

Meta-analysis of HNF1A-MODY3 variants among human population

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Abstract

Background: Previously, numerous case-control studies have highlighted variants responsible for Maturity onset diabetes of young (MODY). However, these studies have been conducted among diverse populations and hence yielded contradictory results. We, therefore, performed a meta-analysis to precisely find the association of SNPs with the disease for the HNF1A gene.

Objective: Meta-analysis of clinically defined studies deciphering mutations in the HNF1A gene responsible for the development of MODY3 was conducted among various populations to determine associations using statistical approaches.

Methods: The curation of 505 research articles published between the years 2000–2021 was carried out. Visualization of data-related protocols and statistical-analysis were conducted, which led to the identification of highly prevalent mutations among different populations (majorly Europe). Further comparison between the frequencies of the control (healthy population) and test (diseased population) dataset generated through curation was performed.

Results: We identified nine MODY3 mutations (rs587776825, rs1169288, rs1800574, rs2464196, rs137853244, rs137853238, rs587780357, rs137853240 and rs137853243) at the genome-wide significance level ($p < 5.0 \times 10^{-8}$). The present study confirmed that the data does not follow a normal distribution. Further, the data was confirmed to be a more homogenous type with frequencies having a significant association with the disease.

Conclusion: This meta-analysis found significant associations of mutations in HNF1A with MODY3, consistent with previous studies. Our findings should help elucidate the mutations in a compiled form responsible for causing MODY3.

Keywords: MODY3; HNF1A; Meta-analysis; Single nucleotide polymorphisms

Introduction

MODY is a noninsulin-dependent diabetes mellitus (NIDDM) that follows an autosomal dominant inheritance pattern [1, 2]. It is characterized by mild fasting hyperglycemia (7 mmol/L on average), with 6.5% median levels of glycosylated hemoglobin [HbA1C], which is present from birth and slowly increases with age, along with the absence of symptoms associated with the autoimmune process or insulin resistance, and the preservation of endogenous insulin secretion [3, 4]. MODY often affects people under the age of 25 and arises from heterozygous mutations in various transcription factors involved in the growth and maturation of pancreatic β -cells [5]. Additionally, mutations in enzymes involved in the β -cell glucose sensing also result in early-onset diabetes [6].

Fajans et al. and Tattersall et al. used the term MODY in the literature for the first time in 1975 to identify a group of patients with familial diabetes who had an autosomal dominant inheritance of a primary impairment in insulin secretion [7]. However, it is challenging to interpret accurate incidence at the global level as MODY is often misdiagnosed as type 1 and type 2 diabetes mellitus [8]. Moreover, since it is a rare disease, its incidence varies among

different populations. For instance, the minimum incidence of this disorder is predicted at 0.4 cases per 100,000 per year among people aged under 18 in Canada [9]. Besides its rare nature, specific communities have been found to have high frequencies, including Pima Indians, the Nauru population, and some others in southern India [10]. For the European population, the prevalence has been enumerated to be 1 per 10,000 in adults and 1 per 23,000 in children [7]. In contrast, the estimated incidence of MODY in children and adolescents under 15 with newly diagnosed diabetes mellitus is 2.4% [11, 12]. According to current data, the prevalence of MODY differs by country and ethnicity, which may be attributed to variations in availability and access to genetic testing facilities [13,14,15].

Molecular genetic studies of MODY families have shown that it is not a single entity but a representation of metabolic, genetic, and clinical heterogeneity that results from mutations affecting a single gene necessary for the proper functioning of the pancreatic beta-cell [16, 17]. Furthermore, it is monogenic diabetes which is the commonest of all diabetes mellitus incidences. Currently, 1–5% of Diabetes mellitus cases are MODY-related [18]. Genetic studies have identified fourteen distinct genes such as HNF4 α -MODY (MODY1), GCK-MODY (MODY2), HNF1 α -MODY (MODY3), HNF-1 β -MODY (MODY5), NEUROD1-MODY (MODY6), KLF11-MODY (MODY7), CEL-MODY (MODY8), PAX4-MODY (MODY9), INS-MODY (MODY10), BLK-MODY (MODY11), ABCC8-MODY (MODY12), KCNJ11-MODY (MODY13) and APPL1-MODY (MODY14) responsible for the etiology of MODY [19, 20].

These various genes stated above with mutation(s) could predispose a person to develop MODY. Such mutations have been highlighted in multiple studies across the globe with the advancement in high-throughput technologies [2, 17, 21,22,23]. Due to this, a great deal of data is generated, making it challenging for doctors to track such mutations. Given the abundance of primary research articles and the functional roles of the HNF1A gene in regulating gene expression for several other genes involved in glucose metabolism, including insulin (INS), glucose transporter (GLUT) 1 and 2, and sodium/glucose co-transporter 2 (SGLT2) [24], we performed a meta-analysis to assess the various SNPs reported in the literature for HNF1A gene and population data to determine association of these SNPs with the disease.

