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Long term treatment with metformin increases the risk of vitamin B-12 deficiency, which results in raised homocysteine concentrations. Vitamin B-12 deficiency is preventable; therefore, our findings suggest that regular measurement of vitamin B-12 concentrations during long term metformin treatment should be strongly considered.

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**Summary**

CLYBL encodes a ubiquitously expressed mitochondrial enzyme, conserved across all vertebrates, whose cellular activity and pathway assignment are unknown. Its homozygous loss is tolerated in seemingly healthy individuals, with reduced circulating B12 levels being the only and consistent phenotype reported to date. Here, by combining enzymology, structural biology, and activity-based metabolomics, we report that CLYBL operates as a citramalyl-CoA lyase in mammalian cells. Cells lacking CLYBL accumulate citramalyl-CoA, an intermediate in the C5-dicarboxylate metabolic pathway that includes itaconate, a recently identified human anti-microbial metabolite and immunomodulator. We report that CLYBL loss leads to a cell-autonomous defect in the mitochondrial B12 metabolism and that itaconyl-CoA is a cofactor-inactivating, substrate-analog inhibitor of the mitochondrial B12-dependent methylmalonyl-CoA mutase (MUT). Our work de-orphans the function of human CLYBL and reveals that a consequence of exposure to the immunomodulatory metabolite itaconate is B12 inactivation.

<http://www.uniprot.org/uniprot/Q8N0X4#pathology_and_biotech>

Mitochondrial citramalyl-CoA lyase indirectly involved in the vitamin B12 metabolism (PubMed:[29056341](http://www.uniprot.org/citations/29056341)). Converts citramalyl-CoA into acetyl-CoA and pyruvate in the C5-dicarboxylate catabolism pathway (PubMed:[29056341](http://www.uniprot.org/citations/29056341)). The C5-dicarboxylate catabolism pathway is required to detoxify itaconate, a vitamin B12-poisoning metabolite (PubMed:[29056341](http://www.uniprot.org/citations/29056341)). Also acts as a malate synthase in vitro, converting glyoxylate and acetyl-CoA to malate (PubMed:[24334609](http://www.uniprot.org/citations/24334609), PubMed:[29056341](http://www.uniprot.org/citations/29056341)). Also acts as a beta-methylmalate synthase in vitro, by mediating conversion of glyoxylate and propionyl-CoA to beta-methylmalate (PubMed:[24334609](http://www.uniprot.org/citations/24334609), PubMed:[29056341](http://www.uniprot.org/citations/29056341)). Also has very weak citramalate synthase activity in vitro (PubMed:[24334609](http://www.uniprot.org/citations/24334609), PubMed:[29056341](http://www.uniprot.org/citations/29056341))

* [(3S)-citramalyl-CoA lyase activity](https://www.ebi.ac.uk/QuickGO/term/GO:0047777) http://www.uniprot.org/citations/29056341
* [magnesium ion binding](https://www.ebi.ac.uk/QuickGO/term/GO:0000287) http://www.uniprot.org/citations/24334609
* [malate synthase activity](https://www.ebi.ac.uk/QuickGO/term/GO:0004474) <http://www.uniprot.org/citations/29056341>
* [regulation of cobalamin metabolic process](https://www.ebi.ac.uk/QuickGO/term/GO:0106064) <http://www.uniprot.org/citations/29056341>

|  |  |
| --- | --- |
| Molecular function | [Lyase](http://www.uniprot.org/keywords/KW-0456), [Transferase](http://www.uniprot.org/keywords/KW-0808) |
| Ligand | [Magnesium](http://www.uniprot.org/keywords/KW-0460), [Metal-binding](http://www.uniprot.org/keywords/KW-0479) |

The protein is absent in 2.7% of the human population due to a loss-of-function polymorphism (rs41281112) that changes Arg-259 to a premature stop codon, leading to loss of the protein product (PubMed:[23754956](http://www.uniprot.org/citations/23754956), PubMed:[24334609](http://www.uniprot.org/citations/24334609)). This polymorphism is associated with reduction of circulating vitamin B12 (PubMed:[23754956](http://www.uniprot.org/citations/23754956), PubMed:[24334609](http://www.uniprot.org/citations/24334609)). The reduction of circulating vitamin B12 is caused by accumulation of citramalyl-CoA, an intermediate in the C5-dicarboxylate metabolic pathway that includes itaconate (PubMed:[29056341](http://www.uniprot.org/citations/29056341)). Itaconate acting as a vitamin B12-poisoning metabolite that inactivates the mitochondrial methylglutaconyl-CoA hydratase (AUH) enzyme (PubMed:[29056341](http://www.uniprot.org/citations/29056341)).

