Familial Colorectal Cancer Type X (FCCTX) and the correlation with various genes—A systematic review

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Abstract

Familial Colorectal Cancer Type X (FCCTX) is a type of hereditary nonpolyposis colorectal cancer in accordance to Amsterdam criteria-1 for Lynch syndrome, with no related mutation in mismatch repair gene. FCCTX is microsatellite stable and is accounted for 40% of families with Amsterdam criteria-1 with a high age of onset. Thus, the carcinogenesis of FCCTX is different compared to Lynch syndrome. In addition to the microsatellite stability and the presence of less predominant tumors in proximal colon, various clinical features have also been associated with FCCTX in comparison with Lynch syndrome such as no increased risk of extra-colonic cancers, older age of diagnosis and higher adenoma/carcinoma rate. Genetic etiology of this type of cancer which is autosomal dominant is unknown. In this review, we focus on the genes and their variants identified in this type of CRC. In order to find out the correlation between FCCTX and various genes database such as PubMed and PMC, search engine such as Google scholar and portals such as Springer and Elsevier have been searched. Based on our literature search, several studies suggest that FCCTX is a heterogeneous type of disease with different genetic variants. Recent studies describe the correlation between FCCTX and genes...
Introduction

Among patients with clinical characteristics of hereditary nonpolyposis colorectal cancers (CRCs), there is a group known as Familial Colorectal Cancer Type X (FCCTX) which is defined based on Amsterdam Criteria-1 (AC1) for Lynch syndrome. Among this type of CRCs, 40% of families met AC1 criteria. Since this type of cancer has no mutation in mismatch repair gene (MMR), the tumors have been characterized as microsatellite stable. Thus, the carcinogenesis of FCCTX is different compared to Lynch syndrome.

In addition to the microsatellite stability and the presence of less predominant tumors in proximal colon, various clinical features have also been associated with FCCTX in comparison with Lynch syndrome such as no increased risk of extra-colonic cancers, older age of diagnosis, and higher adenoma/carcinoma rate. Despite the recent progress in clinical detection of FCCTX, its genetic etiology has remained unknown. FCCTX may be resulted from more than one genetic etiology. Different studies suggest that FCCTX is a heterogeneous disease with various clinical variants. Detection of the genes associated with FCCTX will facilitate the molecular diagnosis of the disease. Current evidence shows that FCCTX families constitute a high heterogeneous group. In this review, we focus on the genes and their variants identified in this type of CRC.

The literature review has been performed in order to find out the correlation between FCCTX and various genes. Only studies published in English were included. Keywords such as “FCCTX” and “genes” have been used to search for all articles related to genetic basis of FCCTX.

In this review, we have searched the most recent published articles. Retrieved articles had original contributions or were review articles.

For each study, the following action was performed: collecting information about the numbers of cases and controls, the genes involved, type of mutations, mutation frequency rate, and the type of analyzing process (for instance, linkage analysis or any other studies). The important inclusion factor was fulfilling Amsterdam criteria. Then all related articles were collected, the results were analyzed and a comparison was made between various genes.

Genes

**BRCA2 gene**

BRCA2 gene has high tumor heterogeneity and it is a good candidate for FCCTX. Some studies show the relation between rare BRCA2 alleles and CRC and the linkage between a marker in BRCA2 gene and familial colorectal families. In addition, in recent studies on 48 FCCTX families, 27 coding sequences and intron/exon boundaries in BRCA2 gene were analyzed and 29 BRCA2 variants including 28 point mutations (14 missense, 12 silent, and 2 intronic) and 1 frameshift mutation were found. The most striking result of this work was the frameshift mutation c.3847_3848delGT p. (Val1283Lysfs*2) detected in 1 FCCTX family. This family, 1 breast cancer, 4 CRCs, and 1 prostate cancer in 2 following generations have been found. Val1283Lysfs*2 mutation is reported in the Breast Cancer Information Core database as pathogenic mutation for Hereditary Breast and Ovarian Cancer Syndrome (HBOC) and it has also been observed in prostate cancer.
In the case of point mutations 4 variants showed cosegregation with disease such as p.(Pro168Thr), c.502C>A, c.5744C>T p.(Thr1915Met), c.927A>G p.(Ser309=), and c.7759C>T p.(Leu2587Phe), which could act as predisposition alleles in these families.12

