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The association of ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) K121Q gene polymorphism with the risk of type 2 diabetes mellitus in European, American, and African populations: A meta-analysis

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ABSTRACT

Introduction: Several studies regarding the association of the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) K121Q gene polymorphism with the risk of type 2 diabetes mellitus (T2DM) showed inconsistent results. This study aimed to investigate the association of *ENPP1* K121Q gene polymorphism with T2DM risk using meta-analysis. The study was limited to the American, European, and African populations.

Methods: PubMed and Embase databases were searched for eligible publications. The following information was extracted from each study: Name of first author, publication year, country of origin, sample size of cases and controls, and size of each allele. The combined odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between *ENPP1* K121Q gene polymorphism and T2DM risk were assessed using random or fixed effect model. A comprehensive meta-analysis (CMA) 2.0 was used to analyze the data.

Results: Nineteen studies (17717 cases/28022 controls) on the association between *ENPP1* K121Q gene polymorphism and T2DM risk were included in this meta-analysis. The results indicated that the *ENPP1* K121Q gene polymorphism was associated with increased T2DM risk (Q vs. K genetic model, OR 95% CI = 1.11 [1.02–1.22], $p = 0.014$; QQ vs. KK + KQ, OR 95% CI = 1.14 [1.01–1.23], $p = 0.039$) and decreased T2DM risk (K vs. Q, OR 95% CI = 0.90 [0.82–1.00], $p = 0.014$; KK vs. KQ + QQ, OR 95% CI = 0.89 [0.80–0.98], $p = 0.024$).

Conclusions: The results indicate that the *ENPP1* K121Q gene polymorphism is associated with the risk of T2DM in the American, European, and African populations.

Keywords: *ENPP1* K121Q gene polymorphism; type 2 diabetes mellitus; genetic polymorphism; meta-analysis; insulin resistance.INTRODUCTION

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes worldwide (1), and includes a complex group of chronic metabolic disorders characterized by hyperglycemia. This is the result of two distinct pathophysiological states: a) defects in insulin secretion caused by impaired pancreatic β-cell function and b) disruption of insulin action caused by insulin resistance in muscle, fat, and the liver (2-4). T2DM is multifactorial in origin with both genetic and environmental factors contributing to its development. The prevalence of T2DM has increased over time. T2DM affected approximately 4% of the world's adult population (1). The World Health Organization (5) estimated that the prevalence and number of people with diabetes in 2014 was 25 million in African, 62 million in American, and 64 million in European population. While DeFronzo (2) reported that approximately 15.6 million persons have T2DM, and about 13.4 million have impaired glucose tolerance in the United States (US), a study by Wild et al. (6) reported that the prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 year. The mortality number caused by T2DM has also increased. The total burden of deaths from high blood glucose1 in 2012 was estimated to be 3.7 million. This number includes 1.5 million diabetes deaths and an additional 2.2 million deaths from cardiovascular diseases, chronic kidney disease, and tuberculosis related to higher-than-optimal blood glucose. The largest number of deaths resulting from high blood glucose occurs in upper-middle income countries (1.5 million) and the lowest number in low-income countries (0.3 million). The mortality rate per 100 000 population was 111.3 in African, 72.6 in American, and 55.7 in European population. The total annual expenditure for T2DM has been increasing. Based on the cost estimates, direct annual cost of diabetes to the world is more than US$ 827 billion (5). T2DM is a complex disorder resulting from an interaction between genes and environment (7). Recently, ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) gene variants have been identified to have a significant functional role in determining susceptibility to T2DM (8).

ENPP1, also known as plasma cell alloantigen 1 (PC1), is an enzyme involved primarily in hydrolysis of ATP at the cell surface (9). The enzyme is highly expressed in adipocytes and is also found in other tissues involved in glucose and lipid metabolism, including liver, skeletal muscle, and β-cells of the pancreas (10). ENPP1 is a transmembrane glycoprotein that down-regulates insulin signaling in cells by inhibiting the tyrosine kinase activity of the insulin receptor (IR), perhaps by interaction with its α-subunit (11). The *ENPP1* is a promising candidate gene for T2DM because it inhibits autophosphorylation of IR and impairs insulin signaling downstream of IR (12). A study found that the 121Q variant had a greater inhibitory action on IR compared to the 121K variant (13).

