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FINAL GRADE PROJECT

INTESTINAL MICROBIOTA IN THE ADENOMA PROGRESSION TO COLORECTAL CANCER: A CROSS-SECTIONAL STUDY

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1. ABBREVIATIONS

AEG	Asociación Española de Gastroenterología
CI	Confidence interval
CIN	Chromosomal instability
CEIC	Comitè d'Ètica i d'Investigació Clínica
CRC	Colorectal cancer
DNA	Deoxyribonucleic acid
FIT	Fecal immunochemical test
FOBT	Fecal occult blood test
HNPCC	Hereditary non polyposis colorectal cancer
HPs	Hyperplastic polyps
IBD	Inflammatory bowel disease
MSI	Microsatellite instability
MSI-H	High microsatellite instability
qPCR	Quantitative real-time PCR
SCD	Societat Catalana de Digestologia
SPs	Serrated polyps
SSA	Sessile serrated adenoma
TSA	Traditional serrated adenoma

2. ABSTRACT

Background: Colorectal cancer is a major health problem worldwide and many efforts have been done to delineate risk factors and develop screening strategies to reduce its incidence and mortality. Colorectal adenomas have been clearly considered preneoplastic lesions due to their potential malignant transformation via the adenoma-carcinoma sequence. Over the last years, intestinal microbiota has been studied in several diseases and it has been hypothesized that colonic microbiota could influence colorectal cancer pathogenesis.

Objective: The goal of this study is to analyse whether there is an association between the fecal microbiota profiling and the presence and progression of colorectal adenomas, detected in population undergoing colonoscopy, to better understand the role of intestinal microbiota in colorectal carcinogenesis.

Design: A cross-sectional study in the Gastroenterology Department at Hospital Universitari Doctor Josep Trueta in Girona, in a period of time of two years.

Participants: General population undergoing screening or diagnostic colonoscopy in the Digestive Endoscopy Unit.

Outcomes: Identification and characterization of intestinal microbiota in stool samples from healthy patients and patients with low and high risk colorectal adenomas.

Key words: Colorectal adenomas, polyp, low risk adenoma, high risk adenoma, colorectal cancer, intestinal microbiota.

3. INTRODUCTION

3.1. BACKGROUND

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world representing the second cause of death from cancer if both genders are considered together. CRC is the third most prevalent cancer in men (746.000 cases in 2012, 10% of the total), after lung (16.7%) and prostatic cancer (15%); and the second in women (614.000 cases in 2012, 9.2% of the total) after breast cancer (25.2%) (1). The global geographic distribution of CRC is not equally distributed and the highest incidence rates are in Western Europe, United States, Australia and New Zeland, whereas the lowest are in Africa and central Asia (1).

In Spain, in 2012, CRC had the highest incidence considering both sexes (32.240 cases, 15%), being the second cause of cancer mortality in our country (14.700 deaths, 14.3% of mortality) (2).

In the European Union overall, mortality for CRC has decreased the last decades and the age-adjusted 5-year relative survival according to EUROCARE-4 was about 56'8%, and slightly higher in Spain (59'9% in men, 64'1% in women) (3). These results are attributable to improvements in diagnosis and treatment and the implantation of screening programmes.

CRC can be classified according its origin in a sporadic or inherited subtype. 70-80% of CRC cases are sporadic and a small proportion corresponds to inherited forms (5-10%). These inherited CRC syndromes include: Hereditary non polyposis colorectal cancer (HNPCC) or Lynch syndrome (2-5% of cases), familial adenomatous polyposis (<1% of cases), MYH-associated polyposis, hamartomatous polyposis syndromes (Peutz-Jeghers syndrome, Juvenile polyposis syndrome) and serrated polyposis syndrome (4). Moreover, up to 20% of CRC cases present familiar aggregation, known

as familiar CRC. Despite the hereditary component is not well established at this moment, CRC in first-degree relatives increases the risk of CRC and its early-onset (5).

Studies about colorectal carcinogenesis identified that some CRC arises from precursor lesions, specifically from some types of colorectal polyps. Colorectal polyps, small clumps of cells rising from the lining of colon and protruding into intestinal lumen, are a common finding in colonoscopies, and according to its histology and risk of malignancy, polyps can be classified as non-neoplastic or as neoplastic:

- Non-neoplastic colorectal polyps are classified as (6):
  - Inflammatory polyps, or pseudopolyps, are common in inflammatory bowel disease (IBD), in infectious or ischemic colitis and in rectal ulcer syndrome, as a result of chronic injury and mucosal regeneration and healing.
  - Hamartomatous polyps are usually solitary, sporadic and do not have malignant potential. Nevertheless, those occurring as part of hamartomatous polyposis syndromes (Peutz-Jeghers syndrome, Juvenile polyposis, Cowden disease) predispose to develop tumours and other clinical disorders.
  - Hyperplastic polyps (HPs) are the most common type of polyps and typically are found in old ages (6^{th}-7^{th} decades). HPs are usually smaller than 5mm, without dysplasia and most commonly found in the left colon and rectum. Recently, they have been classified as a benign subtype of serrated polyps (SPs).
- Neoplastic colorectal polyps harbour a malignant potential, therefore it is necessary to identify and remove them to prevent CRC development:
  - Serrated polyps are an heterogeneous group characterized by its histological appearance in “saw-tooth” and classified into three subgroups: The HPs, mentioned above, without malignant potential transformation (80-90%); and two groups having potential malignant transformation through the serrated pathway of carcinogenesis, the sessile serrated adenoma (SSA) (15-20%) and the traditional serrated adenoma (TSA) (1-6%). SSA and TSA larger than ≥10 mm,

with dysplasia, multiples and in proximal location, have the higher risk of malignancy. If multiple SPs or sized ≥ 10 mm are found, or the patient has a first-degree relative with SPs, serrated polyposis syndrome has to be considered or excluded (7,8).

- Adenomatous polyps are a frequent finding in colonoscopies, with a 20-40% of prevalence in people older than 50 years and its incidence increases with age and in males (8,9). According to its histology, they are subclassified as, tubular (65-80%), villous (5-10%) and tubulo-villous (10-25%). Its histological type, degree of dysplasia and its size determines the risk of malignancy: Tubular adenomas smaller than 1 cm have the lowest risk (<5%), and villous adenomas and larger (≥ 10 mm) have the highest risk (50%) (8). The degree of dysplasia is usually related with size and villous component. The presence of multiple adenomas (≥ 3) also increases the risk of future CRC. An average of 5 to 10 years is required for the malignant transformation into a CRC, and for that reason, endoscopic surveillance has proved to be effective in reducing the incidence of CRC (9).

  Although only a small percentage of adenomas evolve to cancer, it is believed that approximately 80-85% of sporadic CRC develop from adenomatous polyps via the adenoma-carcinoma sequence, also known as the carcinogenic traditional pathway, the chromosomal instability (CIN) or the suppressor pathway. It postulates that molecular events occur in different progressive pathological stages, from normal mucosa, to early and advanced adenoma and finally progress to an invasive carcinoma. Genetic alterations involve mutations in tumour suppressor genes, in proto-oncogenes and chromosomal abnormalities. Mutations in APC are typically the earliest event in the transition from normal epithelium to a small adenoma, followed by K-Ras and SMAD4-SMAD2 mutations, which promote cell growth and prevent apoptosis; and

alterations in p53 occur at the latest stages which are associated with the transition from advanced adenomas to carcinoma (10).

An alternative pathway for colorectal tumorigenesis is the microsatellite instability (MSI) pathway: It is characterized by defects in DNA mismatch repair genes, especially in MLH1 and MSH2, which loss their function and errors are accumulated in microsatellite sequences leading to MSI. It occurs in approximately 15% of sporadic CRC (MSI-H tumours) and these tumours have distinct pathologic and clinical characteristics compared to CIN tumours. That pathway is also the cause of Lynch syndrome or HNPCC (11).

The third carcinogenic pathway, and mentioned above, is the serrated pathway. The sessile serrated pathway is characterized by early mutations in proto-oncogenes and the CpG island methylation phenotype. The hypermethylation of MLH1 lead to MSI and the neoplastic progression of serrated polyps (7).

Understanding colorectal carcinogenesis has been important to increase the knowledge about CRC development. The appearance of these preneoplastic lesions, and ultimately CRC, is influenced by several environmental and personal risk factors. CRC has been considered a multifactorial disease due to the high number of risk factors that have been linked to it, and some of these risk factors are of sufficiently high risk to consider prevention strategies (12,13):

- Age: More than 90% of CRC are diagnosed over the sixth-seventh decade of life. The incidence is more than 50 times higher in people aged 60 to 79 years, compared with those younger than 40 years (14). However, increasing incidence among younger people is of concern, suggesting the involvement of some other risk factors. CRC under 40 years is rare and tend to correspond to hereditary syndromes.
- Family history of CRC or adenomatous polyps: These lesions in two or more first degree relatives, or in one before the age of 50-60, increases the risk of

CRC and screening is recommended at the age of 40, or 10 years earlier than the earliest case. The risk is correlated to the number of relatives affected, the degree of kinship and the age at diagnosis (12).