Materials and methods

The present study employs PRISMA based in silico approach [25], wherein various research articles were gathered and curated to compile SNPs from literature for gene HNF1A to estimate the association of SNPs with the disease.

Literature search strategy

Genetic association studies were identified using specific keywords such as “HNF1A” and “MODY” from PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) from January 1, 2000, to August 27, 2021. The search strategy included multiple queries involving HNF1A or MODY or a combination of both. The search put forth PubMed IDs of relevant research articles which were further scrutinized to extract data concerning SNPs and the population affected. As per the availability of information for SNPs (in the form of dbSNP rs IDs, protein change, or nucleotide change), bioinformatics databases involving Ensembl (for extracting dbSNP IDs) (<https://asia.ensembl.org/index.html>), Mutalyzer (for identifying specific protein change) (<https://mutalyzer.nl/>), ClinVar (to search for dbSNP ID or status of association – benign/pathogenic/unknown significance) (<https://www.ncbi.nlm.nih.gov/clinvar/>) were used.

Finally, the rs IDs were fed to the dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>) to extract associated frequencies of control and test populations.

Eligibility criteria, data extraction, and quality assessment

Primary research articles, including case-control studies, meta-analysis having independent data demonstrating confirmation of MODY3, were considered for scrutiny. However, review articles, research articles related to animal models or cell culture, and having overlapping data were excluded.

The data was extracted from different research articles qualifying eligibility criteria and further subjected to a quality check to identify inconsistencies. The following information was extracted for each included study: Pubmed ID of the research article, SNP information such as nucleotide/protein change or dbSNP rs ID (based on the research article's available information), and ethnicity of the population studied [26]. Furthermore, control and test population frequencies were extracted using specific dbSNP IDs from the dbSNP database. However, since the dbSNPs IDs for all the SNPs were not available, nucleotide or protein change dbSNP IDs were searched using other bioinformatic databases such as ClinVar, Mutalyzer, and Ensembl [26]. Yet, some SNPs were excluded from further statistical analysis since the respective dbSNP IDs were not reported in any of the databases mentioned above.

Meta-analysis using statistical approaches

Association between polymorphisms and the disease was estimated by comparing allele frequencies of control and test populations. Firstly, a 2×2 contingency table was formed, and the populations were grouped in the following manner-

- Group 1 - Asia: South-east Asia, Vietnam, Hmong, China, Korea, Japan, and Taiwan
- Group 2 - Africa: Africa and Africa Americans
- Group 3 - America, Baltimore, California, Mexico, UK, and the US.
- Group 4 - Europe: Germany, Italy, and Poland
- Group 5 - Australia: Australia and New Zealand
- Group 6 - Others

To assess p values using the Fisher exact t-test, the genome-wide significance threshold was considered to be $\leq 5.0 \times 10^{-8}$ to reveal evidence of associations [27, 28]. The quantile-quantile plot (Q-Q plot) and the genomic inflation factor (λ) were used for elucidating distribution characteristics of both reference and altered frequencies among populations [29,30,31,32]. Moreover, the genomic inflation factor (λ) was elucidated to check for the inflation of p values. Furthermore, a scatter plot was plotted to depict the relationship between reference and altered frequencies [30, 33]. Following this, Cochran's Q test and the I^2 statistic were performed to assess heterogeneity in frequencies among different populations [27, 34, 35]. All the statistical analyses were performed using the following softwares - Statistical Package for Social Sciences (SPSS) 23.0, IBM 2009, Python (Jupyter Notebook) version 3.9 (Libraries used: Numpy, Pandas, Matplotlib) and R software (version 3.0.1+).

