Publisher homepage: www.universepg.com, ISSN: 2663-6913 (Online) & 2663-6905 (Print)

https://doi.org/10.34104/ajpab.024.01017

American Journal of Pure and Applied Biosciences

Journal homepage: www.universepg.com/journal/ajpab

Investigating the Impact of ENPP1 Gene's K121Q (RS1044498) Polymorphism in Type 2 Diabetes via an Updated Meta-Analysis

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) occurs due to a complex relationship of genetic, environmental, and physiological factors, encompassing insufficient pancreatic insulin synthesis, peripheral insulin resistance, and diverse molecular pathways. The transmembrane glycoprotein ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) plays a role in insulin regulation, with the K121Q (rs1044498) variant on the ENPP1 gene being a subject of extensive study due to its potential association with T2DM. To comprehensively evaluate this relationship, a meta-analysis was conducted, pooling data from 48 studies retrieved from databases such as PubMed, Google Scholar, Science Direct, and Medline. The analysis, performed using Review Manager Version 5.4.1 and Stata version 14.1, included a total of 24,979 T2DM cases and 33,005 controls. Employing fixed-effects or random-effects models, the combined Odds Ratio (OR) and 95% Confidence Intervals (CIs) were calculated to quantify the connection's magnitude. In the overall population, all genotypic models revealed a statistically noteworthy connotation between ENPP1 and T2DM ($P < 0.05$). Notably, the homozygous model exhibited an OR of 1.53 (95% CI = 1.23-1.90, P = 0.0001), while the heterozygous, dominant, recessive, and allelic models showed ORs of 1.22 (95% CI = 1.08-1.37, P = 0.001), 1.15 (95% CI = 1.11-1.41, P = 0.0003), 1.38 (95% CI = 1.17-1.64, P = 0.0002), and 1.22 (95% CI = 1.10-1.36, P = 0.0003), correspondingly. Subgroup analysis by population indicated no significant correlation between the K121Q polymorphism and T2DM in the African population, while a noteworthy association was detected in both Asian and Caucasian populations, with the heterozygous model lacking significance in the latter. Despite no evidence of publication bias, a notable amount of residual heterogeneity among studies was identified. Sensitivity analysis established the steadiness and dependability of the meta-analysis findings, underscoring the complex nature of the ENPP1 gene's involvement in T2DM across diverse populations.

Keywords: T2DM, *ENPP1* gene, rs1044498, K121Q, Meta-analysis, STATA, and Review manager.

INTRODUCTION:

Diabetes, also referred to as diabetes mellitus, is an enduring health condition that has had a profound and far-reaching effect on the lives of millions of individuals globally. The disease is a collection of disorders that share hyperglycemia as a characteristic. Hyper-

glycemia is caused by low insulin secretion and action, or both (Maraschin, 2013). Based on the figures on diabetes mellitus in the year 2021 from International Diabetes Federation, approximately 537 million individuals, aged between 20 and 79 years, are currently grappling with the disease, translating to one in every 10 people affected by this health condition (Atlas). This figure might reach 643 million by 2030 and 783 million by 2045, according to predictions (Atlas). In addition, diabetes turned out to be the cause of death for 6.7 million people in 2021, equivalent to one death every five seconds (Atlas). If not managed properly, diabetes can lead to severe complications and premature deaths (Rahman *et al.*, 2021). Type 2 diabetes mellitus (T2DM) is markedly further prevalent than type 1 diabetes mellitus (T1DM) or gestational diabetes, accounting for more than 90% of all cases. One of the main characteristics of type 2 diabetes (T2DM) is impaired metabolism of proteins, fats, and carbohydrates as a result of either insulin resistance or inadequate insulin secretion, or both.

In recent decades, our comprehension of the onset and advancement of Type 2 Diabetes Mellitus (T2DM) has undergone rapid expansion. The principal factor driving the disease is the gradual deterioration of insulin secretion by pancreatic β cells (DeFronzo *et al.*, 2015). Type 2 diabetes (T2DM) has its causes rooted in both hereditary and environmental factors. Obesity plays a vital role in T2DM (L Tuck & B Corry, 2010; Habib F., 2022). The Pathophysiological alterations include beta-cell dysfunction, insulin resistance, and chronic inflammation, all of which impede the regulation of blood sugar and hasten onset of micro- and macrovascular problems (DeFronzo, 2009).

Evidence suggests that IR is inherited and is caused by a number of intrinsic factors. Insulin resistance is capable of being identified in patients containing a mutation in the insulin receptor gene. It was recently identified that a glycoprotein with the name ecto-enzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPP1) (ENPP1; likewise referred to as plasma cell glycoprotein 1, PC-1) is strongly expressed in the muscle, skin, and fat of people who have type 2 diabetes. As a consequence of this, insulin signal transduction is disrupted, and insulin resistance is brought about (Maddux & Goldfine, 2000). As a direct consequence of this, type 2 diabetes begins to develop in an organized manner.

Serosa and endoplasmic reticulum membranes contain type II transmembrane glycoprotein ENPP1. The following are possible mechanisms for the ENPP1 121Q allele's induction of IR: (i) changes to the insulin receptor's tyrosine kinase active area affect the serine and threonine autophosphorylation sites, preventing the downstream cascade from receiving insulin signals. (Costanzo *et al.*, 2001); (ii) inhibiting the activity of tyrosine kinase prevents post-receptor signal transduction (Kumakura *et al.*, 1998) as well as (iii) elevated serum insulin levels subsequently induce ENPP1 expression via a system for sending signals quickly and accurately (Menzaghi *et al.*, 2003). The ENPP1 gene, which spans 80 kilobases and is located on chromosome 6q22-23, has 24 introns and 25 exons. The missense mutation at position 121 of the ENPP1 gene (rs1044498) results in a polymorphism known as K121Q. The change in the 121st codon from adenine (A base) to cytosine (C base) results in the matching amino acid sequence being altered to have glutamine (Q) instead of lysine (K) (Grarup *et al.*, 2006). It has been reported that the ENPP1 K121Q (rs1044498) polymorphism has been linked to type 2 diabetes in many countries (Badaruddoza *et al.*, 2015; Hsiao & Lin, 2016; Marchenko *et al.*, 2018; Mtiraoui *et al.*, 2012; Yako *et al.*, 2015). In this analysis, we attempted to get a more precise understanding of the link between ENPP1 (rs1044498) and type 2 diabetes by doing an updated meta-analysis study utilizing the earlier studies that had been done on a range of different ethnic groups. This study through meta-analysis, will help us to summarize the overall association of the SNP with type 2 diabetes. Moreover, to understand the unknown effect size, we can also be able to compare and contrast the findings of several studies, identify patterns among studies, and also find sources of disagreement among those results.

MATERIALS AND METHODS:

Literature Search Strategy

A thorough search of Google Scholar, PubMed, Science Direct, and Medline was conducted up until the end of 2022 to retrieve the literature on the relationship between ENPP1 polymorphisms and T2D susceptibility. The investigation was conducted emplo-

ying the following keywords: (K121Q OR rs1044498 OR polymorphism) AND (ENPP1 OR "PC-1" OR "plasma cell membrane glycoprotein 1" OR "ectonucleotide pyrophosphatase/phosphodies-terase 1") AND (Diabetes OR T2D OR T2DM OR "type 2 diabetes mellitus"). The included research's cited works and other pertinent papers were also read. Alongside we retrieved multiple studies from the previous meta-analysis research.