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<https://www.ncbi.nlm.nih.gov/pubmed/22367966>

Vitamin B12 (VitB12 or cobalamin) is an essential cofactor in several metabolic pathways. Clinically, VitB12 deficiency is associated with pernicious anemia, neurodegenerative disorder, cardiovascular disease and gastrointestinal disease.

We identified four novel genomic loci that were significantly associated with serum level of VitB12 at a genome-wide significance level of 5.00 × 10(-8). CLYBL (13q32; rs41281112; P= 9.23 × 10(-10)), The new loci identified offer new insights into the biochemical pathways involved in determining the serum level of VitB12 and provide opportunities to better delineate the role of VitB12 in health and disease.

The SNP rs41281112 is a non-synonymous SNP found in CLYBL. The substitution of ‘G’ to ‘A’ allele leads to an amino acid change Arg to OPA, a stop codon at amino acid position of 259. Men who carry a homozygous ‘G’ allele for rs41281112 had higher VitB12 levels compared with ‘A’ allele carriers.

The fourth novel region was located on the CLYBL gene at chromosome 13q32. CLYBL encodes citrate lyase beta-like protein. The molecular functions of CLYBL include citrate (pro-3s)-lyase activity, carbon–carbon lyase activity and metal ion binding. The substitution of G to A allele of rs41281112 results in a stop codon. Men carrier AA genotypes had significantly lower level of VitB12, compared with men with AG or GG genotypes. On the basis of the above evidence, we hypothesized that the G to A allele substitution of rs41281112 leads to the early termination of translation of CLYBL protein, which may affect normal functioning of CLYBL protein of metal ion binding and may interfere with ion uptake. This may in turn lead to the malabsorption of VitB12.

AG

88.87%

GG

36.17%

In ME patients, hypomethylation is seen in a majority of certain immune cells [http://dx.doi.org/10.4172/2155-9899.1000228] and of DNA in genes associated with immune cell regulation [https://www.ncbi.nlm.nih.gov/pubmed/25111603/].

In ME/CFS, [hypomethylation](<http://dx.doi.org/10.4172/2155-9899.1000228>), which is greatly affected by the vitamins B12 and folate, is seen in a majority of certain immune cells.

It is strongly recommended that people in this group take an [oral folic acid](<https://www.ncbi.nlm.nih.gov/pubmed/25902009)> supplement on a daily basis to provide blood saturations high enough to be a remedy for good and safe relief in CFS patients. However,

opioid analgesics and other drugs that have to be demethylated as part of their metabolism cause worse MTHRF function.

Vitamins B12 (cobalamin) plays a fundamental role as a cofactor in several metabolic pathways. It also is related to [detoxification](https://www.ncbi.nlm.nih.gov/pubmed/19409980), because it has substantial [antioxidant] (https://www.ncbi.nlm.nih.gov/pubmed/19799418) properties. B12 deficiency is linked with [pernicious anemia, neurodegenerative disorder, cardiovascular disease and gastrointestinal disease]( https://www.ncbi.nlm.nih.gov/pubmed/22367966).

The CSF-B12 level appeared to be generally low, and CSF-homocysteine and CSF-B12 levels correlated significantly with ratings of mental fatigue. The results suggested a blockage of B12 transport over the blood brain barrier.

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Long term treatment with metformin increases the risk of vitamin B-12 deficiency, which results in raised homocysteine concentrations. Vitamin B-12 deficiency is preventable; therefore, our findings suggest that regular measurement of vitamin B-12 concentrations during long term metformin treatment should be strongly considered.

People with the following variants have a slightly reduced effiency of processing folate [(82% of normal](https://www.ncbi.nlm.nih.gov/pubmed/25902009)). In ME/CFS, [hypomethylation](http://dx.doi.org/10.4172/2155-9899.1000228), which is greatly affected by the vitamins B12 and folate, is seen in a majority of certain immune cells. The low B12 and homocysteine levels correlated significantly with ratings of [mental fatigue](<https://www.ncbi.nlm.nih.gov/pubmed/25902009>).