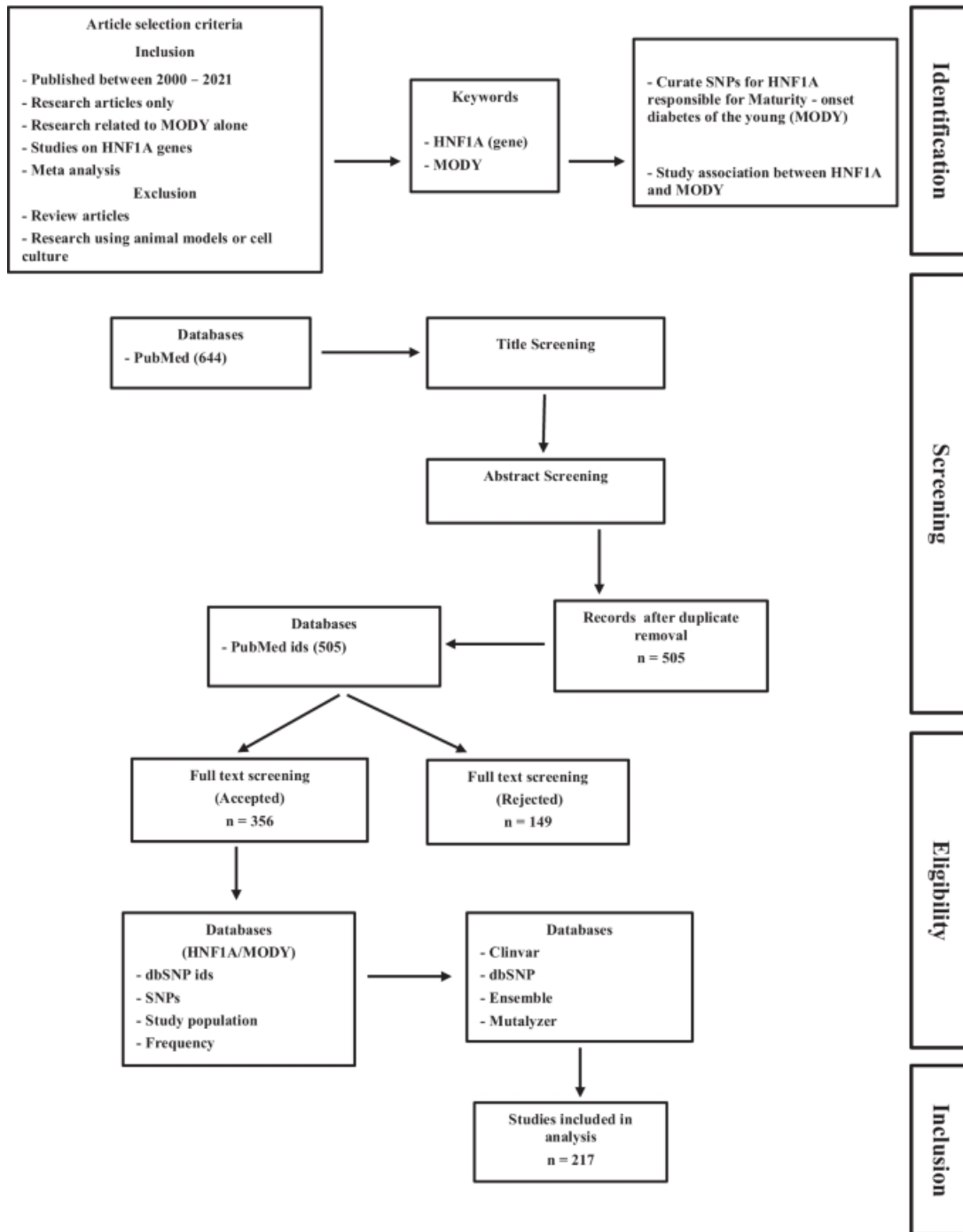


Fig. 1. Schematic representation of PRISMA method employed for selecting and screening research articles for meta-analysis [25]

Results

Characteristics of studies

In all, we included 217 studies in this meta-analysis for the HNF1A gene responsible for MODY development. The detailed characteristics of the studies included are shown in Table 1.

The study selection process is shown in Fig. 1. These polymorphisms were found to occur in frequencies consistent with Hardy–Weinberg equilibrium in the control populations of the vast majority of the published studies [36].

Table 1. Statistical analysis conducted for HNF1A gene- Cochran’s test, Genomic Inflation factor estimation, Fisher Exact test and I² analysis

| dbSNP | Sample size | Altered | Altered Frequency | Cochran’s test | | Genomic inflation factor (Lambda) | Fisher Exact Test <i>p</i> -value | I ² (%) |
|-------------|-------------|---------------------------|-------------------|----------------|----------------|-----------------------------------|-----------------------------------|--------------------|
| | | | | Chi-Square | <i>P</i> Value | | | |
| rs587776825 | 25 | CCCCCCCC/ CCCCC CCC | 0.000293 | 13.52 | 3.61E-10 | 1 | 5.44E-08 | 92 |
| rs1169288 | 20 | A/C | 0.349004 | 2.8 | 6.27E-11 | 1 | 4.09E-12 | 75 |
| rs1800574 | 17 | C/T | 0.016307 | 9.176 | 2.23E-08 | 1 | 2.18E-08 | 76.47 |
| rs2464196 | 14 | G/A | 0.414228 | 6.571 | 0.000004 | 1 | 3.96E-07 | 64.28 |
| rs137853244 | 12 | C/T | 0 | 8.333 | <.001 | 1 | – | – |
| rs137853238 | 10 | G/A | 0 | 3.6 | 0.005 | 1 | – | – |
| rs587780357 | 10 | G/A | 0.000003 | 0 | 0.001565 | 1 | 0.007937 | 90 |
| rs137853240 | 7 | G/A | 0.000001 | 3.571 | 0.008151 | 1 | 0.142857 | 85.71 |
| rs137853243 | 7 | C/T | 0.000004 | 0.143 | 0.008151 | 1 | 0.028571 | 85.71 |

It highlights *p*-values of nine SNPs showing significant association as per genome wide association study for meta-analysis

Genetic variants involved in MODY development

Nine genetic variants in HNF1A gene were identified to be responsible for MODY development such as rs587776825, rs1169288, rs1800574, rs2464196, rs137853244, rs137853238, rs587780357, rs137853240 and rs137853243 (described in Table 1). Majority of studies were of European ethnic groups; however, certain studies have been reported in Asian, African, and American populations (Figs. 2 and 3).