Inclusion and exclusion criteria of study

The subsequent inclusion measures were used to select studies for inclusion in this meta-analysis: (1) case-control studies; (2) consideration of ENPP1 polymorphisms and type 2 diabetes susceptibility; (3) allele and genotype counts in great detail between case and controls; and (4) Value of Hardy-Weinberg Equilibrium (HWE) conforming controls. We technically omitted studies which were basically- i) case studies or reviews that did not include any controls or differentiate case and control data; ii) reports with no available data; and iii) reports that are already on file.

Data extraction

Data extraction was done after the literatures were screened and the inclusion and exclusion criteria were followed. Specifically, the following data were retrieved for each study which included: the list of authors, the year the study was published, the ethnicity of the participants, the sample size, the genotype of each gene variant, and the HWE.

Statistical analysis

Using odds ratios (ORs) and 95% confidence intervals (CIs), the degree of the relationship between ENPP1 rs1044498 polymorphisms and Type 2 Diabetes (T2D) was evaluated. The pooled ORs for ENPP1 rs1044498 (K121Q) K > Q were determined using five distinct genetic models: homozygous (QQ vs. KK), heterozygous (KQ vs. KK), dominant (KQ + QQ vs. KK), recessive (QQ vs. KK + KQ), and allelic (Q vs. K). Heterogeneity was evaluated using I2, with a preference for I2 values exceeding 50% to indicate significant heterogeneity. In instances where I2 exceeded 50%, a random-effects model was the utilized. (DerSimonian & Laird, 1986), and when homogeneity was present ($I^2 \leq 50\%$), we used a fixed-effects model (Mantel & Haenszel, 1959). In addition, subgroup analyses based on ethnicity were carried out to calculate ORs that were specific to each ethnic group. Finally, Begg-Mazumdar's test, Egger's test, and funnel plots were used to evaluate publication bias (Begg & Mazumdar, 1994; Egger, Smith, Schneider, & Minder, 1997), with a P-value for statistical significance of less than 0.05. For each study, to assess the Hardy-Weinberg equilibrium (HWE), a comparison was made between the expected and the observed genotype frequencies of the control group. Using a two-tailed P-value, statistical analyses were done in Stata (StataCorp., College Station, TX, USA) version 14.2, and Review Manager (5.4.1). The cutoff for significance was set at P<0.05.

RESULTS:

Features of the study

A total of forty-four articles were selected after the inclusion and exclusion criteria were applied. The selected articles contained a total of 56 studies. 8 of those studies did not fulfill the HWE value criteria (Abate *et al.*, 2005; Weedon *et al.*, 2006; Willer *et al.*, 2007; Bhatti *et al.*, 2010; Saberi *et al.*, 2011; Barna *et al.*, 2018; Golbon *et al.*, 2018; Gohari-Lasaki *et al.*, 2020) (P<0.05). Therefore, they were excluded from the finalized meta-analysis. The final meta-analysis contained 48 studies from 37 articles comprising 24979 cases and 33005 controls. For the meta-analysis, the features of each study are presented in **Table 2** and **Fig. 1** shows a selection process flowchart.

Table 1: Genotypic and descriptive details according to the chosen study for rs1044498 meta-analysis.

First Author	Year of Publication	Country	Ethnicity	Geno typing Method	Size of Sample		Genotype (case and control)						HWE
					Case	Control	Case			Control			
							KK	KQ	QQ	KK	KQ	QQ	
Pizzuti (Pizzuti *et al.*, 1999)	1999	Italy	Caucasian	PCR-SSCP	132	121	81	47	4	80	39	2	0.2574
Gu (Gu *et al.*, 2000)	2000	Finland and Sweden	Caucasian	PCR-RFLP	392	147	304	80	8	110	36	1	0.286
Du (Du XH, 2002)	2002	China	Asian	PCR-RFLP	217	54	146	65	6	40	12	2	0.3796

Barroso (Barroso *et al.*, 2003)	2003	England	Caucasian	PCR-SSCP	491	509	375	107	9	380	121	8	0.6428
Hamaguchi (Hamaguchi *et al.*, 2004)	2003	Dominican Republic	Caucasian	PCR-RFLP	358	397	66	178	114	97	187	113	0.2611
Abate (Abate *et al.*, 2005) (1)	2005	America	Caucasian	PCR-RFLP	141	717	86	44	11	530	173	14	0.9785
Abate (2)	2005	U.S.A	Asian	PCR-RFLP	121	962	46	72	3	850	106	6	0.1823
Abate (3)	2005	India	Asian	PCR-RFLP	223	456	169	47	7	140	282	34	0
Meyre (Meyre *et al.*, 2005) (1)	2005	Austria	Caucasian	PCR-RFLP	465	732	336	114	15	570	155	7	0.3188
Meyre (2)	2005	French	Caucasian	PCR-RFLP	747	548	525	197	25	405	136	7	0.2378
Bacci (Bacci *et al.*, 2005) (1)	2005	Italy	Caucasian	PCR-RFLP	561	352	393	152	16	260	84	8	0.6946
Bacci (2)	2005	America	Caucasian	PCR-RFLP	408	286	278	115	15	203	74	9	0.4829
Chen (Chen *et al.*, 2006)	2006	China	Asian	PCR-RFLP	1862	844	1515	333	14	681	155	8	0.8025
Lu (Lu, 2006)	2006	China	Asian	PCR-RFLP	119	422	92	26	1	361	59	2	0.8045
Bochenski (Bochenski *et al.*, 2006)	2006	Poland	Caucasian	PCR-RFLP	426	370	328	91	7	286	77	7	0.4983
Grarup (Grarup *et al.*, 2006)	2006	Denmark	Caucasian	MALDI-TOF MS	1386	4770	1037	316	33	3577	1097	96	0.2692
Kubaszek (Kubaszek *et al.*, 2004)	2006	Finland	Caucasian	PCR	97	392	70	25	2	302	83	7	0.6415
Weedon (Weedon *et al.*, 2006)	2006	England	Caucasian	PCR	2287	3846	1691	554	42	2842	949	55	0.0151
Keshavarz (Keshavarz *et al.*, 2006)	2006	Japan	Asian	PCR-RFLP	907	874	727	167	13	703	160	11	0.5802
Gouni-Berthold (Gouni-Berthold *et al.*, 2006)	2006	Germany	Caucasian	PCR-RFLP	402	432	292	102	8	335	91	6	0.949
Lyon (Lyon *et al.*, 2006) (1)	2006	Poland	Caucasian	MALDI-TOF MS	1002	1000	747	240	15	716	263	21	0.5809
Lyon (2)	2006	Scandinavian sib pairs	Caucasian	MALDI-TOF MS	589	501	409	166	14	381	115	5	0.2525
Lyon (3)	2006	Scandinavian trios	Caucasian	MALDI-TOF MS	310	448	235	70	5	332	109	7	0.565
Lyon (4)	2006	Scandinavian	Caucasian	MALDI-TOF MS	473	465	353	111	9	355	103	7	0.8795
Lyon (5)	2006	Sweden	Caucasian	MALDI-TOF MS	501	496	360	127	14	356	126	14	0.4835
Lyon (6)	2006	U.S.A	Caucasian	MALDI-TOF MS	1216	1204	907	288	21	853	319	32	0.7398
Willer (Willer *et al.*, 2007)	2007	Finland	Caucasian	PCR-RFLP	1155	971	853	268	34	755	193	23	0.0137
Meyre (Meyre *et al.*, 2007)	2007	French	Caucasian	PCR-RFLP	316	2005	223	79	14	1438	511	56	0.1956
Seo (Seo *et al.*, 2008)	2008	Korea	Asian	PCR-RFLP	195	1750	164	28	3	1433	302	15	0.8354
Cauchi (Cauchi *et al.*, 2008)	2008	France-Swiss	Caucasian	TaqMan® SNP Genotyping Assays	2783	4326	1979	722	82	3144	1071	111	0.0854
C.B.leitao (Leitão *et al.*, 2008) (1)	2008	Brazil	Caucasian	PCR-RFLP	830	149	520	276	34	91	53	5	0.4148
C.B.leitao (2)	2008	Brazil	African	PCR-RFLP	197	91	51	95	51	20	49	22	0.4601
Achhab (El Achhab *et al.*, 2009)	2009	Morocco	African	PCR	503	412	194	240	69	168	183	61	0.336
Vasudevan (Vasudevan *et al.*, 2009)	2009	Malaysia	Asian	PCR	50	60	39	10	1	44	15	1	0.8284
Ezzidi (Ezzidi *et al.*, 2009)	2009	Tunisia	African	TaqMan SNP Genotyping Assays	809	499	402	311	96	228	205	66	0.0685
Wang (M. Wang *et al.*, 2010)	2010	China	Asian	PCR-RFLP	539	404	429	106	4	340	61	3	0.8844
Bhatti (Bhatti *et al.*, 2010)	2010	India	Asian	PCR-RFLP	328	326	199	129	0	195	131	0	0
Saberi (Saberi *et al.*, 2011)	2010	Iran	Asian	PCR-RFLP	155	377	109	45	1	255	119	3	0.0061