Several polymorphism studies have shown the association between the *ENPP1* K121Q gene polymorphism and the risk of T2DM, however the results were inconsistent. Studies conducted by Rasmussen et al. (14), Abate et al. (15), Meyre et al. (16), Chandalia et al. (17), Willer et al. (18), Bouhaha et al. (19), and Leitao et al. (20) showed that the *ENPP1* K121Q gene polymorphism was associated with increased risk of T2DM. Whereas several other studies (21-32) showed that the *ENPP1* K121Q gene polymorphism had no significant association with increased risk of T2DM. A meta-analysis study is required to determine the actual association of the above-mentioned studies.

This study aimed to investigate the association between the *ENPP1* K121Q gene polymorphism and the risk of T2DM using meta-analysis. The results of this study are expected to be useful for the future treatment and prevention of T2DM. Besides, the study is also expected to be useful as a comparison to other studies on the *ENPP1* K121Q gene polymorphism and T2DM.

METHODS

Study designs

A meta-analysis was conducted to assess the association of the *ENPP1* K121Q gene polymorphism with the risk of T2DM. To achieve this goal, several studies regarding the association between the *ENPP1* K121Q gene polymorphism and the risk of T2DM were collected for calculating combined odds ratio (OR) 95% confidence interval (CI) and

assessed using fixed or random effect model. Article search was conducted in Pubmed and Embase. The study was conducted from January to May, 2016.

Study procedures

The procedures of this study were (1) to identify potentially relevant studies through Pubmed and Embase database search, up to April 20th, 2016, (2) to determine eligibility of the study; the exclusion was performed using several steps, i.e.: (a) by reading the title and abstract, (b) study designs complied with the inclusion criteria, (c) study provided sufficient data to calculate OR 95% CI, (3) collecting abstract and full text data from the studies, (4) collecting the data for calculating OR 95% CI, and (4) analyzing data statistically.

Eligibility criteria and data extraction

The eligibility criteria consisted of predefined inclusion and exclusion criteria. Studies were included in the analysis if they met the following inclusion criteria: (1) case-control; (2) cohort; (3) cross-sectional studies; (4) randomized-controlled trials (RCTs); (5) controlled before-and-after studies; (6) cross-over studies; (7) evaluating the associations of the *ENPP1* K121Q gene polymorphism with the risk of T2DM; and (8) providing sufficient data for calculation of OR 95% CI. Required data were extracted from each study for calculating OR 95% CI. In addition, the following information was extracted from each study: (1) name of first author; (2) year of publication; (3) country of origin; (4) sample size of cases and controls, (5) size of each allele.

Search strategy and literature

PubMed and Embase were searched with no language restrictions, using specified search terms to identify studies published up to April 20th, 2016. The search strategy involved the use of combination of the following key words: (ENPP1 K121Q gene or ectonucleotide pyrophosphatase/phosphodiesterase 1 K121Q gene) and (variant or variation or polymorphism) and (T2DM or type 2 diabetes mellitus). The publication languages were restricted to English. The reference lists of retrieved articles were hand searched. If more than one article was published using the same study data, only the study with the largest sample size was included. We used a scoring system to evaluate the quality of the studies. We used a quality assessment score modified from previous meta-analysis for observational studies. Total scores ranged from 0 (worst) to 9 (best). A study was considered low quality if scores were <6, and high quality if scores were ≥6 (33).

Study variables

1. ENPP1 K121Q gene

ENPP1 is a transmembrane glycoprotein that down-regulates insulin signaling in cells by inhibiting the tyrosine kinase activity of the insulin receptor (11). The measurement results of this variable were each allele in ENPP1 K121Q gene including dominant (K), recessive (Q), dominant homozygous (KK), recessive homozygous (QQ), and heterozygous (KQ). Data were obtained by searching strategy. Nominal scale was used to assess this variable.

2. Risk of T2DM

T2DM is a complex group of chronic metabolic disorders characterized by two distinct pathophysiological states: a) defects in insulin secretion caused by impaired pancreatic β-cell function and b) disruption of insulin action caused by insulin resistance in muscle, fat, and the liver (2-4). The measurement results of this variable were increased or decreased risk of T2DM. The data were obtained by searching strategy. Nominal scale was used to assess this variable.

