

### Annotated table of revision:

| S.No | Reviewer Comments 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Changes done                                                                                                                                                                                                                                                                                                                                                                            |
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| 1.   | This manuscript emphasized the importance of monogenic diabetes research and diagnosis in diverse populations and the genetic heterogeneity of patients with monogenic diabetes among different populations. I have some comments.                                                                                                                                                                                                                                                    | We thank reviewer for the comments.                                                                                                                                                                                                                                                                                                                                                     |
| 2.   | 1) The article changed the topic between MODY and monogenic diabetes throughout the paper, which is confusing. The title and introduction focused on MODY but the sequencing methods was exome sequencing. Since the known MODY genes have been discussed, why use exome sequencing instead of targeted sequencing of MODY genes? If the topic was monogenic diabetes, why not selecting patients for other types of monogenic diabetes such as lipodystrophy and syndromic diabetes? | The reviewer has a good point. It is now known that the MODY phenotype may be caused by mutations in genes other than the 14 listed in OMIM. We have revised the paper to use “monogenic form of diabetes (MFD)” throughout the paper. With falling costs of NGS, it is nowadays less expensive to sequence the whole exome, then focus the bioinformatics on the specific known genes. |
| 3.   | 2) It is now well recognized that KLF11, BLK, and PAX4 are not MODY causing genes, though not reflected in OMIM yet.                                                                                                                                                                                                                                                                                                                                                                  | By our reading of the literature, the question has not been settled, as is reflected in OMIM, and we do not believe that we can completely omit mentioning them. We have inserted a note of caution about these genes in the Discussion (Page 9; line 217-218, Page 10; line 264-266)                                                                                                   |
| 4.   | 3) The detailed ACMG classification criteria of disease-causing variants should be listed.                                                                                                                                                                                                                                                                                                                                                                                            | The missense variants were selected only if predicted disease-causing by the majority of 10 algorithms used (legend to table 1) which satisfies PP3 by ACMG/ AMP criteria). The computational evidence supports deleterious effects on the gene.                                                                                                                                        |

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|    |                                                                                                                                                                       | All variants were selected to be PM2 (absent from databases or rare)<br>(Page 7; line 168-169)                                                                                                                            |
| 5. | 4) The sequencing method and platform should be described.                                                                                                            | Exome sequencing was carried out with 50 Mb Agilent Sure select array and sequencing on Illumina Hi-seq at 50X depth. This has been added in the Methods section.<br>(Page 6; line 146-147)                               |
| 6. | 5) The variants should be listed by their HGVS nomenclatures.                                                                                                         | Changed throughout the manuscript.<br>(Page 3; line 65-69 and other places)                                                                                                                                               |
| 7. | 6) The discussion part mentioned the usage of MODY probability calculator; it would be great to show this part of data while demonstrating the patient's information. | This information is mentioned in Results.<br>(Page 8-9; line 209-213)                                                                                                                                                     |
| 8. | 7) Has any of the enrolled patients been tested for C-peptide?                                                                                                        | Yes, patients from Lahore were tested for fasting c-peptide. All patients selected for exome sequencing were having normal range (0.8-3.8) of fasting c-peptide. This information has been added.<br>Page 6: line 129-130 |
| 9. | Other minor errors:<br>1) The gene name should follow HGNC nomenclature.<br>2) Numbers below ten should be uniformly written as one digit number.                     | Corrected as per HGNC nomenclature<br><br>Corrected                                                                                                                                                                       |

| S.No | Reviewer Comments 2                                                                                                                                                                                                                                                                                                                                    | Changes done                                                                                                       |
|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| 1.   | This article aims to determine the genetic variation and frequency responsible for monogenic diabetes in the Pakistani population. This topic is very interesting and the researchers have come to their own conclusions. However, as the author pointed out, there is need for large scale genetic studies on early onset of diabetes in the country. | We thank the reviewer for encouraging us to proceed for further studies in this population, which we are planning. |
| 2.   | 1. The inclusion criteria of MODY patients should be supplemented according to the standard of clinical diagnosis and treatment.                                                                                                                                                                                                                       | We selected patients having onset of diabetes below 25 years of age and having                                     |

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|    |                                                                                                                                                                                                                          | diabetes as per WHO definition, whose treating physician suspected it might be monogenic, based on either clinical impression, or a calculated high MODY score.                                                                                                                                                                |
| 3. | 2. The word pediatrics in the key words is inappropriate.                                                                                                                                                                | Removed                                                                                                                                                                                                                                                                                                                        |
| 4. | 3. Maturity Onset Diabetes of the young should be spelled completely when it appears for the first time, and if it appears again, it can be referred to as MODY for short.                                               | Per our answer to Reviewer 1, we have replaced MODY with “monogenic form of diabetes”.                                                                                                                                                                                                                                         |
| 5. | 4. How about the treatment? The authors should describe the therapy of the patients.                                                                                                                                     | The treatment details of patients are presented in supplementary table (S1) (Page 21-23; line 559-561)                                                                                                                                                                                                                         |
| 6. | 5. The discussion is simple and needs improvement.                                                                                                                                                                       | We have added some discussion about the need and challenges of identifying candidates for monogenic diabetes in Pakistan. We would be grateful to the reviewer if s/he could point out specific places that need further improvement. (Page 11; 270-272)                                                                       |
| 7. | 6. Supplementary table S1 is the demographic and clinical characteristics of the 28 participants who chose to sequence the exon group. And the article describes 15 patients from Lahore. The two contradict each other. | As shown in Fig. 1 and described in the methods, the 15 Lahore patients were part of the total cohort of 184 patients, from whom we selected the 28 participants. Among these 28, 5 were from Lahore (Fig. 1). (Page 18; line 522)                                                                                             |
| 8. | 7. Supplementary Figure S2 is not clear.                                                                                                                                                                                 | Unfortunately, this figure is the output of the variant calling software and we have no way of changing it to higher resolution. We have revised the legend, to draw the attention of the reader to the one point the figure clearly conveys: the two sets of green marks, one for each variant, showing that they always fall |

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|    |                                                                                                                                                                                            | on a different pair of reads.<br>(Page 20-21; line 554-557) |
| 9. | 8. Ensure the accuracy of the reference lists before submitting the manuscript. Some of the references cited in the paper are older studies, and should be replaced by more recent papers. | Old references are replaced with recent ones.               |

| <b>S.No</b> | <b>Reviewer Comments 3</b>                                                                                                                                                                                                                                                                                                                                                                                                                 | <b>Changes done</b>                 |
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| 1.          | There was a lack of data on monogenic diabetes from Pakistan; therefore this study was designed to determine the genetic variants responsible for monogenic diabetes in the country. The study identified wide spectrum of genetic variants potentially causing monogenic diabetes. The identification of novel variants paved the way for better understanding of genetic landscape and risk factors of monogenic diabetes in the country | We thank reviewer for the comments. |