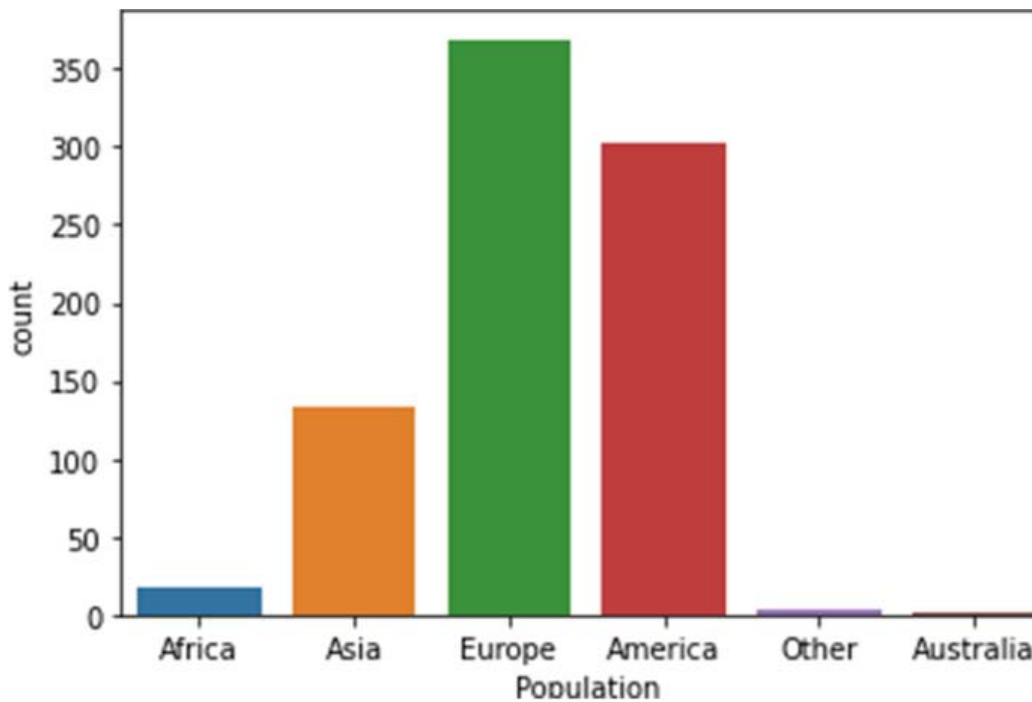
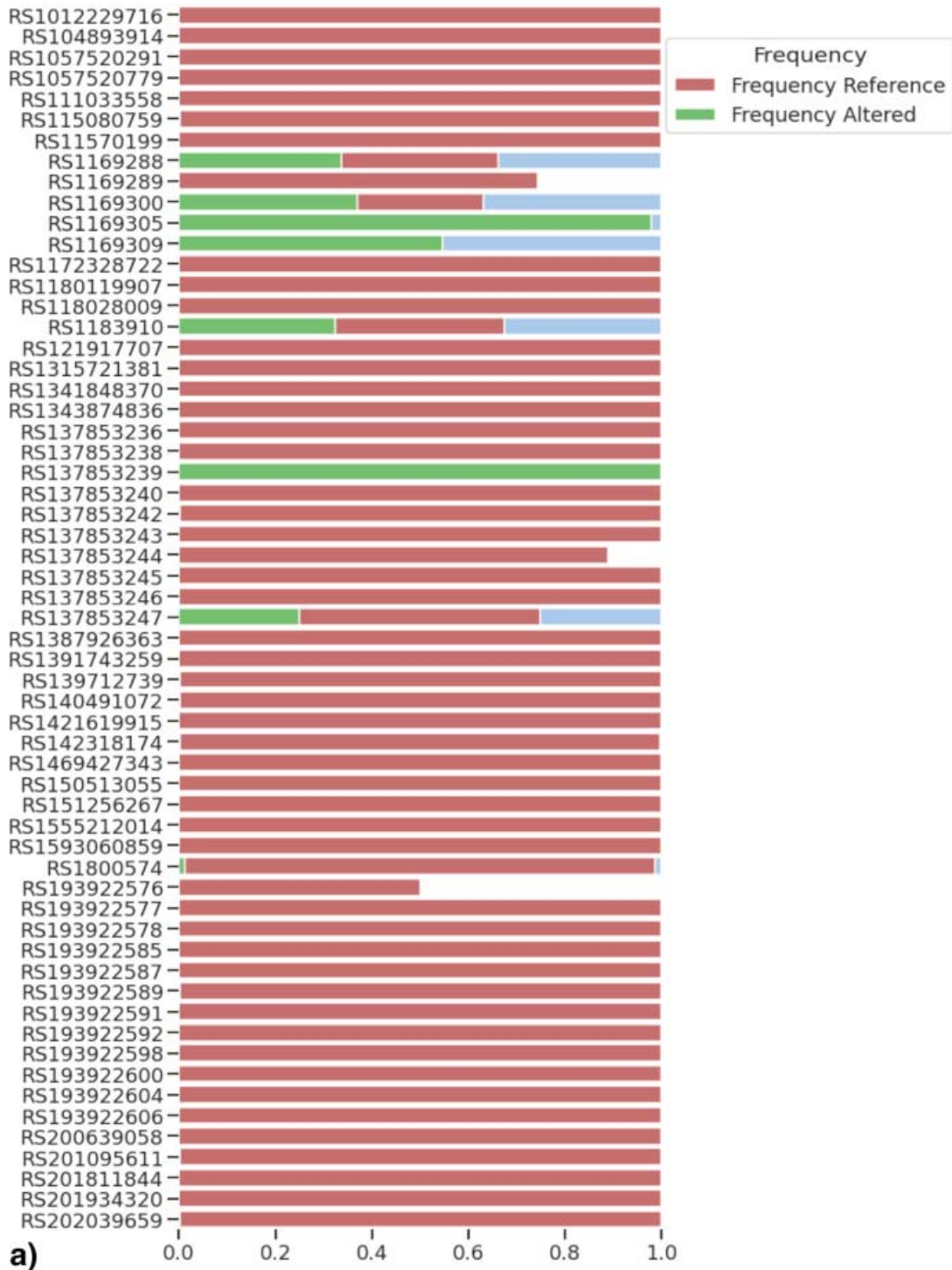