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CLYBL ([Citramalyl-CoA lyase, mitochondrial](<http://www.uniprot.org/uniprot/Q8N0X4#pathology_and_biotech>)) creates a mitochondrial enzyme involved in the vitamin B12 metabolism. It also mediates magnesium ion and metal binding and malate synthase. Vitamins B12 (cobalamin) plays a fundamental role as a cofactor in several metabolic pathways, including [detoxification](<https://www.ncbi.nlm.nih.gov/pubmed/19409980>) due to its substantial [antioxidant] (https://www.ncbi.nlm.nih.gov/pubmed/19799418) properties. Vitamin B12 deficiency is linked with [pernicious anemia, neurodegenerative disorder, cardiovascular disease, gastrointestinal disease]( <https://www.ncbi.nlm.nih.gov/pubmed/22367966>), and [ME/CFS]( <https://www.ncbi.nlm.nih.gov/pubmed/29100069>).

GA

This variant causes an [amino acid change](<https://www.ncbi.nlm.nih.gov/pubmed/22367966>) in the protein and causes it to stop prematurely. The protein may then may bind incorrectly with ions and metals, leading to [malabsorption of B12 and mental fatigue](<https://www.ncbi.nlm.nih.gov/pubmed/25902009>). People with this variant have serum vitamin B12 levels [88.9% lower](https://www.ncbi.nlm.nih.gov/pubmed/22367966) than normal and may have higher homocysteine levels. This variant is also more common in people with [ME/CFS]( <https://www.ncbi.nlm.nih.gov/pubmed/29100069>).

\* Check serum vitamin B12 levels, and consider an [oral or injectable B12](https://www.ncbi.nlm.nih.gov/pubmed/25902009) supplement if low.

\* Be cautious when taking [opioids, duloxetine, pregabalin](<https://www.ncbi.nlm.nih.gov/pubmed/25902009>), and [metformin](<https://www.ncbi.nlm.nih.gov/pubmed/20488910?dopt=Abstract>), which lower B12 levels.

AA

This variant causes an [amino acid change](<https://www.ncbi.nlm.nih.gov/pubmed/22367966>) in the citrate lyase beta-like protein encoded by CLYBL, causing it to stop very prematurely. The protein may then bind incorrectly with ions and metals, leading to [malabsorption of B12](<https://www.ncbi.nlm.nih.gov/pubmed/25902009>). People with this variant have serum vitamin B12 levels [36.2% lower](https://www.ncbi.nlm.nih.gov/pubmed/22367966) than normal. Vitamin B12 deficiency is linked to [anemia, loss of balance, numbness or tingling in the arms and legs, and weakness](https://medlineplus.gov/ency/article/002403.htm). The reduction of circulating vitamin B12 also causes increased levels of [itaconate]( <https://www.ncbi.nlm.nih.gov/pubmed/29056341>), an anti-microbial metabolite and immunomodulator, which in turn deactivates vitamin B12.

This variant is much more common in people with [ME/CFS]( <https://www.ncbi.nlm.nih.gov/pubmed/29100069>). In ME/CFS, [hypomethylation](<http://dx.doi.org/10.4172/2155-9899.1000228>), which is greatly affected by the vitamins B12 and folate (B9), is seen in a majority of certain [immune cells](https://www.ncbi.nlm.nih.gov/pubmed/25111603/). The low B12 causes high homocysteine levels, which correlate significantly with [coronary heart disease, stroke, peripheral vascular disease, hardening of the arteries](https://labtestsonline.org/tests/homocysteine), and [mental fatigue](<https://www.ncbi.nlm.nih.gov/pubmed/25902009>), suggesting a blockage of B12 across the blood brain barrier.

\* Consider a daily [oral folic acid](https://www.ncbi.nlm.nih.gov/pubmed/25902009) supplement combined with oral or injectable B12.

\* Avoid [opioids, duloxetine, pregabalin](<https://www.ncbi.nlm.nih.gov/pubmed/25902009>), [metformin](<https://www.ncbi.nlm.nih.gov/pubmed/20488910?dopt=Abstract>), and other drugs that have to be demethylated.

\* Check homocysteine levels, and consider taking [folate](https://medlineplus.gov/druginfo/natural/1017.html) if elevated.  
\* Watch for eye lens dislocations, unusual (Marfan type) body shape, stroke, blood clotting abnormalities, and low thyroid hormones (hypothyroidism).