

## Chapter 2

# Genetics of Colon Cancer Susceptibility

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**Abstract** Colorectal cancer (CRC) exhibits a strong familial risk with first-degree relatives of cases having a two to three times greater risk of developing CRC than the general population. An estimated 35% of CRC cases are due to genetic factors. Highly penetrant predisposing genes have been identified for several inherited CRC syndromes (e.g., FAP, Lynch syndrome, and juvenile polyposis) through genetic linkage studies. However, despite these considerable successes, mutations in these rare syndromes explain less than 6% of CRCs and only a small fraction of familial risk. While two recently described syndromes, *MUTYH*-associated polyposis, with its pattern of recessive inheritance, and familial CRC type X, account for additional genetic burden, they still account for only a small fraction of CRC risk. In the last few years, considerable effort has been directed toward the identification of common, low-penetrance mutations through the promising approach of genome-wide association studies (GWAS). With respect to CRC, 15 novel disease loci have been identified through GWAS including several genes involved in the TGF $\beta$  signaling pathway. The familial and population risks explained by these loci remain small, but it is expected that additional novel susceptibility markers will be identified as larger ongoing and pooled GWAS are completed. While the role of the majority of susceptibility genes identified through linkage studies and GWAS in energy balance remains unclear, a pattern is emerging of a possible link given that several TGF $\beta$ -related genes have been implicated in energy balance including susceptibility genes identified through linkage analyses or GWAS.

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## 1 Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is an autosomal, dominantly inherited condition with the defining clinical feature of the development of hundreds to thousands of adenomatous polyps throughout the colon in childhood and adolescence [1, 2]. FAP exhibits nearly 100% penetrance [3] with equal gender distribution [4] and accounts for nearly 1% of all colorectal cancers (CRCs) [5]. FAP has a variable degree of clinical expression [6], including attenuated (10–100 polyps), sparse (100–500 polyps), and profuse (>2,000 polyps) forms. Attenuated FAP (AFAP) [7] shows a delayed onset of CRC, occurring on average 12 years later than classic/profuse FAP [8, 9].

Patients with FAP can develop a variety of extracolonic tumors including upper gastrointestinal tract malignancies and cancers of the thyroid, pancreas, biliary tree, brain, and hepatoblastomas [8]. A diagnosis of FAP that also includes medulloblastoma is termed Turcot's syndrome [10], and the association of polyposis with osteomas and desmoid tumors has been referred to as Gardner's syndrome. FAP patients can also develop a variety of extracolonic manifestations, including duodenal and fundic gland polyps or retinal epithelium abnormalities as seen in congenital hypertrophy of retinal pigment epithelium (CHRPE) [11]. Many of these extracolonic manifestations correlate with *APC*-specific mutations (see later in this section).

The gene responsible for FAP, the *Adenomatous Polyposis Coli* (*APC*) gene on chromosome 5q21, was cloned in 1991 following linkage analysis in families with FAP [12–15]. *APC* is a large gene that encodes a protein of 2,843 amino acids [16]. It functions as a tumor suppressor and has been implicated in a number of cell processes [16–18], but the best-characterized role for *APC* is as part of a scaffolding protein complex that negatively regulates Wntless/WNT signaling [16, 19, 20]. This pathway has been reviewed extensively elsewhere [17, 18] and is summarized here only briefly. *APC* and the transcription coregulator  $\beta$ -catenin play central roles in the WNT signaling pathway. In normal cells, in the absence of WNT signaling, *APC*, along with Axin, glycogen synthase kinase 3  $\beta$  (GSK3  $\beta$ ) and casein kinase, recruit  $\beta$ -catenin into a destruction complex where it is phosphorylated by GSK3  $\beta$ , leading to  $\beta$ -catenin degradation by the ubiquitin-mediated proteasome pathway. This cellular process leads to the maintenance of low levels of free cytosolic  $\beta$ -catenin in the cytoplasm. When the WNT signaling pathway is activated the *APC*/Axin/GSK3 $\beta$  complex disassociates, allowing stabilization of cytosolic  $\beta$ -catenin. Accumulated  $\beta$ -catenin associates with T-cell factor (TCF) and lymphoid-enhancer factor (LEF) and the resulting complex enters the nucleus and activates transcription. Once it enters the nucleus the  $\beta$ -catenin/TCF/LEF proteins provide a potent transcriptional complex leading to transactivation of a number of critical genes including *MYC* and *cyclin D1* [18, 21, 22]. Loss of control of this pathway through mutation and inactivation of *APC* leads to aberrant accumulation of  $\beta$ -catenin, and transcriptional activation of  $\beta$ -catenin/TCF/LEF complexes resulting in aberrant activation of target genes [16].

*APC* also participates in a number of other cellular processes related to cytoskeletal organization, in particular microtubule stability [22]. The genetic evidence of the importance of deregulation of the  $\beta$ -catenin signaling pathway in CRC strongly implicates a central role for the WNT/*APC*/ $\beta$ -catenin pathway in CRC development.

More than 800 different disease-causing *APC* germ-line mutations have been reported in FAP [23]. The majority of mutations occur between codons 1250 and 1464 in the 5' region of exon 15, a region known as the mutation cluster region (MCR) [23]. Mutations at codons 1061 and 1309 ("hot spots") account for approximately 11 and 17%, respectively, of all germ-line *APC* mutations [23]. The majority of the remaining mutations occur between codons 200 and 1600 with only a few mutations falling outside this region [16]. The majority of mutations are frameshift or nonsense mutations that lead to an inactive truncated protein product [16, 24]. Approximately 10–30% *APC* mutations are de novo [25]. A common missense mutation (I1307K) in *APC* has also been reported in the Ashkenazi Jewish population [26]. While this missense mutation does not appear to have any effect on *APC* function, carriers do have an increased risk of CRC but not polyposis or any other extra colonic manifestations of FAP [26].

As discussed earlier, there is marked variability in the clinical phenotype of FAP, with severity of disease often correlating with location of the *APC* mutation [27]. For example, mutations in codon 1250 to codon 1464 and particularly codon 1309 mutations correlate with profuse polyposis where symptoms usually occur 10 years earlier than milder forms [28–34]. Mutations at the extreme 5' and 3' ends of the *APC* gene are generally associated with AFAP where patients develop fewer than 100 colon polyps and cancer onset is delayed [6, 35–39].

The appearance of extracolonic manifestations also correlates with the location of *APC* mutation. For example, mutations between codons 1310 and 2011 are associated with the appearance of desmoid tumors [28], with the highest severity occurring between codons 1444/5 and 1580/1 [29, 40–42]. Mutations between codons 140 and 1309 are often associated with the occurrence of papillary thyroid cancer [43], whereas CHRPE is often associated with mutations in codons 457–1444 [12, 44]. Gardner's syndrome involving severe desmoids, osteomas, epidermoid cysts, and upper gastrointestinal polyps is generally associated with *APC* mutations in codons 1403 and 1578 [44, 45]. While no consistent genotype correlation has been found for duodenal adenomas, FAP patients with *APC* mutations in codons 976–1067 have been reported to have a three- to fourfold increased risk [28].

Mouse models support a critical role for *APC* in the development of intestinal neoplasia. Although mice homozygous for inactivated *Apc* are embryonic lethal, mice heterozygous for *Apc* (the *Multiple intestinal neoplasia* or *Min* mouse) invariably develop multiple intestinal tumors [46]. While there are some differences in the tissue specificity and morphogenesis between *Min* mice and FAP, *Min* mice have proven an important model for intestinal tumorigenesis.

## 2 Hereditary Nonpolyposis Colon Cancer/Lynch Syndrome

Hereditary nonpolyposis colorectal cancer (HNPCC) (more commonly referred to as Lynch syndrome) is a clinically heterogeneous disease that has historically been diagnosed based on family history criteria (Amsterdam and Bethesda criteria) that are not very accurate [47–49]. Lynch syndrome is characterized by a high incidence

of CRC and endometrial cancer in families. The lifetime risk for colon cancer in Lynch syndrome subjects is approximately 50–60% [50]. There is increased incidence of extracolonic cancers in both males and females including those of the small bowel, stomach, pancreas, ovary, renal pelvis, ureter, bladder, brain, appendix, liver, bile duct, gall bladder, and skin [49, 51, 52]. Colon cancers arising in Lynch syndrome families have a propensity toward left sidedness with two-thirds arising in the proximal colon [51–53]. These tumors show a variety of common histologic features including tumor-infiltrating lymphocytes, mucinous or signet ring differentiation, and a medullary growth pattern [48, 49, 53, 54].

Like FAP, Lynch syndrome is an autosomal, dominantly inherited condition. However, Lynch syndrome is more challenging to diagnose than FAP because the clinical phenotype is far more varied and more genes are involved. The majority of Lynch syndrome cases are accounted for by mutations in one of four genes (*MSH2*, *MLH1*, *MSH6*, or *PMS2*) involved in DNA mismatch repair (MMR). Of those cases with defective MMR, approximately 80–90% have germ line mutations in one of these genes. The majority of cases are due to mutations in *MSH2* and *MLH1* that play central and critical roles in DNA MMR [55], with *MSH2* forming a heterodimer with *MSH6* (and to a lesser extent *MSH3*), and *MLH1* with *PMS2*.

