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[Continued on next page]

(54) Title: NETWORK-BASED MICROBIAL COMPOSITIONS AND METHODS

(57) Abstract: Provided are therapeutic compositions containing combinations of bacteria, for the maintenance or restoration of a healthy microbiota in the gastrointestinal tract of a mammalian subject, and methods for use thereof.

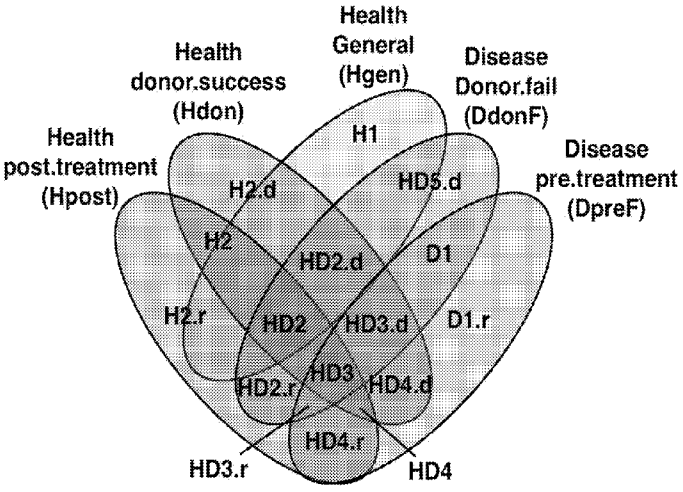

Figure 19

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SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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29 January 2015

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/30817

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 63/00 (2014.01)
CPC - A61K 35/74, A61K 35/742, A01N 63/00, C12R 1/01, C12N 1/20, A23K 1/009, A61K 36/48
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
CPC- A61K 35/74, A61K 35/742, A01N 63/00, C12R 1/01, C12N 1/20, A23K 1/009, A61K 36/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/93.3, 424/93.4, 424/93.41
CPC- A23K 1/007, A61K 31/337, A61K 31/427, A23L 1/0345, A23L 1/3002

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: Seres Health, Inc.; microbiota, dysbiosis, disrupted symbiosis, dis-balance, probiotics; fecal transplant, Clostridium difficile Associated Diarrhea, COAD, Type 2 Diabetes, Obesity, butyrate, propionate, Bacteroides capillosus, Pseudoflavonifractor capillosus.... GenCore 6.4.1: SEQ ID

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/142605 A1 (CUNNINGHAM) 18 October 2012 (18.10.2012) Abstract, Fig 1;	1, 2, 69, 72, (75-82)/(69,72)
A	US 2012/0238468 A1 (Tuk, et al.) 20 September 2012 (20.09.2012) para [103]-[110], claim 1	1, 2, 69, 72, (75-82)/(69,72)

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2014 (30.10.2014)	05 DEC 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/30817

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 62-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-61, 69-82, drawn to a method for treating, preventing, or reducing the severity of a disorder (claims 1-61), a method for producing short chain fatty acids (SCFA) (claims 69-71, 75-82) and catalyzing secondary metabolism of bile acids within a mammalian subject (72-82), said method, comprising: administering to a mammalian subject an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, capable of forming a specified functional network ecology (claims 1-82). Group I+ will be searched to the extent that it reads on the N262.S network ecology, without fee. It is believed that claims 1, 2, 69, 72, 75-82 read on this first named invention. Applicants must indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would read on the N1008, i.e. claims 1-4, 69, 70, 72, 73, 75-82.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 69, 72, (75-82)/(69,72), restricted to the N262.S network ecology

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking:

Group II: claims 83-119, drawn to a pharmaceutical formulation comprising a purified bacterial population comprising a plurality of bacterial entities (claims 83-100) or comprising a purified bacterial population comprising a plurality of bacterial entities/bacterial networks capable of forming germinable bacterial spores (claims 102-119); and a method of inducing engraftment of a bacterial population in the gastrointestinal tract of a human subject, comprising the step of administering to the human subject an orally acceptable pharmaceutical formulation comprising a purified bacterial network, under conditions such that at least i) a subset of the spore-forming bacteria sustainably engraft within the gastrointestinal tract, or ii) at least one type of bacteria not present in the therapeutic composition is augmented within the gastrointestinal tract (claim 101).

Group III: claims 120, 128, drawn to a diagnostic composition for the detection of a dysbiosis, comprising a first detection moiety capable of detecting a first keystone bacterial entity and a second detection moiety capable of detecting a first non-keystone bacterial entity, wherein the keystone bacterial entity and the non-keystone bacterial entity comprise a network, wherein the absence of at least one of the keystone bacterial entity and the non-keystone bacterial entity in a mammalian subject is indicative of a dysbiosis (claim 120), and a kit comprising said composition (claim 128).