Statistical analysis

The correlation of the *ENPP1* K121Q gene polymorphism with the risk of T2DM was estimated by calculating pooled ORs and 95% CI. The significance of pooled ORs was determined by Z tests ($p < 0.05$ was considered statistically significant). A Q test was performed to evaluate whether the heterogeneity existed. Random effect model was used to calculate OR 95% CI if heterogeneity existed ($p < 0.10$). Fixed effect model was used to calculate OR 95% CI if no heterogeneity existed. Publication bias was assessed by Egger's test ($p < 0.05$ was considered statistically significant). Subgroup analyses based on the continent (Europe, America,

and Africa) and sample size (small <400, large ≥400 samples) were also performed. A comprehensive meta-analysis (CMA) 2.0 was used to analyze the data.

RESULTS

Characteristics of the studies

A total of 72 potentially relevant papers were identified based on the search strategy. Among these, 24 papers were excluded because of obvious irrelevance by reading their titles and abstracts. After the full texts were read, 20 papers were excluded because the population was not American, European, or African; three papers were excluded because they did not provide sufficient data for calculating OR with 95% CI. In addition, six reviews were excluded. A flow chart demonstrating the inclusion or exclusion of the studies is displayed as Figure 1. A total of 19 studies were included in the meta-analysis. Twelve studies included European, four studies included American, and three studies included African population. Table 1 describes the characteristics of the studies included in the meta-analysis.

Quantitative data synthesis

A total of 17717 cases and 28022 controls were identified. Overall, the results showed that K vs. Q, KK vs. KQ + QQ, Q vs. K, and QQ vs. KK + KQ genetic models had significant association with the risk of T2DM. While, KQ vs. KK + QQ genetic model had no significant association with the risk of T2DM. The results indicated that the *ENPP1* K121Q gene polymorphism was associated with increased risk (Q vs. K, OR 95% CI = 1.11 [1.02–1.22], $p = 0.014$; QQ vs. KK + KQ, OR 95% CI = 1.14 [1.01–1.23], $p = 0.039$) and decreased risk of T2DM (K vs. Q, OR 95% CI = 0.90 [0.82–1.00], $p = 0.014$; KK vs. KQ + QQ, OR 95% CI = 0.89 [0.80–0.98], $p = 0.024$). Forest plot regarding the correlation of *ENPP1* K121Q gene polymorphism with T2DM is described in Figure 2 for K vs. Q genetic model. Summary ORs and 95% CIs regarding the correlation of *ENPP1* K121Q gene

TABLE 1. Characteristics of studies included in the meta-analysis

Author & Year	Country	Age (years±SD)	Continent	SS	NS		T2DM genotype			Cont genotype		
					T2DM	Cont	KK	KQ	QQ	KK	KQ	QQ
Pizzuti *et al*. 1999	Italy	37.4±13.0	Europe	S	132	121	81	47	4	80	39	2
Gu *et al*. 2000	Italy	54.5±14.1	Europe	L	392	147	304	80	8	110	36	1
Rasmussen *et al*. 2000	Denmark	37.8±8.8	Europe	L	226	356	147	70	9	263	86	7
Barroso *et al*. 2003	England	63.5±0.5	Europe	L	97	392	70	25	2	302	83	7
Kubaszek *et al*. 2004	Finland	69.7±2.8	Europe	S	165	98	143	22	0	85	12	1
Abate *et al*. 2005	USA	47.6±11.7	America	L	485	2099	301	163	21	1484	561	54
Meyre *et al*. 2005	Austria-France	40.4±8.7	Europe	L	1308	900	918	349	41	665	220	15
Bacci *et al*. 2005	Italy	63.0±8.0	Europe	L	2270	1130	1793	448	29	884	229	17
Grarup *et al*. 2006	Denmark	45.9±8.1	Europe	L	1386	4770	1037	316	33	3577	1097	96
Bochenski *et al*. 2006	Poland	54.2±12.1	Europe	L	1386	4770	1037	316	33	3577	1097	96
Lyon *et al*. 2006	USA	59.1±9.7	America	L	4204	4227	3094	1032	78	3079	1062	86
Weedon *et al*. 2006	England	41.0±9.2	Europe	L	2287	3846	1691	554	42	2842	949	55
Chandalia *et al*. 2007	USA	43.1±8.0	America	L	116	967	58	54	4	731	216	20
Willer *et al*. 2007	Finland	63.9±9.8	Europe	L	1155	971	853	268	34	755	193	23
Meyre *et al*. 2007	France	47.0±9.0	Europe	L	316	2005	223	79	14	1438	511	56
Bouhaha *et al*. 2008	Tunisia	52.2±9.6	Africa	S	110	243	32	61	17	106	103	34
Leitao *et al*. 2008	Brazil	56.9±10.0	America	L	1027	240	571	371	85	111	102	27
El-Achhab *et al*. 2009	Morocco	44.5±9.7	Africa	L	503	412	194	240	69	168	183	61
Yako *et al*. 2015	South Africa	56.5±12.6	Africa	L	152	328	41	73	38	81	175	72