- Personal history of IBD: In Ulcerative Colitis and Crohn's disease, the duration of the illness and the extent of the inflammation are the most important determinant factors for CRC (15).
- Smoking: Cigarette smoking is clearly associated with colorectal adenomas, with a relative risk of 2.1 for current smokers, and it is important for development and aggressiveness of adenomas (16). In addition, prospective studies have finally demonstrated a positive association between smoking and CRC incidence in current and past smokers (17) .
- Dietary factors: High fat diet and red and processed meat intake can increase the risk of CRC. The protective benefit of fiber, fruit and vegetables intake is not well demonstrated. Otherwise, it has been suggested that an adequate dietary intake of some micronutrients (folate, calcium, vitamin D) reduces the risk of colon neoplasm, but taking supplements of them is not recommended (12,13).
- Physical activity, obesity and general lifestyle: Lifestyle-related factors have been widely studied in sporadic CRC. Physical inactivity and excess body weight, especially a body mass index >30, in men and central obesity, are linked to CRC by reducing the body's metabolic efficiency and increasing insulin resistance and hyperinsulinism (13,14).

Due to all the above, CRC is particularly suitable for prevention strategies owing to it has high incidence and morbimortality rates representing a major health problem; its natural history is known which allows early diagnosis in asymptomatic stages, there are cost-effective diagnostic methods, and treatment in early stages is more effective. The aim of population screening programmes is to identify early asymptomatic premalignant lesions, basically adenomas, or localised cancers, to reduce the incidence and

mortality of CRC (5,18). Population is classified according its individual risk of suffer CRC in three groups, which determines the screening strategy (12):

1) Low risk group: Age <50 years with no personal or familiar history of adenomas or CRC. In this group, no screening programmes are considered.
2) Medium risk group: Age ≥50 years with no personal or familiar history of adenomas or CRC. Population screening programmes are recommended.
3) High risk group: Personal and/or familiar history of adenomas, CRC, CRC hereditary syndromes and IBD. In these patients specific screening programmes are recommended.

In population screening programmes, different tests are available and all have proven to be cost-effective. Fecal occult blood test annual or biennial, sigmoidoscopy every 5 years and colonoscopy every 10 years are the test used in routine practice (5,12):

- Fecal occult blood testing (FOBT): Fecal quantitative immunochemical test (FIT) is more sensitive, but less specific than the traditional guaiac test, for detection of CRC and advanced adenomas (5). A positive FOBT test requires a colonoscopy and screening with FOBT shows to reduce the mortality rate in a 32% (CI 95%, 18-44%) with annual screening and in a 22% (CI 95%, 7-35%) with biennial screening (19,20). Other stool-based tests were suggested and different stool markers have been analysed, without much success, as potential indicators of adenomas and CRC (calprotectin, mucin abnormalities and exfoliated colonocytes) (21). Stool DNA tests recently emerged and have demonstrated sensitivity in CRC detection, but is necessary to ensure its reliability by defining the best markers (neoplasm-specific DNA alterations) and reduce its costs to be recommended for mass screening (18).
- Flexible sigmoidoscopy: The examination of sigmoid colon and rectum in people between ages 55 and 64 years has demonstrated to be a safe test and to reduce the incidence, especially for distal CRC (50%). Subsequent

colonoscopy has to be indicated if high-risk polyps are found in distal colon due to the increasing risk of synchronous proximal lesions (22).

- Colonoscopy: It is the most sensible and specific screening test, although it is not exempt of risks and colon preparation is required, reducing its acceptability. The greatest advantage is its ability to identify and resect polyps, with a reduction in CRC incidence of 67%, and 80% of CRC could be prevented by polypectomy (12,23).

Recent studies suggest that FIT could be the first-choice test in CRC screening in the average risk population, due to its higher acceptability and accessibility, its similar results on CRC detection to colonoscopy and its lower cost. However, colonoscopy is still considered the gold standard test in CRC prevention, because of its superiority for detection non-advanced and advanced adenomas, and specially, for its possibility of doing polypectomies (23). Nevertheless, further research is necessary to reach an universal agreement about the best strategy (19,24).

During the last years, accumulating evidence that intestinal microbiota is associated with the pathogenesis of several diseases is emerging. Recent studies have started to suggest the contribution of intestinal microbiota in cancer development, including CRC. These studies focus on explore the intestinal microbial composition in healthy and diseased subjects and the alterations in that composition associated with CRC, to support its potential role in colorectal carcinogenesis.

The gastrointestinal tract harbours approximately 100 trillion (10^{14}) microbial cells, grouped in more than 1000 different species or phylotypes, constituting the human intestinal microbiota, with a collective genome known as microbiome (25–27). The establishment of the intestinal microbiota starts just after birth and it is influenced by many factors, such as delivery mode, feeding patterns, introduction of solid food, use of antibiotics and sanitary living conditions. The microbiota composition fluctuates during different colonization stages and its diversity increases until 2.5-3 years of age, when it

resembles the adult microbiota (25,28,29). The adult intestinal microbiota is basically constituted by bacteria, specifically by five phyla: *Bacteroidetes* and *Firmicutes* are the most prevalent divisions representing more than 90% of all microbiota, followed by *Proteobacteria, Actinobacteria* and *Fusobacteria* (26,30). However, interpersonal variability in bacterial species and strains has been observed among individual microbial composition. Despite these differences at species level, it is believed that a set of microbial functions at gene level are common in all individuals, which is known as the "core microbiome" hypothesis. The adult microbiota composition is generally stable, but it is modulated by several factors including age, physiological status, geographical location, environmental factors, diet (high-fat Western diet *vs* high-fiber diet), and use of antibiotics (26,31).

The intestinal microbiota develops in a symbiotic relationship with the host and that symbiosis allows microbiota to develop physiological functions and maintain a healthy balance (32). It is involved in metabolic functions, like dietary energy harvest; in protection against infection by pathologic species due to its colonization resistance; and in development and modulation of immune system (25,31,33).

Dysbiosis is the alteration of intestinal microbiota composition leading to an imbalance between microbiota and the host. It has been associated with numerous diseases like, obesity, diabetes, autoimmune and allergic diseases, IBD, neurological disorders, infections and cancer (25,27,31). However, it is necessary further research to define the role of microbiota in their pathogenesis to establish causal relationships and to investigate if dysbiosis could be the cause, or the consequence, of these diseases (27).

Focusing on cancer risk, several types of cancer are been linked for a long time to infectious agents, representing approximately about 20% of the global. Clear examples are cervical cancer and gastric cancer, induced by *Human Papillomavirus* and *Helicobacter pylori*, respectively (34). Owing to these evidences, the hypothesis that some bacteria could have a potential role in CRC is not illogical. Moreover, the bacterial density in the large intestine reaches its highest concentration, being 12-fold

higher than that in the small intestine (10^{12} vs 10^{2} cells/ml), and suspiciously, there is an estimated 12-fold increase in cancer risk in the large intestine (33,35). Nevertheless, the role of intestinal microbiota in colorectal carcinogenesis is not well characterized and it has to be regarded as a complex multifactorial approach, and an important model is the dysbiosis-inflammation-tumorigenesis. In the initation and progression of colorectal carcinogenesis, the intestinal microenvironment suffers physiological changes, and this altered microenvironment may cause alterations in gut microbiota. In addition, alterations of the gut intestinal barrier, as consequence of increased thigh junction permeability and reduced mucin production, allow bacteria to have direct contact with colon epithelial cells and disrupt the appropriate intestinal immune response. It makes the host more susceptible to opportunistic infections and lead to pathological and persistent intestinal mucosal inflammation. Inflammation is an important mediator of bacteria-induced CRC and chronic inflammation can modify the immune local response, releasing cytokines which might promote tumour development by increasing cell proliferation, promoting angiogenesis and inhibiting apoptosis. Furthermore, bacteria can produce toxins (for example, *Bacteroides fragilis, enteropathogenic Escherichia coli)*; or toxic metabolites as fecapentaenes or superoxide radicals (*Enterococcus faecalis*) which cause DNA damage; or convert dietary and endogenous compounds into genotoxic substances. All these alterations drive the progression towards colorectal malignancy (26,33,36,37).

A new approach, the bacterial driver-passenger model for CRC, has recently emerged. It points out that colonic mucosa of patients at risk of CRC is firstly colonized by "bacterial drivers" (*Bacteroides, Enterobacteriaceae*) which cause inflammation, cell proliferation and produce genotoxic substances which contribute to accumulation of mutations in the adenoma-carcinoma sequence and initiate CRC development. These alterations lead to replacement "drivers" by "passenger bacteria", such as opportunistic pathogens (*Fusobacterium spp.; Streptococcus gallolyticus*, previously known as *Streptococcus bovis* and related with CRC almost 50 years ago), commensal bacteria

(*Coriobacteriaceae family, Roseburia*) or other bacteria with advantages in the tumour niche (35,36,38).

Over the last decade, advances in DNA sequencing technology has been a major advantage in the characterization of microbial communities, because traditional bacterial cultures were limited due to most microbes in intestinal microbiota are obligate anaerobes and were difficult to grow. Amplifying 16S ribosomal DNA from bacteria, allows phylogenetic study which define the lineages present in colorectal samples, and total DNA sequencing *(shotgun metagenomics)* allows an evaluation of the genomic representation in samples which provide functional information of the community (27).

For that reason, in the last years, few studies have been carried out to investigate the intestinal microbiota and colonic dysbiosis associated in patients with CRC and healthy controls. A recent study in the Digestive Department at Hospital Universitari Doctor Josep Trueta in Girona, and the research group in intestinal microbiota of Parc Científic i Tecnològic, in a context of a PhD thesis, has identified differences between intestinal microbiota composition in CRC patients and in healthy subjects, by biopsy samples from tumour and from rectal mucosa, respectively (39).

