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# 1            **Microbial ACBP/DBI-like genes are rare in the human gut** 2            **microbiome and show no links with obesity**

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15

16 Running head: The human gut microbiome rarely encodes ACBP/DBI genes

17

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19

20

## **Abstract**

21 Acyl coenzyme A (CoA) binding protein (ACBP), also called diazepam-binding inhibitor (DBI) is a  
22 phylogenetically conserved protein that is expressed by all eukaryotic species as well as by some  
23 bacteria. Since elevated ACBP/DBI levels play a major role in the inhibition of autophagy, increase  
24 in appetite and lipoanabolism that accompany obesity, we wondered whether ACBP/DBI  
25 produced by the human microbiome might affect host weight. We found that the genomes of  
26 bacterial commensals rarely contain ACBP/DBI homologues, which are rather encoded by  
27 genomes of some pathogenic or environmental taxa that were not prevalent in human feces.  
28 Exhaustive bioinformatic analyses of 1,899 gut samples from healthy individuals refuted the  
29 hypothesis that bacterial ACBP/DBI might affect the BMI in a physiological context. Thus, the  
30 physiological regulation of BMI is unlikely to be affected by microbial ACBP/DBI-like proteins.  
31 However, at the speculative level, it remains possible that ACBP/DBI produced by potential  
32 pathogenic bacteria might enhance their virulence by inhibiting autophagy and hence subverting  
33 innate immune responses.

34

35

## **Importance**

36 Acyl coenzyme A (CoA) binding protein (ACBP) can be encoded by several organisms across the  
37 domains of life, including microbes, and has shown to play major roles in human metabolic  
38 processes. However, little is known about its presence in the human gut microbiome and whether  
39 its microbial counterpart could also play a role in human metabolism. In the present study, we  
40 found that microbial ACBP/DBI sequences were rarely present in the gut microbiome across  
41 multiple metagenomic datasets. Microbes that carried ACBP/DBI in the human gut microbiome  
42 included *Saccharomyces cerevisiae*, *Lautropia mirabilis* and *Comamonas kerstersii*, but these  
43 microorganisms were not associated with body-mass index, further indicating an unconvincing  
44 role for microbial ACBP/DBI in human metabolism.

45

46

## Introduction

47 Acyl coenzyme A (CoA) binding protein (ACBP) is also called diazepam-binding inhibitor (DBI).  
48 In humans and mice, this small (10 kDa) protein plays a dual role, reflecting its double name. As  
49 an intracellular protein, ACBP/DBI binds to medium- and long-chain acyl CoA esters, reducing  
50 their toxicity and facilitating their transport through different subcellular compartments, hence  
51 stimulating lipid metabolism (1–3). As an extracellular protein, ACBP/DBI binds to the peripheral  
52 benzodiazepine receptor (hence displacing the benzodiazepine diazepam from its binding site),  
53 which is the ionotropic gamma-aminobutyric acid (GABA)<sub>A</sub> receptor (GABAAR) possessing  
54 another endogenous ligand,  $\gamma$ -aminobutyric acid, the major inhibitory neurotransmitter (4, 5). In  
55 the central nervous system, ACBP/DBI can be proteolytically cleaved to yield several  
56 neuropeptides, one of which, octadecaneuropeptide (ODN) interacts with a G protein coupled  
57 receptor (GPCR) in the central nervous system (6, 7).

58

59 ACBP/DBI is ubiquitously expressed and can be released from cells through an unconventional,  
60 autophagy-dependent pathway (8). It then acts as a paracrine mediator to inhibit autophagy

61 through an action on GABAAR, which is expressed in many cell types outside of the central  
62 nervous system (9). Hence, antibody-mediated neutralization of extracellular ACBP/DBI offers the  
63 possibility to stimulate autophagy by interrupting a paracrine feedback inhibition loop. In humans,  
64 obesity and metabolic syndrome are associated with elevated ACBP/DBI levels in the plasma  
65 (10), while anorexia nervosa is characterized by abnormally low concentrations of circulating  
66 ACBP/DBI (9, 11). In mice, injection of recombinant ACBP/DBI protein into the peritoneal cavity  
67 or the tail vein causes a GABAAR-dependent increase in feeding. This appetite-stimulatory effect  
68 of ACBP/DBI is also observed for proteins in which the acyl CoA binding moiety has been  
69 mutated. Conversely, injection of a neutralizing antibody blocks feeding responses and  
70 counteracts weight gain or favors weight loss in multiple experimental conditions. These findings  
71 suggest that ACBP/DBI is involved in the pathophysiology of human obesity (12).