In newly replicated DNA, mismatches such as G>T [56] are recognized by *MSH2*–*hMSH6* heterodimers (MutS alpha in yeast), whereas insertion–deletion loops are recognized primarily by *MSH2*–*MSH6* heterodimers, but can also be mediated by the less abundant *MSH2*–*MSH3* (MutS beta) heterodimeric protein complex that appears to function as a backup in the absence of *MSH6*. Loss of *MSH2* therefore leads to the accumulation of aberrant length repeat sequences such as (A)<sub>n</sub> or (CA)<sub>n</sub> and high levels of Microsatellite Instability (MSI). Once the *MSH2*–*MSH6* heterodimer recognizes DNA mismatches, this complex undergoes an ATP-dependent conformational change converting it to a sliding DNA clamp capable of moving away from the repair site [57, 58]. This is followed by the recruitment to the complex of *MLH1*–*PMS2* heterodimers (MutL-alpha) [59]. This is then followed by exonuclease degradation of a few hundred bases of the newly synthesized mutant DNA strand followed by resynthesis of the complementary strand by DNA polymerase. As mutations in *MSH2*, *MLH1*, *MSH6*, and *PMS2* do not appear to account for all MMR deficient cases it is possible that other MMR genes have yet to be identified [59]. A detailed description of the role of these proteins in DNA MMR and their specific roles in Lynch syndrome can be found in several reviews [60, 61].

Defective MMR repair was recognized as the underlying genetic basis for Lynch syndrome following the observation by three independent groups that MSI was a hallmark feature of tumors arising in Lynch syndrome family members [62–65]. MSI, also referred to as a replication error (RER) or “mutator” tumor phenotype [62, 63, 65], occurs as a result of failure to repair of errors in copying during DNA replication. Thousands of microsatellite short tandem repeat DNA sequences (mono-, di-, tri-, or tetranucleotides) exist throughout the human genome, and errors can occur during DNA replication when copying these sequences. Typically such misalignment errors would be repaired by the DNA MMR system. However, in cells with defective MMR repair, these errors are not repaired effectively, and tumor DNAs of

Lynch syndrome family members reveal a “stuttering” (loss or gain of one or more repeats) pattern of microsatellite markers when compared with DNA from normal cells from the same subject. Once it was recognized that the MSI phenotype was similar to the mutational spectrum seen in yeast caused by deletion or mutation of MMR genes, the *MSH2* and *MLH1* genes that account for the majority of Lynch syndrome cases were identified within a year [56, 66].

Germ-line mutations in *MLH1* and *MSH2* account for the majority of mutations found in families with Lynch syndrome with a smaller minority attributable to mutations in *MSH6* and *PMS2*. Germ line testing remains a challenge as mutations can occur throughout any of these relatively large genes and are not localized to any mutation hot spots as in the *APC* gene. *MSH2* consists of 935 amino acids over 16 exons, *MLH1* consists of 756 amino acids over 19 exons, *MSH6* consists of 1,360 amino acids over 10 exons, and *PMS2* consists of 862 amino acids over 15 exons. A wide range of types of mutations has been reported in these genes including missense, nonsense and splice site mutations. In addition, a number of large genomic deletions or rearrangements involving several exons have also been reported [67–73]. Testing for *PMS2* germ-line mutations is not straightforward as there are several highly homologous *PMS2* pseudogenes, the majority of which have homology with at least some of the ten exons at the 3' end of the gene [74–77]. A comprehensive listing of MMR gene mutations can be found on the Mismatch Repair Genes Variant Database [78] and the MMR Gene Unclassified Variants database (<http://www.mmrmissense.net/>), which focuses more on functional assays and other types of data to support the interpretation of the unclassified variants in MMR genes.

Nearly 90% of Lynch syndrome colon tumors exhibit high levels of MSI [62, 65, 79], and there exists a strong correlation between MSI and loss of staining of MMR proteins using immunohistochemistry (IHC). As a result, IHC of the four MMR proteins along with an assessment of family history has been recommended as a starting point for diagnosing Lynch syndrome [79, 80]. However, it should be noted that the sensitivity of IHC staining is not as high as MSI analysis as not all MMR mutations lead to a loss of protein expression [81–83].

While defects in MMR are seen in nearly 15% of CRCs, tumors with MMR germ-line mutations account for less than 5% of all cases. This is because MMR defects are also seen in a subset of “sporadic” CRCs through somatic hypermethylation and inactivation of *MLH1* [84]. “Sporadic” MSI-H tumors share many of the characteristics of those arising in MMR mutation carriers, including a tendency toward a proximal location in the colon and a mucinous phenotype, but they usually occur later in life. Although these cancers generally arise in the absence of a positive family history, a vertical transmission in some families has been reported [85–87].

There is some evidence that *MLH1* and *MSH2* mutation families exhibit different clinical expression. Several studies have been published, with overall findings of greater CRC risk, earlier CRC onset, and fewer extracolonic tumors in *MLH1* mutation carriers compared with *MSH2* mutation carriers [50, 88–95]. Clinically, identification of an MMR gene defect, whether occurring within the context of Lynch syndrome or sporadically, is important as it affects response to some chemotherapeutic agents and ultimately prognosis [96–99].

### 3 MUTYH-Associated Polyposis

Recent studies have identified germ-line mutations in the mutY homologue *MUTYH* (also called *MYH*) with a recessive mode of inheritance associated with high risk of multiple adenomatous polyps (10–1,000) and CRC in up to 50% of *APC*-negative polyposis cases [100–102]. *MUTYH* mutations account for nearly 1% of all CRC cases [103]. The majority of cases are associated with a relatively small number of common variants (around 0.2% population frequency in Caucasians) [104–106]. Biallelic carriers develop multiple polyps by 45–55 years, although this may be an overestimate as large population-based studies have not yet been conducted [103, 105, 107].

The *MUTYH* gene was implicated in CRC risk following the observation in tumors of *APC* mutation-negative multiple polyposis families that the *APC* gene harbored an excess of somatic G:T transversions [100]. Such mutations are hallmarks of oxidative DNA damage. This led Al-Tassan and coworkers to investigate a possible role for a constitutional defect in base excision repair (BER) and the subsequent identification of two germ-line variants (Y179C and G396D) in *MUTYH* that segregated with disease in family members [100]. The majority of *MUTYH* carriers are accounted for by these two common missense mutations (44 and 24%, respectively) with a number of additional rare *MUTYH* missense mutations including some truncating mutations accounting for a small fraction [101–106, 108–112]. The Y179C *MUTYH* variant correlates with a more severe phenotype than G396D, manifesting at an earlier age of onset of polyposis and a greater risk of developing CRC than the Y179C allele [104]. Some studies have suggested that monoallelic *MUTYH* mutations may be associated with an increased risk of CRC, but this remains controversial [102, 104–106, 111, 113–116].

*MUTYH* is involved in BER of DNA damage caused by reactive oxygen species (ROS) produced through cellular metabolism or exposure to ionizing radiation. Among the lesions caused by oxidative DNA damage is 8-oxoguanine (8-oxoG). 8-oxoG is stable and highly mutagenic product prone to post-DNA replication mispairing. *MUTYH* is a DNA glycosylase involved in the identification and removal of mismatched adenines incorporated opposite 8-oxoG during replication. Failure to correct 8-oxoG:A mispairing leads to characteristic G:C to T:A transversions in the next cycle of DNA replication [117, 118]. Two other enzymes, MTH1 and OGG1, also play critical roles in BER [119, 120], but to date no mutations in these genes have been linked convincingly to increased risk of either colorectal polyposis or CRC [121].

There are few discriminatory features to *MUTYH*-related CRC. While CRC can occur throughout the colon in *MUTYH* carriers [104, 105], there is an excess of proximal cancers [101–103, 109, 122]. There are no characteristic histopathology or clinicopathologic features [103–105, 123], and tumors are microsatellite stable [104, 105, 109, 124]. Gastroduodenal polyposis has been observed in nearly 20% of *MUTYH* biallelic carriers [125–127], but this is likely to be an overestimate as these studies were conducted in highly selected polyposis registry families. *MUTYH* variants have been implicated in a number of cancers including lung, breast, gastric, and endometrial cancers. However, there remains no definitive evidence for an elevated risk of such cancers.



## 4 Familial Colorectal Cancer Type X

Over the last few years, there has been growing recognition that many families that fulfill HNPCC Amsterdam I criteria do not harbor an inherited MMR mutation [93, 128]. Growing evidence suggests that this may reflect a separate syndrome.

In a large study using the resources of the Colon Cancer Family Registry [129], Lindor et al. compared 90 Amsterdam I families with MMR-deficient tumors with 71 Amsterdam I families with MMR-proficient tumors and showed that families with MMR-deficient tumors had a statistically significantly elevated risk of developing colorectal, endometrial, gastric, small intestine, and kidney cancers as expected for Lynch syndrome. In contrast, while there was a twofold increased risk of CRC in the families with MMR-proficient tumors, there was no increased risk of any other cancer site [130]. The average age at diagnosis of CRC was also later (61 years) in families with normal MMR compared to families with MMR deficiency (49 years). Based on these data, the authors concluded the normal MMR families that met Amsterdam I should not be considered Lynch syndrome families and coined the name “familial colorectal cancer type X” (FCCTX) [130].