Shi (Shi *et al.*, 2011)	2011	China	Asian	PCR-RFLP	639	885	508	123	8	701	178	6	0.1409
Zhao (Zhao *et al.*, 2011)	2011	China	Asian	PCR-RFLP	1879	2014	1463	393	23	1610	385	19	0.4465
Wang (C.-H. Wang *et al.*, 2012)	2012	China	Asian	PCR-RFLP	416	188	256	129	31	149	34	5	0.0869
Tripathi (Tripathi *et al.*, 2013)	2013	India	Asian	PCR-RFLP	190	210	121	66	3	146	62	2	0.097
Yako (Yako *et al.*, 2015)	2015	South Africa	African	TaqMan SNP Genotyping Assays	152	328	41	73	38	81	175	72	0.219
Badaruddoza (Badaruddoza *et al.*, 2015)	2015	India	Asian	PCR-RFLP	239	211	148	88	3	150	57	4	0.5951
Hsiao (Hsiao & Lin, 2016)	2016	Taiwan	Asian	TaqMan SNP Genotyping Assays	553	960	368	153	32	754	197	9	0.3242
Paramasivam (Darishiani, 2016) (1)	2016	Malaysia	Asian	PCR-RFLP	40	41	18	20	2	22	16	3	0.969
Paramasivam (2)	2016	India	Asian	PCR-RFLP	40	40	13	23	4	17	21	2	0.1606
Paramasivam (3)	2016	China	Asian	PCR-RFLP	40	40	17	20	3	16	22	2	0.1088
Mohamad (Mohamad *et al.*, 2018)	2017	Egypt	Asian	PCR-RFLP	212	170	86	96	30	92	63	15	0.3787
Sumi (Sumi *et al.*, 2017)	2017	India	Asian	PCR-RFLP	160	271	16	104	40	200	63	8	0.2739
Neamati (Neamati *et al.*, 2017)	2017	Iran	Asian	PCR-RFLP	180	310	123	50	7	207	92	11	0.8442
Barna (Barna *et al.*, 2018)	2018	India	Asian	PCR-RFLP	250	250	156	69	25	178	59	13	0.0096
Golbon (Golbon *et al.*, 2018)	2018	Iran	Asian	PCR-RFLP	240	240	60	100	80	86	83	71	0
Sharafshah (Sharafshah *et al.*, 2018)	2018	Iran	Asian	TaqMan SNP Genotyping Assays	533	437	301	215	17	296	121	20	0.0998
Albegali (Albegali *et al.*, 2019)	2019	Pakistan	Asian	PCR-RFLP	161	161	153	7	1	130	29	2	0.7912
Gohari-Lasaki (Gohari-Lasaki *et al.*, 2020)	2020	Iran	Asian	TaqMan assay	290	212	161	117	12	124	83	5	0.037

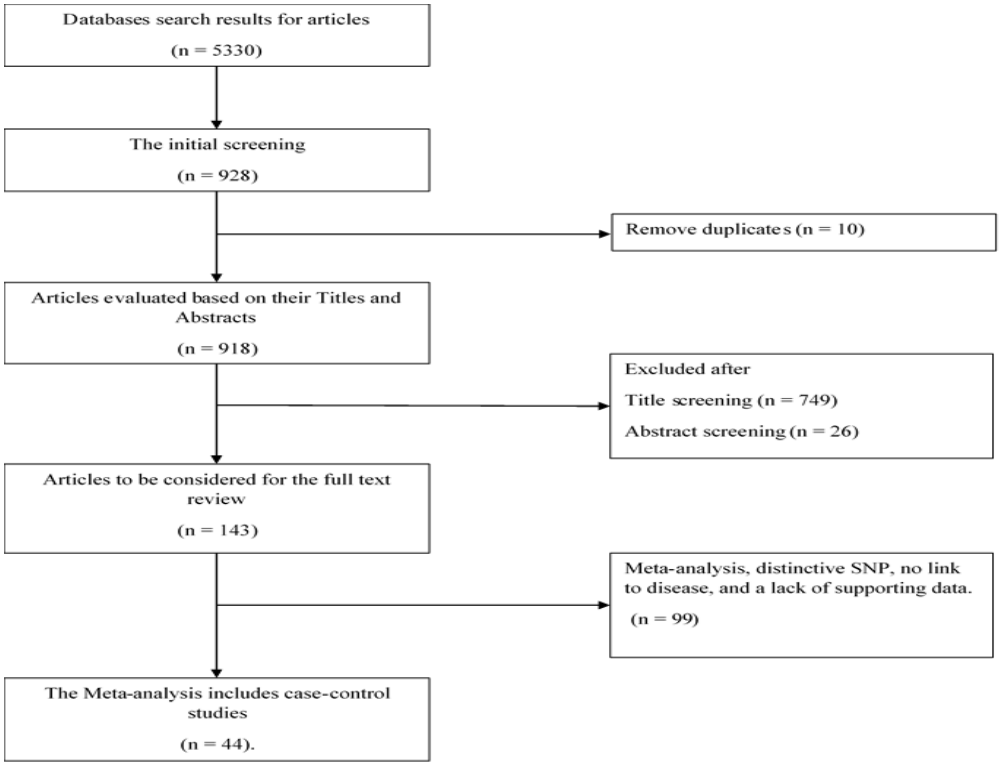

Fig. 1: Schematic representation of the literature review and research selection processes.

