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(54) **Title:** IDENTIFICATION OF SUBJECTS AT RISK OF DEVELOPING IRRITABLE BOWEL SYNDROME

(57) **Abstract:** Described is the use of enterotyping of the gut microbiota for identifying a subject at risk of developing Irritable Bowel Syndrome (IBS) and/or diagnosing a subject suffering from IBS, as well as method for identifying a subject at risk of developing IBS and/or diagnosing a subject suffering from IBS by enterotypes a test sample derived from a subject.



WO 2013/012332 A1

Title: Identification of subjects at risk of developing Irritable Bowel Syndrome

Field of the invention

The present invention is in the field of microbiology and gastrointestinal health, and relates to the use of the gastrointestinal microbiota as a biomarker for intestinal aberrations, notably Irritable Bowel Syndrome.

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Background

The gastro-intestinal tract is colonized since birth by complex communities of microbes, including bacteria, archaea and fungi, that develop in time and space. These microbial communities were collectively termed gut microflora in previous times but are now known as gut microbiota that is of a highly complex nature. (Rajilic-Stojanovic et al. 2007. Environ Microbiol 9: 2125-2136). The gut microbiota is involved in a variety of metabolic functions, such as the processing of food components that are not digested by the host, the synthesis of vitamins and the production of short chain fatty acids. However, in recent years it has been established that gut microbes interact with the host cells resulting in modulation of host processes including gut motility, gut barrier and immune function (Zoetendal et al., 2008. Gut 57: 1605-1615). Hence, aberrations in the gut microbiota can be associated with a variety of functional intestinal disorders, including Inflammatory Bowel Disease (hereinafter also referred to as "IBD") and Irritable Bowel Syndrome (hereinafter also referred to as "IBS").

Recently, the existence of a limited number of well-balanced, defined host-microbial symbiotic states in the gut has been demonstrated (M Arumugam, J Raes, E Pelletier, D Le Paslier, T Yamada, DR Mende, GR Fernandes, J Tap, T Bruls, JM Batto, M Bertalan, N Borruel, F Casellas, L Fernandez, L Gautier, T Hansen, M Hattori, T Hayashi, M Kleerebezem, K Kurokawa, M Leclerc, F Levenez, C Manichanh, HB Nielsen, T Nielsen, N Pons, J Poulain, J Qin, T Sicheritz-Ponten, S Tims, D Torrents, E Ugarte, EG. Zoetendal, J Wang, F Guarner, O Pedersen, WM de Vos, S Brunak, J Doré, MetaHIT Consortium, J Weissenbach, SD Ehrlich, and P Bork (2011) Enterotypes of the human gut microbiome. Nature 473: 174-80). These states have been referred to as "enterotypes". These enterotypes have been shown to be very robust and were detected in data spanning many nations and several continents. Thus, people can be characterized by their gut microbiota analogous to blood type, and can be assigned either enterotype 1, enterotype 2, or enterotype 3. It is known that gut microbiota are quite stable in individuals and can even be restored after perturbations such as antibiotic use (Zoetendal et al. 2008, *supra*). This implies that enterotypes are stable over time.

IBS is a multi-factorial and complex disorder clinically characterized by recurrent episodes of abdominal discomfort or pain, altered bowel habit and urge. Apart from IBD and IBS also other diseases are known to be associated with aberrations in microbiota and these include obesity, the various types of diabetes such as type I diabetes and type II diabetes, 5 Autistic Spectrum Disorder (ASD) related diseases, celiac disease and some forms of cancer (Zoetendal et al, 2008, *supra*).

From all the diseases that affect the gastro-intestinal tract, IBS is the most prevalent functional bowel disorder, that affects up to 20 percent of the general population in the world. Furthermore, IBS is associated with a high rate of absenteeism from work, a significant 10 impairment in quality of life and substantial health care costs. The economic consequences of IBS are substantial. The annual direct and indirect medical costs of IBS management in the US are as high as US\$ 8 billion and \$25 billion, respectively (Horwitz, B. & Fisher, R. The irritable bowel syndrome. N. Engl. J. Med. 344, 1846–1850 (2001)).