72

73 ACBP/DBI is a phylogenetically conserved protein, as ACBP/DBI homologs have been described  
74 in all eukaryotic phyla and even in some bacterial species (13, 14). In the nematode  
75 *Caenorhabditis elegans* and in the insect *Drosophila melanogaster*, ACBP/DBI orthologs  
76 stimulate pharyngeal pumping and mouth hook movement, which are the functional equivalents  
77 of mammalian mastication (15). In the yeast *Saccharomyces cerevisiae*, ACBP/DBI is the only  
78 protein known to be released in response to nutrient or oxidative stress (16, 17). Extracellular  
79 ACBP/DBI stimulates sporulation of yeast in a GPCR-dependent fashion, hence allowing yeast  
80 cells to swarm out to find new food resources (15, 18). Thus, the appetite-stimulatory function of  
81 ACBP/DBI appears to be phylogenetically conserved (19–21).

82

83 Reportedly, the genomes of some bacteria code for ACBP/DBI orthologs (22, 23). It is well known  
84 that human obesity is associated with major shifts in the composition of the intestinal microbiome  
85 (24, 25). Moreover, fecal microbial transplantation (FMT) of the stools from obese (but not lean)

86 individuals into mice can transfer features of obesity and metabolic syndrome, establishing cause-  
87 effect relationships between alterations in the gut microbiome and the obese phenotype (26, 27).

88

89 Intrigued by these observations, we wondered whether specific microbial species in the human  
90 gut might encode for and express ACBP/DBI-like proteins, thus potentially influencing human  
91 metabolism and eating behavior. Here, we report a detailed bioinformatics analysis of ACBP/DBI-  
92 like genes encoded by the human intestinal microbiome and analyze their possible implication in  
93 obesity. We found that ACBP/DBI is mostly encoded by eukaryotes, its presence in bacteria is  
94 mostly limited to pathogenic taxa, and that its rare presence in the human gut is not associated  
95 with alterations in the body mass index (BMI).

96

## 97 **Results**

98

### 99 **ACBP/DBI-like proteins are rarely encoded into members of the human microbiome**

100 To assess whether microbial ACBP/DBI ortholog genes could potentially contribute to  
101 microbiome-dependent gut metabolism, we first looked for their presence in 99,211 microbial  
102 genomes from NCBI as of January 2019. Using an initial set of 1,098 UniRef-annotated  
103 orthologous ACBP/DBI sequences (see **Methods**) to search these genomes, we found ACBP to  
104 be present in 3,635 of them, encompassing 1,668 unique TaxIDs, with the majority belonging to  
105 *Proteobacteria* (89% of genomes). Species with the largest number of genomes encoding ACBP  
106 showed it to be part of the core genome of several known pathogens from the *Burkholderia* genus,  
107 as well as *Saccharomyces cerevisiae* and *Ralstonia solanacearum* (**Table 1**). While  
108 *Saccharomyces cerevisiae* can be found in the human gut (28, 29) - although usually at low  
109 abundance - the bacterial taxa in NCBI containing ACBP are at best very rare members of the  
110 human microbiome.

111

112 Because genomic sequencing captures only a limited fraction of the human microbiome diversity  
113 (30–33), we proceeded by searching homologous sequences of known ACBP genes in  
114 metagenome-assembled genomes (MAGs). We screened 154,000 MAGs previously recovered  
115 from the human microbiome sampled from almost 10,000 individuals spanning diverse geography  
116 and lifestyle (**Supplementary Table 1**). We found only 129 out of the 154,000 MAGs (0.08%) to  
117 encode ACBP, belonging to 14 species-level genome bins (SGBs). One of these SGBs was  
118 classified as *Deinococcus-Thermus* and another as *Chitinophagaceae*, whereas the remaining  
119 12 all belonged to *Proteobacteria*, with the closest known taxa being again *Burkholderia* or taxa  
120 linked with sample-processing contamination such as *Ralstonia* or *Acidovorax* (34). This  
121 exploration of microbial genomes and MAGs thus highlights a lack of ACBP/DBI ortholog genes  
122 in microbes of putative relevance in the human microbiome.