Overall, at this moment, no specific bacterial species have been clearly defined as risk factors for CRC and is not defined if the presence of specific bacteria in samples is suggestive of their role in tumour progression or is the consequence of tumour changes. However, some studies have revealed specific alterations in CRC microbiota: One study found a higher abundance of *Fusobacterium spp.* in colon tumours (40). These findings led to another study with the purpose of analysing the presence of *Fusobacterium* in patients with colorectal adenomas, not taking into account the adenoma type, and it showed a higher abundance of *Fusobacterium* in adenoma cases (41).

A recent study, using stool samples, has analysed bacterial species and also metabolites in feces from CRC patients and healthy controls, obtaining a more functional approach. *Bacteroides, Prevotella, Ruminococcus, Dorea, Dialister spp.* and *Megamonas spp.* were more abundant in healthy controls, whereas *Akkermansia muciniphila*, a mucin-degrading bacteria, and *Citrobacter farmeri,* a group with *N*-acetyltransferase activity involved in activation of carcinogens, were over-represented in CRC patients. Some metabolites (short chain fatty acids, specific amino acids, glycerol) were found in different concentrations in both groups of patients, and it can lead to identify CRC metabolic biomarkers (42).

Until today, these intestinal microbiota changes have been studied in different sample types. Stool samples are the most common method, followed by mucosal biopsies and rectal swabbing. All techniques have their advantages and drawbacks that have to be taken into account and standards for accurate sampling are needed, not forgetting that it has to be the most practical and suitable method (43).

3.2. JUSTIFICATION

The high incidence and mortality of CRC has become a major health problem in our country. A better knowledge of the natural history of sporadic CRC has allowed us to identify precursor lesions, the adenomatous polyps, which suffer a multistep malignant transformation progress during years and turn into a carcinoma. It is proved that environmental and personal factors are involved in it, but CRC causes are not completely established yet. Despite this, the contribution of intestinal microbiota in CRC is increasing. Many researches are focusing on trying to identify specific bacteria in colorectal cancers; however, it is not clear which bacteria are involved and their role in early stages of colorectal neoplastic transformation. A previous study has tried to describe mucosal adherent bacterial, by colorectal biopsies, between patients with adenoma and patients without adenoma. Higher abundance of *Proteobacteria* and lower abundance of *Bacteroidetes* were observed in adenoma cases, and other specific differences at genus level, suggesting that these changes can be involved in adenoma development and recommending extending the research in this field (44).

We wish to go further in this field and analyse the bacterial alterations in these malignant transformation, and we specially consider that it is interesting to characterize bacterial changes, in stool samples, according to the malignant risk of these precursor lesions, in simple or low risk adenomas, in advanced or high risk adenomas; and in patients without precursor lesions. Understanding that microbiota dysbiosis in feces from patients with preneoplastic lesions, could be used to identify early bacterial markers in order to develop new non-invasive screening tests for CRC.

4. BIBLIOGRAPHY

1. Globocan 2012. Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 [Internet]. Lyon: International Agency for Research on Cancer; 2014 [cited 2014 Sep 29]. Available from: http://globocan.iarc.fr/Default.aspx

2. SEOM. Las Cifras del Cáncer en Espanya 2014 [Internet]. Madrid: Sociedad Española de Oncología Médica; 2014 [cited 2014 Oct 31]. Available from: http://www.seom.org/es/prensa/el-cancer-en-espanyacom/104582-el-cancer-en-espana-2014#content

3. Brenner H, Francisci S, de Angelis R, Marcos-Gragera R, Verdecchia A, Gatta G, et al. Long-term survival expectations of cancer patients in Europe in 2000-2002. Eur J Cancer [Internet]. 2009 Apr [cited 2014 Oct 21];45(6):1028–41. Available from: http://www.sciencedirect.com/science/article/pii/S0959804908009106

4. Stigliano V, Sanchez-Mete L, Martayan A, Anti M. Early-onset colorectal cancer: A sporadic or inherited disease? World J Gastroenterol [Internet]. 2014 Sep 21 [cited 2014 Oct 4];20(35):12420–30. Available from: http://www.wjgnet.com/1007-9327/pdf/v20/i35/12420.pdf

5. Castells A. Colorectal cancer screening. Gastroenterol Hepatol [Internet]. 2011 Oct [cited 2014 Sep 29];34 Suppl 2:60–6. Available from: http://www.sciencedirect.com/science/article/pii/S021057051170022X

6. Kumar V, Abbas A, Fausto N AJ. The Gastrointestinal Tract. Robbins and Cotran's Pathologic Basis of Disease. Philadelphia: Saunders Elsevier; 2010. p. 815–20.

7. Carballal S, Moreira L, Balaguer F. Serrated polyps and serrated polyposis syndrome. Cirugía española [Internet]. 2013 Mar [cited 2014 Sep 16];91(3):141–8. Available from: http://zl.elsevier.es/es/revista/cirugia-espanola-36/linkresolver/polipos-serrados-sindrome-poliposis-serrada-90193798

8. Shussman N, Wexner SD. Colorectal polyps and polyposis syndromes. Gastroenterol Rep [Internet]. 2014 Feb [cited 2014 Oct 22];2(1):1–15. Available from: http://gastro.oxfordjournals.org/content/2/1/1.full.pdf+html

9. Hassan C, Quintero E, Dumonceau J-M, Regula J, Brandão C, Chaussade S, et al. Post-polypectomy colonoscopy surveillance: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy [Internet]. 2013 Oct 12 [cited 2014 Oct 16];45(10):842–51. Available from: https://www.thieme-connect.com/products/ejournals/html/10.1055/s-0033-1344548#N69007

10. Leslie A, Carey FA, Pratt NR, Steele RJC. The colorectal adenoma-carcinoma sequence. Br J Surg [Internet]. 2002 Jul [cited 2014 Sep 29];89(7):845–60. Available from: http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2168.2002.02120.x/pdf

11. Imai K, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis [Internet]. 2008 Apr [cited 2014 Sep 16];29(4):673–80. Available from: http://carcin.oxfordjournals.org/content/29/4/673.full.pdf+html

12. Castells A, Marzo-Castillejo M, Mascort J, Amador F, Andreu M, Bellas B et al. Guía de práctica clínica. Prevención del cáncer colorrectal. [Internet]. Barcelona: Asociación Española de Gastroenterología, Sociedad Española de Medicina de Familia y Comunitaria y Centro Cochrane Iberoamericano; 2009 [cited 2014 Oct 11]. Available from: http://www.guiasalud.es/GPC/GPC_494_colorrectal_(2009).pdf

13. Tárraga P, Solera J, Rodríguez-Montes J. Primary and Secondary Prevention of Colorectal Cancer. Clin Med Inisghts Gastroenterol [Internet]. 2014;7:33–46. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4116379/pdf/cgast-7-2014-033.pdf

14. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg [Internet]. 2009 Nov [cited 2014 Oct 17];22(4):191–7. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2796096/pdf/ccrs22191.pdf

15. E RK. Colorectal cancer in inflammatory bowel disease: The risk, pathogenesis, prevention and diagnosis. World J Gastroenterol [Internet]. 2014 [cited 2014 Oct 19];20(29):9872–81. Available from: http://www.wjgnet.com/1007-9327/pdf/v20/i29/9872.pdf

16. Botteri E, Iodice S, Raimondi S, Maisonneuve P, Lowenfels AB. Cigarette smoking and adenomatous polyps: a meta-analysis. Gastroenterology [Internet]. 2008 Mar [cited 2014 Oct 31];134(2):388–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18242207

17. Liang PS, Chen T-Y, Giovannucci E. Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. Int J Cancer [Internet]. 2009 May 15 [cited 2014 Oct 28];124(10):2406–15. Available from: http://onlinelibrary.wiley.com/doi/10.1002/ijc.24191/pdf

18. Lansdorp-Vogelaar I, von Karsa L. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First Edition--Introduction. Endoscopy [Internet]. 2012 Sep [cited 2014 Oct 21];44 Suppl 3(S 03):SE15–30. Available from: https://www.thieme-connect.com/DOI/DOI?10.1055/s-0032-1308898

19. Castells A. Colorectal cancer screening: reaffirming the past and resolutely advancing toward the future. Gastroenterol Hepatol [Internet]. 2014 May [cited 2014 Oct 31];37(5):277–9. Available from: http://www.sciencedirect.com/science/article/pii/S0210570513003178

20. Aasma Shaukat, Steven J. Mongin, Mindy S. Geisser, Frank A. Lederle, John H. Bond JSM and TRC. Long-Term Mortality after Screening for Colorectal Cancer. N Engl J Med [Internet]. 2013 [cited 2014 Oct 11];369:1106–14. Available from: http://www.nejm.org/doi/full/10.1056/NEJMoa1300720

21. Osborn NK, Ahlquist D. Stool screening for colorectal cancer: Molecular approaches. Gastroenterology. 2005 Jan;128(1):192–206.

