes 1 and 2 (BRCA1 and BRCA2), and the rearranged during transfection proto-oncogene RET which is responsible for multiple endocrine neoplasia type 2 are discussed. In all cases emphasis is given to the data available on the Greek population.

Correspondence to: G. Nasioulas, Ph.D., Head, Molecular Biology Research Center HYGEIA “Antonis Papayiannis”, Kifissias Ave. & Erythrou Stavrou str, 151 23 Maroussi, Athens; Tel: +30 210 686 7932, Fax: +30 210 686 7933, e-mail: g.nasioul@hygeia.gr

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In this review the focus is on the syndromes of inherited colorectal cancer (CRC), breast and/or ovarian cancer and multiple endocrine neoplasia type 2 (MEN2) in the Greek population.

**Method**

When investigating inherited disorders the first step is the compilation of a detailed family tree of the patients and collection of specimens of whole blood. In our laboratory DNA is extracted from the whole blood by standard methods. Mutation detection for point mutations and small insertions / deletions is carried out by cycle sequencing using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, USA). Analysis of the sequencing products is carried out by electrophoresis on a fluorescent automated DNA Sequencer (ABI Prism® 310, Applied Biosystems, Foster City, USA) (5, 6, 12, 18). Sequences obtained are aligned, using Sequencher® PC software (Gene Codes, USA), with normal sequences from Genbank and examined for the presence of mutations. If no point mutations or small insertions / deletions are identified, screening is carried out for the detection of large genomic rearrangements using the recently described method of Multiplex Ligation-dependent Probe Amplification (MLPA, MRC-Holland, The Netherlands) (12, 18). If a large genomic rearrangement is identified, the exact nature is characterized by additional methods such as long-PCR, fluorescent in situ hybridization (FISH) restriction mapping and Southern blotting. Characterization of the mutation helps in confirming its pathogenicity and in developing an accurate diagnostic test for relatives of the proband (8, 12, 18).

**Inherited Colorectal Cancer**

*Familial adenomatous polyposis (FAP).* CRC is the third most common type of cancer. The majority of cases are sporadic. Approximately 10% of all CRC cases are inherited, comprising a series of rare syndromes, which are mostly transmitted in an autosomal dominant manner.

The best known type of inherited CRC FAP is characterized by multiple polyps in the colorectal tract and a very high penetrance that reaches 90%. If polyps are left untreated 100% of the patients develop colorectal cancer.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Type of cancer</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited breast and / or ovarian cancer syndrome</td>
<td>Mainly breast and ovaries, in males prostate and pancreas</td>
<td>BRCA1, BRCA2, CHEK2</td>
</tr>
<tr>
<td>Li-Fraumeni</td>
<td>Sarcoma, breast, brain, leukemia</td>
<td>p53, CHEK2</td>
</tr>
<tr>
<td>Cowden Syndrome</td>
<td>Breast, thyroid, endometrium, etc</td>
<td>PTEN</td>
</tr>
<tr>
<td>Ataxia telangiectasia</td>
<td>Leukemia, lymphoma</td>
<td>ATM</td>
</tr>
<tr>
<td>HNPCC</td>
<td>Mainly CRC, endometrium, ovaries, urinary tract, stomach, pancreas</td>
<td>hMLH1, hMSH2, hMSH6, hPMS1</td>
</tr>
<tr>
<td>FAP</td>
<td>Mainly CRC, more rarely brain, thyroid and liver</td>
<td>APC, MYH</td>
</tr>
<tr>
<td>Hereditary gastric cancer</td>
<td>Stomach</td>
<td>CDH1</td>
</tr>
<tr>
<td>Peutz-Jeghers Syndrome</td>
<td>Carcinomas of the gastrointestinal tract, breast, testicular cancer, gynecological malignancies</td>
<td>STK11</td>
</tr>
<tr>
<td>Juvenile Polyposis</td>
<td>CRC, stomach</td>
<td>SMAD4, BMPRIA</td>
</tr>
<tr>
<td>Neurofibromatosis 1</td>
<td>Neurofibrosarcomas, pheochromocytoma, optical glioma, meningioma</td>
<td>NF1</td>
</tr>
<tr>
<td>Neurofibromatosis 2</td>
<td>Vestibular neuromas</td>
<td>NF2</td>
</tr>
<tr>
<td>Xeroderma pigmenos</td>
<td>Skin cancer, melanoma, leukemia</td>
<td>XPA, B, C, D, E, F, G POLH</td>
</tr>
<tr>
<td>Von Hippel-Lindau</td>
<td>Hemangioblastoma of the retina</td>
<td>VHL</td>
</tr>
<tr>
<td>Wilms Syndrome</td>
<td>Wilms tumors</td>
<td>WT1</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>Retinoblastoma, osteosarcoma</td>
<td>RB1</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 1</td>
<td>Adenomas of the pituitary glands and parathyroid</td>
<td>MEN1</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 2</td>
<td>Myeloid carcinoma of the thyroid, pheochromocytoma, hyperplasias of the parathyroid</td>
<td>RET</td>
</tr>
</tbody>
</table>