The diagnosis of IBS is based on aberrant bowel functions using the so called Rome 15 criteria and three subtypes of IBS are discriminated, including the constipation (IBS-C), diarrhea (IBS-D) and alternating constipation/diarrhea (IBS-A) subtypes (Thompson et al., 1989. Gastroenterology 130: 1552-1556; Longstreth et al., 2006. Gastroenterology 130: 1480-1491). While the diagnosis of IBD is based on non-invasive diagnostic procedures as the presence of inflammatory biomarkers in the blood, imaging diagnostics and endoscopic 20 observations (including histology of mucosal specimens), IBS is much harder to diagnose. Nowadays, IBS is only diagnosed by exclusion of IBD and other (bowel) disorders (such as celiac disease, colorectal cancer, ovarian cancer, diverticulosis, gallstones, food allergies, bacterial infections, intestinal parasites, endometriosis and lactose malabsorption) and is dependent on an anamnesis as laid down in the Rome criteria. This makes the diagnosis of 25 IBS a rather undefined 'exclusion diagnosis' and relatively expensive. Hence there is a great need to develop biomarkers that are indicative of IBS, as is confirmed by the US National Institute of Health that states that no test for IBS is known (<http://digestive.niddk.nih.gov/ddiseases/pubs/ibs/>). Specifically, reliable non-invasive biomarkers are needed to develop a diagnostic test for IBS. These biomarkers can be used to 30 diagnose IBS but also may be instrumental in identifying subjects at increased risk of developing IBS. Moreover, the identification of such biomarkers may lead to the discovery and development of new and innovative therapeutic or preventive interventions for IBS.

The pathophysiologic pathway of IBS is unknown, and diagnostic procedures, among other by blood analysis, endoscopy, histology and radiologic procedures, do not reveal any 35 common structural abnormalities in the digestive tract. While for a long time IBS has been considered a psychosomatic aberration, in recent years support has been provided for the involvement of biological and hereditary factors concerning the hypersensitivity of the brain-

gut axis. Recent studies provide several lines of evidence that support a relation between intestinal microbiota and IBS. In various cases IBS is triggered in previously healthy individuals by acute GI tract infection (gastro-enteritis) by external microbiota resulting in the so called post-infective IBS: up to 25% of patients with acute GI tract infection develop IBS.

5 During these infections the intestinal function and microbiota composition is affected. In several cases successful treatment of IBS has been shown by the consumption of pre- and probiotics that are all known to affect the intestinal microbiota composition and function (Spiller, 2009. Aliment Pharmacol Ther 28: 385-396). Finally, there are observations that IBS subjects in comparison with healthy individuals show deviations in intestinal microbiota
10 composition or metabolites. However, no clear picture emerges from these studies as to what are the specific microbes or microbial groups that differ between IBS and healthy subjects.

WO 2011/043654, which is herein incorporated by reference, describes a method for diagnosing and/or subtyping IBS in a test sample, which method uses the increased or decreased level of specific gut microbes in IBS patients compared to healthy subjects to
15 establish a diagnosis of IBS.

There is a need in the art to identify subjects having a propensity to develop IBS, allowing early detection of an increased risk of IBS and allowing intervention to prevent IBS from developing. Additionally, there is a need for an easy method to allow a diagnosis of IBS.

20 **Summary of the invention**

The present invention provides for the use of enterotyping of the gut microbiota for identifying a subject at risk of developing Irritable Bowel Syndrome (IBS) and/or diagnosing a subject suffering from IBS.

In an embodiment, assignment of the gut microbiota of a subject to enterotype 3 is
25 indicative of an increased risk of developing IBS.

The invention is also concerned with a method for identifying a subject at risk of developing IBS and/or for diagnosing a subject suffering from IBS, said method comprising the step of enterotyping the gut microbiota of said subject.

Said method may further comprise the steps of: a) providing a test sample of a
30 subject; b) determining the enterotype of said subject from said test sample; and c) determining whether the enterotype of said subject is indicative of an increased risk of developing IBS.

In an embodiment, a determination of enterotype 3 is indicative of an increased risk of developing IBS.