USA: United States of America, SS: Sample size, L: Large (≥ 400 samples), S: Small (< 400 samples), NS: Number of samples, T2DM: Type 2 diabetes mellitus, Cont: Control

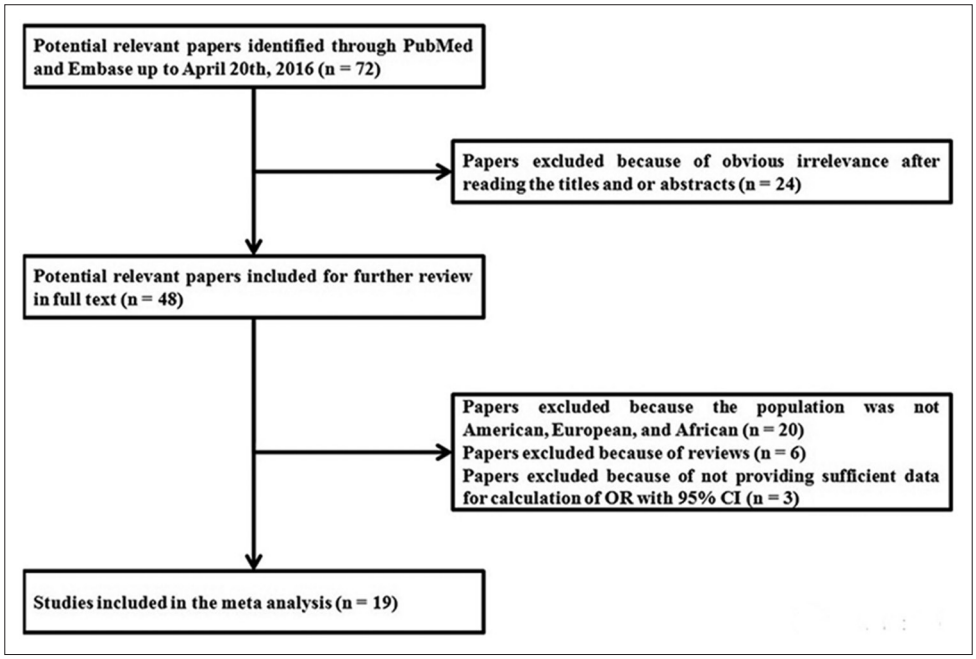

FIGURE 1. Selection of articles for inclusion in the meta-analysis

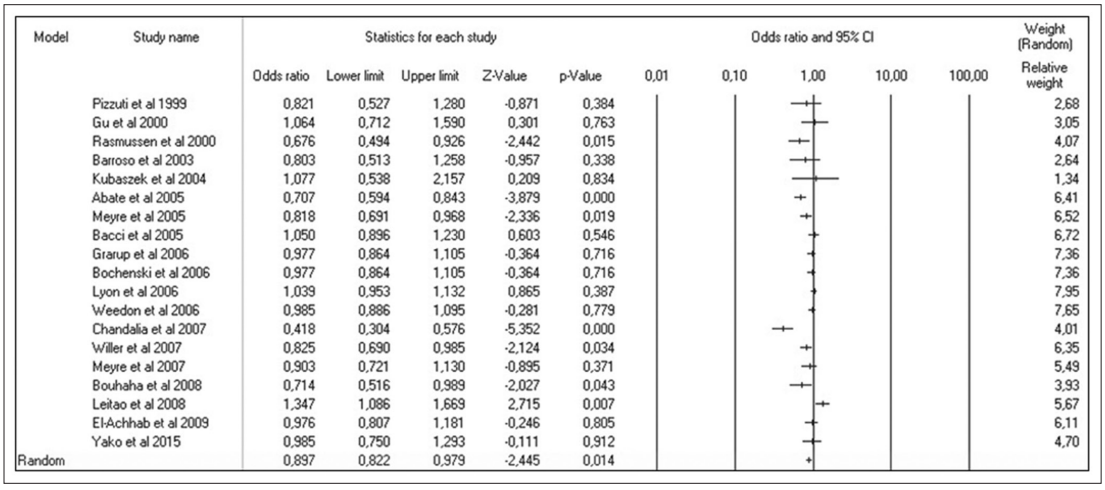

FIGURE 2. Meta-analysis of the association between the ENPP1 K121Q gene polymorphism and the risk of T2DM (K vs. Q allele)

polymorphism and T2DM are described in Table 2. In the subgroup analysis, the *ENPP1* K121Q gene polymorphism was associated with the risk of T2DM in three genetic models of the Europe continent subgroup (K vs. Q p = 0.019; Q vs. K p = 0.019; QQ vs. KK + KQ p = 0.005), in two genetic models of the small sample subgroup (KK vs. KQ + QQ p = 0.029; KQ vs. KK + QQ p = 0.045), and in three genetic models of the large sample subgroup (K vs. Q p = 0.033; Q vs. K p = 0.033; QQ vs. KK + KQ p = 0.043). However, the *ENPP1* K121Q gene polymorphism had no significant association with the risk of T2DM in two genetic models of the Europe continent subgroup (KK vs. KQ + QQ p = 0.095; KQ vs. KK + QQ p = 0.453), all genetic models of the America continent subgroup (K vs.

TABLE 2. Summary ORs and 95% CIs of the association between the *ENPP1* K121Q gene polymorphism and the risk of T2DM