Fig. 2. Graphical illustration of SNPs for MODY3 reported in literature among different populations

Barplot from Altered and Referenced Genes



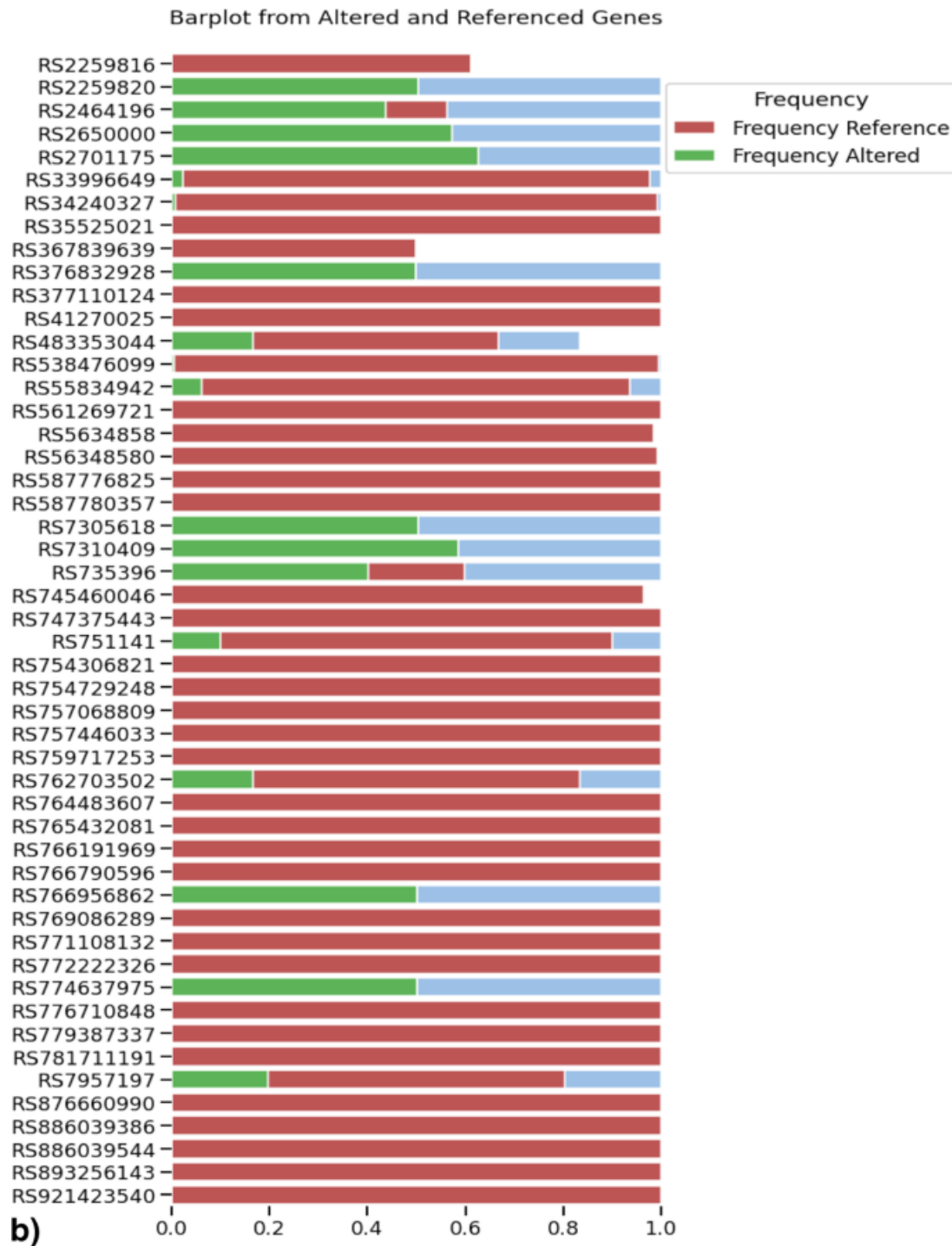


Fig. 3. a and b: Boxplot depicting different SNPs reported across various studies from the year 2000–2021