123

#### 124 **Phylogenetic modelling of ACBP/DBI is highly taxonomically consistent**

125 To better assess the sequence diversity of the ACBP/DBI gene, we phylogenetically modelled its  
126 sequence variants found in human MAGs and reference genomes from NCBI across different  
127 organisms. This analysis revealed very distinct eukaryotic *versus* microbial ACBP/DBI  
128 sequences, despite the relatively short alignment length used for phylogenetic inference (**Figure**  
129 **1**). This distinct pattern between the two domains was also seen when using pairwise nucleotide  
130 identities calculated from multiple sequence alignments (**Supplementary Figure 1**). We found  
131 ACBP to be widespread across the domains of life, with ACBP sequences found in eukaryotic  
132 phyla including *Streptophyta*, *Arthropoda*, *Nematoda*, *Ascomycota* and *Chordata* and present in  
133 10 different bacterial phyla. Some taxa such as the genera *Daphnia* and *Variovorax* exhibited  
134 clearly defined clades, whilst other taxa such as the phyla *Arthropoda* and *Actinobacteria*  
135 displayed more diverse and paraphyletic phylogenies. The bacterial genera *Burkholderia* and

136 *Paraburkholderia* showed a clearly defined subtree. ACBP sequences belonging to MAGs  
137 recovered from the human microbiome were widespread across the phylogeny but always  
138 maintained a consistent taxonomic structure. This adherence between phylogeny and taxonomy  
139 for ACBP/DBI suggests vertical evolutive trajectories for this gene, as a comparison between  
140 prokaryotic phylogenies built at the whole genome level was highly consistent with the  
141 phylogenetic tree constructed for the ACBP/DBI gene (**Supplementary Figure 2**), with very  
142 limited evidence (if any) of horizontal transfer events, and consequently a low likelihood that yet-  
143 to-be-characterized taxa not captured by our analysis carry ACBP/DBI ortholog genes.

144

#### 145 **ACBP/DBI is rarely found in human gut microbiomes**

146 To further investigate whether the few ACBP/DBI-positive genomes and MAGs recovered from  
147 the human microbiome could potentially contribute to gut metabolism, we evaluated their  
148 prevalence across 7,698 human gut metagenomes present in the curatedMetagenomicData R  
149 package (35), spanning different countries, age categories and health conditions (**Figure 2A**,  
150 **Supplementary Table 2**). We found that the majority of MAGs belonging to these SGBs were  
151 very rarely found in samples across different datasets, with two known SGBs classified as  
152 *Acidovorax* sp 12322\_1 (kSGB 12676) and *Cupriavidus metalidurans* (kSGB 12928) achieving  
153 the highest overall prevalence (0.3%).

154

155 Since MAGs rely on the success of metagenomic assembly and binning and thus may miss some  
156 low-abundance or hard-to assemble taxa, we further screened unbinned contigs as well as the  
157 raw reads for each sample. The use of unbinned contigs (assembled reads) indeed led to an  
158 increase in the overall prevalence of ACBP/DBI positive samples but this number remained low  
159 (0.6% - **Figure 2B**). When we aligned raw metagenomic reads to the set of retrieved ACBP/DBI

160 sequences we further observed an increase in the overall relatively low prevalence across  
161 samples (1.79%), although we cannot exclude that some of the hits are false positives that inflate  
162 the prevalence estimation. Notably, some datasets such as CM\_madagascar from a non-  
163 Westernized society (30) and VincentC\_2016 comprising fecal microbiome of 98 hospitalized  
164 patients treated with antibiotics and that used laxatives (36), showed a higher prevalence of  
165 ABCP/DBI in their raw metagenomes when compared to others, 19.64% and 25.76%,  
166 respectively. On the contrary, 35 datasets (83%) had a prevalence of 0%.

167

168 This analysis thus reinforces the very low prevalence of ABCP/DBI-positive taxa and of the  
169 ABCP/DBI gene in the human gut microbiome which appears inconsistent with a hypothesis of a  
170 role of this microbial gene variant in human metabolism. Moreover, the taxonomy assignments of  
171 the species (from MAGs and NCBI genomes) found to encode ACBP/DBI and occasionally  
172 present in some gut microbiome datasets (**Figure 2A**) points at sample contamination as a  
173 potential source for those taxa. Indeed, *Pseudoxanthomonas*, *Acidovorax*, *Comamonas*, *Delftia*,  
174 *Ralstonia* and *Cupriavidus* have been previously described as common reagent and laboratory  
175 contaminants (34).