No.	Alleles	Parameter	All	Continent			Sample size	
				Europe	America	Africa	S	L
1	K vs. Q	OR	0.90	0.94	0.82	0.92	0.78	0.90
		95% CI	0.82-1.00	0.89-0.99	0.57-1.17	0.80-1.06	0.61-1.00	0.83-0.99
		p	0.014	0.019	0.276	0.264	0.053	0.033
		p_H	< 0.001	0.245	< 0.001	0.230	0.560	< 0.001
		p_E	0.152	0.048	0.353	0.090	< 0.001	0.154
2	KK vs. KQ+QQ	OR	0.89	0.95	0.78	0.84	0.70	0.90
		95% CI	0.80-0.98	0.90-1.01	0.50-1.21	0.58-0.94	0.51-0.96	0.81-1.00
		p	0.024	0.095	0.263	0.346	0.029	0.061
		p_H	< 0.001	0.303	< 0.001	0.064	0.291	< 0.001
		p_E	0.181	0.044	0.430	0.262	0.140	0.178
3	KQ vs. KK+QQ	OR	1.10	1.02	1.29	1.14	1.37	1.08
		95% CI	0.99-1.21	0.96-1.08	0.86-1.94	0.80-1.64	1.01-1.88	0.98-1.19
		p	0.062	0.453	0.217	0.472	0.045	0.136
		p_H	< 0.001	0.505	< 0.001	0.051	0.465	< 0.001
		p_E	0.163	< 0.001	0.391	0.258	< 0.001	0.162
4	Q vs. K	OR	1.11	1.06	1.22	1.08	1.27	1.10
		95% CI	1.02-1.22	1.01-1.12	0.85-1.75	0.94-1.25	1.00-1.63	1.01-1.21
		p	0.014	0.019	0.276	0.264	0.053	0.033
		p_H	< 0.001	0.245	< 0.001	0.230	0.560	< 0.001
		p_E	0.152	0.048	0.353	0.090	< 0.001	0.154
5	QQ vs. KK+KQ	OR	1.14	1.29	1.07	1.03	1.12	1.14
		95% CI	1.01-1.23	1.08-1.54	0.71-1.60	0.80-1.34	0.63-2.01	1.00-1.29
		p	0.039	0.005	0.752	0.804	0.693	0.043
		p_H	0.350	0.765	0.056	0.659	0.481	0.251
		p_E	0.085	< 0.001	0.310	< 0.001	< 0.001	0.120