Statistical analysis

A Q-Q plot was constructed to identify frequencies deviation from normality. Two graphs depicting reference and altered frequencies were obtained (shown in Fig. 4). The graph for Q-Q plot frequencies illustrated the blue data points in the middle right; they were primarily found below the expected standard distribution line. The points on the outer edges were above the

expected normal distribution line, forming a v-shape. These findings indicates that the data is right-skewed compared to the normal curve. For altered frequencies Q-Q plot, the blue data points obtained depicts opposite patterns to that of reference frequencies Q-Q plot. The second graph indicates a left-skewed curve compared to the normal curve because the edges were under the graph as the middle left was over the graph. Therefore, both the graphs do not fit a normal curve as they are both skewed, suggesting that they deviate from normality.

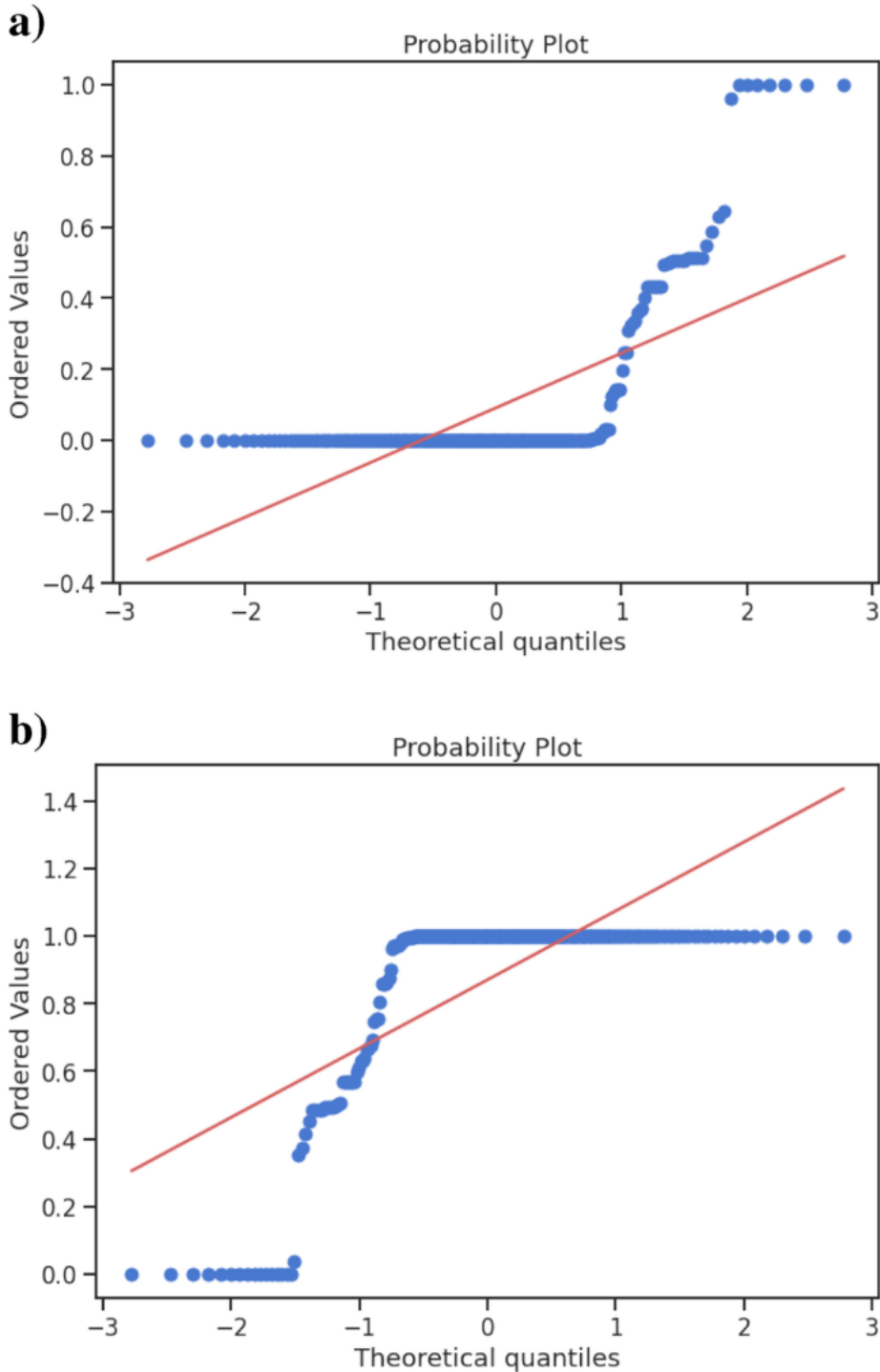


Fig. 4. Q-Q plot of **a)** reference frequencies **b)** altered frequencies of HNF1A gene