HNPCC: hereditary non-polyposis colorectal cancer, FAP: familial adenomatous polyposis, CRC: colorectal cancer.
Frequently, patients with FAP develop other tumors including brain, thyroid and liver tumors. Other extracolonic manifestations of FAP include desmoid tumors (Gardner syndrome), cysts in the sebaceous glands and the jawbones and congenital hypertrophy of the retinal pigment epithelium (CHRPE) (9). Despite its strong selective disadvantage, the incidence of FAP is maintained by the frequency of de novo mutations, which account for about 30% of all cases.

An inherited predisposition to CRC may also be caused by other rarer syndromes such as juvenile polyposis, caused by mutations in the homologue of mothers against decapentaplegic drosophila, 4 (SMAD4) and the gene encoding for bone morphogenetic protein receptor-1A (BMPRIA), Peutz-Jeghers syndrome (Serine/threonine protein kinase 11 – STK11 gene) (10), and MutY human homologue - associated FAP transmitted in an autosomal recessive manner (11).

HNPCC is the most common inherited syndrome predisposing to CRC, accounting for 5-10% of the total CRC (12, 13). HNPCC segregates in an autosomal dominant manner and it is caused by germline mutations in a group of genes encoding proteins involved in the DNA mismatch repair (MMR) pathway. At least five genes of the pathway, namely homo sapiens mutL homologue 1 (hMLH1) and mutS homologues 2 and 6 (hMSH2 and hMSH6) and the postmeiotic segregation increased genes 1 and 2 (hPMS1 and hPMS2), have been implicated in HNPCC (14-16). However, the majority of mutations (~ 90%) have been identified in hMLH1 (~ 50%) and hMSH2 (~ 40%). In HNPCC families, affected individuals have inherited a germline mutation, which leads to loss of function of one of the MMR genes. Malignant transformation is the result of a second, somatic mutation in the patient’s cells. HNPCC is characterized by early-onset CRC (mean age at diagnosis ~ 45 years) and an increased incidence of cancer in other organs such as the endometrium, stomach, small bowel, ovary, hepatobiliary tract, renal pelvis and ureter (13, 17).

As was the case for FAP, the only available data on the Greek population has originated from our group (12). We have carried out genetic analysis of the MMR genes hMLH1 and hMSH2 in 140 patients from 37 families with a clinical diagnosis of HNPCC (Figure 2). A pathogenic mutation has been identified in 10 (27%) of the families including a 2.2 kb deletion encompassing exon 3 of the hMSH2 gene. In contrast to other populations (16) the majority of mutations have been identified in the hMSH2 gene (60%). Furthermore, five of the
identified mutations have not been previously described in the literature (12).

Our center has become the reference center for inherited CRC genetic diagnosis in Greece and has made an enormous effort to record all Greek CRC families (5, 6, 9, 12).

Hereditary Breast and Ovarian Cancer

Breast cancer is the most common malignancy in women and a major health problem in developed countries. It has been shown that the majority of hereditary breast / ovarian carcinomas can be attributed to mutations in two genes, breast cancer genes 1 and 2 (BRCA1 and BRCA2). The lifetime risk of developing breast or ovarian cancer for mutation carriers is 60-80% and 20-40%, respectively. The average age of breast cancer onset for mutation carriers is 42 years old, that is 20 years earlier than the average female population in the USA and W. Europe (18, 19). BRCA1 encodes for a protein, which seems to act as a transcription regulator and also have a role in the maintenance of genomic integrity. The major role of the protein encoded by BRCA2 seems to be in DNA damage repair. It is estimated that 6-7% of breast cancer and 10% of ovarian cancer in the general population is caused by mutations in one of the two genes.

Our group and that of Yannoukakos’s laboratory (20-22) have been carrying out mutation screening for the BRCA 1 and 2 genes in the Greek population for the past ten years. In our group a pathogenic mutation has been identified in 15.7% of the 70 families examined, eight families with a BRCA1 mutation including a deletion that encompasses 3.2 kb and three families with a BRCA2 mutation (18) (Figure 3). These results are in agreement with those of Yannoukakos et al. where deleterious mutations in one of the BRCA genes were identified in 20% of the families tested (20-22).