A number of studies have now been published that support these findings and strongly imply that FCCTX should be regarded as a distinct syndrome(s) rather than a missed diagnosis of Lynch syndrome [131–133]. In support of this, FCCTX cases are more likely to be diagnosed at a later age than Lynch syndrome cases despite having a similar incidence of adenomas, are less likely to develop multiple primary tumors, and tumors are less likely to have Lynch syndrome characteristics such as a propensity toward right-sidedness, or a mucinous or tumor-infiltrating lymphocyte pathology [113, 134–136]. While the molecular phenotype of FCCTX tumors appears to differ from that of Lynch syndrome tumors, the phenotype does not appear to be distinct from that of sporadic CRC [137, 138].

FCCTX is likely to be a heterogenous group including families with a chance aggregation of CRC, families with an undiagnosed syndrome such as *MUTYH*-associated polyposis [113] or MSI-variable families [139], and families with an as yet to undiscovered syndrome.

## 5 Hamartomatous Polyposis and Other Rare Syndromes

Several familial syndromes have been described that are characterized by multiple hamartomatous polyps in the intestinal tract including Cowden disease, Peutz–Jeghers syndrome, and juvenile polyposis syndrome. Hamartoma refers to an excessive focal overgrowth and distorted architecture of cells and tissues native to the organ in which it occurs. These rare syndromes are all inherited in an autosomal dominant fashion, and specific genetic mutations have been identified. A more extensive review of these syndromes has recently been published [140].

Cowden disease is an autosomal dominant disease characterized by intestinal hamartomas, facial trichilemmomas, oral papillomas, goiter, and esophageal glycogenic

acanthosis [141–143] with an estimated incidence of 1 in 200,000. Breast and thyroid cancer risk is also pronounced in Cowden disease, with CRC developing in up to 10% of patients. Cowden disease and several related syndromes such as Bannayan–Ruvalcaba–Riley syndrome, proteus syndrome, and proteus-like syndrome are all associated with germ-line mutations in the *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) gene. Clinical features include benign and malignant neoplasms of the thyroid, breast, uterus, and skin as well as hamartomatous intestinal polyps [144].

*PTEN* modulates G1 cell cycle progression through negatively regulating the survival signal mediated by the phosphatidylinositol 3-kinase (PI3K)/AKT pathway [145]. Inactivation of *PTEN* through mutation or deletion leads to the activation of AKT [146], increased cell proliferation and reduced apoptosis. Germ-line mutations in *PTEN* have been identified in approximately 80% of subjects diagnosed with Cowden syndrome. *PTEN* promoter mutations may account for at least another 10% of Cowden cases [147], and the remaining cases may arise from as yet undiscovered mutations in *PTEN* [148]. There appears to be a different pattern of mutation in Bannayan–Ruvalcaba–Riley syndrome cases. *PTEN* germ-line mutations account for 50–60% of patients, and large genomic deletions or rearrangements of exons of *PTEN* have been reported in Bannayan–Ruvalcaba–Riley syndrome patients but not Cowden syndrome patients. In addition, *PTEN* promoter mutations are uncommon in Bannayan–Ruvalcaba–Riley syndrome patients [143, 147, 149].

Peutz–Jeghers syndrome is a rare (approximately 1 in 200,000) autosomal dominant disorder characterized by the presence of pigmentation of the lips, buccal mucosa, hands, and feet; hamartomatous polyps throughout the gastrointestinal tract; and increased risk for gastrointestinal, breast, ovarian, and testicular cancers [150, 151]. The cumulative risk is around 30% for CRC and 50% for breast cancer [6].

Nearly half of Peutz–Jeghers cases are due to germ-line mutations in *STK11/LKB1* [152, 153]. *STK11/LKB1* is a serine–threonine kinase that phosphorylates and activates AMP-activated protein kinase an essential positive regulator the mTOR pathway that is also implicated in the *PTEN* hamartomatous syndrome [146]. Genotype–phenotype correlation suggests that patients with Peutz–Jeghers, who have a truncation mutation in *STK11/LKB1*, have a significantly earlier age of onset than those who have a missense mutation or when no mutation is detected in *STK11/LKB1* [154]. There are some families with Peutz–Jeghers syndrome that did not show linkage to the *STK11/LKB1* chromosomal region suggesting genetic heterogeneity of this disease [155, 156].

Juvenile polyposis syndrome is a rare (1 in 100,000 births) autosomal dominant condition. It is characterized by juvenile polyps, which are distinctive hamartomas that have a smooth surface and are covered by normal colonic epithelium [157]. The polyps may affect not only the colon and rectum but also the proximal gastrointestinal tract. The clinical diagnosis consists of the following criteria: more than five juvenile polyps of the colorectum, or multiple juvenile polyps throughout the gastrointestinal tract, or any number of juvenile polyps and a family history of juvenile polyps [158]. The lifetime risk approaches 60% and patients are also at risk of developing cancers of the stomach and small intestine [159]. Germ-line mutations



in the TGF $\beta$  signaling genes *SMAD4/MADH4* and *BMPRIA* account for around 20% of juvenile polyposis cases each [160–164]. More recently, mutations have been identified in a third gene, *ENG*, but the frequency remains unknown [165, 166]. Clinically, patients with an *SMAD4/MADH4* mutation are more likely to develop large gastric polyps than those with a *BMPRIA* mutation and these patients usually have a family history of upper gastrointestinal polyposis [36, 167].

Hereditary mixed polyposis syndrome (HMPS) is characterized by colonic polyps of mixed hyperplastic, adenomatous, and occasional juvenile types that eventually lead to the development of CRC [168]. The syndrome is similar to FAP in that it is an autosomal dominantly inherited condition. However, unlike the excessive number of adenomas seen in FAP, the polyps in HMPS are fewer in number, of mixed histology, and appear to be confined to the large bowel. Using a linkage approach, the *BMPRIA* gene was identified and an 11-bp deletion in the *BMPRIA* gene found in one family [168]. *BMPRIA* mutations were later confirmed in other families [169, 170].

Germ-line mutations in *BMPRIA* have been previously associated with a subset of juvenile polyposis syndrome patients [36, 161, 162]. However, the phenotypic features of the two families in this study differ from JPS. Just as germ-line mutations in *APC* can cause diverse phenotypic manifestations including those of Turcot and Gardner syndromes, it is perhaps not surprising that mutations in *BMPRIA* could be responsible for two different syndromes.

## 6 Genome-Wide Association Studies and Low-Penetrance Mutations

Over the last 5 years, genome-wide association studies (GWAS) have provided a powerful new approach for identifying susceptibility loci. Rather than focusing on the highly penetrant rare mutations described above, GWAS focus on the identification of common, low-penetrance mutations. As with linkage studies, GWAS represents an agnostic survey of the genome, but unlike linkage analyses that use a relatively small number of markers to screen cancer-dense families, GWAS employs SNP arrays containing hundreds of thousands of SNPs to screen relatively large populations. GWAS have only become possible in recent years due to major technological advances in the development of genotyping platforms that allow cost-effective high throughput genotyping of large sample sets. This approach has begun to reveal novel findings that are improving our understanding of the contribution of common alleles to risk of many complex genetic disorders including CRC.

GWAS have met with unprecedented success for a range of complex diseases [171]. As of the second quarter of 2011, there have been 1449 published genome-wide associations (at  $p < 5 \times 10^{-8}$ ) for 237 traits [172] and this number is expected to increase substantially over the next few years. With respect to CRC, as of January 2011, 15 novel disease loci have been identified in European populations [173–180]. Table 2.1 summarizes the

**Table 2.1** Published GWA studies of CRC<sup>a</sup>

Study reference	Initial sample <sup>b</sup>	Genotyping platform [SNPs]	SNP	Gene/region	OR
Houlston, Nat Genet [180]	3,334/4,638	Illumina [various]	rs6691170	1q41	1.06
			rs6687758	1q41	1.09
			rs10936599	3q26.2	0.93
			rs11169552	12q13.13	0.92
			rs7136702	12q13.13	1.06
			rs4925386	20q13.33	0.93
Houlston, Nat Genet [179]	1,902/1,929	Illumina [up to 548,586]	rs961253-A	20p12.3	1.12
			rs4444235-C	14q22.2	1.11
			rs10411210-C	19q13.11	1.15
			rs9929218-A	16q22.1	1.10
			rs4939827-T	18q21 SMAD7	1.20
			rs7014346-A	8q24	1.19
Tenesa, Nat Genet [181]	981/1,002	Illumina [541,628]	rs3802842-C	11q23	1.11
			rs10795668-A	10p14	1.27
			rs16892766-A	8q23.3 EIF3H	1.25
			rs6983267	8q24.21	1.24
			rs4779584	15q13.3	1.23
			rs4939827	18q21.1	1.18
Tomlinson, Nat Genet [177]	922/927	Illumina [547,647]	rs4939827-T	18q21 SMAD7	1.16
			rs6983267-G	8q24	1.21
			rs10505477-A	8q24	1.17
			rs719725	9p24	1.14

<sup>a</sup> NHGRI GWAS database (<http://www.genome.gov>). All studies in Caucasians

<sup>b</sup> Cases/controls

published findings from these studies, and without exception the risks conferred have been low with odds ratios between 1.1 and 1.3 [173–180]. To date these studies have been limited to individuals of European ancestry.