Further, a scatter plot (shown in Fig. 5) was used to visualize trends between two sets (continuous) of data. The scatter plot depicted the relationship between altered and reference frequencies when plotted against encoded population data (Y). The plot pattern revealed reference frequencies to be slightly different from altered frequencies among all the populations undertaken by the study.

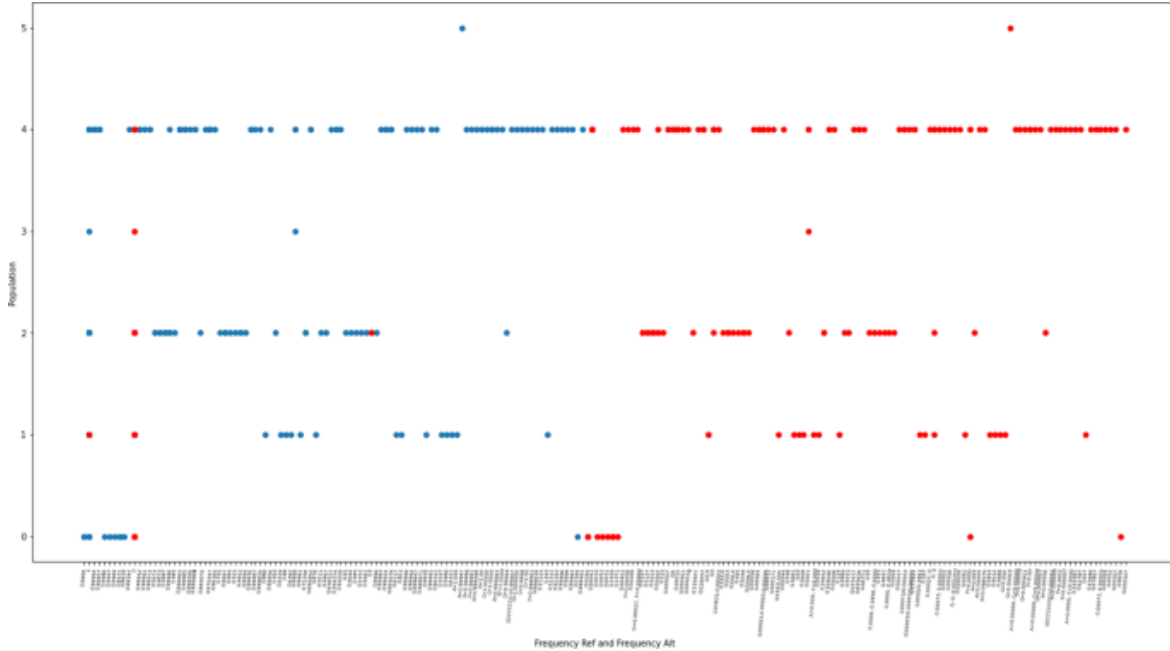


Fig. 5. Scatter plot indicating differences among reference and altered frequencies for HNF1A gene. The numerical values indicates different populations, such as 0- Africa, 1- America, 2- Asia, 3- Australia, 4- Europe and 5- others respectively

Following this, Cochran’s Q test was performed, which revealed homogeneity between the reference and altered frequencies. The p value was tested using the threshold given by the Genome-Wide Association Studies (5×10^{-8}). The required hypothesis was as:

- H_0 : There is homogeneity between the reference & altered frequencies. That is, proportion in reference & alternative group is same.

vs

- H_1 : There is heterogeneity between the reference & altered frequencies. That is, proportion in reference & alternative group is unequal.

Based on the results obtained, six SNPs were more homogenous, but three frequencies had a p value below the threshold. Moreover, system bias was investigated, which might have been present in the association results. Therefore, the genomic inflation factor calculation was carried out, also known as lambda gc (λ_{gc}). The genomic inflation factor is expressed as a ratio of the median of the empirically observed distribution of the test statistic to the expected median, which helps decipher both system bias and inflation of data [33]. The advantage is that it can be calculated from z-scores, chi-square statistics, or p values, thus, p values were considered while doing calculations. The λ_{gc} values obtained for all the mutations was 1, indicating that there were no system biases and our results are valid. If the λ_{gc} value should

have been greater than 1, then some systematic biases would have been present, which needs to be corrected.

Later I^2 test was performed, which indicated the percentage of total variability due to true heterogeneity. As per the GWAS standard, heterogeneity is revealed if $I^2 > 50\%$. Based on the values of I^2 obtained (reported in Table 1), it can be concluded that there is some heterogeneity in control & test groups for all populations.