Multiple Endocrine Neoplasia Type 2 (MEN 2)

MEN 2 is an autosomal dominant inherited syndrome whose main characteristic is a strong predisposition to the development of endocrine tumors. Based on the affected tissue MEN 2 has been subdivided to three groups: MEN 2A - 69-90% of MEN 2 cases; MEN 2B - 5% and Familial medullary thyroid cancer - FMTC (5-35%). In all three groups medullary thyroid cancer (MTC) is the main characteristic (23, 24).

MEN 2A is characterized by MTC (95% of cases), pheochromocytoma (50%) and hyperparathyroidism (20-30%). MEN 2B is characterized by MTC (100%), pheochromocytoma (50%), mucinous neuroa, intestinal ganglioneuroma and Marfan-like constitution. FMTC is characterized by a strong predisposition to MTC without associated problems of the parathyroid or the adrenal medulla. MEN 2 is caused by mutations in the rearranged during transfection proto-oncogene (RET), which encodes for a tyrosine kinase transmembrane receptor. The syndrome is characterized by high penetrance (92%). Inherited MTC accounts for 20-25% of all MTC cases, the remainder being sporadic (23, 24).
As far as the Greek population is concerned there have been only two reports of familial analysis of the RET proto-oncogene (23, 24). In the first report published in 1998 (23) a pathogenic mutation was identified in 66.67% (8/12 families). In the second report the RET proto-oncogene was examined in 43 patients from 17 families. A mutation in exon 10 of the gene was identified in seven families and one in exon 11 in two families (Figure 4) (24).

Preimplantation Genetic Diagnosis

Individuals who are affected by a genetic disease or carry a mutation are at an increased risk of transmitting this to their offspring. With increased understanding of the underlying basis of inherited disorders, the ability to screen individuals for a specific disorder has become possible. This identifies mutation carriers who can then receive effective genetic counseling and management.

Despite the obvious advantages to couples at risk of having an affected child, prenatal diagnosis of an established pregnancy suffers from a major drawback, namely the need for terminating a fetus diagnosed as affected. For some couples termination of an established pregnancy is unacceptable on moral or religious grounds. This is especially true for non life-threatening or late onset disorders. This led to the advent of PGD, a very early form of prenatal diagnosis carried out before embryonic implantation. Couples requesting PGD have to go through routine in vitro fertilization (IVF) procedures, although they are usually fertile, in order to generate a large number of embryos for genetic testing. Genetic analysis is performed within 24 hours allowing the selection of unaffected embryos for transfer to the mother’s uterus within the same IVF cycle (25). In theory, PGD could be offered for any disorder of which the underlying molecular basis is known. In fact, in the period between January 1997 and May 2001, more than 1,500 clinical PGD cycles were carried out in 25 European and Australian centers, resulting in 215 pregnancies and 117 babies born (26). These cycles cover a wide spectrum of disorders, including chromosomal abnormalities and a number of late onset disorders, such as inherited predisposition to cancer (FAP, Von Hippel-Lindau syndrome, retinoblastoma, Li-Fraumeni syndrome, neurofibromatosis types I and II and familial posterior fossa brain tumour) (27-29) or non-life threatening, but nevertheless incapacitating disorders such as fragile X and Crouzon syndrome (30-33).

Conclusion

The results of genetic testing for inherited cancer predisposition syndromes in the Greek population have revealed that a combination of methods capable of detecting both single point substitutions and small insertions/deletions...
in addition to large genomic rearrangements is necessary as a gross rearrangement has been identified in one family each in FAP, HNPCC and inherited breast/ovarian cancer families (8, 12, 18).

Another interesting point emerging from the study of the Greek population is the fact that in contrast to other populations no founder mutations have been identified in FAP and HNPCC making genetic testing of these conditions more time consuming and expensive. In contrast, a common founder mutation in the BRCA1 gene, 5382insC, accounts for 45% of the mutations identified in Greek breast/ovarian cancer families (22).

Today, the genetic basis of a large portion of inherited cancer predisposition syndromes is known. In such cases, patients can benefit from genetic testing and genetic counseling. For confirmed carriers closer inspection is required while there is no need for clinical examinations for non-carriers. Early close inspection of carriers may significantly improve their life quality and expectancy. Furthermore, carriers can opt for genetic testing of their fetus (prenatal diagnosis) or embryos (PGD) in order to prevent transmission of the disorder to their offspring.

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References


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