Enrichment of Motilin Receptor Loss-of-Function Variants in Gastroparesis

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INTRODUCTION: **Gastroparesis is a serious medical condition characterized by delayed gastric emptying and symptoms of nausea, vomiting, bloating, fullness after meals, and abdominal pain.**

METHODS: **To ascertain the genetic risk factors for gastroparesis, we conducted the largest thus far whole-genome sequencing study of gastroparesis. We investigated the frequency and effect of rare loss-of-function variants in patients with both idiopathic and diabetic gastroparesis enrolled in a clinical study of gastroparesis.**

RESULTS: **Among rare loss-of-function variants, we reported an increased frequency of a frameshift mutation p.Leu202ArgfsTer105, within the motilin receptor gene, variant rs562138828 (odds ratio 4.9). We currently replicated this finding in an independent large cohort of gastroparesis samples obtained from patients participating in the ongoing phase III gastroparesis clinical study.**

DISCUSSION: **Motilin receptor is an important therapeutic target for the treatment of hypomotility disorders. The identified genetic variants may be important risk factors for disease as well as may inform treatments, especially those targeting motilin receptor.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A780 and http://links.lww.com/CTG/A781.

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Gastroparesis is a potentially serious medical condition characterized by delayed gastric emptying and symptoms of nausea, vomiting, bloating, fullness after meals, and abdominal pain (1). The incidence of gastroparesis ranges from 6.3 to 17.2 per 100,000 person-years (2). The pathophysiology of gastroparesis is complex and probably involves neuromuscular dysfunction and sensory neuropathy, resulting in delayed gastric emptying, nausea, and pain (1). There are no reported genetic risk factor for gastroparesis (Figure 1).

To ascertain the genetic risk factors for gastroparesis, we conducted a large whole-genome sequencing (WGS) study of gastroparesis. We investigated the frequency and effect of rare loss-of-function (LOF) variants in patients with idiopathic and diabetic gastroparesis enrolled in a phase II clinical study (VP-VLY-686-2301) (3). Inclusion criteria included patients with idiopathic or diabetic gastroparesis with moderate to severe nausea, delayed gastric emptying, daily 50% worst average nausea score $\geq$3, and GCSI nausea score $\geq$ 3 at screening (see Study Demographics, Supplementary Methods, Supplementary Digital Content 1, http://links.lww.com/CTG/A780). The genetic data set consisted of 119 WGS samples. Among rare LOF variants, we reported an increased frequency of a frameshift mutation p.Leu202ArgfsTer105, within the motilin receptor (*MLNR*) gene (rs562138828). Motilin is a 22 amino acid peptide hormone expressed throughout the gastrointestinal tract (4). The motilin receptor is a heterotrimeric guanosine triphosphate–binding protein G-protein–coupled receptor. The protein encoded by *MLNR* is a motilin receptor, which is a member of the G-protein–coupled receptor 1 family. Motilin receptor is an important therapeutic target for the treatment of hypomotility disorders (5). In our study, we report an increased frequency of the rs562138828 frameshift mutation with minor allele frequency (MAF) of 0.0168 as compared to 0.0036 AF in gnomAD (6) (P value $<$ 0.002; Table 1). We reported 4 female carriers of the variant of interest (2 diabetic, 2 idiopathic) with an average body mass index (BMI) of 34.1.

We have also replicated this finding in an independent large cohort of gastroparesis samples obtained from patients participating in an ongoing phase III gastroparesis clinical study (VP-VLY-686-3301). The data set consisted of 894 WGS samples from screened subjects. Subjects included male and female adults aged 18–70 years with a diagnosis of diabetic or idiopathic gastroparesis.