So what candidate genes have been identified through CRC GWAS? Of the 15 SNPs identified to date, 6 map to regions that include TGF $\beta$  signaling pathway genes, a pathway that previously has been implicated in CRC. These include *SMAD7* [174, 179], *GREM1* [176], *RHPN2*, the bone morphogenetic protein genes *BMP2* and *BMP4* [179], and most recently *LAMA5* that is required for the production of noggin, a secreted BMP antagonist [180]. TGF $\beta$  proteins play critical roles in proliferation, differentiation, cell migration, adhesion, and extracellular matrix (ECM) production [182, 183], and also energy balance (see below), and several lines of evidence support a key role for the TGF $\beta$  pathway in CRC susceptibility. For example rare, high-penetrance variants in other TGF $\beta$ -related genes (*SMAD4* and the BMP receptor *BMPRIA/ALK3*) have been reported for juvenile polyposis [36, 161, 162] and for HMPs [169, 170]. In addition, somatic mutations of *SMAD4* and the TGF $\beta$  receptor *TGFBR2* have been identified in CRC tumors. The cancer initiation properties of TGF $\beta$  seem to be distinct from those of progression, as activation of the TGF $\beta$  signaling pathway leads to enhanced tumor growth and increased metastatic potential [184].

In addition to TGF $\beta$  signaling candidates, there are intriguing findings for some of the other CRC loci identified through GWAS. For example, 8q24 is a gene desert region that has been identified as a risk locus for several different cancers including CRC [173, 175, 185–188]. While no known genes map to this region, the *MYC* oncogene maps within 300–400 kb of several independently associated SNPs. Replication, sequencing, and fine-mapping studies of this locus have identified rs6983267 as the most promising variant for functional assessment in CRC and other cancers [189]. This SNP lies in a sequence that is highly conserved across vertebrates and is predicted to have regulatory function [189]. Its relative proximity to *MYC* makes it plausible that it may disrupt a putative enhancer. However, while *MYC* is often amplified in CRC, this variant has not been found to correlate with *MYC* expression in CRC tumors or lymphoblastoid cell lines [190], although tissue-specific long-range chromatin loops between putative enhancer elements in this region and *MYC* have been shown [191]. Many of the other associated loci (e.g., 9p24, 10p14, 11q23.1, 18q23, and 20p12.3) also lie in intergenic or gene desert regions with no known biological relevance.

It is important to note that any candidate genes identified through GWAS, including those belonging to the TGF $\beta$  signaling pathway, have not yet been confirmed as causal, and there is growing emphasis on dissecting the functional consequences of GWAS findings [192]. One of the challenges for GWAS is that they rarely identify the causal variant or gene, as the SNPs that are included on commercial SNP arrays are chosen to capture regions of linkage disequilibrium (LD) identified through the HapMap project [193] rather than for any functional or putative functional role. As a result, the nearest gene mapping adjacent to an associated SNP may not be the causal gene. Considerable work is needed before functionality can be assigned to any susceptibility SNPs. This is not a trivial task as most effect sizes

are relatively small and the functional effect of any causal SNP is likely to be subtle. In addition, the majority of disease-associated SNPs identified through GWAS map to intergenic regions or gene “deserts” such as the 8q24 region [194] described above, suggesting that they affect regulatory elements such as enhancers, posing even greater challenges. Undoubtedly, a large amount of work will be needed to clarify the biological implications of these associations.

Only limited data are available with regards to the epidemiological characteristics of GWAS associations. Rs3802842 at 11q23 and rs4939827 (*SMAD7*) have been reported to be more strongly associated with rectal cancer than colon cancer [178]. No differences in risk have been reported by tumor molecular subtypes for the published variants, with the exception of rs4444235 (*BMP4*) for which the association was found to be significantly stronger for MMR proficient than deficient tumors [179]. Low-penetrance susceptibility alleles may function as modifier genes contributing to the severity of CRC in high-risk subjects. In two large studies of Lynch syndrome, two GWAS hits (rs16892766 on 8q23.3 and rs3802842 on 11q23.1) were significantly associated with an increased CRC risk in these patients [195, 196].

The familial and population risks explained by CRC GWAS loci remain small accounting for less than 10% of overall inherited risk and less than 1% of familial risk [179], and as a result they are not yet useful for risk prediction. However, it is expected that risk prediction will improve as additional susceptibility alleles are identified once ongoing, larger and pooled GWAS analyses as well as studies in other ethnic populations are completed [173–180]. In terms of risk, studies suggest that around 100 SNPs would be required to achieve 80% accuracy of prediction of CRC genetic risk [181], accounting for ~17% of the phenotypic variance providing useful predictive value. This does not take into account the contribution of rare or private variants and their effect on risk are unknown. It will take several years to more fully comprehend the impact of rare variants on CRC risk as these types of studies can only be accomplished through next generation sequencing GWAS that are just being contemplated.

It is clear that CRC etiology has a very strong environmental component [197, 198] and there are several ongoing studies examining the relationship between lifestyle risk factors for CRC and interactions with the risk alleles identified through GWAS (gene  $\times$  environment interactions). Pooling of GWAS data through collaborative efforts should improve power to detect both gene  $\times$  environment and gene  $\times$  gene interactions [199].

## 7 CRC Susceptibility Genes and Energy Balance

As discussed above, while promising progress has been made in identifying CRC susceptibility genes through linkage analyses and GWAS, the susceptibility alleles identified to date still only account for a small fraction of CRC risk. Despite this, a growing understanding of the genetic etiology of CRC is beginning to emerge as

a significant number of susceptibility genes or candidate susceptibility genes belong to the TGF $\beta$ /BMP superfamily, including *SMAD4*, *BMPRIA/ALK3*, *SMAD7*, *GREM1*, *RHPN2*, *BMP2*, *BMP4*, and *LAMA5*. The TGF $\beta$  family of proteins is a well-known key regulator of many biological processes, and several lines of evidence implicate TGF $\beta$ 1 signaling in energy balance. A review of the role of TGF $\beta$  in regulating adiposity and energy expenditure was recently published [200].

TGF $\beta$  is a negative regulator of adipogenesis, promoting preadipocyte proliferation while simultaneously inhibiting differentiation [201], a process augmented by SMAD7 (and SMAD6), a negative regulator of TGF $\beta$  signaling. TGF $\beta$  may also influence adipogenesis indirectly through upregulation of WNT signaling, a cascade that also inhibits adipocyte differentiation [202]. That APC mutations lead to the activation of WNT signaling may also implicate APC in energy balance. TGF $\beta$ 1 expression also correlates with body mass index and visceral fat obesity, which along with insulin resistance, plays a central role in metabolic syndrome [203–207], and elevated serum TGF $\beta$ 1 levels are associated with incident type 2 diabetes [208]. These findings are supported by observations in genetically engineered mice [209].

Several lines of evidence also support a role for BMPs in adipogenesis [210]. BMPs appear to play dual roles in this process. The candidate CRC susceptibility gene *BMP4* is best recognized for its role in the earliest stages of white adipocyte differentiation [211, 212]. BMP4 promotes the formation of white adipocytes in a dose-dependent manner in mouse embryonic stem cells [211, 213] a finding supported by mouse studies [214]. Several lines of evidence suggest that BMP4 is an important risk factor for metabolic syndrome [215, 216]. BMP4 was associated with increased adiposity [217], recognized as being essential for energy balance [218], and white fat differentiation [212, 214, 219]. Serum BMP4 levels also correlated with body mass index, waist circumference, waist-to-hip ratio, triglycerides, HDL cholesterol, and fasting plasma insulin [216]. *BMP4* mRNA expression has also been shown to correlate with obesity in ob/ob transgenic mice [219].

The CRC candidate susceptibility gene *BMP2* has also been implicated in adipogenesis both as a pro- and anti-adipogenic protein. BMP2 has been shown to promote osteoblast differentiation while suppressing adipocyte development [220]. In contrast, BMP2 can also stimulate adipocyte differentiation [221–223].

The cellular response to BMP2 and BMP4 is mediated by ligand binding to cell surface receptors including *BMPRIA* [224, 225], a gene that has been implicated in both HMPs and JPS patients. BMPRIA has been shown to be involved in adipocyte differentiation in vitro [105]. BMPRIA has been strongly implicated in obesity, where *BMPRIA* mRNA expression was elevated in patients with obesity, type 2 diabetes, and components of metabolic syndrome including body mass index, body mass, and waist-to-hip ratio [216]. Furthermore, *BMPRIA* mRNA levels were elevated in adipose tissues of obese and overweight adults and three SNP variants in the *BMPRIA* gene were associated with increased body mass index [225].

A pattern is, therefore, emerging of a possible link between some CRC susceptibility genes and energy balance that warrants further investigation. Based on growing evidence of a link between TGF $\beta$ -related genes, CRC susceptibility and the

development of features of metabolic syndrome, modulation of TGF $\beta$  signaling may represent a valuable therapeutic approach in at-risk individuals.