Meta-analysis

Table 2 and **Fig. 2** illustrated that K>Q polymorphism in ENPP1 (K121Q) is related with an enhanced risk of developing type 2 diabetes. Overall, population susceptibility to type 2 diabetes was strongly correlated with the polymorphism. Each genetic variation was linked to an enhanced chance of evolving into type 2 diabetes, and the associations were statistically significant. (QQ vs. KK: OR = 1.53, 95% CI = 1.23-1.90, P = 0.0001; KQ vs. KK: OR = 1.22, 95% CI = 1.08-1.37, P = 0.001; KQ + QQ vs. KK: OR = 1.15, 95% CI = 1.11-1.41, P = 0.0003; QQ vs. KK + KQ: OR = 1.38, 95% CI = 1.17-1.64, P = 0.0002; and Q vs. K; OR = 1.22, 95% CI = 1.10-1.36, P = 0.0003) (P<0.05). In the African population, the ENPP1 rs1044498 K>Q polymorphism was not shown to be related with an enhanced risk of evolving into type 2 diabetes, according to the subgroup analysis (P>0.05), but there was a link corresponding to the Asian population in all genetic models (**Table 2**) and the Caucasian population for four genetic models (Homozygous, Dominant, Recessive and Allelic model) where P value was less than 0.05. However, there was no evidence of any associations between the heterozygous model and type 2 diabetes in the Caucasian group (P>0.05). Egger's test and Begg-Mazumdar's test below (**Table 3**), as well as the funnel plot (**Fig. 3**), were utilized in this study to investigate whether or not each genetic model had been affected by publication bias. However, these analyses and figures did not reveal any signs of publishing bias. A leave-one-out investigation was completed to determine the impact of each individual study on the pooled odds ratios (**Fig. 4**). The results showed that the pooled effect did not change much when a study was taken out of a meta-analysis and the remaining studies were used instead. This demonstrated the stability and dependability of the meta-analysis's findings.

Table 2: Meta-analysis of the association between rs1044498 and T2DM.

Genetic model	Evaluation of associations					
	Odds Ratio (OR)	95% Confidence Interval (CI)	P value	Model	I^2 (%)	P value
Homozygous						
Overall	1.53	1.23-1.90	0.0001	Random	69%	<0.00001
Asian	1.93	1.16-3.24	0.01	Random	78%	<0.00001
Caucasian	1.29	1.12-1.49	0.0004	Fixed	38%	0.04
African	0.92	0.73-1.15	0.45	Fixed	0%	0.88
Heterozygous						
Overall	1.22	1.08-1.37	0.001	Random	85%	<0.00001
Asian	1.49	1.13-1.96	0.004	Random	92%	<0.00001
Caucasian	1.04	0.99-1.10	0.12	Fixed	22%	0.17
African	0.93	0.79-1.10	0.40	Fixed	0%	0.39
Dominant						
Overall	1.25	1.11-1.41	0.0003	Random	87%	<0.00001
Asian	1.53	1.15-2.02	0.003	Random	93%	<0.00001
Caucasian	1.06	1.01-1.12	0.02	Fixed	44%	0.01
African	0.93	0.80-1.08	0.34	Fixed	0%	0.51
Recessive						
Overall	1.38	1.17-1.64	0.0002	Random	53%	<0.0001
Asian	1.64	1.11-2.42	0.01	Random	62%	<0.0001
Caucasian	1.24	1.08-1.42	0.002	Fixed	29%	0.10
African	0.97	0.79-1.19	0.80	Fixed	0%	0.72
Allelic						
Overall	1.22	1.10-1.36	0.0003	Random	88%	<0.00001
Asian	1.40	1.09-1.79	0.008	Random	93%	<0.00001
Caucasian	1.10	1.02-1.18	0.01	Random	55%	0.001
African	0.96	0.86-1.07	0.42	Fixed	0%	0.68

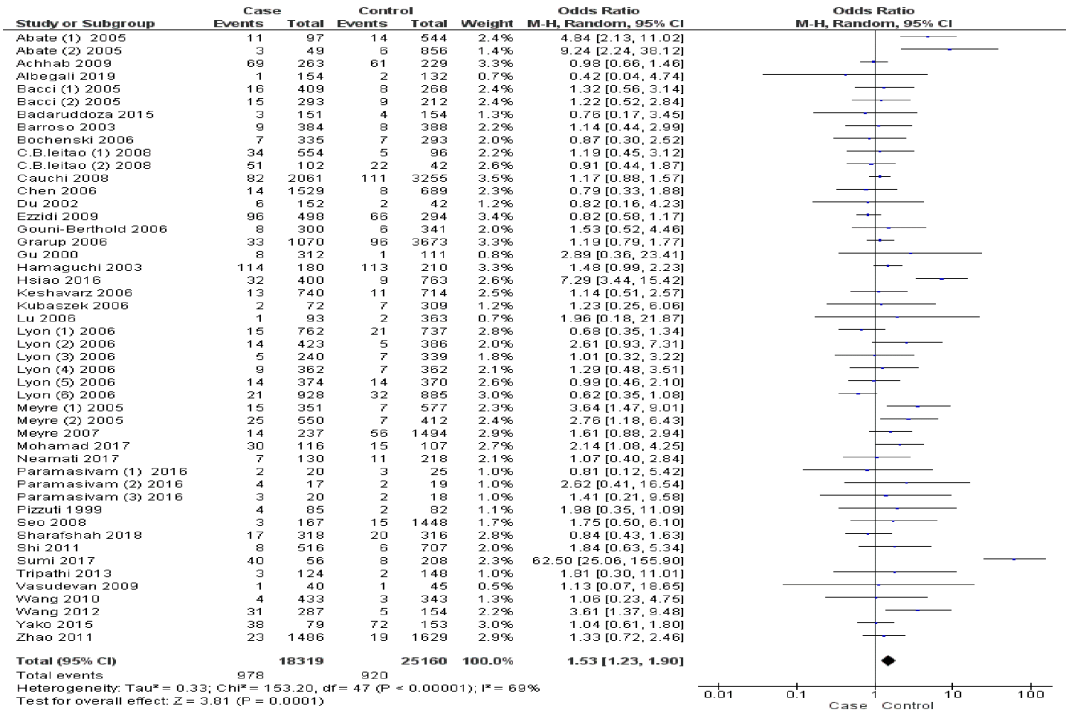

Fig. 2: Forest plot of type 2 diabetes and rs1044498 polymorphism for homozygous model (QQ vs KK).

Table 3: Evolution of Publication Bias.

Genetic model	Evaluation of Publication Bias (P value)	
	Begg-Mazumdar's test	Egger's test
Homozygous	0.081	0.079
Heterozygous	0.062	0.089
Dominant	0.057	0.069
Recessive	0.182	0.069
Allelic	0.053	0.131

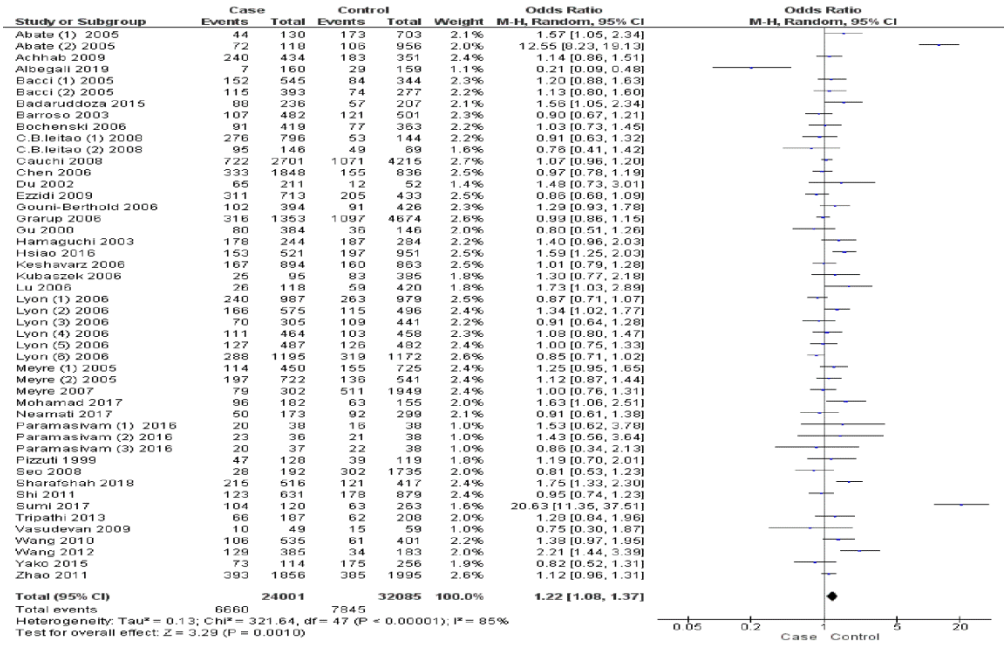

Fig. 3: Forest plot of type 2 diabetes and rs1044498 polymorphism for heterozygous model (KQ vs KK).