22. Atkin WS, Edwards R, Kralj-Hans I, Wooldrage K, Hart AR, Northover JMA, et al. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. Lancet [Internet]. 2010 May 8 [cited 2014 Jul 21];375(9726):1624–33. Available from: http://www.sciencedirect.com/science/article/pii/S014067361060551X

23. Quintero E, Castells A, Bujanda L, Cubiella J, Salas D, Llanas Á et al. Colonoscopy versus Fecal Immunochemical Testing in Colorectal-Cancer Screening. N Engl J Med [Internet]. 2012 [cited 2014 Oct 11];366:697–706. Available from: http://www.nejm.org/doi/full/10.1056/NEJMoa1108895

24. Andreu García M, Marzo M, Mascort J, Quintero E, García-Alfonso P, López-Ibor C, et al. Prevención del cáncer colorrectal. Gastroenterol Hepatol [Internet]. 2009 Mar [cited 2014 Oct 16];32(3):137–9. Available from: http://zl.elsevier.es/es/revista/gastroenterologia-hepatologia-14/articulo/prevencion-del-cancer-colorrectal-13133854

25. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell [Internet]. 2012 Mar 16 [cited 2014 Jul 9];148(6):1258–70. Available from: http://www.sciencedirect.com/science/article/pii/S0092867412001043

26. Candela M, Guidotti M, Fabbri A, Brigidi P, Franceschi C, Fiorentini C. Human intestinal microbiota: cross-talk with the host and its potential role in colorectal cancer. Crit Rev Microbiol [Internet]. 2011 Mar [cited 2014 Sep 29];37(1):1–14. Available from: http://informahealthcare.com/doi/pdf/10.3109/1040841X.2010.501760

27. Wu GD, Lewis JD. Analysis of the human gut microbiome and association with disease. Clin Gastroenterol Hepatol [Internet]. 2013 Jul [cited 2014 Oct 10];11(7):774–7. Available from: http://www.cghjournal.org/article/S1542-3565(13)00596-X/pdf

28. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. Front Immunol [Internet]. 2014 Sep 5 [cited 2014 Sep 10];5(September):1–18. Available from: http://journal.frontiersin.org/Journal/10.3389/fimmu.2014.00427/full

29. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol [Internet]. 2014 Jan [cited 2014 Oct 9];5(September):494. Available from: http://journal.frontiersin.org/Journal/10.3389/fmicb.2014.00494/full

30. Robles Alonso V, Guarner F. Linking the gut microbiota to human health. Br J Nutr [Internet]. 2013 Jan [cited 2014 Oct 6];109 Suppl:S21–6. Available from: http://journals.cambridge.org/download.php?file=%2FBJN%2FBJN109_S2%2FS0007114512005235a.pdf&code=4e94bf129d836b7de051a6bcc1b17fc3

31. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature [Internet]. 2012 Sep 13 [cited 2014 Jul 14];489(7415):220–30. Available from: http://www.nature.com/nature/journal/v489/n7415/full/nature11550.html

32. Hagland HR, Søreide K. Cellular metabolism in colorectal carcinogenesis: Influence of lifestyle, gut microbiome and metabolic pathways. Cancer Lett [Internet]. 2014 Mar 12 [cited 2014 Aug 12]; Available from: http://www.sciencedirect.com/science/article/pii/S0304383514001335

33. Boleij A, Tjalsma H. Gut bacteria in health and disease: a survey on the interface between intestinal microbiology and colorectal cancer. Biol Rev Camb Philos Soc [Internet]. 2012 Aug [cited 2014 Oct 24];87(3):701–30. Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-185X.2012.00218.x/pdf

34. Zur Hausen H. The search for infectious causes of human cancers: where and why. Virology [Internet]. 2009 Sep 15 [cited 2014 Oct 10];392(1):1–10. Available from: http://www.sciencedirect.com/science/article/pii/S0042682209003419

35. Sobhani I, Amiot A, Le Baleur Y, Levy M, Auriault M-L, Van Nhieu JT, et al. Microbial dysbiosis and colon carcinogenesis: could colon cancer be considered a bacteria-related disease? Therap Adv Gastroenterol [Internet]. 2013 May [cited 2014 Oct 24];6(3):215–29. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3625019/pdf/10.1177_1756283X12473674.pdf

36. Collins D, Hogan AM, Winter DC. Microbial and viral pathogens in colorectal cancer. Lancet Oncol [Internet]. 2011 May [cited 2014 Oct 16];12(5):504–12. Available from: http://www.sciencedirect.com/science/article/pii/S1470204510701868

37. Zhu Y, Michelle Luo T, Jobin C, Young HA. Gut microbiota and probiotics in colon tumorigenesis. Cancer Lett [Internet]. 2011 Oct 28 [cited 2014 Oct 23];309(2):119–27. Available from: http://www.sciencedirect.com/science/article/pii/S0304383511003405#

38. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. Nat Rev Microbiol [Internet]. Nature Publishing Group; 2012 Aug [cited 2014 Sep 18];10(8):575–82. Available from: http://search.proquest.com/docview/1029874665/FEDD6B9224374C77PQ/17?accountid=15295

39. Mas de Xaxars Rivero T. Descripció i quantificació de la microbiota intestinal associada al càncer colorectal [Internet]. Girona: Universitat de Girona; 2012. Available from: http://dugi-doc.udg.edu/handle/10256/7289

40. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res [Internet]. 2012;22:292–8. Available from: http://genome.cshlp.org/content/22/2/292.long

41. McCoy AN, Araújo-Pérez F, Azcárate-Peril A, Yeh JJ, Sandler RS, Keku TO. Fusobacterium is associated with colorectal adenomas. PLoS One [Internet]. 2013 Jan [cited 2014 Oct 20];8(1):e53653. Available from: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0053653

42. Weir TL, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. White BA, editor. PLoS One [Internet]. Public Library of Science; 2013 Jan [cited 2014 Oct 14];8(8):e70803. Available from: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0070803

43. Budding AE, Grasman ME, Eck A, Bogaards J a, Vandenbroucke-Grauls CMJE, van Bodegraven A a, et al. Rectal swabs for analysis of the intestinal microbiota. PLoS One [Internet]. 2014 Jan [cited 2014 Nov 6];9(7):e101344. Available from: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0101344

44. Shen XJ, Rawls JF, Randall T, Burcal L, Mpande CN, Jenkins N, et al. Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. Gut Microbes [Internet]. 2014 [cited 2014 Nov 8];1(3):138–47. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2927011/pdf/gmic0103_0138.pdf

45. Martinez-Medina M, Aldeguer X, Gonzalez-Huix F. Abnormal Microbiota Composition in the Ileocolonic Mucosa of Crohn ' s Disease Patients as Revealed by Polymerase Chain Reaction Y Denaturing Gradient Gel Electrophoresis. Inflamm Bowel Dis. 2006;12(12):1136–45.

5. HYPOTHESIS

- Intestinal microbiota is involved in the pathogenesis of colorectal cancer; therefore it should be altered at some point of the natural history of colorectal neoplasia.

- There might be differences in the intestinal microbiota profile of healthy subjects and of patients with colorectal preneoplastic lesions, in particular colorectal adenomas.

  Intestinal microbiota identified in stool samples could be different between low risk and high risk adenomatous polyps.

6. OBJECTIVE

- This study aims to analyse whether there is an association between the fecal microbiota profiling and the presence and progression of colorectal adenomas, detected in population undergoing colonoscopy, to better understand the role of intestinal microbiota in colorectal carcinogenesis.

7. MATERIALS AND METHODS

7.1. STUDY DESIGN

This study is designed as a cross-sectional study, with the purpose of study the association between preneoplastic colorectal lesions with different risk of malignant progression and intestinal microbiota changes. It will be performed in the Gastroenterology Department at Hospital Universitari de Girona Doctor Josep Trueta in a period of time of two years.

7.2. POPULATION

The study population include adult patients undergoing screening or diagnostic colonoscopies, in the Digestive Endoscopy Unit at Hospital Universitari Doctor Josep Trueta.

7.3. INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA:

- Patients aged 18 years or over.
- Patients without pathologic findings in the colonoscopy.
- Patients with ≥ 1 or < 10 adenomatous polyps found and removed.
- Patients who are able to cooperate in the study.

EXCLUSION CRITERIA:

- Active antibiotic treatment or within the month prior to the colonoscopy.
- Immunosuppressed patients or receiving immunosuppressive treatment.
- Patients treated with chemotherapy and/or radiotherapy in the previous six months.
- Patients with intestinal Inflammatory Bowel Disease.

- Prior intestinal surgery or surgical intestinal resection.
- Finding of hyperplastic or serrated polyp.
- Finding of malignant colorectal polyp (carcinomatous cells invading the *muscularis mucosae*).
- CRC presently or personal history of CRC.
- Intestinal polyposis syndromes:
  - Familial adenomatous polyposis
  - MYH-associated polyposis
  - Peutz-Jeghers syndrome
  - Juvenile polyposis syndrome
  - Hyperplastic polyposis or serrated polyposis syndrome
- Hereditary non polyposis colorectal cancer (Lynch syndrome).

7.4. SAMPLE

SAMPLING METHOD

A consecutive non-probabilistic sampling will be performed as population undergo the colonoscopy procedure and sample recruitment will take place during six months.

Patients with no pathological findings in the colonoscopy and meeting the inclusion and exclusion criteria of the study will be invited to participate in it just after the colonoscopy. The information document and informed consent will be given to participants. Patients with polyps removed in the colonoscopy will be cited in gastroenterology outpatient hospital visit. According to histopathological results, patients with adenomatous polyps and meeting inclusion and exclusion criteria will be invited to participate in the study and information document with informed consent will be given to them.

SAMPLE SIZE

In the Digestive Endoscopy Unit of our centre, approximately 1.500 colonoscopies are performed in six months. According to data from Pathology Department, in the first six months of 2014, 154 low-grade adenomas and 49 high-grade adenomas were diagnosed when removed polyps were analysed.

Our sample size was defined taking into account these data and the number of patients analysed in other previous similar studies about intestinal microbiota realized in our Gastroenterology Department (45). We designed it as an exploratory study and we include 15 patients for each group (n=15) with a total sample size of 45 patients (n=45).