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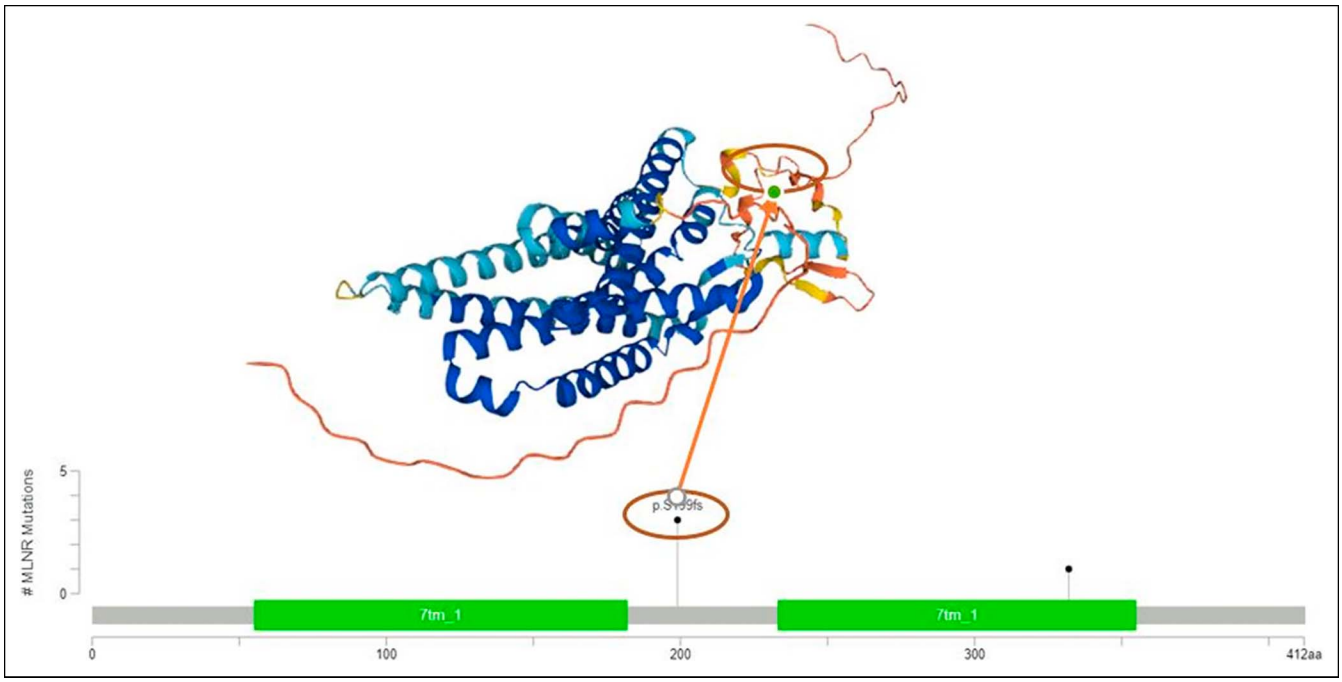

Figure 1. Location of the variant of interest (rs562138828) within the motilin receptor gene and within the motilin receptor protein (Uniprot ID 043193; obtained from Alphafold [9]).

Subjects who later enrolled in the study had evidence of delayed gastric emptying and moderate to severe nausea.

In this replication data set, we confirm an increased frequency of this frameshift mutation (rs562138828) within *MLNR* gene. Altogether, we report 13 cases of 894 in the gastroparesis cohort vs a frequency of 13 of 1,912 in the control cohort (MAF: cases 0.007; MAF: controls 0.003) and odds ratio (OR) 2.15. As this variant has increased allelic frequency in the African American (AA) population per gnomAD (6) controls, we inspected the frequencies in this group alone: AA MAF (10/203) cases: 0.024 vs AA MAF (11/514) controls 0.01, OR: 2.36 (relative risk: 1.71, P value < 0.02). Noteworthy is the fact that an equal number of mutation carriers were observed among the idiopathic and diabetic subgroups of patients, suggesting that the observed frameshift MLNR mutation may confer a risk to the development of gastroparesis regardless of etiology.