**SEMA4A gene**

SEMA4A is a membrane-bound class 4 semaphorin receptor family.16-18 Semaphorins, functionally act in physiological and developmental procedures; additionally an association between semaphorins and their receptors with malignant disorders has been found.19,20 Recent study on Austrian patients with FCCTX shows that only variant p.val78met of this gene could be observed in all patients. When samples were analyzed for copy-number alterations, they observed amplification on **SEMA4A** locus.21 In comparison with wild-type protein, clinical variant V78M shows highly related to activated MAPK/Erk and PI3K/Akt signaling pathway. Inheritance pattern of the germline variant V78M was autosomal dominant with incomplete penetrance.21 In this variant, tumor development occurred at a higher age compared to the patients affected with classical Lynch syndrome. In this study and in 53 FCCTX patients, 2 more **SEMA4A** mutations, p.Gly484Ala and p.Ser326Phe have been found. Also, the single nucleotide polymorphism (SNP) p.Pro682Ser has been detected. This single nucleotide polymorphism is highly related to the FCCTX phenotype showing enhanced risk of CRC.

Additional genetic factors, environmental parameters and lifestyle modifiers are necessary to establish the malignant phenotype. Such a genetic modifier might be **MUTYH** where the heterozygous germline variant R217H was found in 2 V78M carriers affected by CRC.22

**KRAS gene**

In a study by Sanchez-de-Abajo et al, performed on 28 tumors dissected from 17 different families affected with FCCTX, genetic analysis of **KRAS** shows that the mutation rates are similar to Lynch syndrome and sporadic CRCs. These mutations were disproportionately in codon 12 compared to equal representation between codons 12 and 13 in Lynch syndrome.23 Also in a study performed by Sánchez-Tome et al24 on 22 FCCTX affected families, mutational analysis of **KRAS** show variants that was similar to the above-mentioned findings. But in another study by Francisco et al25 on 15 FCCTX families, mutational analysis of exon 2 in **KRAS** gene, show **KRAS** somatic mutations in 11/24 (46%) of the FCCTX tumors. Also FCCTX tumors showed the highest frequency of mutations in codon 12 (ie, 91%) compared to Lynch syndrome or sporadic MSI-H tumors.25

**BRAF gene**

In addition to the above-mentioned mutations, in a study by Sanchez-de-Abajo et al similar mutation in **KRAS** and **BRAF** has also been found in 3.6% of patients, and was not different from Lynch syndrome and sporadic CRCs. Thus, between the Type X families and the sporadic microsatellite-stable cancer, the changes in the RAS/RAF signaling pathway appeared similar, leading the authors to propose that some Type X families are classified as such due to the chance of aggregation of sporadic cases.1 Similarly, in the Sánchez-Tome et al study, in all FCCTX tumors screened for **BRAF** mutations, high-resolution melting analysis has been used to find the mutations on exon 15 of the gene (including the hotspot V600E).23 **BRAF** mutations were identified in 3 out of 23 tumors and again it was shown that **BRAF** mutations are similar to those described for CRC in general.26

**APC gene**

The situation of **APC** gene is like 2 other genes including **KRAS** and **BRAF.**27 However in a study by Francisco et al,25 in order to detect somatic mutations in exon 15 of **APC**, the mutation cluster region was examined by automated sequencing. They found the p.E1317Q mutation in 1 out of 10 tumor suppressor gene (TSG) which correlate to loss of function and all the affected relatives were positive for the germline missense mutation. Also they identified truncated **APC** somatic mutations in (39% of) tumors. Loss of heterozygosity (LOH) of the **APC** locus was found in 42% of tumors that resulted in the detection of **APC** somatic mutations (either truncated mutations or LOH) in 15/24 (62%) of the FCCTX tumors. Four missense mutations (p.E1353D, p.E1374K, p.Q1429H, and p.N1473T) were also
detected in 3 distinct tumors, although the p.E1374K mutation has been already detected in combination with LOH.28