7.5. VARIABLES. METHODS OF MEASUREMENT.

- **VARIABLE A: Colorectal adenomas in the studied groups**

- Colonoscopy procedure: Patients undergoing colonoscopy are going to do an adequate bowel preparation to ensure a proper examination of the entire mucosa. Colon cleansing can be done with different compounds following manufacturer's guidelines [polyethylenglicol substances (*Bohm®), biphosphate based (Fosfosoda®)*, sodium sulphate + oxide magnesium *(Citrafleet®)*]. In our centre, the quality of colon preparation is evaluated according to the Boston Bowel Preparation Scale *(Annex 1)*.

Endoscopists will perform a meticulous inspection along the colon up to ileocecal valve. Polypoid lesions observed during the procedure will be removed, using forceps or loops depending on polyp's size. The endoscopic appearance of the polyp is not a good indicator of its histological nature. The Paris endoscopic classification is used to make a first description and classification of the polyp according its superficial appearance *(Annex 2)*. Other specific technique to enhance the detail of mucosal surface, to identify

lesions and to classify them based on pit-patterns of colonic mucosa described by Kudo, is the chromoendoscopy (*Annex 2).*

- For the final histological diagnosis, excised polyps will be sent, placed in sterile containers with formaldehyde, to the Pathology Department for histopathological analysis.

According to colonoscopy outcomes, we are going to classify the patients in two main groups:

- Group 1: Negative colonoscopy: No pathological findings.
- Group 2: Polyps in the colonoscopy: Polypectomy procedure.

According to histopathological analysis of removed polyps, the second group will be divided into three categories, according to the following criteria:

1) Low Risk Adenomas or simple adenomas:

- Adenoma < 10 mm in size.
- Adenoma with low grade dysplasia.
- Tubular adenoma.

2) High Risk Adenomas or advanced adenomas:

- Adenoma ≥ 10 mm in size.
- Adenoma with high grade dysplasia.
- Villous architecture adenoma.

3) Non adenomatous polyps: These will be excluded of our study.

- Hyperplastic polyps
- Serrated polyps

The variable will be defined according to the presence or not of colorectal adenomas and the studied groups will be the next:

1) Patients with no pathological findings (group 1)
2) Patients with low risk adenomas (group 2)
3) Patients with high risk adenomas (group 3)

- **VARIABLE B: Outcome**

Our outcome variable is the intestinal microbiota composition in feces by stool samples. It is going to be measured in the three subgroups.

Stool samples are going to be collected by patients themselves, fifteen days after the colonoscopy procedure, in containers for stool samples. Patients are going to bring the sample to Gastroenterology Department at Hospital Universitari Doctor Josep Trueta, where stool samples will be labelled and stored at -20ºC freezer service by the researchers.

The microbiological analysis will be performed by specialized Microbiological Service at Parc Científic i Tecnològic de la Universitat de Girona, as follows:

Stool samples are going to be stored at -80ºC freezer until sample preparation for the analysis. Stool samples DNA extraction will be performed with Nucleospin® Soil Kit (Macherey-Nagel GmbH&Co) according to the instructions recommended by the manufacturer. The DNA concentration obtained will be quantified by Qubit®BR assay Kit in the Fluorometer Qubit® 2.0.

From extracted DNA, the 16S rRNA will be amplified by Polymerase Chain Reaction (PCR) using universal bacterial primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-CGG TTA CCT TGT TAC GAC TT-3'). PCR will be performed with a thermal cycler GeneAmp® (model 9700 Applied Biosystems) with a specific step-down cycling program. The product will be visualized by gel electrophoresis on agarose gels.

A massive sequencing of the whole bacterial genome of the sample will be done by next-generation sequencing (NGS) using Illumina MiSeq® System, together with a phylogenetic analysis to identify both main phylogroups and specific phylotypes. With that analysis we obtain the prevalence of different bacterial sequences. Once identified bacterial sequences which are significantly different between the groups, specific primers and probes will be

designed for quantitative determination of bacterial markers using quantitative real-time PCR (qPCR). Results will be compared among groups using relative numeric indexes. Significant differences observed will be validated in future studies to confirm the findings.

- **COVARIATES**

These variables have to be taken into account to interpret the outcomes due to their influence on intestinal microbiota composition and adenomas development. Covariates with significant differences will be analysed with a multivariate analysis. We also want to take into account some other interest variables which be useful to describe our study population and that could be used for a deeper analysis in our research *(Annex 3)*.

- Age: years
- Gender: male or female
- Body mass index: kilos/metre^2
- Ethnic group: Caucasian, African, Asian
- Smoking: non-smoker, smoker, ex-smoker
- Family history of CRC: Yes/No
- Rectal bleeding and/or anaemia: Yes/No

7.6. DATA COLLECTION AND STUDY CIRCUIT

Personal from the Gastroenterology Department and Endoscopy Unit, previously informed and coordinated for the study; together with the Pathology Department, the researchers of gastroenterologist service and the participants in the study, will work together for data collection following a specific circuit for the study, explained below:

1. Endoscopists: According to clinical history of patients and colonoscopy findings, they will detect patients who can be included in the study.

   - Patients with no lesions: They will be informed about the study and the information document and informed consent will be given by endoscopists. Nurses will be given them a container for stool sample collection with the collection instructions.

   - Patients with polyps removed: They will have a next visit with the gastroenterologist who requested the colonoscopy in three weeks.
2. Pathologists: The analysis of polyps removed and the histopathological report will be performed in a period of about two weeks.
3. Three weeks after the colonoscopy, patients with polyps removed will be informed by the gastroenterologist about the histological characteristics of polyp and about the surveillance endoscopic control if it is necessary. The physician will select patients with low and high risk adenomas who can participate in the study and the information document, the informed consent and a container for stool sample collection will be given to them.
4. All participants will bring the stool samples to the Digestive Endoscopy Unit. Researchers will perform the stool sample collection (labelling and storage), collect signed informed consent and give participants a data collection sheet that they fill with personal and clinical data. These data, together with data from clinical history, will be used to describe and analyse characteristics of participants. *(Annex 3)*.
5. Stool samples will be sent for their analysis to Microbiological Service at Parc Científic i Tecnològic de la UdG.

8. STATISTICAL ANALYSIS

Descriptive analysis:

In the context of our project, we cannot technically define our variables as independent and dependent due to the study is a descriptive cross-sectional study. However, for the analysis, we will define the variable consisting of polyp lesions in the three studied groups as the independent variable and the variable consisting of microbiota profile as the outcome variable. Independent variable in this study will be measured as a nominal qualitative variable. The outcome will be measured firstly, as a nominal qualitative variable (bacteria phylotypes) and, in a secondary analysis, as a quantitative continuous variable (nº of bacteria). For qualitative or categorical variables, results will be expressed as percentages. For quantitative variables, assuming that they are not normally distributed, median will be estimated. In case that they follow a normal distribution, arithmetic mean and typical deviation will be calculated.

Bivariate analysis:

- Percentages for categorical variables will be shown in a contingency table and chi-square test (χ^2) will be used to compare bacterial species between three subgroups.
- To compare quantities of microorganisms (nº), we assume a non-normal distribution and non-parametrical tests will be used. To compare variables with ≥ 3 categories, Kruskal-Wallis test will be performed to test differences. In case that we finally found that it follows a normal distribution, we will use parametrical tests as the analysis of variance to check significance distances between groups.

Multivariate analysis:

The analysis will be adjusted for covariates statistically significant ($p<0'05$). Multinomial logistic regression analysis will be performed to assess the association between the three clinical groups and the percentages of different types of bacteria phylotypes in order to adjust for potential confounders.

9. ETHICAL CONSIDERATIONS

This study will be conducted according to the ethical principles established by World Medical Association in the *Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects.* The research protocol must be presented and submitted for consideration, guidance and approval by the *Clinical Research Ethical Committee* (*CEIC*, "*Comitè Ètic d'Investigació Clínica"*) at Hospital Universitari Doctor Josep Trueta before the study begins, and at the end of the study, the final report must also be submitted to the CEIC.

According to "*Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal",* personal and clinical information of participants will be confidential and only used for the purpose of the research. Moreover, all data will be analysed anonymously.

All participants will be personally informed by researchers and an information document about the study will be given to them *(Annex 4.1.).* Participants will have to sign voluntarily the informed consent *(Annex 4.2.)* before being included in the study after receiving the appropriate information about procedures, according to *"Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica".* Written information about the colonoscopy procedure and informed consent before the colonoscopy *(Annex 5)* is also necessary. If participants authorize the introduction of their stool sample if it is necessary, in the IdibGi BioBank, the information document and informed consent *(Annex 6)* have also to be given to them.

10. STUDY LIMITATIONS

Analysing our study, we detected and took into account some limitations which interfere in the research. The most relevant limitations are explained below:

1. Our study is designed as an exploratory study due to there are no earlier studies which investigate the microbiota changes in stool samples at preneoplastic colorectal stages, in healthy people and in patients with colorectal low and high risk adenomas. This exploratory study uses a limited sample size which we consider that it is enough to answer our research question. If significant differences in bacterial composition are found between the groups, a next study with a larger sample will be necessary to validate our findings and to make definitive and generalizable conclusions.

2. Owing to ethical limitations and the natural history of sporadic CRC, our study has to be design as a cross-sectional study. Colorectal adenomas have to be removed due to their potential malignant transformation and no longitudinal study can be done. The purpose of the study is to investigate, at one time point, if there are associations between these preneoplastic colorectal lesions and intestinal microbiota changes, but it is no possible to infer causality.