176

#### 177 **Lack of correlation between ACBP/DBI-positive species and body mass index**

178 Although we found a low prevalence of ACBP/DBI encoding members in the human gut  
179 microbiome, theoretically there could still be a possibility that low-prevalent low-abundance taxa  
180 can somehow contribute to human gut metabolism. To evaluate a possible link between microbial  
181 ACBP/DBI ortholog genes and obesity, we performed a meta-analysis of correlations between  
182 species-level abundances and BMI as a read-out using 1,899 gut samples from healthy  
183 individuals curated within the *curatedMetagenomicData* (35) effort (**Figure 3, Table 2**). We found  
184 14 taxa to be significantly associated with BMI (random effects model FDR <0.1, **Supplementary**



185 **Table 3**), with species such as *Flavonifractor plautii*, *Coprococcus comes* and *Blautia*  
186 *hydrogenotrophica* associated with increased BMI, in line with previous reports (37, 38). We also  
187 found species associated with decreased BMI, which included *Oscillibacter* sp 57\_20, *Alistipes*  
188 *shahii* and *Odoribacter splanchnicus*, as previously described (39). However, these 14 species  
189 significantly associated with BMI were all ACBP/DBI-negative. Within the limited panel of  
190 ACBP/DBI-positive species at least occasionally found in the gut microbiome, only  
191 *Saccharomyces cerevisiae*, *Lautropia mirabilis* and *Comamonas kerstersii* were sufficiently  
192 prevalent in these samples to perform the meta-analysis but showed no significant associations  
193 (q-values >0.8, **Figure 3**). These results indicate that species found to encode ACBP/DBI in the  
194 human gut microbiome do not show associations with BMI.

195

## 196 **Discussion**

197 ACBP/DBI plays a major role in the control of appetite and metabolism through a phylogenetically  
198 conserved pathway that is conserved in yeast, nematodes, insects and mammals (15, 20, 21, 40).  
199 Intrigued by the observation that ACBP/DBI is a highly conserved protein that is even encoded  
200 by some bacteria, as well as by the link between human obesity and the gut microbiome, we  
201 investigated the prevalence of ACBP/DBI in intestinal commensals and their potential correlation  
202 with the body mass index.

203

204 The bioinformatic analyses presented in this paper based on extensive available metagenomic  
205 datasets suggest that ACBP/DBI producing bacterial species are rather rare in the human  
206 microbiome and are mostly produced by eukaryotic species (as exemplified by the yeast *S.*  
207 *cerevisiae*) and environmental or potentially pathogenic bacteria (exemplified by *Comamonas*  
208 *kerstersii* that can cause peritonitis, bacteremia and sepsis (41–43)), as well as potential sample

209 contaminants. Indeed, the presence of ACBP/DBI producing species in the human gut appears  
210 relatively rare. Moreover, we did not find any correlation between the presence of ACBP/DBI  
211 encoding species and BMI across a large cumulative dataset comprising 1,899 samples from  
212 healthy gut metagenomes. These results refute the hypothesis that the production of ACBP/DBI  
213 by the gut microbiome might affect whole body metabolism, at least in the context of the normal  
214 microbiome.

215

216 Despite our findings, it could still be possible that microbes that are strongly associated with the  
217 mucosal tissue in the upper intestinal tract (and that hence would be grossly underrepresented in  
218 fecal samples) might have some local or systemic effects. It is also noteworthy to mention that  
219 the lack of an association between ACBP/DBI gene carriage and obesity found here did not take  
220 into account gene expression levels, which could be relevant as they might not mirror gene  
221 presence and/or abundance patterns. Moreover, in the context of infections, bacterial ACBP/DBI  
222 might exert some physiological effects on the host. However it is unclear whether prokaryotic  
223 ACBP/DBI orthologues possess similar functions as those present in yeast or other eukaryotes,  
224 despite previous work showing strong conservation of amino acids at the majority of sites  
225 determined to be important for ACBP structure and function across phyla (22). ACBP/DBI inhibits  
226 autophagy (9, 19), and autophagy is a potent mechanism to eliminate intracellular bacteria (44),  
227 meaning that the subversion of autophagy (also called xenophagy) might contribute to the  
228 virulence of pathogenic species. Thus, *Streptococcus pneumoniae* degrades the essential  
229 autophagy protein ATG14 to assure its survival in host cells (45); while *Salmonella typhimurium*  
230 targets the V-ATPase-ATG16L1 axis to avoid xenophagy (46), just to mention a few examples. In  
231 view of these premises, it might be interesting to generate recombinant ACBP/DBI protein  
232 encoded by bacterial species and to evaluate them for their autophagy-inhibitory and metabolic  
233 effects.