OR: Odds ratio, CI: Confidence interval, *p:* *p* value based on between-study Z test, p_H: *p* value based on Q test between-study heterogeneity, p_E: *p* value based on between-study Egger's test, L: Large (≥ 400 samples), S: Small (< 400 samples), T2DM: Type 2 diabetes mellitus

Q p = 0.276; KK vs. KQ + QQ p = 0.263; KQ vs. KK + QQ p = 0.217; Q vs. K p = 0.276; QQ vs. KK + KQ p = 752), all genetic models of the Africa continent subgroup (K vs. Q p = 0.264; KK vs. KQ + QQ p = 0.346; KQ vs. KK + QQ p = 0.472; Q vs. K p = 0.264; QQ vs. KK + KQ p = 804), three genetic models of the small sample subgroup (K vs. Q p = 0.053; Q vs. K p = 0.053; QQ vs. KK + KQ p = 0.693), and two genetic models of the large sample subgroup (KK vs. KQ + QQ p = 0.061; KQ vs. KK + QQ p = 0.136).

Source of heterogeneity

Evidence for heterogeneity (p < 0.10) between studies was found in four genetic models (K vs. Q p_H < 0.001; KK vs. KQ + QQ p_H < 0.001; KQ vs. KK + QQ p_H < 0.001; Q vs. K p_H < 0.001) and one genetic model had no heterogeneity (QQ vs. KK + KQ p_H = 0.350). Therefore, four genetic models in this study were assessed using random effect model, and one genetic model was assessed using fixed effect model. Summary evidence of heterogeneity regarding the correlation of *ENPP1* K121Q gene polymorphism with T2DM is described in Table 2. In the subgroup analysis, evidence for heterogeneity was found in all genetic models of the America continent subgroup (K vs. Q p_H < 0.001; KK vs. KQ + QQ p_H < 0.001; KQ vs. KK + QQ p_H < 0.001; Q vs. K p_H < 0.001; QQ vs. KK + KQ p_H = 0.056), two genetic models of the Africa continent subgroup

(KK vs. KQ + QQ p_H = 0.064; KQ vs. KK + QQ p_H = 0.051), four genetic models of the large sample subgroup (K vs. Q p_H < 0.001; KK vs. KQ + QQ p_H < 0.001; KQ vs. KK + QQ p_H < 0.001; Q vs. K p_H < 0.001). Therefore, random effect model was used to calculate OR 95% CI in these subgroups. While, no evidence for heterogeneity was found in all genetic models of the Europe continent subgroup (K vs. Q p_H = 0.245; KK vs. KQ + QQ p_H = 0.303; KQ vs. KK + QQ p_H = 0.505; Q vs. K p_H = 0.245; QQ vs. KK + KQ p_H = 0.765), three genetic models of the Africa continent subgroup (K vs. Q p_H = 0.230; Q vs. K p_H = 0.230; QQ vs. KK + KQ p_H = 0.659), all genetic models of the small sample subgroup (K vs. Q p_H = 0.560; KK vs. KQ + QQ p_H = 0.291; KQ vs. KK + QQ p_H = 0.465; Q vs. K p_H = 0.560; QQ vs. KK + KQ p_H = 0.481), and one genetic model of the large sample size subgroup (QQ vs. KK + KQ p_H = 0.251). Therefore, fixed effect model was used to calculate OR 95% CI in these subgroups.