11. STUDY CHRONOGRAM

TASKS	2014				2015												2016												PERSONAL
	S	O	N	D	G	F	M	A	M	J	J	A	S	O	N	D	G	F	M	A	M	J	J	A	S	O	N	D	
TASK 1: PREPARATION AND COORDINATION PHASE																													
- Protocol elaboration and evaluation																													Main researcher CEIC
- Coordination of the research team																													All research team
TASK 2: FIELD WORK AND DATA COLLECTION																													
- Patients recruitment																													Gastroenterology team Clinical researchers
- Sample collection																													
- Probe design in the laboratory																													Microbiologist researcher
- Setting up of PCR																													
- DNA extraction and amplification																													
- Analysis of microbiological profile (NGS) - Quantitative real-time PCR (qPCR)																													
TASK 3: DATA ANALYSIS AND FINAL EVALUATION																													
- Statistical analysis																													Statistician
- Analysis and interpretation of results																													Main researcher Clinical researchers
- Final report elaboration																													
TASK 4: PUBLICATION AND DISSEMINATION																													
- Scientific publications - Attendance to SCD, AEG congresses*																													Main researcher Gastroenterology team

* SCD: Societat Catalana de Digestologia / AEG: Asociación Española de Gastroenterología

12. BUDGET

EXPENSES	COSTS (€)
1. Personal expenses	
2. Executive expenses	
Material acquisition	
- Stool containers (x 45 samples)	2,50 €
- DNA extraction kits	1.500 €
- PCR reagents	2.000 €
- Molecular biology reagents (Proteinase K, buffers, agarose, etc.)	1.200 €
- Other consumables: Gloves, test tubes, PCR plates, tips.	1.000 €
Services procurement	
- Next Generation Sequencing + phylogenetic study (x45 samples)	3.600 €
- Probes and primers design + bacterial quantification (qPCR)	4.000 €
- Statistical Analysis (x25h, per 35€/h)	875 €
3. Publication and dissemination expenses	
- Scientific publications	1.500 €
- Attendance to scientific meetings and congresses: SCD, AEG	600 € = 1.200€
TOTAL:	16.877,5 €

13. CLINICAL AND HEALTH CARE IMPACT

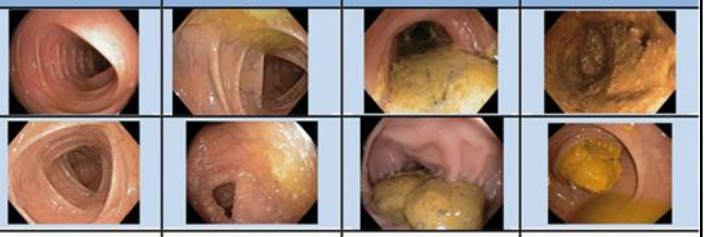
If the results obtained in our study are relevant and show that different microorganisms and in different amounts, are present in healthy subjects and in patients with different stages of colorectal adenomas, we might guess that microbiota suffers changes in parallel to CRC development, and not only it is altered when cancer is present, as it has been demonstrated until today.

Further research is necessary to confirm possible findings and studies are needed to define if some bacteria could be used as markers of oncogenic transformation.

With increasing knowledge in this field, new screening strategies could be considered in order to detect people who are at risk of CRC and reduce the incidence and mortality of that neoplasia. The determination of fecal microbiota could be in a near future an initial screening test, especially for these patients with high risk adenomas, in which we hypothesize that more changes in microbiota profiling should have. This new screening tests should be compared to the FOBT, the most widely used test nowadays: a test well accepted by general population and available in the different levels of health care.

14. ANNEXES

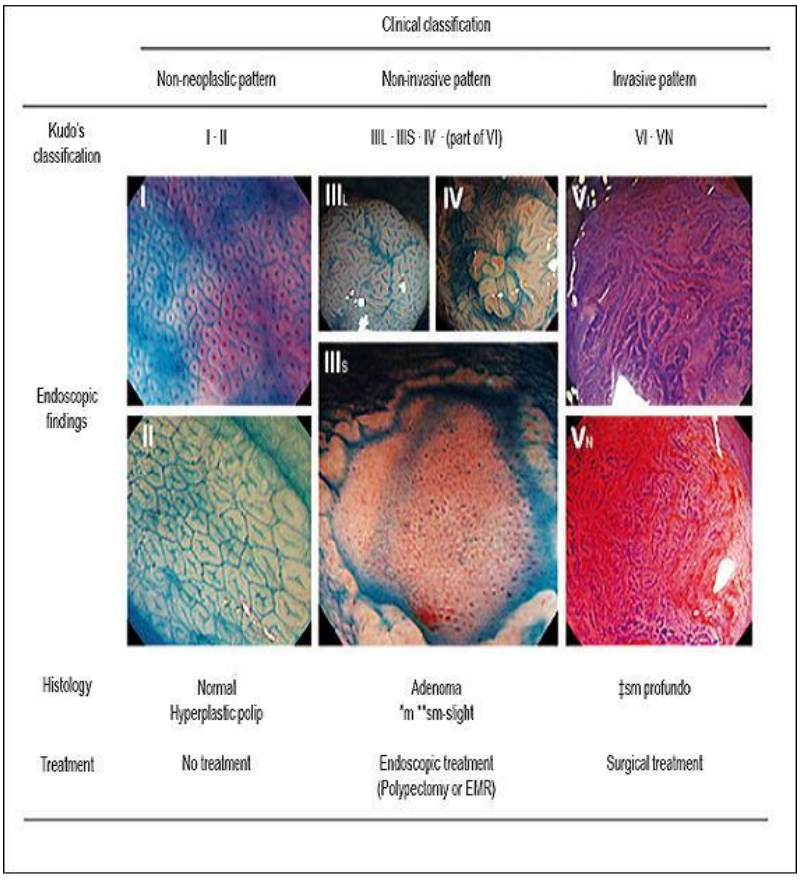
ANNEX 1. Boston Bowel Preparation Scale (BBPS)

BBPS		3	2	1	0
3=Excellent 2=Good 1=Poor 0=Inadequate					
LC	☐	☐	☐	☐	☐
TC	☐	☐	☐	☐	☐
RC	☐	☐	☐	☐	☐
BBPS= ☐					

ANNEX 2. Endoscopic appearance: Paris Classification / Kudo Pit Pattern Classification

Endoscopic appearance	Paris class		Description
Protruded lesions	Ip		Pedunculated polyps
	Ips		Subpedunculated polyps
	Is		Sessile polyps
Flat elevated lesions	0-IIa		Flat elevation of mucosa
	0-IIa/c		Flat elevation with central depression
Flat lesions	0-IIb		Flat mucosal change
	0-IIc		Mucosal depression
	0-IIc/IIa		Mucosal depression with raised edge

** Endoscopic appearance: Paris Classification*



** Kudo Pit Pattern Classification*

ANNEX 3. Data collection sheet (to refill by the participant and clinical researchers)

PARTICIPANT CODE: _____

- **Age** (years): _____
- **Gender**: ☐ Female ☐ Male
- **Ethnicity**: ☐ Caucasian ☐ African ☐ Asian
- **Height** (cm): _____ **Weight** (kg): _____
- **Body Mass Index** (kg/m^2): _____ ☐ ≤ 18'5 kg/m^2 ☐ 18'5-24'99 kg/m^2 ☐ 25-29'99 kg/m^2 ☐ ≥ 30 kg/m^2
- **Reason for colonoscopy:** ☐ Screening
  ☐ Control Reason: _____
  ☐ Clinical symptoms: ☐ Rectal bleeding ☐ Anaemia ☐ Others: _____
- **Medical history:**
  - **Personal diseases**: _____
  - **Specific gastrointestinal disorders**: _____
  - **Family history of colorectal cancer**: _____
- **Current treatments**: _____
- **Smoking:** ☐ Non-smoker ☐ Smoker (___cig/d) ☐ Ex-smoker

ANNEX 4.

4.1. Information document for the study

FULL D'INFORMACIÓ PEL PARTICIPANT

INVESTIGADORS PRINCIPALS: Virginia Piñol, Anna Brujats, Mariona Serra, Anna Bahí.

CODI DEL PROJECTE: _____

1) **Generalitats del projecte**: El present estudi serà dut a terme pel servei de Digestiu de l'Hospital Universitari de Girona Doctor Josep Trueta, en un període de temps aproximat de dos anys. El projecte de recerca ha estat valorat i aprovat pel Comitè Ètic d'Investigació Clínica de l'Hospital Doctor Josep Trueta. Els participants en l'estudi col·laboraran en la recollida de mostres aportant una mostra de la seva femta.

2) **Objectius i finalitats de l'estudi**: Amb aquest estudi es pretén estudiar el perfil microbiològic en mostres de femta de pacients amb colonoscòpia sense troballes patològiques, i de pacients amb troballa de pòlips adenomatosos de baix risc i d'alt risc de progressió neoplàsica, per tal d'identificar possibles marcadors bacterians que puguin servir com a eina de cribratge i detecció precoç del càncer colorectal.

3) **Participació**: La seva participació en l'estudi és totalment voluntària. El participant és lliure d'abandonar l'estudi si així ho desitja en qualsevol moment, sense necessitat de justificacions i sense que aquest fet afecti la seva assistència sanitària. La participació en l'estudi és totalment gratuïta i no s'obtindrà cap compensació econòmica per la participació.

4) **Confidencialitat i protecció de dades**: S'adoptaran les mesures per garantir la confidencialitat de les seves dades en compliment de la *Llei Orgànica* 15/1999 i les dades recollides seran gestionades de forma anònima i només utilitzades amb fins d'investigació. També es garantiran els principis establerts per la *Llei d'Investigació Biomèdica* 14/2007.