234

235 The appetite-stimulatory effects of ACBP/DBI are lost in mice that bear a phenylalanine (F) to  
236 isoleucine (I) substitution at position 77 in the N-terminal domain of the gamma2 subunit of  
237 GABAAR (10, 47), supporting the contention that this neurotransmitter receptor is responsible for  
238 the obesogenic activity of DBI. ACBP/DBI is a GABAAR antagonist, while GABA is a GABAAR  
239 agonist. Of note, GABA, the natural agonist of GABAAR can be produced by a series of bacteria.  
240 Reportedly, oral administration of GABA-producing *Lactobacillus brevis* strains reduces the  
241 abundance of mesenteric adipose tissue, enhances insulin secretion following glucose challenge  
242 and improves plasma cholesterol clearance (48). Hence, it is possible that, beyond their  
243 documented effects on depression (49, 50), GABA-producing bacteria might affect whole-body  
244 metabolism including appetite control. This hypothesis will be actively investigated by our  
245 laboratories.

246

## 247 **Materials and Methods**

### 248 **Identification of ACBP/DBI sequences and phylogenetic tree reconstruction**

249 To obtain a more comprehensive set of ACBP/DBI sequences we downloaded amino acid  
250 sequences that matched the keyword "ACBP" from UniProt90 (51), mapped their identifiers to  
251 those of the European Molecular Biology Laboratory's coding sequences using UniParc and used  
252 the resulting DNA sequences to search, using BLASTn (52), all 99,211 microbial genomes  
253 available in NCBI, that included the whole set of 17,607 microbial species (16,959 bacteria, 648  
254 archaea) available as of January 2019 and 154,723 metagenome assembled genomes (MAGs)  
255 from (30). Matching queries were filtered to include only alignments with >70% identity, alignment  
256 length >100nt and an e-value <1x10<sup>-5</sup>. We found no evidence that more permissive minimum  
257 alignment lengths lead to increased ACBP/DBI detection.

258

259 To build a phylogenetic tree of the known and metagenomically retrieved sequences, we clustered  
260 sequences at 97% sequence identity using UCLUST (parameters: '-id 0.97') (53) and aligned  
261 centroid cluster sequences using MAFFT (parameters: '--localpair --maxiterate 1000') (54). We  
262 removed gappy regions and ACBP/DBI sequences with insufficient aligned positions from the  
263 multiple sequence alignment using Jalview (55), resulting in 240 nucleotides of aligned positions  
264 and 1,223 sequences. The tree was built using fastTree (parameters: '-mlacc 2 -slownni -spr 4 -  
265 fastest -mlnni 4 -no2nd -nt') (56) and refined with RAxML (parameters: '-m GTRGAMMA -t') (57).  
266 GraPhlAn (58) was used for tree annotation and visualization.

267

268 We used PhyloPhlAn 3 (59) to build a phylogeny on 3,490 reference prokaryotic genomes and  
269 129 MAGs (which we found to contain ACBP/DBI) using the parameters '-diversity high --accurate --  
270 force\_nucleotides' and the set of up to 400 PhyloPhlAn genome markers. We compared trees built using  
271 PhyloPhlAn 3 and ACBP/DBI (with the aforementioned methods) in terms of their normalized  
272 pairwise branch lengths and used the *tqDist* (60) function available in the R quartet package to  
273 compare their quartet distances using a random sampling of 477 genomes repeated 1000 times.

274

### 275 **Search of ACBP/DBI sequences in human gut metagenomes**

276 The prevalence of both known and unknown species-level genome bins (kSGBs and uSGBs)  
277 from a previous repository (30) with ACBP/DBI encoding MAGs was calculated using 7,698  
278 human gut metagenomes present in the curatedMetagenomicData (cMD) version 1.16.0 R  
279 package (35). A given sample was deemed positive if a MAG belonging to the ACBP/DBI  
280 encoding SGB was found.

281

282 We used the set of retrieved ACBP/DBI sequences to search, using BLASTn, all contigs  
283 assembled from human gut metagenomes available in cMD. Samples were considered to be

284 positive for ACBP if any of their contigs had a significant hit (>70% identity, alignment length  
285 >100nt and an e-value <1x10<sup>-5</sup>).

286

287 We aligned raw reads from these gut metagenomes to the set of retrieved ACBP/DBI sequences  
288 using bowtie2 (61). Resulting BAM files were filtered to keep only alignments with >50nt of  
289 matching positions and were used to calculate the breadth of coverage of each sequence using  
290 Samtools (62) and VCF utils (63). Samples' whose metagenome presented ACBP/DBI sequences  
291 with breadth >80% were considered positive.