Moreover, given the large sample size in the replication set, we expanded the analysis to other MLNR rare nonsynonymous variants. We furthermore report an increased MAF for nonsynonymous variant rs372763744 in *MLNR*. The variant has a global MAF of 0.005 in gnomAD, which varies across ethnicities. It is highest among South Asian (MAF 0.02) and European (MAF 0.008), and much lower among East Asian (MAF 0.0006) and African/African American (MAF 0.0005). Hence, we did the analysis only for our largest set of European ancestry: MAF cases 0.01 (8/342) vs MAF controls 0.004 (8/962). We also report on a single homozygote case for this variant observed in our gastroparesis cohort, with only a single other case reported in the entire gnomAD WGS (15,264 genotypes). We also reported on an additional extremely rare singleton *MLNR* nonsynonymous variant cases not present in our control set (see Supplementary Material for full list, Supplementary Digital Content 2, http://links.lww.com/CTG/A781), suggesting an enrichment of mutations in *MLNR* when ancestries are accounted for in the burden analysis.

Table 1. Allelic frequencies for variant of interest

Group	Cases	Controls	MAF[a]	OR (CI)
Cohort I	4	115	0.0168	4.92 (1.78–13.57)
gnomAD controls	113	15,541	0.0036	P value 0.0021
All cases combined	13	881	0.0073	2.17 (1.0031–4.7067)
Vanda controls	13	1899	0.0034	P value 0.0491

Allelic frequencies for variant of interest (rs562138828) in cases and controls for both gastroparesis cohorts. The variant is enriched in all the tested gastroparesis samples in comparison with internal and gnomAD controls.
CI, confidence interval; MAF, minor allele frequency; OR, odds ratio.

Whole-genome sequencing of gastroparesis patient samples showed enrichment of rare variants in the *MLNR* in cases compared with controls. These findings may be of direct relevance to treatment because individuals with the identified mutation may respond differently to gastroparesis treatments especially those targeting the *MLNR* protein such as motilin receptor agonists. Interestingly, previous experiments examined the mutations within predicted intracellular loop regions of *MLNR* and their effects on motilin- and erythromycin-stimulated activity (7). The authors identified functionally relevant residues: deletions of receptor residues 63–66, 135–137, and 296–301 that each resulted in significant loss of intracellular calcium responses on stimulation by motilin and erythromycin (7). It is noteworthy that our identified frameshift causes out-of-frame translation starting at amino acid 202 (leucine), leading to premature termination at 260. This would affect the empirically derived, important residues 296–301. The other identified missense variants in our cohort did not fall within the above residues. This identified variant is precisely predicted to cause stop-gain because of frameshift variant,

in the new, out-of-frame, reading frame of *MLNR*. It has not been reported previously in literature. Follow-up experiments expressing the variant of interest would constitute follow-up analysis. As functional motilin system is absent in rodents, these species are not commonly used for translational studies; hence, the expression of the LOF variants nor lessons from knockout mice are limited. Recent studies examine the usefulness of human *MTLR*-overexpressing transgenic mice that could be used for such follow-up experiments (8). Moreover, further studies are warranted to explore whether there are other rare LOF loci, leading to or predisposing to gastroparesis, because currently, these rare variants are explaining only a small proportion of patients with gastroparesis.

The present analysis was focused on enrichment of loss-of-function variants. Large genomewide association studies based on all the collected data are warranted. These further analyses will potentially help discern any other genetic underpinnings of gastroparesis. It may be the case that several individuals are predisposed because of rare consequential variants, but the majority of genetic risk is due to polygenic effect similar to the risk of type II diabetes that is best explained by polygenic risk score spanning many loci across genes. Variants elsewhere in the *MLNR* gene, either in noncoding regions including control regions and intron regulatory elements as well as missense mutations, may further contribute to the genetic risk of gastroparesis. The identified LOF variants within the region can serve as a risk factor for disease as well as 1 day may inform treatments.

CONFLICTS OF INTEREST

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