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Definitions

The term “enterotype” is well known from the publication by Arumugam et al. (2011), *supra*. It refers to a characteristic gastrointestinal microbial community of which only a limited number exist across individuals. The enterotype is characteristic for an individual, in line with
 5 gut microbiota being quite stable in individuals and capable of being restored even after perturbation. It may be compared to the blood type, of which also a limited number exist, and which is also characteristic for an individual. For further details on enterotypes we refer to the publication by Arumugam et al. (2011), *supra*, which is herein incorporated by reference.

Presently, 3 of such enterotypes have been identified. Enterotype 1 is enriched in

10 *Bacteroides*, which co-occurs with *Parabacteroides*, *Acidaminococcus*, *Roseburia*, *Faecalibacterium*, *Anaerostipes*, and *Clostridiales*. Enterotype 2 is enriched in *Prevotella* and the co-occurring *Desulfovibrio*, *Streptococcus*, *Enterococcus*, and *Lachnospiraceae*. Enterotype 3 is enriched in *Ruminococcus* and co-occurs with *Akkermansia*, *Allistipes*, *Klebsiella*, *Escherichia/Shigella*, *Dialister*, *Mitsuokella*, *Methanobrevibacter*, *Eggerthella*,
 15 *Ruminococcaceae*, *Subdoligranulum*, *Coprococcus*, *Collinsella*, *Blautia*, *Eubacterium* and *Dorea*. Table 3 of WO 2011/043654, which is herein incorporated by reference, discloses accession numbers for 16S rRNA genes for said genera.

The term “enterotyping” as used herein refers to determining the enterotype, i.e., a characteristic gastrointestinal microbial community as defined above. Enterotypes may be
 20 determined in a variety of ways. They have been discovered based on deep metagenome sequencing of DNA isolated from fecal samples that was cloned and characterized by the so-called Sanger sequencing, providing long about 500 nucleotide reads (Arumugam et al. 2011, *supra*). Similarly, fecal metagenome libraries characterized by Illumina sequencing that generate short approximately 50 nucleotide reads were used to derive enterotypes
 25 (Arumugam et al. 2011, *supra*). Moreover, enterotypes were also found using information derived from fragments of the 16S rRNA genes that were characterized by pyrosequencing, an approach that generated a few hundred nucleotide long reads (Arumugam et al. 2011, *supra*). In addition, information generated by hybridization of rRNA gene sequences derived from fecal samples to the Human Intestinal Tract (HIT)Chip, a phylogenetic microarray, was
 30 described to generate the enterotypes (Arumugam et al. 2011, *supra*). Finally, also specific groupings of conserved proteins were found to allow discrimination between enterotypes. Hence, sequence information of metagenomes as well as 16S rRNA genes, phylogenetic microarray analysis, or proteome information can be used to classify a subject’s fecal microbiota into enterotypes. Additionally, transcriptome and metabolome analysis can be
 35 used to classify a subject’s fecal microbiota into enterotypes. Moreover, as in the original description of the enterotypes (Arumugam et al. 2011, *supra*) a listing is provided of microbes that contribute to various extent to the enterotypes, this information can be used by those

skilled in the art to design various ways to determine enterotypes. Presently, 3 of such enterotypes have been identified (i.e., enterotype 1, 2, and 3), with characteristic gastrointestinal microbial communities as indicated above.

In the context of the invention, a subject may be an animal or a human being. In principle, any subject could be diagnosed using the method of the invention. The diagnosis method may be applied as often as necessary in a subject. Preferably, the subject is a human being. A "healthy subject" as referred to herein does not suffer from any conditions or diseases of the gastrointestinal tract such as IBS, and preferably does not suffer from any conditions or diseases.

