Whole Exome Sequencing Among 26 Patients With Indeterminate Acute Liver Failure: Response to Letter to the Editor

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Clinical and Translational Gastroenterology 2020;11:e00187. https://doi.org/10.14309/ctg.0000000000000187

We have read with great interest the letter by Heddar and Misrachi and have drafted the following response.

Our study used gnomAD version 2.1.1 for hg38 genome build to annotate these variants because this was the most current version of gnomAD available during the course of this study, version 3 was published in October 2019, whereas this study was submitted to Clinical and Translational Gastroenterology in June 2019 and was published online in October 2019. We stand by our use of version 2, and we would like to emphasize an insight regarding gnomAD that Heddar and Misrachi seemed to have overlooked. In the FAQ page of this software, it recommends not switching from version 2: “The gnomAD v2 call set contains fewer whole genomes than v3 but also contains a very large number of exomes that substantially increase its power as a reference in coding regions. Therefore, gnomAD v2 is still our recommended dataset for most coding regions analyses” (https://gnomad.broad-institute.org/faq#should-i-switch-to-the-latest-version-of-gnomad). This is very pertinent because we performed whole exome sequencing in our study. Furthermore, this version has reported 4 of 12 variants at very low allele frequency (last column in table below), contrary to the high AF suggested by Heddar and Misrachi.

The 12 variants corresponding to 11 genes were represented in a significantly higher prevalence in our study population based on the Fisher exact test.

1. All variants were annotated with allele frequencies from the following databases:
2. ExAC (r0.3.GRCh38: 60,706 unrelated individuals from 17 disease-specific and population genetic studies, excluding individuals affected by a severe pediatric disease)
3. 1000G (20130502.GRCh38: integrated set of SNPs, indels, MNPs, long insertions and deletions, copy number variations, and other types of structural variations discovered and genotyped in 2,504 unrelated individuals), and
4. Mayo Clinic Biobank (funded by a Mayo Clinic initiative for Individualized Medicine to assist investigators throughout the institution in obtaining “normal” samples to serve as controls for their patient populations, 982 whole genome samples)

Furthermore, as explained in our study, we divided 26 patients into the following 2 groups: 8 patients who survived spontaneously from their acute liver failure (ALF) episode of indeterminate

### Table 1. Variants found to be significantly associated with indeterminate acute liver failure in our 26 patients

| chr | pos   | Ref | alt | Gene   | dbSNP.ID | Rvtests output | v2.1.1.exome.gnomAD.AF |
|-----|-------|-----|-----|--------|----------|----------------|------------------------|
|     |       |     |     |        |          | Ctrl.AF | Case.AF | Pvalue | v2.1.1.exome.gnomAD.AF |
| 10  | 46330066 | C   | G   | ANTXRL | rs7091749 | 0       | 0.277778 | 0.02177 | NA |
| 11  | 1016887 | G   | A   | MUC6   | rs776572312 | 0.375 | 0.083333 | 0.017629 | 1.99701e-05 |
| 14  | 22634064 | A   | G   | OR6J1  | rs1753430 | 0.125 | 0.444444 | 0.030609 | NA |
| 18  | 11689670 | C   | CGGCCCT | GNAL   | rs201898548, rs531745431 | 0.1875 | 0       | 0.025339 | 8.42043e-02 |
| 18  | 63712604 | G   | T   | SERPINB11 | rs4940595 | 0.4375 | 0.777778 | 0.024961 | NA |
| 1   | 12719616 | C   | T   | AADACL3 | rs3010877 | 0.3125 | 0.055556 | 0.02281 | NA |
| 1   | 150578851 | G | A   | MCL1   | rs11580946 | 0.1875 | 0       | 0.025339 | 8.42152e-03 |
| 22  | 42126611 | C   | G   | CYP2D6 | rs1135840 | 0.75 | 0.305565 | 0.005608 | NA |
| 22  | 42141186 | C   | T   | CYP2D7 | rs56404506 | 0.4375 | 0.138889 | 0.031057 | NA |
| 22  | 42141587 | G   | A   | CYP2D7 | rs1800754 | 0.75 | 0.25 | 0.001624 | NA |
| 6   | 43021675 | CGCGGG | C   | RRP36 | rs200886831, rs551189349, rs753769770 | 0.0625 | 0.333333 | 0.043973 | 4.19229e-02 |
| 9   | 34372875 | G   | C   | KIAA1161 | rs4879782 | 0.3125 | 0.055556 | 0.02281 | NA |
etiology and 18 patients with the same diagnosis who died or underwent liver transplantation. The 12 variants reported in Table 1 of our study were found to have significant group-specific (spontaneous survivors vs death or liver transplantation) variant distributions by performing genetic association analysis using the Rvtests package (the Fisher exact association model was used to determine significance of variant association statistics).

Given the small cohort size of our pilot study, we intend to expand our study to a larger population with ALF of indeterminate etiology and compare their variants distribution with patients with ALF associated with viral hepatitis, ALF associated with drug-induced liver injury, and autoimmune ALF. We hope that these studies may provide an insight into the mechanism(s) underlying ALF and specifically ALF with indeterminate etiology.

**CONFLICTS OF INTEREST**
**Guarantor of the article:** Jorge Rakela, MD. **Specific author contributions:** J.R. is accepting full responsibility for the conduct of the study. He had access to the data and had control of the decision to publish. J.R., he led the group in planning the response to the letter to the editor, interpreting data, and drafting the response to the letter to the editor. M.K.D. reviewed collected data and the analysis performed using gnomAD version 2 and 3. S.B., expert in bioinformatics analysis reviewed our response and verify accuracy of data. Ms. Dehankar and Mr. Baheti participated in the writing and editing of the letter to the editor as well.

**Financial support:** Grant 18-0011260; Mayo Clinic Center for Individualized Medicine. **Potential competing interests:** None to report.

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