**MMR and MGMT genes**

In the study by Francisco et al25 in 18 colorectal samples including 7 adenomas and 11 carcinomas from the FCCTX families, promoter methylation analysis of DNA repair genes using the methylation-specific multiplex ligation-dependent probe amplification was performed. While hypermethylation of at least 1 of the MMR genes was observed in about 28% of the cases, MGMT hypermethylation was found in 44% of FCCTX tumors. For MGMT, hypermethylation (either of MGMT or MMR genes) appeared to be more common among TSG loss positive tumor in comparison to the TGS loss negative group, and in the case of the MMR genes, none of the TGS loss negative tumors presented methylation.25,29 Interestingly, Francisco et al could also find that the type of KRAS mutations differed according to the MGMT methylation status so that C-T transitions were significantly more frequent among MGMT methylated tumors compared to the unmethylated ones.

**BMPR1A gene**

**BMPR1A** encodes a type I bone morphogenetic protein receptor that belongs to a family of serine/threonine kinases.30 Earlier studies have proven that BMPR1A mutations are the cause of 20% of juvenile polyposis syndrome and 50% of hereditary mixed polyposis syndrome in families.31,32 In a study, 18 families from the Hereditary Colorectal Cancer Registry of Finland, all fulfilling the AC1 and having no MMR defects in tumor tissue or in the germline were investigated by genetic linkage analysis, mutation analysis of candidate genes, and by molecular studies of tumor tissues.33 Genotyping with microsatellite markers resulted in a high limit of detection score for marker D10S1686 residing on 10q23 locus. All 11 exons and the intron-exon boundaries of BMPR1A were sequenced. One individual revealed a germline mutation consisting of an in-frame deletion of 3 nucleotides (AGA) at 264 nucleotide position in exon 3 (c.264-266del, with p.Glu88del a predicted protein), which cosegregated with colon carcinoma and/or adenoma in the family. Remaining families were screened for BMPR1A alterations, a splice site mutation (c.68-10_6814del) was found in 1 family. The overall frequency of pathogenic BMPR1A mutations was 2/18 families (11%).

Patients in 2 families were examined and it was documented that the rate of polyps did not fundamentally differ from typical screening consequences of MMR gene mutation-positive patients in Lynch syndrome. No juvenile polyps, polyps with mixed histology, serrated adenomas, or sessile-serrated polyps/adenomas were identified.33,34

**RPS20 gene**

In a study by Nieminen et al35 performed on 26 FCCX families, genetic linkage analysis, exome sequencing, tumor studies, and functional investigations of 4 generations of a FCCTX family led to the identification of a truncated germline mutation in RPS20, which encodes a component (S20) of the small ribosomal subunit and is a new colon cancer predisposition gene. Since this study was subsequently in progress (in the recent study related to the role of BMPR1A gene in FCCTX), patients with germline mutations in bone morphogenetic protein receptor type IA were omitted. A novel germline mutation (c. 147 dupA) in the RPS20, was found in 7 CRC-affected patients. The mutation leads to a frameshift and premature truncated protein (p.Val50SerfsX23) and was associated with a defect in preribosomal RNA maturation. Their findings show that mutations in a gene encoding a ribosomal protein can predispose individuals to microsatellite-stable colon cancer.35 The product of RPS20 is required during the late steps of 18S ribosomal RNA (rRNA) formation.36 RPS20 encodes a ribosomal protein with a component of the 40S subunit.35 When RPS20 function normally, it can bind to Mdm2 and activate p53 tumors suppressor protein.37

Among 80 genes in the ribosomal protein gene family, at least 11 of them are recognized to be mutated in Diamond-Blackfan anemia, a form of pure red cell aplasia that has a dominant pattern of inheritance with some features like growth retardation and congenital anomalies. No such features exist in colon cancer patients.38-40 It is shown that haploinsufficiency for RPS19 or RPS20 in mice play a role in stabilizing p53.41 discoveries make them speculate that cell type-specific effects of RPS20
haploinsufficiency might have a function in RPS20-associated colon tumor formation in human cancers.42

**Unrelated genes**

Although some studies have been performed on relation between BRCA2, SEMA4, KRAS, BRAF, APC, BMPR1A, and RPS20 and FCCTX cancer, no specific variants have been discovered. Similarly, about NTS gene, its variant does not demonstrate considerable difference comparing to normal population.