The term "test sample" is preferably a biological sample. As used herein, a "biological sample" refers to a biological tissue or biological fluid from a subject. A variety of samples can be useful in practicing the invention including, for example, faeces, an intestinal sample, blood, serum, plasma, urine, breath (exhaled air), DNA, salivary fluid, ascite fluid, and the like. For example, microbial metabolites may be found in the urine, blood, fecal water or extracts of fecal material or exhaled air. It is known that specific host-microbe interactions occur in the human body and hence it is feasible that host genomic sequences may be correlated with specific enterotypes. The term "intestinal sample" refers to all samples that originate from the intestinal tract, including, without limitation, feces samples, rectal swap samples, but also samples obtained from other sites in the intestinal tract, such as mucosal biopsies, as was shown previously (Zoetendal et al 2002 . Appl. Environ. Microbiol. 68:3401-7 and Kerkhoffs et al., 2009, *supra*). A test sample may be obtained from any subject, such as a subject suffering from IBS, a healthy subject, a subject with unknown diagnosis of IBS, or a subject with complaints related to the gastro-intestinal tract. The test sample may have been processed; for example, DNA and/or RNA and/or protein may have been isolated from feces samples, rectal swap samples, or samples obtained from other sites in the intestinal tract.

The term "gut microbiota" as used herein refers to the microorganisms that inhabit the digestive tract (also referred to as "gut" or "gastrointestinal tract").

Detailed description of the invention

By comparing IBS patients with healthy subjects the present inventors have demonstrated that IBS patients carried almost exclusively microbiota belonging to enterotype 3 (hereinafter also referred to as "ES3") (98.4 %) which is significantly higher (p value 0.0004) than that found in healthy subjects. Since it is unlikely that a transition between enterotypes may occur, it is hypothesized that individuals with an ES3 microbiota have a propensity to develop one of the specific types of IBS. As such, it is possible to determine the risk for IBS by determining the enterotype. Specifically, subjects with enterotype 3 have a higher risk for IBS than subjects with other enterotypes. Subjects at increased risk for IBS may be monitored

for development of IBS, and may be subjected to intervention preventing the development of IBS.

Thus, the present invention provides for use of enterotyping of the gut microbiota for identifying a subject at risk of developing Irritable Bowel Syndrome (IBS) and/or diagnosing a subject suffering from IBS. In an embodiment, said subject has an increased risk of developing IBS. Said use is carried out *ex vivo*, i.e., on an *ex vivo* test sample.

As subjects suffering from IBS are known to have enterotype 3 exclusively, it is obvious that subjects having enterotype 3 have a propensity to develop IBS. Thus, determining the enterotype results in identification of a subject at risk of developing IBS. Subjects having enterotype 3 may have an increased risk of developing IBS compared to subjects having enterotype 1 or enterotype 2. Enterotyping may therefore exclude subjects having enterotype 1 or enterotype 2 from running a risk of developing IBS.

The method of the present invention may also be used in diagnosing a subject suffering from IBS. For example, determining the enterotype may be a first step in diagnosing a subject suffering from IBS. Subjects having enterotype 1 or enterotype 2 may be excluded from receiving a diagnosis of IBS. Subjects having enterotype 3 may in a second step be subjected to further IBS diagnosis methods, for example, those described in WO 2011/ 043654 to unequivocally establish a diagnosis of IBS. The enterotype is preferably determined using an *ex vivo* sample as described above in the definitions section.

The present invention also provides for a method for identifying a test sample derived from a subject at risk of developing IBS and/or from a subject suffering from IBS.

As described above in the definitions section, the enterotype may be determined in various way which have been set out in Arumugam et al. 2011, *vide supra*.

For example, the enterotype may be determined by determining the level of bacteria belonging to the enterotype genera. One of the most researched microbial nucleic acids is that of the 16S rRNA. This 16S rRNA, also known as small subunit (SSU) RNA, is encoded by an approximately 1500 bp gene that is present in a variable number of copies, usually 1-10 per microbial genome. The nucleotide sequence of the 16S rRNA genes is frequently used in diagnostics as it shows differences between microbial species. In fact 16S rRNA gene sequences are instrumental in defining the taxonomic position of microbes. Moreover, these 16S rRNA sequences may also identify microbes that have not yet been cultured but are only known because of the presence of a 16S rRNA gene sequence. In case this gene sequence differs significantly (usually less than 98 % similarity) from the 16S rRNA gene sequence of a known species, this is indicated as a new phylotype (a new species consisting of microbes that have not been cultured yet). However, a growing number of microbes are brought into culture and otherwise described by sequence analysis of their complete or partial genomes. Up to now over several thousands of microbial genomes have been sequenced and are

publicly available (see <http://genomesonline.org> or <http://www.ncbi.nlm.nih.gov>). Many more are to follow either after their isolation or from metagenome projects that aim to sequence the entire microbial DNA present in an ecosystem, such as Human Microbiome Project aiming to determine the metagenome of the human microbiota (see <http://nihroadmap.nih.gov/hmp/>).