Group IV: claims 121-126, a method of altering a microbiome population present in a mammalian subject, comprising the steps of determining the presence of an incomplete network of bacterial entities in the gastrointestinal tract of the mammalian subject, and introducing to the gastrointestinal tract of the mammalian subject an effective amount of one or more supplemental bacterial entities not detectable in the gastrointestinal tract of the mammalian subject prior to such administration, under conditions such that the incomplete network is completed, thereby altering the microbiome population (claims 121-124); a method for detection and correction of a dysbiosis in a mammalian subject in need thereof, comprising the steps of: providing a fecal sample from the mammalian subject comprising a plurality of bacterial entities; contacting the fecal sample with a first detection moiety capable of detecting a first bacterial entity present in a network; detecting the absence of the first bacterial entity in the fecal sample, thereby detecting a dysbiosis in the mammalian subject; and administering to the mammalian subject a composition comprising an effective amount of the first bacterial entity (claims 125-126).

Group V: claim 127, drawn to a system for predicting a dysbiosis in a subject, the system comprising: a storage memory for storing a dataset associated with a sample obtained from the subject, wherein the dataset comprises content data for at least one network of bacterial entities; and a processor communicatively coupled to the storage memory for determining a score with an interpretation function wherein the score is predictive of dysbiosis in the subject.

The inventions listed as Groups I+, II through V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of each invention of Group I+ is a specific network ecology recited therein, because 1) network ecologies were known in the art at the time of the invention (please see a paper by Faust, et al. Titled "Microbial Co-occurrence Relationships in the Human Microbiome" (PLoS Comput Biol 2012, 8(7): e1002606); pg 2, col 1, "This ecological network proved to contain few highly connected (hub)organisms and was, like most biological networks, scale-free"; pg 5, Figure 2 and its legend, "Significant co-occurrence and co-exclusion relationships among the abundances of clades in the human microbiome. A global microbial interaction network capturing 1,949 associations among 452 clades at or above the order level in the human microbiome, reduced for visualization from the complete network in Figure S1. Each node represents a bacterial order, summarizing one or more genus-level phylotypes and family-level taxonomic groups.... for a full network of all phylotypes and clades, see Figure S1. A high degree of modularity is apparent within body areas (skin, urogenital tract, oral cavity, gut, and airways) and within individual body sites, with most communities forming distinct niches across which few microbial associations occur"; Abstract, "a global network of 3,005 significant co-occurrence and co-exclusion relationships between 197 clades occurring throughout the human microbiome. This network revealed strong niche specialization, with most microbial associations occurring within body sites and a number of accompanying inter-body site relationships. Microbial communities within the oropharynx grouped into three distinct habitats, which themselves showed no direct influence on the composition of the gut microbiota"), and 2) the claimed network ecologies comprise distinct sets of bacteria known at the time of the invention, and no significant structural similarities can readily be ascertained among the claimed network ecologies (see for example, instant application, pg 475, Table 14a).

Another special technical feature of each invention of Group I+ is a specific clade recited therein, because clades were known in the art at the time of the invention (please see Faust, et al., pg 2, col 2, "Among the 726 taxa and 884 clades in the HMP [Human Microbiome Project] data"), and 2) the claimed clades comprise distinct sets of bacteria known at the time of the invention, and therefore, no significant structural similarities can readily be ascertained among the claimed clades (see for example, instant application, pg 475, Table 14a).

Another special technical feature of each invention of Group I+ is a specific OTU [Operational Taxonomic Unit] recited therein, because OTUs were known in the art at the time of the invention (please see a paper by Peterson, et al. Titled "Metagenomic approaches for defining the pathogenesis of inflammatory bowel diseases" (Cell Host Microbe 2008, 3(6):417-27); pg 418, Figure 1, and it's legend, "Bacterial SSU rRNA Gene-Based Surveys of the Gut Microbiota... (B) Analysis of diversity in the human gut microbial community based on surveys of a limited number of humans. Collector's curves of richness are shown. 16S rRNA gene sequences were "binned" into operational taxonomic units (OTUs), which are widely assumed to represent "strain-level" taxa when the OTU contains sequences with >99% pairwise sequence identity (%ID)")"), and 2) the claimed OTUs comprise distinct sets of bacteria known at the time of the invention, and therefore, no significant structural similarities can readily be ascertained among the claimed clades (see for example, instant application, pg 475, Table 14a).

********** See Supplemental Sheet to continue **********

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking and the Preceding Supplemental Sheet:

Finally, another special technical feature of each invention of Group I+ is a specific bacterium characterized by its 16S rRNA sequence recited therein, because the claimed bacteria are distinct and were known in the art at the time of the invention (please see GenBank HQ819637.1 titled "Uncultured organism clone ELU0180-T56-S-NIPCRAMgANa_000311 small subunit ribosomal RNA gene, partial sequence" (30 July 2012) [Retrieved from the Internet 21 August 2014: < http://www.ncbi.nlm.nih.gov/nuccore/HQ819637>] disclosing a bacterium having 16S rRNA sequence comprising a nucleotide sequence 99.9% identical to the claimed 16S rRNA sequence SEQ ID NO:674 (nucleotides 53-1423, 99.9% identity).