## References

1. Bulow S (1987) Familial polyposis coli. *Dan Med Bull* 34:1–15
2. Bussey HJ (1970) Gastrointestinal polyposis. *Gut* 11:970–978
3. Wennstrom J, Pierce ER, McKusick VA (1974) Hereditary benign and malignant lesions of the large bowel. *Cancer* 34(suppl):850–857
4. Rozen P, Macrae F (2006) Familial adenomatous polyposis: the practical applications of clinical and molecular screening. *Fam Cancer* 5:227–235
5. Lipton L, Tomlinson I (2006) The genetics of FAP and FAP-like syndromes. *Fam Cancer* 5:221–226
6. de la Chapelle A (2004) Genetic predisposition to colorectal cancer. *Nat Rev Cancer* 4:769–780
7. Knudsen AL, Bisgaard ML, Bulow S (2003) Attenuated familial adenomatous polyposis (AFAP). A review of the literature. *Fam Cancer* 2:43–55
8. Giardiello FM, Offerhaus JG (1995) Phenotype and cancer risk of various polyposis syndromes. *Eur J Cancer* 31A:1085–1087
9. Lynch HT, Smyrk T, McGinn T et al (1995) Attenuated familial adenomatous polyposis (AFAP). A phenotypically and genotypically distinctive variant of FAP. *Cancer* 76:2427–2433
10. Hamilton SR, Liu B, Parsons RE et al (1995) The molecular basis of Turcot's syndrome. *N Engl J Med* 332:839–847
11. Chen CS, Phillips KD, Grist S et al (2006) Congenital hypertrophy of the retinal pigment epithelium (CHRPE) in familial colorectal cancer. *Fam Cancer* 5:397–404
12. Bodmer WF, Bailey CJ, Bodmer J et al (1987) Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 328:614–616
13. Groden J, Thliveris A, Samowitz W et al (1991) Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 66:589–600
14. Kinzler KW, Nilbert MC, Vogelstein B et al (1991) Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* 251:1366–1370
15. Nishisho I, Nakamura Y, Miyoshi Y et al (1991) Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 253:665–669
16. Fearnhead NS, Britton MP, Bodmer WF (2001) The ABC of APC. *Hum Mol Genet* 10:721–733
17. Bienz M (2002) The subcellular destinations of APC proteins. *Nat Rev Mol Cell Biol* 3:328–338
18. Nathke I (2004) APC at a glance. *J Cell Sci* 117:4873–4875
19. Goss KH, Groden J (2000) Biology of the adenomatous polyposis coli tumor suppressor. *J Clin Oncol* 18:1967–1979
20. Nathke IS (2004) The adenomatous polyposis coli protein: the Achilles heel of the gut epithelium. *Annu Rev Cell Dev Biol* 20:337–366
21. Watson SA (2001) Oncogenic targets of beta-catenin-mediated transcription in molecular pathogenesis of intestinal polyposis. *Lancet* 357:572–573
22. Nathke I (2006) Cytoskeleton out of the cupboard: colon cancer and cytoskeletal changes induced by loss of APC. *Nat Rev Cancer* 6:967–974
23. Nieuwenhuis MH, Vasen HF (2007) Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): a review of the literature. *Crit Rev Oncol Hematol* 61:153–161
24. Galiatsatos P, Foulkes WD (2006) Familial adenomatous polyposis. *Am J Gastroenterol* 101:385–398



25. Guillem JG, Smith AJ, Calle JP, Ruo L (1999) Gastrointestinal polyposis syndromes. *Curr Probl Surg* 36:217–323
26. Laken SJ, Petersen GM, Gruber SB et al (1997) Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nat Genet* 17:79–83
27. Lefevre JH, Parc Y, Svrcek M et al (2009) APC, MYH, and the correlation genotype-phenotype in colorectal polyposis. *Ann Surg Oncol* 16:871–877
28. Bertario L, Russo A, Sala P et al (2003) Multiple approach to the exploration of genotype-phenotype correlations in familial adenomatous polyposis. *J Clin Oncol* 21:1698–1707
29. Caspari R, Friedl W, Mandl M et al (1994) Familial adenomatous polyposis: mutation at codon 1309 and early onset of colon cancer. *Lancet* 343:629–632
30. Enomoto M, Konishi M, Iwama T, Utsunomiya J, Sugihara KI, Miyaki M (2000) The relationship between frequencies of extracolonic manifestations and the position of APC germline mutation in patients with familial adenomatous polyposis. *Jpn J Clin Oncol* 30:82–88
31. Ficari F, Cama A, Valanzano R et al (2000) APC gene mutations and colorectal adenomatosis in familial adenomatous polyposis. *Br J Cancer* 82:348–353
32. Gayther SA, Wells D, SenGupta SB et al (1994) Regionally clustered APC mutations are associated with a severe phenotype and occur at a high frequency in new mutation cases of adenomatous polyposis coli. *Hum Mol Genet* 3:53–56
33. Nagase H, Miyoshi Y, Horii A et al (1992) Correlation between the location of germ-line mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis patients. *Cancer Res* 52:4055–4057
34. Nugent KP, Phillips RK, Hodgson SV et al (1994) Phenotypic expression in familial adenomatous polyposis: partial prediction by mutation analysis. *Gut* 35:1622–1623
35. Brensinger JD, Laken SJ, Luce MC et al (1998) Variable phenotype of familial adenomatous polyposis in pedigrees with 3' mutation in the APC gene. *Gut* 43:548–552
36. Friedl W, Uhlhaas S, Schulmann K et al (2002) Juvenile polyposis: massive gastric polyposis is more common in MADH4 mutation carriers than in BMPR1A mutation carriers. *Hum Genet* 111:108–111
37. Sieber OM, Segditsas S, Knudsen AL et al (2006) Disease severity and genetic pathways in attenuated familial adenomatous polyposis vary greatly but depend on the site of the germline mutation. *Gut* 55:1440–1448
38. Soravia C, Berk T, Madlensky L et al (1998) Genotype-phenotype correlations in attenuated adenomatous polyposis coli. *Am J Hum Genet* 62:1290–1301
39. Walon C, Kartheuser A, Michils G et al (1997) Novel germline mutations in the APC gene and their phenotypic spectrum in familial adenomatous polyposis kindreds. *Hum Genet* 100:601–605
40. Davies DR, Armstrong JG, Thakker N et al (1995) Severe Gardner syndrome in families with mutations restricted to a specific region of the APC gene. *Am J Hum Genet* 57:1151–1158
41. Friedl W, Caspari R, Sengteller M et al (2001) Can APC mutation analysis contribute to therapeutic decisions in familial adenomatous polyposis? Experience from 680 FAP families. *Gut* 48:515–521
42. Gebert JF, Dupon C, Kadmon M et al (1999) Combined molecular and clinical approaches for the identification of families with familial adenomatous polyposis coli. *Ann Surg* 229:350–361
43. Cetta F, Montalto G, Gori M, Curia MC, Cama A, Olschwang S (2000) Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. *J Clin Endocrinol Metab* 85:286–292
44. Giardiello FM, Petersen GM, Piantadosi S et al (1997) APC gene mutations and extraintestinal phenotype of familial adenomatous polyposis. *Gut* 40:521–525
45. Dobbie Z, Spycher M, Mary JL et al (1996) Correlation between the development of extracolonic manifestations in FAP patients and mutations beyond codon 1403 in the APC gene. *J Med Genet* 33:274–280
46. Moser AR, Pitot HC, Dove WF (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* 247:322–324
47. Vasen HF, Mecklin JP, Khan PM, Lynch HT (1991) The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum* 34:424–425

48. Rodriguez-Bigas MA, Boland CR, Hamilton SR et al (1997) A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 89:1758–1762
49. Umar A, Boland CR, Terdiman JP et al (2004) Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 96:261–268
50. Dunlop MG, Farrington SM, Carothers AD et al (1997) Cancer risk associated with germline DNA mismatch repair gene mutations. *Hum Mol Genet* 6:105–110
51. Lynch HT, Smyrk T (1996) Hereditary nonpolyposis colorectal cancer (Lynch syndrome). An updated review. *Cancer* 78:1149–1167
52. Lynch HT, Smyrk TC, Watson P et al (1993) Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 104:1535–1549
53. Lynch HT, de la Chapelle A (2003) Hereditary colorectal cancer. *N Engl J Med* 348:919–932
54. Gryfe R, Kim H, Hsieh ET et al (2000) Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 342:69–77
55. Seifert M, Reichrath J (2006) The role of the human DNA mismatch repair gene hMSH2 in DNA repair, cell cycle control and apoptosis: implications for pathogenesis, progression and therapy of cancer. *J Mol Histol* 37:301–307
56. Leach FS, Nicolaides NC, Papadopoulos N et al (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75:1215–1225
57. Blackwell LJ, Martik D, Bjornson KP, Bjornson ES, Modrich P (1998) Nucleotide-promoted release of hMutSalph $\alpha$  from heteroduplex DNA is consistent with an ATP-dependent translocation mechanism. *J Biol Chem* 273:32055–32062
58. Gradia S, Acharya S, Fishel R (2000) The role of mismatched nucleotides in activating the hMSH2-hMSH6 molecular switch. *J Biol Chem* 275:3922–3930
59. Jiricny J, Marra G (2003) DNA repair defects in colon cancer. *Curr Opin Genet Dev* 13:61–69
60. Peltomaki P (2003) Role of DNA mismatch repair defects in the pathogenesis of human cancer. *J Clin Oncol* 21:1174–1179
61. Modrich P (2006) Mechanisms in eukaryotic mismatch repair. *J Biol Chem* 281:30305–30309
62. Aaltonen LA, Peltomaki P, Mecklin JP et al (1994) Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 54:1645–1648
63. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 363:558–561
64. Peltomaki P, Aaltonen LA, Sistonen P et al (1993) Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 260:810–812
65. Thibodeau SN, Bren G, Schaid D (1993) Microsatellite instability in cancer of the proximal colon. *Science* 260:816–819
66. Fishel R, Lescoe MK, Rao MR et al (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 75:1027–1038
67. Gill S, Lindor NM, Burgart LJ et al (2005) Isolated loss of PMS2 expression in colorectal cancers: frequency, patient age, and familial aggregation. *Clin Cancer Res* 11:6466–6471
68. Charbonnier F, Raux G, Wang Q et al (2000) Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments. *Cancer Res* 60:2760–2763
69. Nakagawa H, Yan H, Lockman J et al (2002) Allele separation facilitates interpretation of potential splicing alterations and genomic rearrangements. *Cancer Res* 62:4579–4582
70. Wagner A, Barrows A, Wijnen JT et al (2003) Molecular analysis of hereditary nonpolyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene. *Am J Hum Genet* 72:1088–1100