**NTS and RASSF9 gene**

NTS (neurotensin) is a significant regulatory hormone that affects many facets of gastrointestinal role including motility, secretion, and mucosal growing. RASSF9 is a member of the family of RAS-associated domain-containing proteins whose members have appropriate tumor suppressor roles. A linkage analysis on 22 Spanish families affected with Lynch syndrome showed that 2p24.3, 4q13.1, 4q31.21, and 12q21.2-q21.31 loci are associated with FCCTX and a family-specific analysis approved a better NPL-score for 12q21.2-q21.31.24 Eight (STR) markers within the 12q21.2-q21.31 were genotyped; candidate causal genes were amplified by PCR and sequenced. Between more than 50 genes at 12q21 locus, RASSF9, and NTS have been chosen as the best candidates because of their potential involvement in carcinogenesis and colorectal epithelium development.43-48 Both genes were sequenced in all affected members, 2 variants were found in NTS gene in 2 families including a missense mutation in exon 2 and a deletion in 3′-UTR region but no variants were found in RASSF9 gene.45 Nevertheless, the frequency that was detected in controls, in addition to an in silico study, ruled out a probable deleterious effect for both genes.49-51

**GALNT12 gene**

GALNT12 encodes a key enzyme that has a function in the first step of mucin type O-glycosylation, this gene defined as a candidate gene for hereditary CRC but not specifically for FCCTX based on genomewide association studies. This gene is located in 9q22-33 near to a CRC linkage pick and germline missense variants that decrease the enzymatic activity of the protein and have been identified in CRC patients. Seguí et al52 sequenced coding regions of the gene in 103 probands, but they found no functionally relevant mutations. This shows that GALNT12 is a major CRC susceptibility gene but it is not a major contributor of FCCTX.

**Complementary studies**

In this section, we review more studies on FCCTX regarding its relation to other genes. Domínguez-Valentín et al studied gene expression profile of 37 FCCTX tumors. They found upregulation of 1059 genes that a number of genes \( n = 16 \) including: GNAS, P2RY2, RAMP2, MC1R, VIP, F2R, F2RL2, EDN1, GRM8, GNAAZ, GNG11, GNG12, HCR, and PTGER1 were associated with the G-protein coupled receptor pathway. In addition, validation study using qRT-PCR was performed using 5 genes associated with cancer, the results show increased expression of AXIN2 and MYC in FCCTX tumors, reduced expression of NDUFA9 in these tumors (and also in sporadic MMR proficient tumors).53

There is also another alteration identified in FCCTX. For example in a study by Christina Therkildsen et al, array-based comparative genomic hybridization was applied to 23 CRC tumors with FCCTX. FCCTX tumors showed genomic complication with common gains on chromosomes 17, 19, and 20q and losses of 15, 8p, and 18 loci. Gains of genetic material in 2 distinct loci including 20q12-13.12 and 20q13.2-13.32, was observed in 65% of the FCCTX tumors. Gain on chromosome 20q and loss on chromosome 18 notably differentiated CRCs associated with FCCTX from Lynch syndrome.54

Some studies show that in these families 2 single molecular pathways are involved. In one of them there is no TSG, loss of genes loci, and promoter methylation, in other pathway there is such an
alteration for some TSG loci including \textit{DCC}, \textit{TP53}, \textit{APC}, and \textit{SMAD4}, somatic mutations of \textit{KRAS} and \textit{APC}, and \textit{MGMT} promoter methylation.\textsuperscript{25}