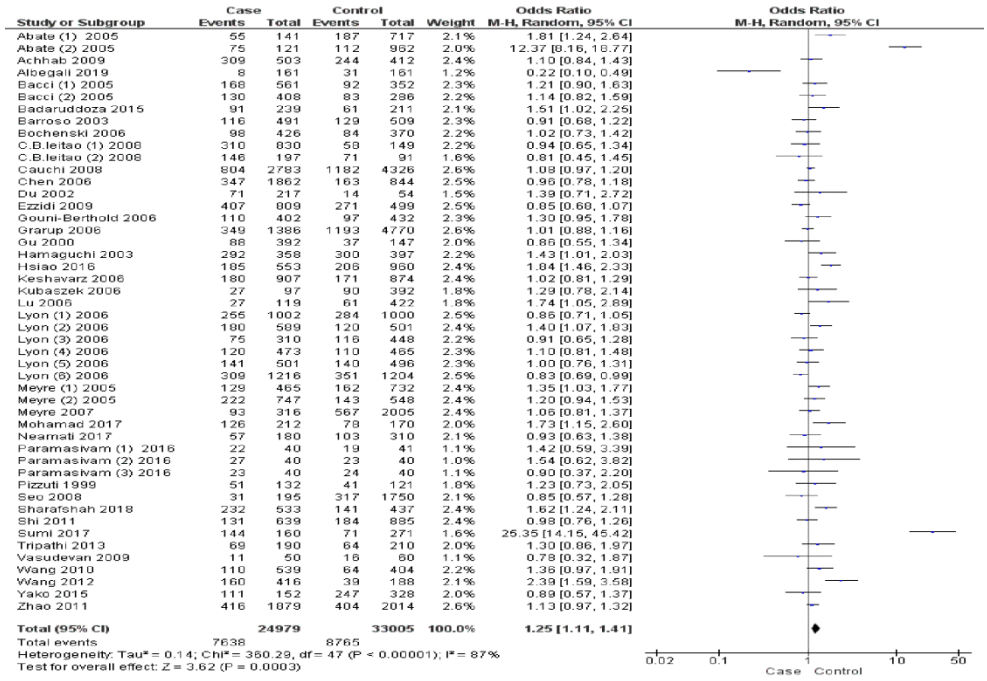

Fig. 4: Forest plot of type 2 diabetes and rs1044498 polymorphism for dominant model (KQ+QQ vs KK).

DISCUSSION:

The worldwide spread of Type 2 Diabetes Mellitus (T2DM) is a big cause for concern in terms of public health. Approximately 6.9 million adults in Bangladesh are living with diabetes. Deaths related to diabetes make up 3% of the total mortality rate in the country (Yasmin *et al.*, 2020). Probability of developing T2DMover time increases in those inflicted with insulin resistance (IR) (Bacci *et al.*, 2005). A connection between insulin resistance and the ENPP1 gene has been found by (Bacci *et al.*, 2005). Based on these findings, we choose this gene to include in this meta-analysis.

There are a total of 24979 cases representing type 2 diabetes patients and 33005 healthy people serving as controls in this study. These case-control were collected from 48 case-control studies that were published in 37 articles. According to the findings of the meta-analysis, the rs1044498 polymorphism in ENPP1 shows a noteworthy connection to type 2 diabetes. In each of the models, the rs1044498 polymorphism in ENPP1 was observed to be associated with an enhanced risk of evolving into type 2 diabetes. The odds ratio (OR) for the homozygous model was 1.53 (95% confidence interval = 1.23-1.90, P = 0.0001), while the odds ratio (OR) for the heterozygous model was 1.22 (95% confidence interval = 1.08-1.37, P = 0.001). The odds ratio (OR) for the dominant model was 1.15 (95% confidence interval = 1.11-1.41), P = 0.0003; for the recessive model, the rs1044498 polymorphism was 1.38 (95% confidence interval = 1.17-1.64), P = 0.0002; and for the allelic model, the OR was 1.22 (95% confidence interval = 1.10-1.36), P = 0.0003 (P<0.05). According to the heterogeneity (I^2) among the studies, we generated forest the plots to quantify the findings. These plots were produced using either a model with random effects or a fixed effect. If the I^2 value was lower than 50%, the fixed effect model was favored over the random effect model (DerSimonian & Laird, 1986; Higgins & Thompson, 2002; Mantel & Haenszel, 1959).

It was found that there was heterogeneity among the studies and resolved it using subgroup analysis. Except for the African population, all other models showed considerable heterogeneity. However, we recognized that a limited number of studies from African ethnicity were found from databases included in this meta-analysis (**Table 1**) and we expect that including more research from this region in future may produce a different conclusion. In every genetic model for Asian populations, the ENPP1 rs1044498 mutation was found to have a highly substantial link with type 2 diabetes. In the case of the Caucasian population, the association could be detected in the homozygous,

dominant, recessive, and allelic model; not in the heterozygous model. This confirms what has been found in prior research (McAteer *et al.*, 2008; Tang *et al.*, 2014).

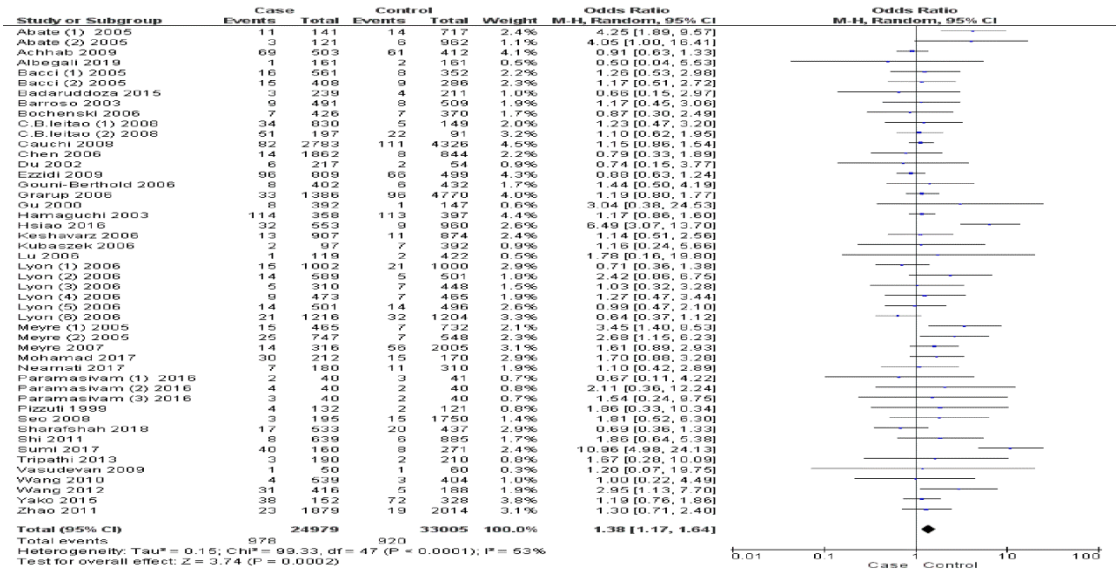

Fig. 5: Forest plot of type 2 diabetes and rs1044498 polymorphism for recessive model (QQ vs KK+KQ).