71. Wijnen J, van der Klift H, Vasen H et al (1998) MSH2 genomic deletions are a frequent cause of HNPCC. *Nat Genet* 20:326–328
72. Yan H, Papadopoulos N, Marra G et al (2000) Conversion of diploidy to haploidy. *Nature* 403:723–724
73. Casey G, Lindor NM, Papadopoulos N et al (2005) Conversion analysis for mutation detection in MLH1 and MSH2 in patients with colorectal cancer. *JAMA* 293:799–809
74. Nicolaides NC, Carter KC, Shell BK, Papadopoulos N, Vogelstein B, Kinzler KW (1995) Genomic organization of the human PMS2 gene family. *Genomics* 30:195–206
75. Nicolaides NC, Kinzler KW, Vogelstein B (1995) Analysis of the 5' region of PMS2 reveals heterogeneous transcripts and a novel overlapping gene. *Genomics* 29:329–334
76. Nakagawa H, Lockman JC, Frankel WL et al (2004) Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. *Cancer Res* 64:4721–4727
77. De Vos M, Hayward BE, Picton S, Sheridan E, Bonthron DT (2004) Novel PMS2 pseudo-genes can conceal recessive mutations causing a distinctive childhood cancer syndrome. *Am J Hum Genet* 74:954–964
78. Woods MO, Williams P, Careen A et al (2007) A new variant database for mismatch repair genes associated with Lynch syndrome. *Hum Mutat* 28:669–673
79. Cunningham JM, Kim CY, Christensen ER et al (2001) The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. *Am J Hum Genet* 69:780–790
80. Lindor NM, Burgart LJ, Leontovich O et al (2002) Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 20:1043–1048
81. Wahlberg SS, Schmeits J, Thomas G et al (2002) Evaluation of microsatellite instability and immunohistochemistry for the prediction of germ-line MSH2 and MLH1 mutations in hereditary nonpolyposis colon cancer families. *Cancer Res* 62:3485–3492
82. Hampel H, Frankel WL, Martin E et al (2005) Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 352:1851–1860
83. Hampel H, Frankel WL, Martin E et al (2008) Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol* 26:5783–5788
84. Herman JG, Umar A, Polyak K et al (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 95:6870–6875
85. Gazzoli I, Loda M, Garber J, Syngal S, Kolodner RD (2002) A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the MLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. *Cancer Res* 62:3925–3928
86. Miyakura Y, Sugano K, Akasu T et al (2004) Extensive but hemiallelic methylation of the hMLH1 promoter region in early-onset sporadic colon cancers with microsatellite instability. *Clin Gastroenterol Hepatol* 2:147–156
87. Suter CM, Martin DI, Ward RL (2004) Germline epimutation of MLH1 in individuals with multiple cancers. *Nat Genet* 36:497–501
88. Goecke T, Schulmann K, Engel C et al (2006) Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC Consortium. *J Clin Oncol* 24:4285–4292
89. Kastrinos F, Stoffel EM, Balmana J, Steyerberg EW, Mercado R, Syngal S (2008) Phenotype comparison of MLH1 and MSH2 mutation carriers in a cohort of 1,914 individuals undergoing clinical genetic testing in the United States. *Cancer Epidemiol Biomarkers Prev* 17:2044–2051
90. Vasen HF, Wijnen JT, Menko FH et al (1996) Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* 110:1020–1027
91. Parc Y, Boisson C, Thomas G, Olschwang S (2003) Cancer risk in 348 French MSH2 or MLH1 gene carriers. *J Med Genet* 40:208–213
92. Farrington SM, Lin-Goerke J, Ling J et al (1998) Systematic analysis of hMSH2 and hMLH1 in young colon cancer patients and controls. *Am J Hum Genet* 63:749–759

93. Bisgaard ML, Jager AC, Myrholm T, Bernstein I, Nielsen FC (2002) Hereditary non-polyposis colorectal cancer (HNPCC): phenotype-genotype correlation between patients with and without identified mutation. *Hum Mutat* 20:20–27
94. Vasen HF, Stormorken A, Menko FH et al (2001) MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. *J Clin Oncol* 19:4074–4080
95. Peltomäki P, Gao X, Mecklin JP (2001) Genotype and phenotype in hereditary nonpolyposis colon cancer: a study of families with different vs. shared predisposing mutations. *Fam Cancer* 1:9–15
96. Ribic CM, Sargent DJ, Moore MJ et al (2003) Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 349:247–257
97. Vilar E, Scialtiti M, Balmana J et al (2008) Microsatellite instability due to hMLH1 deficiency is associated with increased cytotoxicity to irinotecan in human colorectal cancer cell lines. *Br J Cancer* 99:1607–1612
98. Bertagnoli MM, Niedzwiecki D, Compton CC et al (2009) Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol* 27:1814–1821
99. Kim GP, Colangelo LH, Wieand HS et al (2007) Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. *J Clin Oncol* 25:767–772
100. Al-Tassan N, Chmiel NH, Maynard J et al (2002) Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. *Nat Genet* 30:227–232
101. Sieber OM, Lipton L, Crabtree M et al (2003) Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med* 348:791–799
102. Croitoru ME, Cleary SP, Di Nicola N et al (2004) Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. *J Natl Cancer Inst* 96:1631–1634
103. Nielsen M, Joerink-van de Beld MC, Jones N et al (2009) Analysis of MUTYH genotypes and colorectal phenotypes in patients with MUTYH-associated polyposis. *Gastroenterology* 136:471–476
104. Lubbe SJ, Di Bernardo MC, Chandler IP, Houlston RS (2009) Clinical implications of the colorectal cancer risk associated with MUTYH mutation. *J Clin Oncol* 27:3975–3980
105. Cleary SP, Cotterchio M, Jenkins MA et al (2009) Germline MutY human homologue mutations and colorectal cancer: a multisite case-control study. *Gastroenterology* 136:1251–1260
106. Tenesa A, Campbell H, Barnetson R, Porteous M, Dunlop M, Farrington SM (2006) Association of MUTYH and colorectal cancer. *Br J Cancer* 95:239–242
107. Sampson JR, Dolwani S, Jones S et al (2003) Autosomal recessive colorectal adenomatous polyposis due to inherited mutations of MYH. *Lancet* 362:39–41
108. Enholm S, Hienonen T, Suomalainen A et al (2003) Proportion and phenotype of MYH-associated colorectal neoplasia in a population-based series of Finnish colorectal cancer patients. *Am J Pathol* 163:827–832
109. Wang L, Baudhuin LM, Boardman LA et al (2004) MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. *Gastroenterology* 127:9–16
110. Venesio T, Molatore S, Cattaneo F, Arrigoni A, Risio M, Ranzani GN (2004) High frequency of MYH gene mutations in a subset of patients with familial adenomatous polyposis. *Gastroenterology* 126:1681–1685
111. Farrington SM, Tenesa A, Barnetson R et al (2005) Germline susceptibility to colorectal cancer due to base-excision repair gene defects. *Am J Hum Genet* 77:112–119
112. Poulsen ML, Bisgaard ML (2008) MUTYH associated polyposis (MAP). *Curr Genomics* 9:420–435