A growing database of over a million microbial 16S rRNA sequences can be found in publicly available databases such as <http://www.arb-silva.de> (Pruesse et al., 2007. Nucleic Acid Res. 35:7188) and <http://rdp.cmu.mse.edu> (Cole et al., 2008. Nucleic Acids Res. 35 (Database issue): D169-D172). It has been well-established that the 16S rRNA sequence
5 contains a limited number of variable regions of several dozens of nucleotides, termed V1-V8, that are targets for developing nucleic acid probes, PCR primers or LCR probes. By analyzing the variable regions in the microbes that are found in the human intestinal tract, it was observed that the most diagnostic information for developing nucleic acid probes were the V1 and V6 regions (Rajilic-Stojanovic et al., 2009, *supra*). Hence, based on the sequences of
10 these variable regions a total of over 3,699 unique oligonucleotide probes of around 16-30 nucleotides have been developed that are present on the so called Human Intestinal Tract (HIT) Chip, a phylogenetic microarray (Rajilic-Stojanovic et al 2009, *supra*). These oligonucleotides are called HIT probes. Hybridization to the HIT probes can be used to deduce what microbe is present and allows its taxonomic identification at different level, the
15 most important ones including genus-like groups (sequence similarity > 90% - so called level 2 groups) and phylotype-like groups (sequence similarity > 98% - so called level 3 groups) (Rajilic-Stojanovic et al 2009, *supra*).

The level of bacteria belonging to enterotype 1, enterotype 2 and/or enterotype 3 may be measured by determining the level of specific nucleic acid sequences in said test sample,
20 which nucleic acid sequences are preferably 16S rRNA gene sequences of said one or more bacteria, more preferably one or more variable regions of said 16S rRNA gene sequences, e.g., one or more of the variable regions V1 and/or V6 of said 16S rRNA gene sequences.

The disclosed microbial groups as well as oligonucleotide probes differentiating between microbial groups can serve as biomarkers for subjects at risk of developing IBS
25 alone or in combination. A biomarker, or biological marker, is in general a substance used as an indicator of a biologic state. Biomarkers can include a variety of stable macromolecular molecules, including nucleic acids, proteins or lipids but also metabolites or a combination thereof. Of particular interest are nucleic acids, including DNA and RNA, that are present in the intestinal microbiota as they are stable but can be isolated easily. However, also proteins
30 encoded by the said DNA can be considered useful biomarkers, notably when they are stable.

Starting from the microbial groups and bacteria described herein, persons skilled in the art can deduce LCR, PCR or hybridization probes to specifically discriminate subjects at risk

of developing IBS and/or suffering from IBS from healthy subjects using intestinal microbiota as target. An affected intestinal bacteria as used in this context means either more or less prevalent in subjects at risk of developing IBS as compared to subjects not at risk of developing IBS (i.e., those having enterotypes 1 or 2).

In an embodiment, an assignment of the gut microbiota of a subject to enterotype 3 is indicative of an increased risk of developing IBS. The assignment of the enterotype can be done as shown by Arumugam et al. (2011), *supra*, which is herein incorporated by reference, and which is discussed above in the definitions section.

5 In another aspect, the present invention pertains to a method for identifying a subject at risk of developing IBS and/or for diagnosing a subject suffering from IBS, said method comprising the step of enterotyping the gut microbiota of said subject. The method may comprise the steps of: a) providing a test sample of a subject; b) determining the enterotype of said subject from said test sample; and c) determining whether the enterotype of said
10 subject is indicative of an increased risk of developing IBS. In the method of the invention, a determination or assignment of enterotype 3 is indicative of an increased risk of said subject developing IBS.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

15 It will be clear that the above description is included to illustrate some embodiments of the invention, and not to limit the scope of protection. Starting from this disclosure, many more embodiments will be evident to a skilled person which are within the scope of protection and the essence of this invention and which are obvious combinations of prior art techniques and the disclosure of this patent.