Three other meta-analysis studies on the same polymerphism issue were conducted in the distant past (Li, 2012; McAteer *et al.*, 2008; Tang *et al.*, 2014). However, there were severe limitations in those meta-analyses that we have addressed in ours. The meta-analysis study on this particular polymorphism published in 2008 was centered around only the European studies (McAteer *et al.*, 2008). Another meta-analysis was conducted in 2012 with the Chinese studies on the polymorphism only (Li, 2012). Both of these studies were conducted only on a selective ethnicity; not on all the ethnic populations available. However, a meta-analysis study in 2014 considered studies on all available ethnic studies (Tang *et al.*, 2014). They carried out a meta-analysis on 51 studies retrieved from 40 articles. But major drawbacks can be pointed out from that study. For instance, they undertook the meta-analysis with four genotypic models in view whereas in our study, we executed a meta-analysis for five genotypic models. Also, they used some insufficient study data, some of which contained no control data present at all. In our updated meta-analysis, we excluded insufficient study data so that our data are more acceptable and reliable despite being quantitatively compromised. Moreover, in our meta-analysis, the studies included are of the most recent years to allow our results to be more up-to-date and robust. Our research revealed symmetry in the funnel plot, indicating that there was no publishing bias. However, funnel plot alone is not an effective tool for assessing publication bias. We also conducted Begg Mazumdar and Egger tests and found no proof of publication bias across the analysis (P>0.05). Furthermore, sensitivity analysis demonstrated the consistency and reliability of our analysis. Because of the limited size of the sample, the connection between the ENPP1 rs1044498 variant with the African population was not established. This result may change if more genetic association studies with large sample data from African populations are incorporated, which was considered the first limitation of this meta-analysis.

However, the limitation mentioned may serve more as a direction through which the study can be further sophisticated. Including more subjects would strengthen the sample pool. To attain a more comprehensive comprehension of the connection between the ENPP1 gene and the threat of developing type 2 diabetes, it is suggested that further investigations be carried out to examine additional single nucleotide polymorphisms (SNPs) of this gene. These future studies would serve to more precisely elucidate the function of ENPP1 in the progress of type 2 diabetes and could shed light on the mechanisms by which specific SNPs of the genemay increase an individual's predisposition to developing the disease.

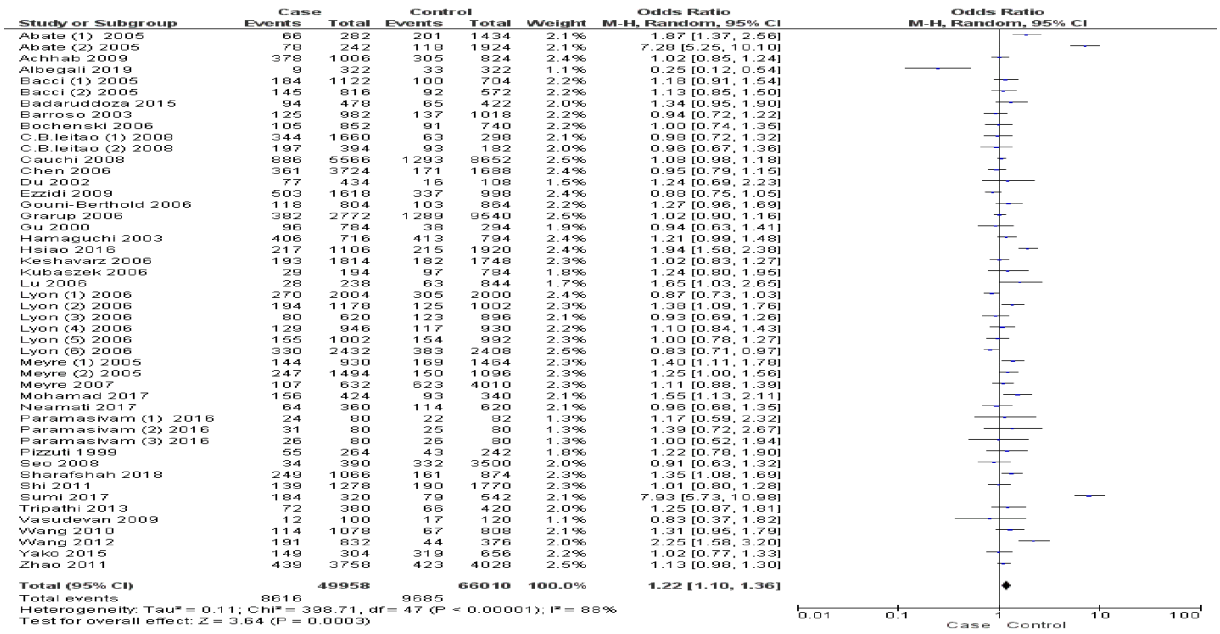

Fig. 6: Forest plot of type 2 diabetes and rs1044498 polymorphism for allelic model (Q vs K).