113. Jenkins MA, Croitoru ME, Monga N et al (2006) Risk of colorectal cancer in monoallelic and biallelic carriers of MYH mutations: a population-based case-family study. *Cancer Epidemiol Biomarkers Prev* 15:312–314
114. Jones N, Vogt S, Nielsen M et al (2009) Increased colorectal cancer incidence in obligate carriers of heterozygous mutations in MUTYH. *Gastroenterology* 137:489–494, 94 e1; quiz 725–6
115. Fleischmann C, Peto J, Cheadle J, Shah B, Sampson J, Houlston RS (2004) Comprehensive analysis of the contribution of germline MYH variation to early-onset colorectal cancer. *Int J Cancer* 109:554–558
116. Gismondi V, Meta M, Bonelli L et al (2004) Prevalence of the Y165C, G382D and 1395delGGA germline mutations of the MYH gene in Italian patients with adenomatous polyposis coli and colorectal adenomas. *Int J Cancer* 109:680–684
117. Lu AL, Li X, Gu Y, Wright PM, Chang DY (2001) Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem Biophys* 35:141–170
118. Yamane A, Shinmura K, Sunaga N et al (2003) Suppressive activities of OGG1 and MYH proteins against G:C to T:A mutations caused by 8-hydroxyguanine but not by benzo[a]pyrene diol epoxide in human cells in vivo. *Carcinogenesis* 24:1031–1037
119. Tudek B (2007) Base excision repair modulation as a risk factor for human cancers. *Mol Aspects Med* 28:258–275
120. Frosina G (2007) Tumor suppression by DNA base excision repair. *Mini Rev Med Chem* 7:727–743
121. Dallosso AR, Dolwani S, Jones N et al (2008) Inherited predisposition to colorectal adenomas caused by multiple rare alleles of MUTYH but not OGG1, NUDT1, NTH1 or NEIL 1, 2 or 3. *Gut* 57:1252–1255
122. Croitoru ME, Cleary SP, Berk T et al (2007) Germline MYH mutations in a clinic-based series of Canadian multiple colorectal adenoma patients. *J Surg Oncol* 95:499–506
123. O'Shea AM, Cleary SP, Croitoru MA et al (2008) Pathological features of colorectal carcinomas in MYH-associated polyposis. *Histopathology* 53:184–194
124. Lipton L, Halford SE, Johnson V et al (2003) Carcinogenesis in MYH-associated polyposis follows a distinct genetic pathway. *Cancer Res* 63:7595–7599
125. Aretz S, Uhlhaas S, Goergens H et al (2006) MUTYH-associated polyposis: 70 of 71 patients with biallelic mutations present with an attenuated or atypical phenotype. *Int J Cancer* 119:807–814
126. Nielsen M, Poley JW, Verhoef S et al (2006) Duodenal carcinoma in MUTYH-associated polyposis. *J Clin Pathol* 59:1212–1215
127. Nielsen M, Hes FJ, Nagengast FM et al (2007) Germline mutations in APC and MUTYH are responsible for the majority of families with attenuated familial adenomatous polyposis. *Clin Genet* 71:427–433
128. Wijnen JT, Vasen HF, Khan PM et al (1998) Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. *N Engl J Med* 339:511–518
129. Newcomb PA, Baron J, Cotterchio M et al (2007) Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol Biomarkers Prev* 16:2331–2343
130. Lindor NM, Rabe K, Petersen GM et al (2005) Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. *JAMA* 293:1979–1985
131. Renkonen E, Zhang Y, Lohi H et al (2003) Altered expression of MLH1, MSH2, and MSH6 in predisposition to hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 21:3629–3637
132. Schieman U, Muller-Koch Y, Gross M et al (2004) Extended microsatellite analysis in microsatellite stable, MSH2 and MLH1 mutation-negative HNPCC patients: genetic reclassification and correlation with clinical features. *Digestion* 69:166–176
133. Mueller-Koch Y, Vogelsang H, Kopp R et al (2005) Hereditary non-polyposis colorectal cancer: clinical and molecular evidence for a new entity of hereditary colorectal cancer. *Gut* 54:1733–1740

134. Dove-Edwin I, de Jong AE, Adams J et al (2006) Prospective results of surveillance colonoscopy in dominant familial colorectal cancer with and without Lynch syndrome. *Gastroenterology* 130:1995–2000
135. Valle L, Perea J, Carbonell P et al (2007) Clinicopathologic and pedigree differences in amsterdam I-positive hereditary nonpolyposis colorectal cancer families according to tumor microsatellite instability status. *J Clin Oncol* 25:781–786
136. Llor X, Pons E, Xicola RM et al (2005) Differential features of colorectal cancers fulfilling Amsterdam criteria without involvement of the mutator pathway. *Clin Cancer Res* 11:7304–7310
137. Abdel-Rahman WM, Ollikainen M, Kariola R et al (2005) Comprehensive characterization of HNPCC-related colorectal cancers reveals striking molecular features in families with no germline mismatch repair gene mutations. *Oncogene* 24:1542–1551
138. Sanchez-de-Abajo A, de la Hoya M, van Puijenbroek M et al (2007) Molecular analysis of colorectal cancer tumors from patients with mismatch repair proficient hereditary nonpolyposis colorectal cancer suggests novel carcinogenic pathways. *Clin Cancer Res* 13:5729–5735
139. Minoo P, Baker K, Goswami R et al (2006) Extensive DNA methylation in normal colorectal mucosa in hyperplastic polyposis. *Gut* 55:1467–1474
140. Chen HM, Fang JY (2009). Genetics of the hamartomatous polyposis syndromes: a molecular review. *Int J Colorectal Dis* 24:865–874
141. Liaw D, Marsh DJ, Li J et al (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 16:64–67
142. Marsh DJ, Coulon V, Lunetta KL et al (1998) Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. *Hum Mol Genet* 7:507–515
143. Marsh DJ, Kum JB, Lunetta KL et al (1999) PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum Mol Genet* 8:1461–1472
144. Eng C (2000) Will the real Cowden syndrome please stand up: revised diagnostic criteria. *J Med Genet* 37:828–830
145. Machama T, Dixon JE (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol* 9:125–128
146. Inoki K, Corradetti MN, Guan KL (2005) Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet* 37:19–24
147. Zhou XP, Waite KA, Pilarski R et al (2003) Germline PTEN promoter mutations and deletions in Cowden/Bannayan-Riley-Ruvalcaba syndrome result in aberrant PTEN protein and dysregulation of the phosphoinositol-3-kinase/Akt pathway. *Am J Hum Genet* 73:404–411
148. Nelen MR, Padberg GW, Peeters EA et al (1996) Localization of the gene for Cowden disease to chromosome 10q22–23. *Nat Genet* 13:114–116
149. Pilarski R, Eng C (2004) Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. *J Med Genet* 41:323–326
150. Foley TR, McGarrity TJ, Abt AB (1988) Peutz-Jeghers syndrome: a clinicopathologic survey of the “Harrisburg family” with a 49-year follow-up. *Gastroenterology* 95:1535–1540
151. Bourke B, Broderick A, Bohane T (2006) Peutz-Jeghers syndrome and management recommendations. *Clin Gastroenterol Hepatol* 4:1550; author reply
152. Hemminki A, Markie D, Tomlinson I et al (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* 391:184–187
153. Jenne DE, Reimann H, Nezu J et al (1998) Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet* 18:38–43
154. Amos CI, Keitheri-Cheteri MB, Sabripour M et al (2004) Genotype-phenotype correlations in Peutz-Jeghers syndrome. *J Med Genet* 41:327–333
155. Giardiello FM, Brensinger JD, Tersmette AC et al (2000) Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 119:1447–1453
156. Yoon KA, Ku JL, Choi HS et al (2000) Germline mutations of the STK11 gene in Korean Peutz-Jeghers syndrome patients. *Br J Cancer* 82:1403–1406



157. Brosens LA, van Hattem WA, Jansen M, de Leng WW, Giardiello FM, Offerhaus GJ (2007) Gastrointestinal polyposis syndromes. *Curr Mol Med* 7:29–46
158. Jass JR, Williams CB, Bussey HJ, Morson BC (1988) Juvenile polyposis—a precancerous condition. *Histopathology* 13:619–630
159. Chow E, Macrae F (2005) A review of juvenile polyposis syndrome. *J Gastroenterol Hepatol* 20:1634–1640
160. Howe JR, Roth S, Ringold JC et al (1998) Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* 280:1086–1088
161. Howe JR, Bair JL, Sayed MG et al (2001) Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. *Nat Genet* 28:184–187
162. Zhou XP, Woodford-Richens K, Lehtonen R et al (2001) Germline mutations in BMPR1A/ALK3 cause a subset of cases of juvenile polyposis syndrome and of Cowden and Bannayan-Riley-Ruvalcaba syndromes. *Am J Hum Genet* 69:704–711
163. Aretz S, Stienen D, Uhlhaas S et al (2007) High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome. *J Med Genet* 44:702–709
164. van Hattem WA, Brosens LA, de Leng WW et al (2008) Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis. *Gut* 57:623–627
165. Sweet K, Willis J, Zhou XP et al (2005) Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. *JAMA* 294:2465–2473
166. Howe JR, Haidle JL, Lal G et al (2007) ENG mutations in MADH4/BMPR1A mutation negative patients with juvenile polyposis. *Clin Genet* 71:91–92
167. Sayed MG, Ahmed AF, Ringold JR et al (2002) Germline SMAD4 or BMPR1A mutations and phenotype of juvenile polyposis. *Ann Surg Oncol* 9:901–906
168. Cao X, Eu KW, Kumarasinghe MP, Li HH, Loi C, Cheah PY (2006) Mapping of hereditary mixed polyposis syndrome (HMPS) to chromosome 10q23 by genomewide high-density single nucleotide polymorphism (SNP) scan and identification of BMPR1A loss of function. *J Med Genet* 43:e13
169. O’Riordan JM, O’Donoghue D, Green A et al (2011) Hereditary mixed polyposis syndrome due to a BMPR1A mutation. *Colorectal Dis* 12:570–573
170. Cheah PY, Wong YH, Chau YP et al (2009) Germline bone morphogenesis protein receptor 1A mutation causes colorectal tumorigenesis in hereditary mixed polyposis syndrome. *Am J Gastroenterol* 104:3027–3033
171. Manolio TA (2010) Genomewide association studies and assessment of the risk of disease. *N Engl J Med* 363:166–176
172. Hindorff LA, Junkins HA, Hall PN, Mehta JP, Manolio TA (2011). A Catalog of published genome-wide association studies. Available at: [www.genome.gov/gwastudies](http://www.genome.gov/gwastudies).
173. Zanke BW, Greenwood CM, Rangrej J et al (2007) Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. *Nat Genet* 39:989–994
174. Broderick P, Carvajal-Carmona L, Pittman AM et al (2007) A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. *Nat Genet* 39:1315–1317
175. Tomlinson I, Webb E, Carvajal-Carmona L et al (2007) A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. *Nat Genet* 39:984–988
176. Jaeger E, Webb E, Howarth K et al (2008) Common genetic variants at the CRAC1 (HMPS) locus on chromosome 15q13.3 influence colorectal cancer risk. *Nat Genet* 40:26–28
177. Tomlinson IP, Webb E, Carvajal-Carmona L et al (2008) A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23.3. *Nat Genet* 40:623–630
178. Tenesa A, Farrington SM, Prendergast JG et al (2008) Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. *Nat Genet* 40:631–637