The inventions of Groups I+ and II do not include the technical feature of a diagnostic composition for the detection of a dysbiosis, as required by Group III, or determining the presence of an incomplete network of bacterial entities in the gastrointestinal tract of the mammalian subject, as required by Group IV, or a system for predicting a dysbiosis in a subject, the system comprising: a storage memory for storing a dataset associated with a sample obtained from the subject and a processor communicatively coupled to the storage memory for determining a score with an interpretation function, as required by Group V.

The inventions of Group I+ do not include the technical feature of a pharmaceutical formulation comprising a purified bacterial population comprising a plurality of bacterial entities or consisting essentially of a bacterial network capable of forming germinable bacterial spores, as required by Group II.

The inventions of Group II do not include the technical feature of a method of treating or preventing a condition by administering to a mammalian subject an effective amount of a therapeutic bacterial composition (claims 1-82), as required by Group I+.

The inventions of Groups IV and V do not include the technical feature of diagnostic composition for the detection of a dysbiosis, as required by Group III.

The inventions of Groups III and IV do not include the technical feature of a system for predicting a dysbiosis in a subject, the system comprising: a storage memory for storing a dataset and a processor communicatively coupled to the storage memory, as required by Group V.

The inventions of Groups III and V do not include the technical feature of determining the presence of an incomplete network of bacterial entities in the gastrointestinal tract of the mammalian subject, as required by Group IV.

Common Technical Features
The inventions of Groups I+, II, and IV share the technical feature of a method of altering a microbiome population present in a mammalian subject, by administering to the gastrointestinal tract of the subject an effective amount of one or more supplemental bacterial entities not detectable in the gastrointestinal tract of the mammalian subject prior to such administration, under conditions such that the incomplete network is completed, thereby altering the microbiome population. However, this shared technical feature does not represent a contribution over prior art as being anticipated by a paper titled "Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut" by Petrof, et al. (Microbiome 09 January 2013, 1(1):3) (hereinafter "Petrof").

Petrof discloses a method of altering a microbiome population present in a mammalian subject (Abstract, "Results: Both patients were infected with the hyper virulent C. difficile strain, ribotype 078. Following stool substitute treatment, each patient reverted to their normal bowel pattern within 2 to 3 days and remained symptom-free at 6 months. The analysis demonstrated that rRNA sequences found in the stool substitute were rare in the pre-treatment stool samples but constituted over 25% of the sequences up to 6 months after treatment"), by administering to the gastrointestinal tract of the subject an effective amount of one or more supplemental bacterial entities not detectable in the gastrointestinal tract of the mammalian subject prior to such administration (Abstract, "a stool substitute preparation, made from purified intestinal bacterial cultures derived from a single healthy donor, to treat recurrent C. difficile infection that had failed repeated standard antibiotics. Thirty-three isolates were recovered from a healthy donor stool sample. Two patients who had failed at least three courses of metronidazoleor vancomycin underwent colonoscopy and the mixture was infused throughout the right and mid colon"), under conditions such that the incomplete network is completed, thereby altering the microbiome population (pg 10, col 1, "Patient 1 actually had a very diverse microbiome at the outset but still suffered from severe recurrent CDI. Sequences identical to those of the stool substitute bacteria were initially rare in the pre-treatment samples for both patients (<7%), but became transiently abundant and constituted over 25% of the sequences up to 6 months after stool substitute treatment was given... we conclude that some of the administered bacteria are stably colonizing the colon"; also Abstract, 'Results"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Groups I+ share the technical feature of a method treating, preventing, or reducing the severity of a disorder, comprising: administering to a mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria or the purified bacterial preparation capable of forming a network ecology. However, this shared technical feature does not represent a contribution over prior art as being anticipated by Petrof, as above.

Petrof discloses a method of treating a disorder (Abstract, recurrent Clostridium difficile infection), comprising:
administering to a mammalian subject in need thereof an effective amount of a therapeutic bacterial composition (Abstract, "a stool substitute preparation, made from purified intestinal bacterial cultures derived from a single healthy donor, to treat recurrent C. difficile infection that had failed repeated standard antibiotics. Thirty-three isolates werer ecovered from a healthy donor stool sample. Two patients who had failed at least three courses of metronidazoleor vancomycin underwent colonoscopy and the mixture was infused throughout the right and mid colon"),