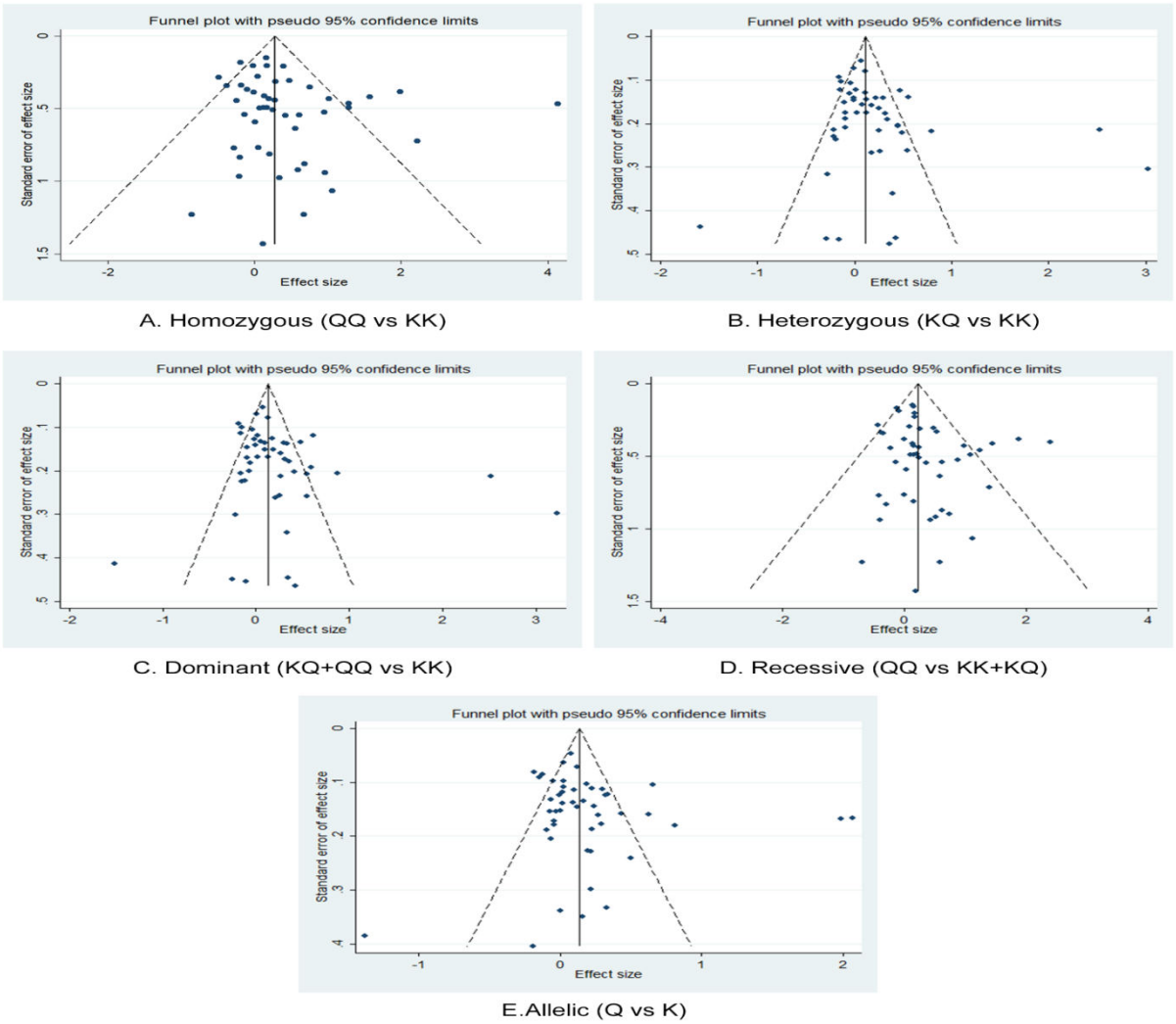

Fig. 7: Examination of publication bias using funnel plots.

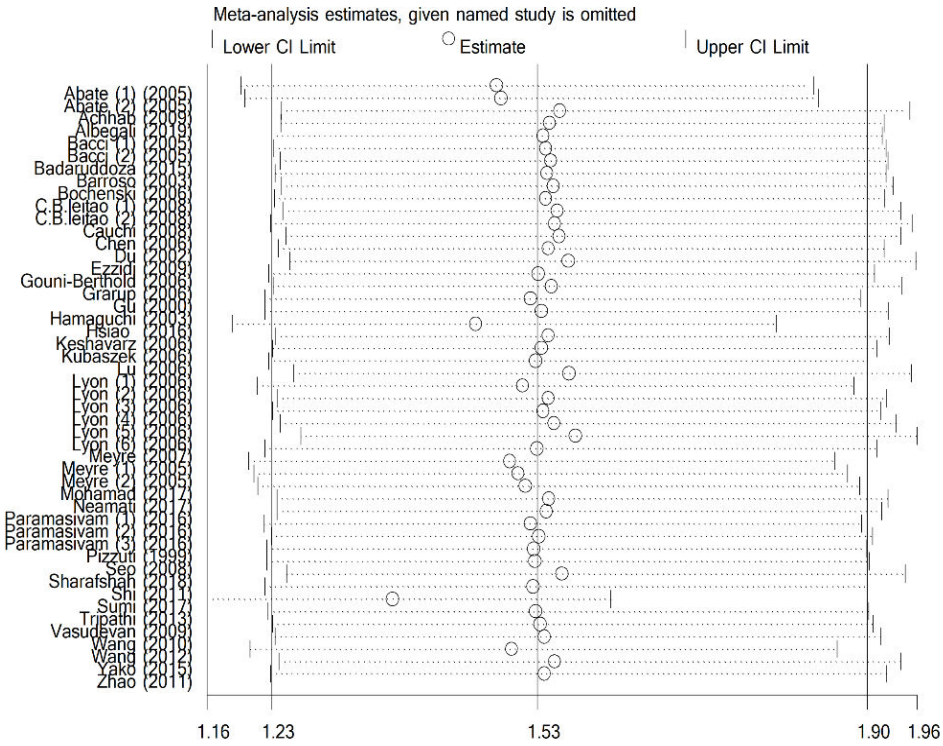

Fig. 8: Sensitivity analysis plot for the Homozygous model (QQ vs. KK) of the association between the rs1044498 polymorphism and type 2 diabetes.

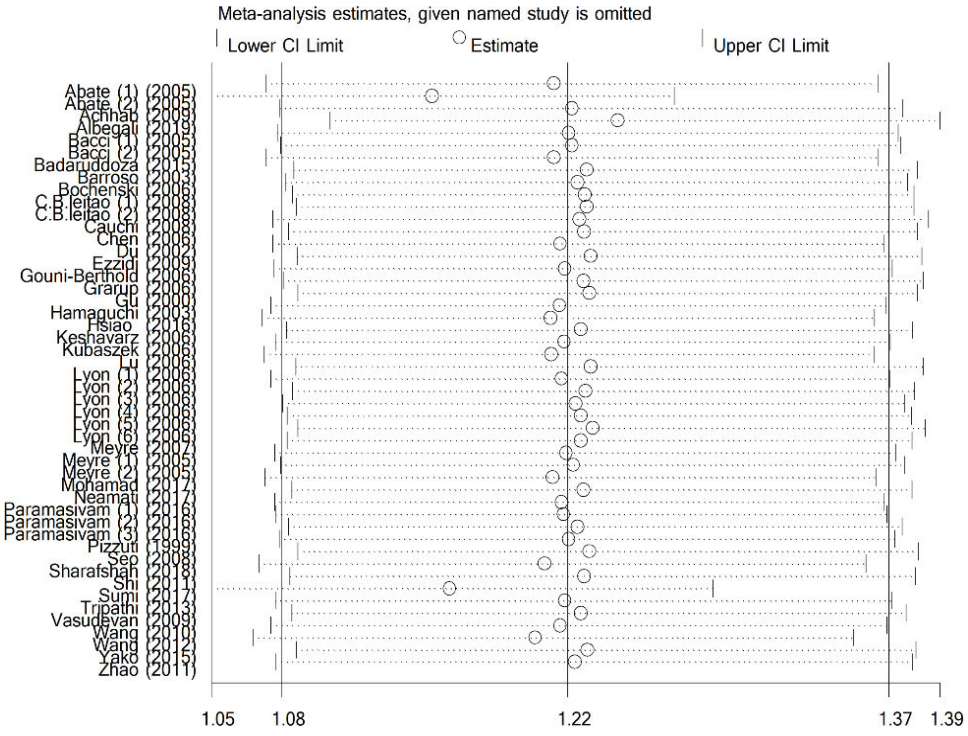

Fig. 9: Sensitivity analysis plot for the Heterozygous model (KQ vs. KK) of the association between the rs1044498 polymorphism and type 2 diabetes.