292

### 293 **Correlations between BMI and species' abundances**

294 We used the PREDICT 1 dataset comprising 1,001 healthy individuals from the UK and 97 from  
295 the US (38), as well as publicly available datasets collected in cMD and profiled with version 3 of  
296 MetaPhlAn (64, 65). Of the 57 datasets available, we selected those that had samples with the  
297 following characteristics: (i) gut samples collected from healthy adult individuals at first collection  
298 ("days\_from\_first\_collection"=0 or NA), (ii) samples with age, sex and BMI data available. Outlier  
299 samples were removed if their BMI value was outside 3.5 and 7.5 times the interquartile range  
300 (IQR) of samples meeting the above criteria (IQR = 5.03). Only datasets with at least 50 samples  
301 were considered: Asnicar\_2020\_UK (953 samples out of 1,001), Asnicar\_2020\_US (92 samples  
302 out of 97) (38), CosteaPI\_2017 (82 samples out of 279) (66), DhakanDB\_2019 (80 samples out  
303 of 110) (67), HansenLBS\_2018 (57 samples out of 208) (68), JieZ\_2017 (140 samples out of 385)  
304 (39), SchirmerM\_2016 (437 samples out of 471) (69), and ZellerG\_2014 (58 samples out of 199)  
305 (70).

306

307 For each species, Spearman's correlations with BMI were computed using the *pcor.test* function  
308 from the *ppcor* R package controlling for age and sex. Resulting correlations were used as input

309 to the *metacor* function from the *meta* R package using Fisher's Z transformation of correlations  
310 and the Paule-Mandel estimator of between-study variance in the random effects model. P-values  
311 from the random-effects model were corrected using false discovery rate (FDR) through the  
312 Benjamini-Hochberg procedure, which are reported in the figure as q-values. We report q-values  
313 of ACBP/DBI-carrying taxa found in these datasets, as well as species with FDR <0.1.