In a recent study, Villacis et al assessed copy-number variations (CNVs) in 45 separate FCCTX patients. By analyses with 2 different microarray platforms, they revealed 35 rare CNVs covering 67 known genes in 22 patients. They found gains (\textit{GALNT6} and \textit{GALNT11}) and losses (\textit{SEMA3C}) including the same gene families related to CRC predisposition among the rare CNVs. By segregation analysis performed on 4 relatives from 1 family, they suggested the involvement of \textit{GALNT11} and \textit{KMT2C} in those at risk of developing CRC.\textsuperscript{55}

Other studies showed the association of hypomethylation in long interspersed nucleotide element-1 (LINE-1) with familial CRC, including FCCTX.\textsuperscript{56,57} In addition in recent studies candidate genes have been suggested, such as \textit{CENPE}, \textit{CDH18}, \textit{GREM1}, \textit{BCR}, \textit{KIF24}, \textit{ZNF367}, \textit{HABP4}, \textit{GABBR2}, and \textit{BMP4}.\textsuperscript{58}

Also in the above-mentioned study by Melas et al\textsuperscript{59} in addition to \textit{APC}, germline exome sequencing was performed on DNA samples from 41 patients with FCCTX and a mutation in \textit{MSH6} was found.

Furthermore, it is documented that there is a phenotypic overlap between FCCTX and other identified genetic syndromes such as PPAP (Polymerase proofreading associated polyposis) syndrome in which the exonuclease domain of \textit{POLE} (encoding DNA polymerase \(\varepsilon\)) or \textit{POLD1} (encoding DNA polymerase \(\delta\)) is mutated in the germline.\textsuperscript{60-62}

There is an association between CRC and coronary artery disease and both probably develop through common risk factors such as chronic inflammation mechanisms.\textsuperscript{63} For example Runt-related transcription factor 2 (RUNX2) as a transcription factor, was detected in the nuclei of the colon carcinoma cells.\textsuperscript{64} This transcription factor is mainly associated with osteogenesis and osteogenesis is mediated by oxidative stress.\textsuperscript{65-67}

\textbf{Results}

Although the number of papers used in this review are adequate, but majority of them just study the clinical differences between Lynch syndrome and FCCTX, and among these articles a limited numbers potentially could help us about genetic basis of FCCTX. Almost 12 genes were studied in all of these papers where the mutations of 10 genes and hypermethylation of 2 genes were addressed. In the newest study in 2015, \textit{BRCA2} mutations were considered in FCCTX so we concluded that \textit{BRCA2} gene probably has a significant role in genetic basis of FCCTX in comparison to other genes. According to other articles based on the mutation frequency rate, \textit{KRAS}, \textit{APC}, \textit{NTS}, \textit{BRAF}, \textit{BMPR1A}, and \textit{RPS20} are associated with FCCTX. Hypermethylation of \textit{MMR} and \textit{MGMT} also should not be ignored.

The Table describes the name of gene, the number of patients examined for each gene in the literature and the frequency rate of each mutation. Also in the Figure a comparison between the frequency rates of mutations found for each gene is presented.