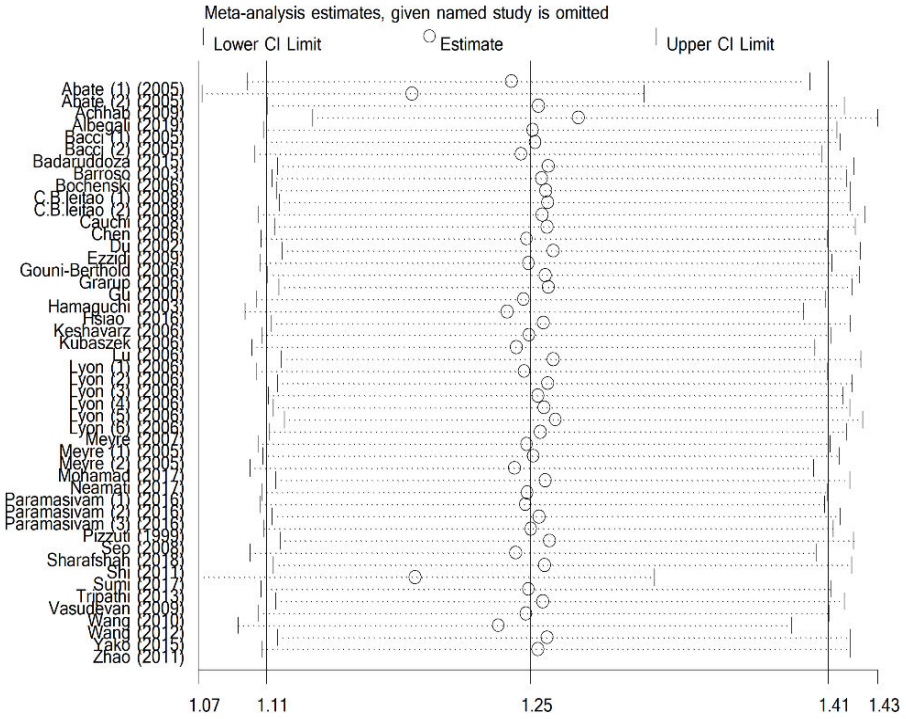

Fig. 10: Sensitivity analysis plot for the Dominant model (KQ+QQ vs. KK) of the association between the rs1044498 polymorphism and type 2 diabetes.

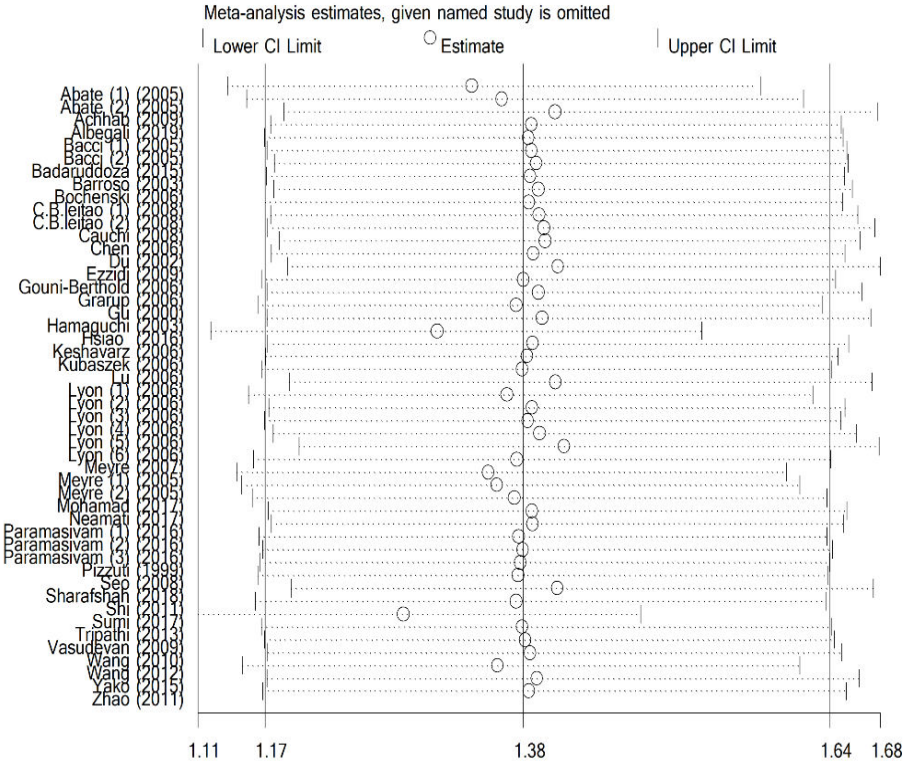

Fig. 11: Sensitivity analysis plot for the Recessive model (QQ vs. KK+KQ) of the association between the rs1044498 polymorphism and type 2 diabetes.

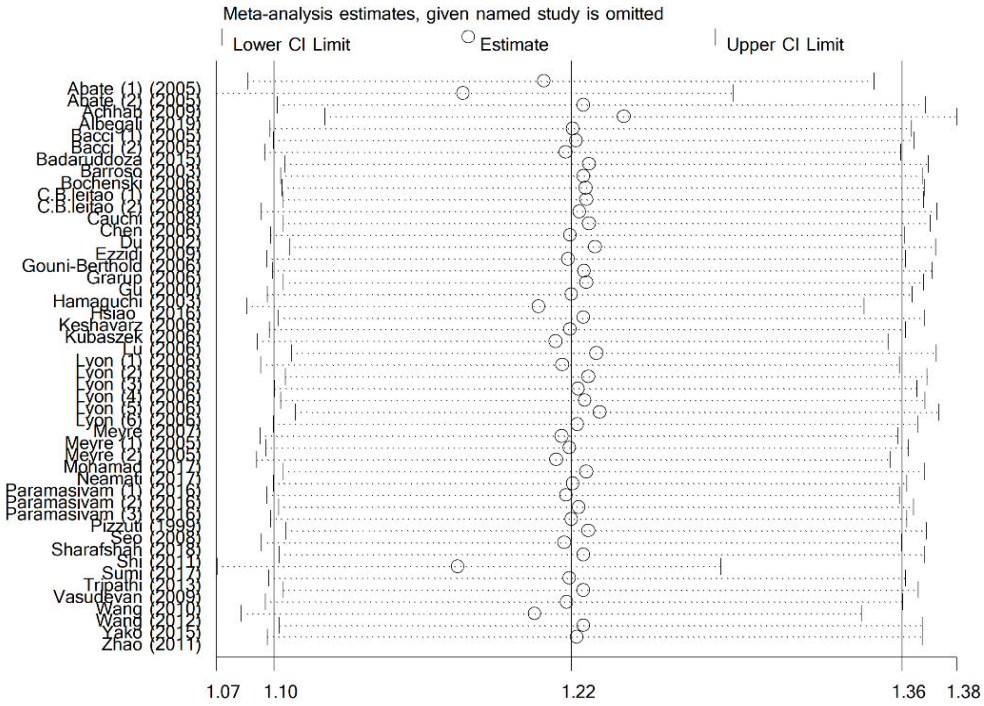

Fig. 12: Sensitivity analysis plot for the Allelic model (Q vs. K) of the association between the rs1044498 polymorphism and type 2 diabetes.

CONCLUSION:

This meta-analysis report supports the connection between the ENPP1 rs1044498 polymorphism and Type 2 diabetes, especially in Caucasian and Asian populations. The significance of the ENPP1 gene has been proposed in the initiation of Type 2 diabetes. To completely comprehend the mechanism by which this gene contributes to the onset of T2DM and to identify potential therapeutic targets, more rigorous research into the avenue is necessary. Overall, the data could help create strategies to prevent or treat T2DM in people with this genetic variant. To verify and understand this preliminary finding in the Caucasian and Asian populations, large-scale prospective studies may be required.

ACKNOWLEDGEMENT:

The authors thank to the Department of Biotechnology and Genetic Engineering, Noakhali Science and Technology University for providing the opportunity to conduct the research.

CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest.

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Citation: Akter F, Rahman I, Supti DA, Kader MA, Munim MA, Tarin RJ, Afroz S, Tonmoy MIQ, Alam MR, and Hossain MA. (2024). Investigating the impact of enpp1 gene's k121q (rs1044498) polymorphism in type 2 diabetes via an updated meta-analysis. *Am. J. Pure Appl. Sci.*, **6**(1), 1-17.
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