20

Examples

Example 1. Enterotype determination of the fecal microbiota of IBS and healthy subjects

25 Fecal samples were obtained from a of a total of 62 IBS subjects and a total of 46 healthy individuals that were matched for age, gender and body mass index. Microbial DNA was isolated from these fecal samples following the method of Ahlroos & Tynkynnen (Ahlroos T, Tynkynnen S. Quantitative strain-specific detection of *Lactobacillus rhamnosus* GG in human faecal samples by real-time PCR J Appl Microbiol. 2009 Feb;106(2):506-14) and used for
30 profiling using the HITChip phylogenetic microarray using 3699 distinct HIT probes as described (Rajilic-Stojanovic et al., 2009, *supra*). By comparing the obtained HITChip profiles with that of subjects with known enterotypes (Aramugam et al., 2011, *supra*) using a Random Forest algorithm a correlation between the profiles and the enterotype could be made. The

results indicated that of the healthy subjects 8 (17.4 %) could be classified as Enterotype 1 (ET1), 1 (2.2 %) as Enterotype 2 (ET2) and 37 (80.4 %) as Enterotype 3 (ET3). These results corroborate the presence of 3 enterotypes in healthy subjects as obtained in the first description of the enterotypes (Aramugam et al., 2011, *supra*) where the HITChip analysis was applied to 22 subjects and it was found that of these 2 (9.1 %) belonged to ET1, 6 (27.3 %) belonged to ET2 and 14 (63.6 %) belonged to ET3. Of great interest is the result that when using the same approach it was discovered that all but one (belonging to ET1) of the IBS patients carried microbiota belonging to ET3 (98.4 %) which is significantly higher (p value 0.0004) than that found in healthy subjects analyzed in this study (78.6 %). Since it is unlikely that a transition between enterotypes may occur (Aramugam et al., 2011, *supra*), it demonstrates that subjects with a microbiota that can be classified as belonging to ET3 have either IBS or have a propensity to develop IBS. This finding could significantly improve the early diagnostics and possibly treatment of IBS.

CLAIMS

1. Use of enterotyping of the gut microbiota for identifying a subject at risk of developing Irritable Bowel Syndrome (IBS) and/or diagnosing a subject suffering from IBS.

2. Use according to claim 1, wherein assignment of the gut microbiota of a subject to
5 enterotype 3 is indicative of an increased risk of developing IBS.

3. A method for identifying a subject at risk of developing IBS and/or for diagnosing a subject suffering from IBS, said method comprising the step of enterotyping the gut microbiota of said subject.

10

4. A method according to claim 3, said method comprising the steps of:

a) providing a test sample of a subject;

b) determining the enterotype of said subject from said test sample; and

c) determining whether the enterotype of said subject is indicative of an increased risk

15 of developing IBS.

5. A method according to any one of claims 3 or 4, wherein a determination of enterotype 3 is indicative of an increased risk of developing IBS.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/043654 A1 (AAK PATENT B V [NL]; TUK LAMBERTUS [NL]; DE VOS WILLEM MEINDERT [NL]) 14 April 2011 (2011-04-14) cited in the application	1,3,4
A	claim 1	2,5
A	----- ARUMUGAM MANIMOZHIYAN ET AL: "Enterotypes of the human gut microbiome", NATURE (LONDON), vol. 473, no. 7346, May 2011 (2011-05), pages 174-180, XP055018562, cited in the application abstract page 179, column 1, paragraph 3 ----- -/--	1-5



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2012/050519

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	RAJILIC-STOJANOVIC MIRJANA ET AL: "Global and Deep Molecular Analysis of Microbiota Signatures in Fecal Samples From Patients With Irritable Bowel Syndrome", GASTROENTEROLOGY, vol. 141, no. 5, November 2011 (2011-11), pages 1792-1801, XP055018598, page 1799, column 1, paragraph 3 - column 2, paragraph 1 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2012/050519

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011043654 A1	14-04-2011	CA 2776420 A1	14-04-2011
		EP 2486143 A1	15-08-2012
		US 2012238468 A1	20-09-2012
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