\textbf{Discussion}

FCCTX is a type of HNPCC in which tumors are microsatellite stable with no \textit{MMR}-related mutations. Genetic factors have an important function in CRC susceptibility, although the heritability of this tumor is almost 35\%.\textsuperscript{68} The lifetime risk of CRC is 5%-6%, defined as without personal history or family history of CRC and above the age of 50.\textsuperscript{69} Even though the FCCTX is a relatively recent notion, a short study has already recognized some clinical and molecular differences between FCCTX families and those with a \textit{MMR} deficient system.\textsuperscript{4} Despite the fact that the clinical identification of FCCTX has improved in recent years, its genetic etiology remained unknown.\textsuperscript{3} The definition of FCCTX is still controversial. Originally, FCCTX collectively describes cases of CRC that meet clinical AC1, but whose tumors are \textit{MMR} proficient as assessed by microsatellite instability testing.\textsuperscript{4} However, some studies of FCCTX have also included \textit{AC2} families with MMR-stable tumors.\textsuperscript{25,34,52,54} The study by Nieminen et al\textsuperscript{15} was based on FCCTX families, which fulfilled the AC1/2 or the
Bethesda criteria without MMR defects in tumor tissue or in the germline. In recent years, some studies on genetic basis of FCCTX have been performed and different genes were introduced as candidates for FCCTX. For instance in Garre et al.\textsuperscript{12} study BRCA2 mutations were analyzed and 29 BRCA2 variants were found; including 28 point mutations and 1 frameshift mutation, or in another study by Sánchez-Tome et al.\textsuperscript{24} linkage analysis showed that 12q21.2-12q21.31 loci, is the region with the highest NPL-score, and contains more than 50 genes that among them, RASSF9 and NTS were considered good candidates. Both genes were sequenced in all affected members of the linked families, and 2 variants in NTS gene were found. Similarly different studies on different genes have been done. Based on these studies frequency of the mutation is 60% for BRCA2, 45.5% for KRAS, 39% for APC, 21.5% for NTS, 13% for BRAF, 11% for BMPR1A, and 7% for RPS20. For RASSF9 gene, despite the importance of the loci, no variant has been found, although this could be a remarkable clue for focusing on other members of RASSF family and GALNT12. The methylation analysis of MGMT and MMR shows the methylation rates of 44% and 28%, respectively. Considering the fact that BRCA2 gene has high mutation rate of 60% and since it is one of the most crucial DNA repair genes, which its role in different cancers is demonstrated in many articles, we could conclude that in comparison with

### Table

Summary of selected studies on FCCTX, including reported genes and variants. Frequency rate of each mutation is based on the total number of patients examined.

<table>
<thead>
<tr>
<th>References</th>
<th>Genes</th>
<th>Cases</th>
<th>Clinical variants and frequency rate (%)</th>
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<tr>
<td>Garre et al.\textsuperscript{12}</td>
<td>BRCA2</td>
<td>48 FCCTX probands</td>
<td>29 Variants: 28 point mutations (14 missense, 12 silents, and 2 introns) (58%) 1 Frameshift mutation (2%)</td>
</tr>
<tr>
<td>Sánchez-Tome et al.\textsuperscript{24}</td>
<td>NTS</td>
<td>22 FCCTX families</td>
<td>Missense alteration in exon 2 (1.5%) Deletion in the 3 UTR region (20%)</td>
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<tr>
<td>Sánchez-Tome et al.\textsuperscript{24}</td>
<td>RASSF9</td>
<td>22 FCCTX families</td>
<td>No variant Somatic mutation in exon 2 (46%) Somatic mutation in exon 12 (91%)</td>
</tr>
<tr>
<td>Francisco et al.\textsuperscript{25}</td>
<td>KRAS</td>
<td>15 FCCTX families</td>
<td>No variant Somatic alteration in exon 2 (46%) Somatic alteration in exon 12 (91%)</td>
</tr>
<tr>
<td>Francisco et al.\textsuperscript{25}</td>
<td>APC</td>
<td>15 FCCTX families</td>
<td>Mutation (39%) LOH (62%)</td>
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<td>Francisco et al.\textsuperscript{25}</td>
<td>MMR and MGMT</td>
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<td>Nieminen et al.\textsuperscript{33}</td>
<td>BMPR1A</td>
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<td>Nieminen et al.\textsuperscript{35}</td>
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<td>Somatic mutation (7%) Somatic mutations (2.7%)</td>
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**Fig.** Frequency rate of mutations found for each gene in FCCTX. (Color version of figure is available online.)
other genes, this gene probably would have the biggest role in this type of cancer. Assessing other genes, their importance has been evaluated based on frequency of mutations, the type of mutations, and their effect that has on the protein function.

We have systematically reviewed the correlation between FCCTX and various related genes. The good features of the present paper are the review of the related articles in the literature that analyzed genetic basis of FCCTX. All candidate genes are collected in this article, thus for the researchers it is a good opportunity to choose which gene could be the best one for the susceptible cases. However this review has some limitations: for example since the molecular mechanism and genetic basis of FCCTX are not well defined, it is difficult to choose a single gene as a definite candidate. Moreover the future studies could clarify most of the complexities. It is likely that more genes related to this syndrome will be found in the near future.

References