5) **Tasca del participant en la recollida de mostres**: El participant haurà de recollir una mostra de femta seguint les instruccions recomanades i com a mínim quinze dies després de la colonoscòpia, en el recipient per recollida de femta que se li proporcionarà. Caldrà que entregui la mostra al servei de Digestiu (5ª planta) de l'hospital el mateix dia de la recollida, i en cas de no ser possible, caldrà que mantingui la mostra a la nevera fins a la seva entrega. La mostra li serà recollida pels investigadors i correctament etiquetada amb el codi que se li haurà assignat per l'estudi.

6) **Resultats i beneficis de la investigació**: El pacient està en el seu dret de ser informat dels resultats de la investigació, així i com es respectarà la seva voluntat de no ser informat respecte aquests. Els beneficis derivats de la investigació, tan poden beneficiar al participant com a altres persones, i aquests seran adequadament utilitzats per assolir els objectius de l'estudi i serviran de base per futures investigacions en aquest àmbit.

Gràcies per la seva participació.

4.2. Informed consent

CONSENTIMENT INFORMAT

Declaració del participant:

Jo, _____

- He llegit la fulla informativa sobre l'estudi que se m'ha entregat.
- He pogut fer totes les preguntes necessàries respecte l'estudi.
- He rebut suficient informació sobre l'estudi.
- He estat informat per l'investigador.....de les implicacions i finalitats de l'estudi.
- Entenc que la meva participació és voluntària.
- Entenc que les mostres obtingudes seran etiquetades amb un codi per tal de mantenir la confidencialitat de les meves dades.
- Entenc que d'acord amb la Llei de Biomedicina 14/2007 d'Investigació Biomèdica la mostra sobrant de l'estudi serà utilitzada per futurs projectes relacionats amb aquest projecte, o amb la malaltia, o bé destruïda, segons la meva voluntat.
- Entenc que puc revocar el meu consentiment de participació a l'estudi, sense haver de donar justificacions i sense afectar la meva assistència sanitària.

❖ Accepto que els investigadors principals del projecte puguin contactar amb vostè si en un futur es considera oportú? ☐ Sí ☐ No

❖ Lliurement, dono la meva conformitat per participar en l'estudi amb mostres de femta? ☐ Sí ☐ No

❖ Autoritzo, que en cas de sobrant de les meves mostres, aquestes siguin utilitzades en investigacions futures relacionades amb l'estudi? ☐ Sí ☐ No

❖ Autoritzo, que en cas de sobrant de les meves mostres, siguin introduïdes al Biobanc de l'hospital? ☐ Sí ☐ No

Firma del participant

Data: __ / __ / __

Firma de l'investigador

Data: __ / __ / __

ANNEX 5. Colonoscopy informed consent

Hospital Universitari de Girona Doctor Josep Trueta

Institut Català de la Salut

Primer cognom
Segon cognom
Nom
Data de naixement **Sexe**
NHC **DNI**
CIP
Episodi origen

Consentiment informat

Cognoms i nom de la persona responsable quan el pacient sigui menor o incapaç de donar el seu consentiment **DNI*** **Relació amb el/la pacient***

Nom del procediment

Colonoscòpia

1. Descripció del procediment

L'endoscòpia digestiva baixa (colonoscòpia) és un examen visual de la mucosa del còlon (intestí gros). Per a realitzar-la s'ha d'introduir a través de l'anús una sonda òptica, llarga i flexible, nomenada colonoscopi. Si és necessari, durant l'exploració s'agafaran petites mostres de teixit (biòpsies) per analitzar-les amb un microscopi. Se li administraran sedants perquè tingui poques molèsties.
Aquesta exploració es realitza sota sedació moderada - profunda per endoscòpia digestiva diagnòstica o terapèutica.

2. Riscos Generals

Qualsevol exploració, tractament o intervenció quirúrgica presenta uns riscs generals. El més greu és la possibilitat d'una parada cardíaca. Altres complicacions són les hemorràgies i les infeccions. En cas d'urgència vital, caldrà actuar sobre aquestes complicacions amb els mitjans oportuns per al bé del pacient, dels quals s'informarà (sempre que les circumstàncies ho permetin) el malalt o la persona que en sigui responsable

3. Riscos específics d'aquest procediment

Colonoscòpia:
Perforació 0,1%
Hemorràgia 0,5 - 7%
Infecció 0,5 -7%
Riscos greus 0,1%
Sedació:
Hiposaturació d'oxigen - 046% (<80%)
Bradicàrdia - 0,21%
Broncoaspiracions - 0,03%
Laringoespasme - 0,03%
Convulsions - 0,035
Transtorns neurològics - 0,0002%
Complicacions totals - 0,8%

4. Riscos Personalitzats

Atesa la meva situació clínica i les meves circumstàncies personals, els meus riscos, que m'han explicat i he entès perfectament, poden dur a alguna complicació durant el procediment.
Si així fos, dono el meu consentiment per què es modifiqui el procediment previst i es pugui resoldre el meu problema.

Data impressió **Hora impressió** Pàgina 1 de 2

Hospital Universitari de Girona Doctor Josep Trueta

Institut Català de la Salut

Primer cognom
Segon cognom
Nom
Data de naixement **Sexe**
NHC **DNI**
CIP
Episodi origen

Consentiment informat

5. Possibles alternatives

Suggerències del malalt

7. Autorització

He rebut la suficient informació verbal i/o escrita i he llegit el full informatiu sobre l'exploració, sedació, tractament, i/o intervenció quirúrgica que em realitzaran.
He pogut fer preguntes sobre aquest procediment.
Puc canviar d'opinió en qualsevol moment, abans de la realització del procediment, si així ho crec convenient He comprès la informació que m'ha estat donada, i per això conscientment autoritzo que es porti a terme el procediment.
Aquest consentiment es formula d'acord amb el que estableix la Llei 21/2000 de 29 de desembre publicada en el DOGC núm. 3303 de l'11 de gener de 2001.

Servei **Cognoms i nom del metge que informa** **Número de col.legiat**
GASTROENTEROLOGIA- AP

Signatura del/la pacient o responsable **Data** **Signatura del metge que informa**

☐ Accepta

☐ No accepta

Data impressió **Hora impressió**

Pàgina 2 de 2

ANNEX 6. Biobank information document and informed consent

FULL D'INFORMACIÓ AL PACIENT

OBTENCIÓ I UTILITZACIÓ DE MOSTRES BIOLÒGIQUES I DADES CLÍNIQUES PER INVESTIGACIÓ MÈDICA I CONSERVACIÓ EN UN BIOBANC

A l'Hospital Universitari de Girona Dr Josep Trueta (HUGJT) i/o altres Centres Hospitalaris adscrits, igual que en la majoria d'hospitals, a més de l'assistència als pacients, es realitza investigació biomèdica. La finalitat d'aquesta investigació és progressar en el coneixement de les malalties i en la seva prevenció, diagnòstic i tractament. Aquesta investigació biomèdica requereix recollir dades clíniques i mostres biològiques de pacients i donants sans per a analitzar-los i obtenir conclusions amb l'objectiu de conèixer millor les malalties i avançar cap al seu diagnòstic i/o tractament. Les mostres i dades clíniques obtingudes per al diagnòstic o control de les malalties, una vegada utilitzades amb aquesta finalitat, resulten també útils i necessàries per a la investigació. De fet, molts dels avenços científics obtinguts en aquests últims anys en medicina són fruit d'aquest tipus d'estudis.

Sol·licitem la seva autorització per a l'obtenció d'una mostra biològica addicional que se li extraurà a l'HUGJT i/o altres Centres Hospitalaris adscrits, amb la finalitat de dipositar-la al Biobanc IDIBGI, així com la seva autorització per utilitzar la informació clínica associada a aquest material biològic per prosseguir amb la investigació biomèdica.

Seguint el que estableix la Llei 14/2007, d'Investigació Biomèdica, la Llei Orgànica 15/1999, de Protecció de Dades Personals, i les seves normes de desenvolupament, li sol·licitem que llegeixi detingudament aquest document d'informació i el consentiment informat que se li adjunta al final per a la seva firma, si està d'acord en participar en aquesta proposta.

FINALITAT DE LA INVESTIGACIÓ: Progressar en el coneixement de les malalties.

La finalitat de la investigació és millorar el nostre coneixement de les malalties. Les mostres, les dades clíniques i analítiques i les proves d'imatge s'utilitzaran per a la recerca biomèdica.

MOSTRES BIOLÒGIQUES I INFORMACIÓ ASSOCIADA: Les mostres obtingudes es custodiaran i conservaran en el Biobanc IDIBGI fins la seva extinció.

Es guardarà i disposarà de la mostra biològica addicional de ***femta*** per a realitzar estudis d'investigació biomèdica, sense que aquest fet li causi molèsties addicionals. La donació d'aquestes mostres cedides al Biobanc IDIBGI no impedirà que vostè o la seva família puguin usar-les, quan sigui necessari per motius de salut. Les mostres i la informació associada a aquestes es custodiaran i conservaran al Biobanc (banc de mostres biològiques) IDIBGI, fins a la seva extinció.

Aquest Biobanc és un establiment sense ànim de lucre i inscrit en el *Registro Nacional de Biobancos* dependent de l'*Instituto de Salud Carlos III* amb la referència B.0000872, que acull col·leccions organitzades de mostres biològiques i informació associada en les condicions i garanties de seguretat que exigeix la legislació anteriorment referida i els codis de conducta aprovats per els Comitès d'Ètica. Les esmentades mostres i la seva informació associada queden disponibles per aquells investigadors que ho sol·licitin al Biobanc.