314

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332

### 333 **Competing interests**

334 The authors declare that they have no competing interests.

335

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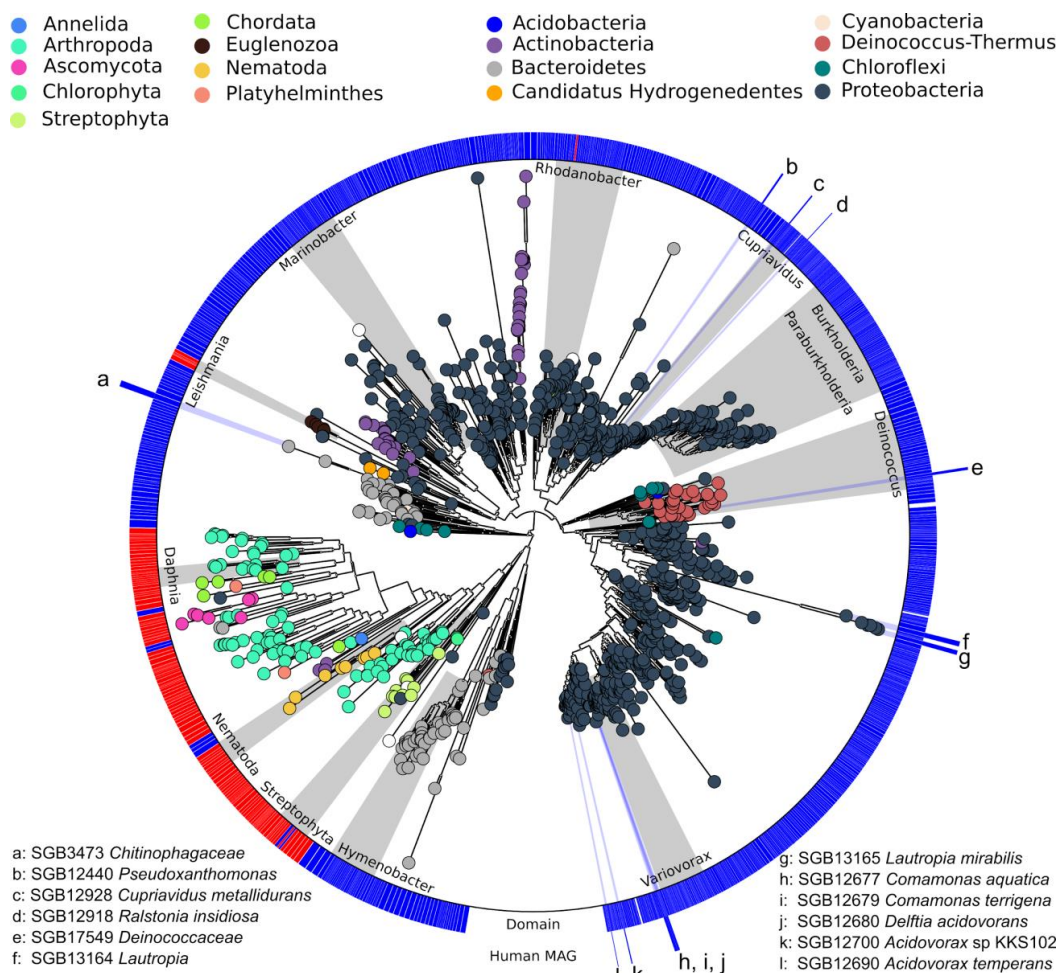
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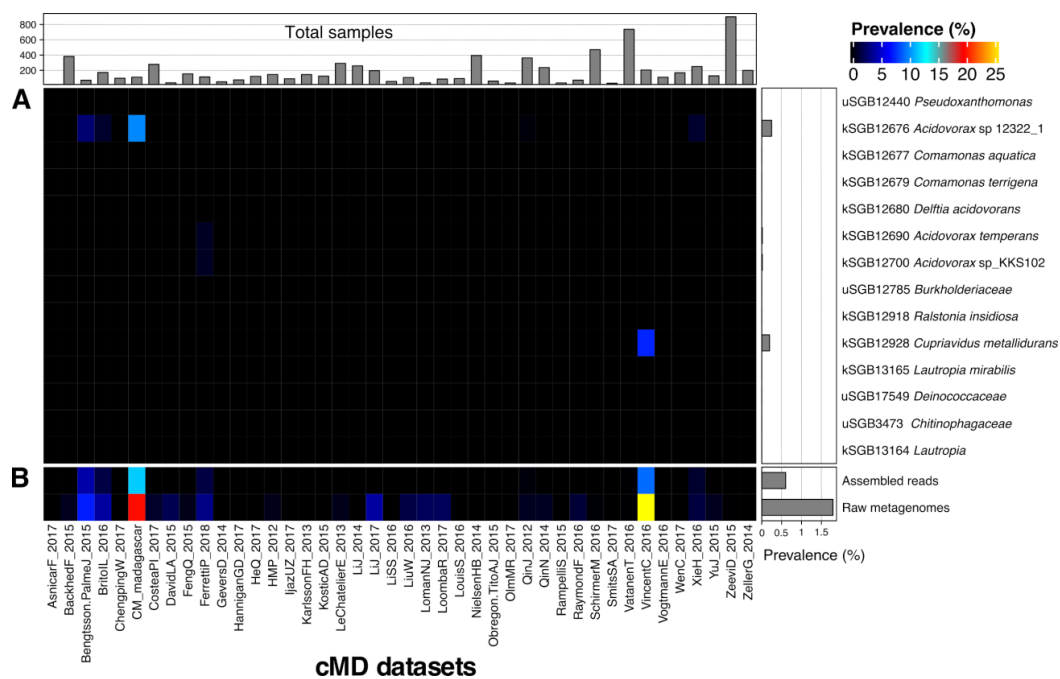