********** See Supplemental Sheet to continue **********

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking and the Preceding Supplemental Sheet:

said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria or the purified bacterial preparation capable of forming a network ecology (pg 10, col 1, "Patient 1 actually had a very diverse microbiome at the outset but still suffered from severe recurrent CDI. Sequences identical to those of the stool substitute bacteria were initially rare in the pre-treatment samples for both patients (<7%), but became transiently abundant and constituted over 25% of the sequences up to 6 months after stool substitute treatment was given... we conclude that some of the administered bacteria are stably colonizing the colon", wherein "bacteria are stably colonizing the colon" is synonymous with "bacterial preparation capable of forming a network ecology"; also Abstract, 'Results"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Group I+ share the technical feature of a method for catalyzing secondary metabolism of bile acids within a mammalian subject. However, this shared technical feature does not represent a contribution over prior art as being obvious over a paper titled "Cholesterol-Lowering Probiotics as Potential Biotherapeutics for Metabolic Diseases" by Kumar, et al. (Experimental Diabetes Research 2012, Volume 2012, Article ID 902917) (hereinafter "Kumar").

Kumar discloses administering to said mammalian subject in need thereof a therapeutic bacterial composition, said therapeutic bacterial composition comprising an isolated bacteria or a purified bacterial preparation capable of forming a bacterial functional pathway capable of forming a functional network ecology (pg 2, col 1, "Lactic acid bacteria (LAB) with active bile salt hydrolase (BSH) or products containing them have been suggested to lower cholesterol levels through interaction with host bile salt metabolism.... Lactobacilli with BSH activity have an advantage to survive and colonize the lower small intestine where the enterohepatic cycle takes place, and therefore BSH activity may be considered as an important colonization factor [8]. Sanders [9] proposed the mechanism based on the ability of certain probiotic lactobacilli and bifidobacteria to deconjugate bile acids enzymatically, increasing their rates of excretion.... The use of such orally applied microorganisms (probiotics) is a major aim of the concept of functional food... Lactobacillus plantarum, the predominating Lactobacillus species on oral and intestinal human mucosa, has shown the ability to survive the passage through the human gastrointestinal tract and to establish itself for at least a shorter time in the intestine after consumption", wherein "bile salt hydrolysis" is synonymous with "catalyzing secondary metabolism of bile acids") and "Lactobacillus plantarum, the predominating Lactobacillus species on oral and intestinal human mucosa, has shown the ability to survive the passage through the human gastrointestinal tract and to establish itself for at least a shorter time in the intestine after consumption" is synonymous with "capable of forming a functional network ecology").
Kumar does not disclose an effective amount of the therapeutic bacterial composition. It would have been obvious to one of ordinary skill in the art how to determine, in the course of routine experimentation and with a reasonable expectation of success, an effective amount of the therapeutic bacterial composition.
Kumar does not disclose in an embodiment a therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation. However, Kumar does disclose a plurality of bacteria capable of bile salt hydrolysis (pg 2, Table 1). One of ordinary skill in the art would have been motivated to combine, in the course of routine experimentation and with a reasonable expectation of success, the probiotic organisms with BSH activity disclosed by Kumar (pg 2, Table 1), to maximize efficiency of bile salt hydrolysis, thereby achieving the claimed method for catalyzing secondary metabolism of bile acids within a mammalian subject. As said technical feature would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Groups I+ share the technical feature of a method for producing short chain fatty acids (SCFA) within a mammalian subject. However, this shared technical feature does not represent a contribution over prior art as being obvious over Kumar, as above.

Kumar obviates a method comprising: administering to said mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria of the purified bacterial preparation capable of forming one or a plurality of bacterial functional pathways, the one or plurality of bacterial functional pathways capable of forming a functional network ecology (pg 2, col 1, pg 2, Table 1, also see the immediately preceding paragraph).
Kumar does not specifically disclose that administering of said therapeutic bacterial composition results in producing short chain fatty acids (SCFA) within a mammalian subject. However, said limitation is inherently present in Kumar as follows:
The therapeutic composition of Kumar comprises 8 species of Lactobacillus and 6 species of Bifidobacterium (pg 2, Table 1).
Nyangale, et al. ("Gut microbial activity, implications for health and disease: the potential role of metabolite analysis" (J Proteome Res. 2012, 11(12):5573-85) discloses that Lactobacillus and 6 Bifidobacterium form an ecology network capable of producing propionic acid, a SCFA (pg 5579, col 1, several genera are responsible for the productionof SCFA... Other SCFA including acetate and propionate are produced by Bacteroides-prevotella group and Bifidobacterium spp."; pg pg 557, Fig 2, "Transformation of glucose and amino acids into SCFA and some species involved"). Thus, 8 species of Lactobacillus and 6 species of Bifidobacterium (pg 2, Table 1) are bacteria producing SCFA within a mammalian subject. As said technical feature would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Groups I+, II through V therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note re item 4:
Claims 62-68 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.