Qualsevol estudi d'investigació per al qual se sol·liciti la utilització d'aquestes dades o mostres haurà de disposar sempre de l'aprovació del Comitè d'Ètica de la Investigació Clínica (CEIC) competent, que vetllarà per a què els investigadors desenvolupin els seus estudis seguint sempre les més estrictes normes ètiques i legals. A més, el comitè científic del Biobanc garantirà que els projectes siguin d'excel·lència científica. La investigació biomèdica és actualment un fenomen global, de manera que ocasionalment aquestes mostres podran ser cedides a grups d'investigació fora d'Espanya, sempre que compleixin els requisits de la legislació espanyola i ho aprovin els corresponents comitès.

A partir de les mostres donades, en els casos en que la investigació ho requereixi, es realitzaran estudis genètics, i a partir d'ells es pot obtenir informació sobre la seva salut i la dels seus familiars. Sempre s'actuarà vetllant per la protecció d'aquesta informació (apartat protecció de dades).

En el cas de ser necessària alguna mostra addicional, la institució sanitària es podria posar en contacte amb vostè per a sol·licitar-li novament la seva col·laboració. En aquest cas se li informarà dels motius i se li sol·licitarà de nou el seu consentiment.

PROTECCIÓ DE DADES I CONFIDENCIALITAT: Les mostres es conservaran codificades.

Les dades personals que es recullin seran obtingudes, tractades i emmagatzemades complint en tot moment el deure del secret, d'acord amb la legislació vigent en matèria de protecció de dades de caràcter personal.

La identificació de les mostres biològiques del Biobanc serà sotmesa a un procés de codificació. A cada mostra se li assigna un codi d'identificació, que serà l'utilitzat per els investigadors. Només el personal autoritzat per el Biobanc podrà relacionar la seva identitat amb els citats codis. Mitjançant aquest procés els investigadors que sol·licitin mostres al Biobanc no podran conèixer cap dada que reveli la seva identitat. De la mateixa manera, encara que els resultats obtinguts de la investigació realitzada amb les seves mostres es publiquin en revistes científiques, la seva identitat no serà facilitada. En aquells estudis en els quals no es prevegin resultats potencialment útils per a la seva salut, i d'acord amb el corresponent Comitè d'Ètica, les mostres i dades podran ser anonimitzades, és a dir, no hi haurà cap possibilitat de tornar a associar la mostra amb la seva identitat.
Les seves mostres i dades clíniques associades a les mateixes passaran a formar part del fitxer del Biobanc, inscrit en l'Agència de Protecció de Dades sota la responsabilitat de l'Institut d'Investigació Biomèdica de Girona (IDIBGI).
Vostè podrà exercir els seus drets d'accés, rectificació, cancel·lació i objecció, així com obtenir informació sobre l'ús de les seves mostres i dades associades, dirigint-se a:

DIRECCIÓ DEL BIOBANC IDIBGI	Avinguda de França s/n
Hospital Universitari de Girona Dr. Josep Trueta	17007 Girona
Biobanc@IDIBGI.org	Tlfn. 972 940 282

CARÀCTER ALTRUISTA DE LA DONACIÓ: La cessió de mostres biològiques que vostè realitza al Biobanc IDIBGI és gratuïta: La donació té per disposició legal caràcter altruista, per la qual cosa vostè no obtindrà ni ara ni en el futur cap benefici econòmic de la mateixa, ni tindrà drets sobre possibles beneficis comercials dels descobriments que es puguin aconseguir com a resultats de la investigació biomèdica.

PARTICIPACIÓ VOLUNTÀRIA: La seva negativa NO repercutirà en la seva assistència mèdica, present o futura: La seva participació és totalment voluntària. Si firma el consentiment informat, confirmarà que desitja participar. Pot negar-se a participar o retirar el seu consentiment en qualsevol moment posterior a la firma sense haver d'explicar els motius i que això repercuteixi en la seva assistència mèdica, present o futura.

COST i RISCOS ASSOCIATS: La seva donació no li suposa CAP cost.
L'extracció de la mostra no suposarà cap cost econòmic per a vostè. En el cas d'una extracció de sang, el risc per a la seva salut és molt petit, però pot incloure les molèsties habituals d'una extracció de sang: dolor de molt poca importància, pell contusionada, sagnat per on entra l'agulla o l'ansietat davant les agulles. Es prendran precaucions per a evitar aquests inconvenients. En el cas d'una mostra de **femta**, l'extracció es realitzarà en el context assistencial., de manera que no afegeix cap risc addicional per a vostè. Mai es realitzarà una intervenció exclusivament per a l'obtenció de mostres per a investigació.

REVOCACIÓ DEL CONSENTIMENT: Si vostè decideix firmar aquest consentiment podrà també cancel·lar-lo lliurement. Això comportarà la destrucció de les seves mostres.
Si en un futur vostè volgués anul·lar el seu consentiment, les seves mostres biològiques serien destruïdes i les dades associades a les mateixes serien retirades del Biobanc. També podria sol·licitar l'anonimització de les mostres, de manera que en aquest cas s'eliminaria la relació entre les seves dades personals (que revelen la seva identitat) i les seves mostres biològiques i dades clíniques associades. Els efectes d'aquesta cancel·lació o anonimització no es podrien estendre a la investigació que ja s'hagi realitzat. Si desitgés cancel·lar el consentiment, ho hauria de sol·licitar per escrit a la Direcció del Biobanc IDIBGI, a l'adreça anteriorment mencionada.

INFORMACIÓ SOBRE ELS RESULTATS DE LA INVESTIGACIÓ: Se li proporcionarà informació si vostè la desitja rebre.
En el cas que vostè ho demani expressament, el Biobanc podrà proporcionar informació sobre quines són les investigacions en què s'han utilitzat les seves mostres i dels resultats globals d'aquestes investigacions, excepte en el cas de cancel·lació o anonimització. Els mètodes utilitzats en investigació biomèdica solen ser diferents dels aprovats per a la pràctica clínica, per el que no han de ser considerats amb valor clínic per a vostè. Malgrat això, en el cas que aquestes investigacions proporcionin dades que poguessin ser clínica o genèticament rellevants per a vostè i interessar a la seva salut o a la seva família, li seran comunicats si així ho estima oportú. Així mateix, podria donar-se el cas que s'obtingui informació rellevant per a la seva família. En aquest supòsit, li correspondrà a vostè decidir si vol o no que aquesta informació li sigui comunicada. En cas afirmatiu, ha de consignar-ho a la casella que apareix al final d'aquest document. Si vostè no desitja aquesta informació, tingui en compte que la llei estableix que, quan la informació obtinguda sigui necessària per a evitar un greu perjudici per a la salut dels seus familiars biològics, un Comitè d'experts estudiarà el cas i haurà de decidir si és convenient informar als afectats o als seus representants legals.

Si us plau, pregunti al personal sanitari que li ha comunicat aquesta informació sobre qualsevol dubte que pugui tenir, ara o en el futur, en relació a aquest consentiment. Així mateix, pot comentar els seus dubtes al seu metge, que el posarà en contacte amb el personal sanitari autoritzat.

Moltes gràcies per la seva col·laboració. **BIOBANC IDIBGI**

CONSENTIMENT INFORMAT

OBTENCIÓ I UTILITZACIÓ DE MOSTRES BIOLÒGIQUES I DADES CLÍNIQUES PER INVESTIGACIÓ MÈDICA I CONSERVACIÓ EN UN BIOBANC

Si ha comprès la informació que se li ha proporcionat en el document informatiu, resolt qualsevol dubte que pogués tenir i decideix col·laborar amb el Biobanc IDIBGI en els termes abans explicats, si us plau, llegeixi i firmi a continuació aquest full:

Qui signa el present document autoritza a l'HUGJT i/o altres Centres Hospitalaris adscrits a obtenir la mostra biològica addicional de ***FEMTA*** per tal que puguin ser incorporada al Biobanc IDIBGI, el qual podrà emmagatzemar i utilitzar científicament tant la informació clínica i assistencial del seu historial mèdic com les proves d'imatge i les mostres biològiques obtingudes, amb la finalitat de desenvolupar projectes d'investigació biomèdica, sempre que aquests comptin amb l'obligada aprovació del Comitè d'Ètica d'Investigació competent.

Confirmo que:

1. Autoritzo que la mostra biològica cedida i la informació clínica associada s'utilitzi en investigacions:
   Nacionals: ☐ SÍ ☐ NO Internacionals: ☐ SÍ ☐ NO
2. Desitjo que se'm comuniqui la informació derivada de la investigació que realment sigui rellevant i aplicable per a la meva salut o la de la meva família:
   ☐ SÍ ☐ NO Telèfon o email de contacte.....
3. Autoritzo a ser contactat en el cas de necessitar més informació o mostres biològiques addicionals:
   ☐ SÍ ☐ NO Telèfon o email de contacte.....
4. He expressat el meu desig de que se'm respectin les següents excepcions respecte a l'objectiu i mètodes de les investigacions:
   .....
   .....

DONANT	PERSONA QUE INFORMA	☐ TESTIMONI[1]/ ☐ TUTOR[2]
Nom	*Nom*	*Nom*
Cognoms	*Cognoms*	*Cognoms*
DNI	*DNI*	*DNI*
Edat		*Relació amb el donant:*
Signatura	*Signatura*	*Signatura*

[1] Autoritzat pel donant
[2] Representant legal

A ....., a .....de.....de.....

Arribada la majoria d'edat, el donant té dret a l'anul·lació del consentiment. En cas que no l'exerceixi, es considerarà que l'actual document de consentiment continua vigent.