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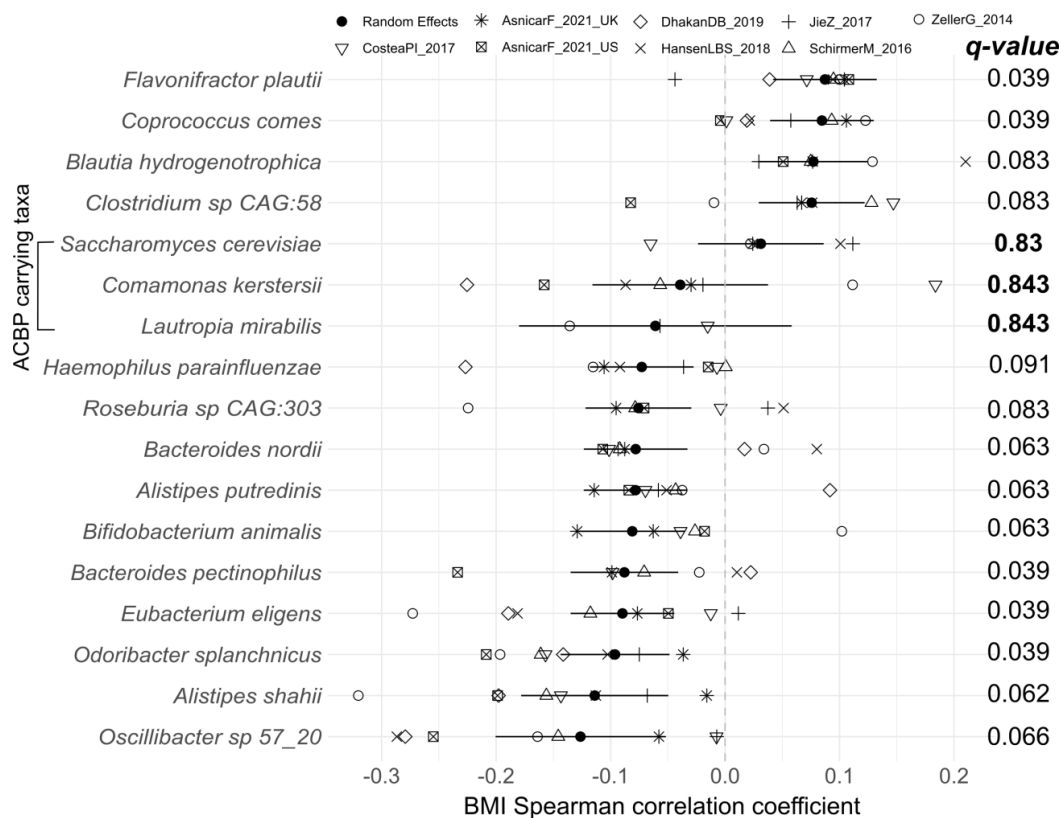
**Figure 1. Whole phylogeny of the ACBP/DBI gene sequences across kingdoms and phyla.** The tree was built using 1,223 ACBP/DBI nucleotide sequences retrieved from UniProtKB, reference genomes from NCBI and human metagenome assembled genomes (MAGs) belonging to species-level genome bins (SGBs) from Pasolli et al. 2019 (see **Methods**). Sequences were clustered at 97% identity prior to multiple sequence alignment and the tree was built using 240nt of aligned positions.

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**Figure 2. ACBP/DBI is rare in human gut metagenomes.** Prevalence of ACBP encoding SGBs from the human microbiome (Panel A) for all datasets available in curatedMetagenomicData representing 7,698 metagenomic samples from the human gut. Panel B reports the prevalence of assembled reads (contigs) with a significant hit to ACBP sequences and metagenomic reads that map to ACBP sequences with a breadth of coverage >80% (see **Methods**).



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**Figure 3. ACBP/DBI carrying taxa present in the human gut show no significant associations with BMI.** We performed a meta-analysis of partial correlations (adjusted for age and sex) between species abundances and BMI across 1,899 samples from healthy gut metagenomes using a random effects model. Meta-analysis p-values were corrected for multiple hypothesis testing correction using the false discovery rate (q-values). ACBP-carrying species are shown, as well as species whose FDR <10%.

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574 **Table 1.** Top 10 species with the largest number of ACBP/DBI encoding genomes based on  
575 available reference genomes

Taxonomy	# of genomes with ACBP (% positive from genomes searched)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia pseudomallei	663 (100%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia ubonensis	291 (100%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia cenocepacia	242 (99.1%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia multivorans	198 (100%)
Eukaryota;Ascomycota;Saccharomycetes;Saccharomycetales;Saccharomycetaceae;Saccharomyces;Saccharomyces cerevisiae	109 (93.9%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia cepacia	98 (100%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Ralstonia;Ralstonia solanacearum	80 (100%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia stagnalis	64 (100%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia mallei	56 (100%)
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia;Burkholderia vietnamiensis	44 (100%)

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578 **Table 2.** Demographic information of gut samples from healthy individuals used in the meta-  
579 analysis

Dataset name	n	Age (years)			BMI (kg/m <sup>2</sup> )			Sex (n)	
		Mean	Min	Max	Mean	Min	Max	Female	Male
AsnicarF_2021_UK	953	45.6	18.5	65.9	25.3	18.7	40.0	686	267
AsnicarF_2021_US	92	42.5	22.3	65.9	25.9	18.8	38.8	61	31
CosteaPI_2017	82	50.6	29	75	27.4	20.0	38.0	52	30
DhakanDB_2019	80	35.6	19	71	23.6	19.2	36.4	42	38
HansenLBS_2018	57	48.7	22.4	65.4	28.5	21.3	35.1	30	27
JieZ_2017	140	61.0	38	107	23.7	18.8	32.1	76	64
SchirmerM_2016	437	27.8	18	75	22.9	18.8	34.4	246	191
ZellerG_2014	58	61.0	25	84	24.8	20.0	34.0	31	27

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