Following this, Fisher's exact test was computed with a 95% confidence region to determine whether the reference frequencies are associated with altered frequencies across the globe. The required hypothesis is as follow:

- H_0 : *There is no association between reference frequencies and altered frequencies*

vs

- H_1 : *There is association between reference frequencies and altered frequencies*

P value (probability values) was used to measure the strength of evidence against the null hypothesis (H_0). For F-test, the smaller the p value obtained, the more substantial the evidence against the null hypothesis. If the p value is less than the significance level (0.05), the null hypothesis is rejected; otherwise, it will be accepted [29]. All the F-Test p values were found to be less than 0.05, excluding rs137853240, where the p value (0.1428) was greater than the significance level (0.05). These findings indicated a chance of association between reference frequencies and altered frequencies across all the regions considered in this study.

All the statistical analyses performed revealed significant associations in reference and altered frequency values, suggesting that mutations/altered DNA bases can have detrimental effects on the population. In conclusion, homogeneity was observed among these frequencies, and analysis was performed for each test reported in Table 1.

Discussion

Extensive sample-based epidemiological studies have illustrated the relationship between gene polymorphisms and several complex diseases. Similarly, in this meta-analysis, 9 SNPs of the HNF1A gene were associated with MODY3 (as per data curated and statistical analysis). Various previous genetic studies have deciphered SNPs of the HNF1A gene among different populations [2, 17, 21,22,23]. To the best of our knowledge, this is the first study providing a collective assessment of genetic mutations of the HNF1A gene in the context of MODY3. This information could provide insights into the pathogenic mechanisms and the relationship between polymorphisms and the disease. It is well-known that MODY is a monogenic form of diabetes mellitus, characterised by autosomal dominant inheritance and pancreatic β -cell dysfunction [37]. MODY results from heterozygous mutations in various transcription factors involved in the development and maturation of pancreatic β -cells [38]. Among all the transcription factors involved, mutations in the HNF1A gene are the most common cause of this disease [5]. Since it is a genetic disease, it is often misdiagnosed as Type 1 diabetes (T1DM) or Type 2 diabetes (T2DM). Hence, a reference is needed that includes all the mutations and the affected population to help the medics diagnose MODY accurately. Therefore, this meta-analysis was conducted to facilitate the identification of SNPs of MODY,

particularly for the HNF1A gene reported in various populations, and decipher the relationship of polymorphisms with the disease.

Furthermore, the characterization and identification of disease variations can aid in the discovery of new biological and etiological processes. Individual etiological processes may enable preventive and therapeutic approaches in complex diseases to be tailored to people based on their genetic profiles, laying the groundwork for personalised medicine. In this regard, hypothesis-free techniques, such as GWAS, appear to be the most promising. At the moment, it seems prudent to concentrate on determining the significance of previously discovered genetic variations. Because common SNPs associated with MODY and discovered by GWAS may reflect rare genetic variations with significant effects, it appears appropriate to sequence the regions around highly significant and replicated genomic regions to detect rare variants. Follow-up in vitro and in vivo research could determine the functional significance of these HNF1A variations.

In the present study, 9 SNPs HNF1A-MODY were identified, namely, rs587776825, rs1169288, rs1800574, rs2464196, rs137853244, rs137853238, rs587780357, rs137853240, and rs137853243. Various statistical analyses on the curated data led us to draw the following conclusions. Firstly, Q-Q plots depicted that control and test population frequencies deviate from normality. Additionally, the scatter plot illustrated the relationship between the two frequencies and suggested that both the frequencies are somewhat different. Further, Cochran's Q test revealed that the population frequencies were homogenous except for two dbSNPs, i.e., rs1169288 and rs587776825 (suggesting they have been reported among different populations). The I² test, performed to assess heterogeneity, revealed that heterogeneity was present among all the dbSNPs. For the association analysis, Fisher exact t-test was performed, which interpreted all the *p* values below the threshold (excluding rs137853240), suggesting that the reference and altered frequencies are associated among different populations while is not associated for rs137853240.

Some limitations of this meta-analysis should be considered when interpreting the results. Even though a statistical test did not reveal it, publication bias is an issue in all meta-analyses. Negative studies are less likely to be published, resulting in overestimating effects. Furthermore, non-significant genetic connections may have been underreported in peer-reviewed papers. As a result, the current study's effect estimations should be evaluated with caution. Furthermore, the overall results were based on individual *p* values, but a more precise assessment should account for other potentially dubious characteristics such as age, BMI, and environmental factors.

To summarise, this study suggests a significant homogeneity between the reference and the altered frequencies obtained from different populations, and some association exists between them. The homogeneity was found as substantial SNPs of HNF1A have been reported for the European population. Thus, to characterize the HNF1A gene, more detailed studies are required in different populations. For future studies, gene-gene and gene-environment interactions should also be considered [39].

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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