179. Houlston RS, Webb E, Broderick P et al (2008) Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. *Nat Genet* 40:1426–1435
180. Houlston RS, Cheadle J, Dobbins SE et al (2010) Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26.2, 12q13.13 and 20q13.33. *Nat Genet* 42:973–977
181. Tenesa A, Dunlop MG (2009) New insights into the aetiology of colorectal cancer from genome-wide association studies. *Nat Rev Genet* 10:353–358
182. Massague J, Seoane J, Wotton D (2005) Smad transcription factors. *Genes Dev* 19:2783–2810
183. Massague J (2008) TGFbeta in cancer. *Cell* 134:215–230
184. Blobel GC, Schiemann WP, Lodish HF (2000) Role of transforming growth factor beta in human disease. *N Engl J Med* 342:1350–1358
185. Amundadottir LT, Sulem P, Gudmundsson J et al (2006) A common variant associated with prostate cancer in European and African populations. *Nat Genet* 38:652–658
186. Freedman ML, Haiman CA, Patterson N et al (2006) Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. *Proc Natl Acad Sci USA* 103:14068–14073
187. Haiman CA, Patterson N, Freedman ML et al (2007) Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet* 39:638–644
188. Haiman CA, Le Marchand L, Yamamoto J et al (2007) A common genetic risk factor for colorectal and prostate cancer. *Nat Genet* 39:954–956
189. Yeager M, Xiao N, Hayes RB et al (2008) Comprehensive resequence analysis of a 136 kb region of human chromosome 8q24 associated with prostate and colon cancers. *Hum Genet* 124:161–170
190. Pomerantz MM, Beckwith CA, Regan MM et al (2009) Evaluation of the 8q24 prostate cancer risk locus and MYC expression. *Cancer Res* 69:5568–5574
191. Ahmadiyeh N, Pomerantz MM, Grisanzio C et al (2010) 8q24 prostate, breast, and colon cancer risk loci show tissue-specific long-range interaction with MYC. *Proc Natl Acad Sci USA* 107:9742–9746
192. Editorial (2010) On beyond GWAS. *Nat Genet* 42:551
193. The International HapMap Consortium (2003) The International HapMap Project. *Nature* 426:789–796
194. Hindorf LA, Sethupathy P, Junkins HA et al (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci USA* 106:9362–9367
195. Talseth-Palmer BA, Brenne IS, Ashton KA et al (2011) Colorectal cancer susceptibility loci on chromosome 8q23.3 and 11q23.1 as modifiers for disease expression in lynch syndrome. *J Med Genet* 48:279–284
196. Wijnen JT, Brohet RM, van Eijk R et al (2009) Chromosome 8q23.3 and 11q23.1 variants modify colorectal cancer risk in Lynch syndrome. *Gastroenterology* 136:131–137
197. Le Marchand L, Wilkens LR (2008) Design considerations for genomic association studies: importance of gene-environment interactions. *Cancer Epidemiol Biomarkers Prev* 17:263–267
198. Potter JD (1999) Colorectal cancer: molecules and populations. *J Natl Cancer Inst* 91:916–932
199. Evans DM, Marchini J, Morris AP, Cardon LR (2006) Two-stage two-locus models in genome-wide association. *PLoS Genet* 2:e157
200. Zamani N, Brown CW (2011) Emerging roles for the transforming growth factor- $\beta$  superfamily in regulating adiposity and energy expenditure. *Endocr Rev* 32(3):387–403
201. Derynck R, Akhurst RJ (2007) Differentiation plasticity regulated by TGF- $\beta$  family proteins in development and disease. *Nat Cell Biol* 9:1000–1004
202. Ross SE, Hemati N, Longo KA et al (2000) Inhibition of adipogenesis by Wnt signaling. *Science* 289:950–953
203. Sciarretta S, Ferrucci A, Ciavarella GM et al (2007) Markers of inflammation and fibrosis are related to cardiovascular damage in hypertensive patients with metabolic syndrome. *Am J Hypertens* 20:784–791
204. Rosmond R, Chagnon M, Bouchard C, Bjorntorp P (2003) Increased abdominal obesity, insulin and glucose levels in nondiabetic subjects with a T29C polymorphism of the transforming growth factor- $\beta$ 1 gene. *Horm Res* 59:191–194

205. Spencer M, Yao-Borengasser A, Unal R et al (2010) Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. *Am J Physiol Endocrinol Metab* 299:E1016–E1027
206. Alessi MC, Bastelica D, Morange P et al (2000) Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. *Diabetes* 49:1374–1380
207. Porreca E, Di Febbo C, Vitacolonna E et al (2002) Transforming growth factor-beta1 levels in hypertensive patients: association with body mass index and leptin. *Am J Hypertens* 15:759–765
208. Herder C, Zierer A, Koenig W, Roden M, Meisinger C, Thorand B (2009) Transforming growth factor-beta1 and incident type 2 diabetes: results from the MONICA/KORA case-cohort study, 1984–2002. *Diabetes Care* 32:1921–1923
209. Samad F, Pandey M, Loskutoff DJ (1998) Tissue factor gene expression in the adipose tissues of obese mice. *Proc Natl Acad Sci USA* 95:7591–7596
210. Tseng YH, Kokkottou E, Schulz TJ et al (2008) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454:1000–1004
211. Taha MF, Valojerdi MR, Mowla SJ (2006) Effect of bone morphogenetic protein-4 (BMP-4) on adipocyte differentiation from mouse embryonic stem cells. *Anat Histol Embryol* 35:271–278
212. Bowers RR, Kim JW, Otto TC, Lane MD (2006) Stable stem cell commitment to the adipocyte lineage by inhibition of DNA methylation: role of the BMP-4 gene. *Proc Natl Acad Sci USA* 103:13022–13027
213. Dani C, Smith AG, Dessolin S et al (1997) Differentiation of embryonic stem cells into adipocytes in vitro. *J Cell Sci* 110(Pt 11):1279–1285
214. Tang QQ, Otto TC, Lane MD (2004) Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proc Natl Acad Sci USA* 101:9607–9611
215. Matsuzawa Y, Funahashi T, Nakamura T (1999) Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann N Y Acad Sci* 892:146–154
216. Son JW, Kim MK, Park YM et al (2011) Association of serum bone morphogenetic protein 4 levels with obesity and metabolic syndrome in non-diabetic individuals. *Endocr J* 58(1):39–46
217. Huang H, Song TJ, Li X et al (2009) BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proc Natl Acad Sci USA* 106:12670–12675
218. Mohamed-Ali V, Pinkney JH, Coppack SW (1998) Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord* 22:1145–1158
219. Bowers RR, Lane MD (2007) A role for bone morphogenetic protein-4 in adipocyte development. *Cell Cycle* 6:385–389
220. Skillington J, Choy L, Derynck R (2002) Bone morphogenetic protein and retinoic acid signaling cooperate to induce osteoblast differentiation of preadipocytes. *J Cell Biol* 159:135–146
221. Chen D, Ji X, Harris MA et al (1998) Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J Cell Biol* 142:295–305
222. Sottile V, Seuwen K (2000) Bone morphogenetic protein-2 stimulates adipogenic differentiation of mesenchymal precursor cells in synergy with BRL 49653 (rosiglitazone). *FEBS Lett* 475:201–204
223. Hata K, Nishimura R, Ikeda F et al (2003) Differential roles of Smad1 and p38 kinase in regulation of peroxisome proliferator-activating receptor gamma during bone morphogenetic protein 2-induced adipogenesis. *Mol Biol Cell* 14:545–555
224. ten Dijke P, Yamashita H, Sampath TK et al (1994) Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. *J Biol Chem* 269:16985–16988
225. Bottcher Y, Unbehauen H, Klötting N et al (2009) Adipose tissue expression and genetic variants of the bone morphogenetic protein receptor 1A gene (BMPRI1A) are associated with human obesity. *Diabetes* 58:2119–2128



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