Potential publication bias

Using Egger's test, no publication bias was detected (K vs. Q p_E = 0.152; KK vs. KQ + QQ p_E = 0.181; KQ vs. KK + QQ p_E = 0.163; Q vs. K p_E = 0.152; QQ vs. KK + KQ p_E = 0.085). Summary Egger's test regarding the correlation of *ENPP1* K121Q gene polymorphism with T2DM is described in Table 2. In the subgroup analysis, no publication bias was detected in all genetic models of the America continent subgroup (K vs. Q p_E = 0.353; KK vs. KQ + QQ p_E = 0.430; KQ vs. KK + QQ p_E = 0.391; Q vs. K p_E = 0.353; QQ vs. KK + KQ p_E = 0.310), four genetic models of the Africa continent subgroup (K vs. Q p_E = 0.090; KK vs. KQ + QQ p_E = 0.262; KQ vs. KK + QQ p_E = 0.258; Q vs. K p_E = 0.090), one genetic model of the small sample size subgroup (KK vs. KQ + QQ p_E = 0.140), and all genetic models of the large sample size subgroup (K vs. Q p_E = 0.154; KK vs. KQ + QQ p_E = 0.178; KQ vs. KK + QQ p_E = 0.162; Q vs. K p_E = 0.154; QQ vs. KK + KQ p_E = 0.120). On the other hand, publication bias was detected in all genetic models of the Europe continent subgroup (K vs. Q p_E = 0.048; KK vs. KQ + QQ p_E = 0.044; KQ vs. KK + QQ p_E < 0.001; Q vs. K p_E = 0.048; QQ vs. KK + KQ p_E < 0.001), one genetic model of the Africa continent subgroup (QQ vs. KK + KQ p_E < 0.001), and four genetic models of the small sample size subgroup (K vs. Q p_E < 0.001; KQ vs. KK + QQ p_E < 0.001; Q vs. K p_E < 0.001; QQ vs. KK + KQ p_E < 0.001).

DISCUSSION

Overexpression of ENPP1 has been implicated in the pathology of a number of diseases, including T2DM. ENPP1 is a transmembrane glycoprotein that inhibits the tyrosine kinase activity of IR and down-regulates insulin signaling in cells (11) through inhibiting the autophosphorylation of IR and impairing insulin signaling downstream of IR (12). A study found that the 121Q variant has a greater inhibitory action on IR than does the 121K variant (13). Because of the effects of ENPP1 on IR signaling, a series of studies have focused on the contribution of polymorphisms within *ENPP1* cluster genes to the T2DM risk. However, results have been contradictory. This study reported the association of *ENPP1* K121Q gene polymorphism with T2DM risk, although the power of this meta-analysis was limited due to the size and heterogeneity of studies.

The results indicated that the *ENPP1* K121Q gene polymorphism was associated with increased (Q vs. K, OR 95% CI = 1.11 [1.02–1.22], p = 0.014; QQ vs. KK + KQ, OR 95% CI = 1.14 [1.01–1.23], p = 0.039) and decreased T2DM risk (K vs. Q, OR 95% CI = 0.90 [0.82–1.00], p = 0.014; KK vs. KQ + QQ, OR 95% CI = 0.89 [0.80–0.98], p = 0.024). Summary of ORs 95% CIs, correlation, heterogeneity, and Egger's test regarding the correlation of *ENPP1* K121Q gene polymorphism with T2DM is described in Table 2, while study characteristics are described in Table 1. Forest plot regarding the correlation of *ENPP1* K121Q gene polymorphism with T2DM is described in Figure 2 for K vs. Q model. Previous similar meta-analysis studies reported the correlation of *ENPP1* K121Q gene polymorphism with the risk of T2DM in distinct populations. Li (34) conducted a meta-analysis study regarding the association of the *ENPP1* K121Q gene polymorphism with the risk of T2DM in Chinese population. He found that the *ENPP1* K121Q gene polymorphism was in correlation with T2DM susceptibility (OR 95% CI = 1.29 [1.09–1.53] p = 0.003) and the Q allele of the *ENPP1*

K121Q gene might be the risk factor for T2DM in the Chinese population. Jing et al. (35) conducted a study regarding the association of polymorphisms in five candidate genes with the risk of T2DM in Chinese population. They found that the Q allele of *ENPP1* K121Q gene increased the risk of T2DM in Chinese population (OR 95% CI = 1.41 [1.13–1.76] p = 0.003). However, they did not provide the results of various genetic model analyses. It was probably because of the fact that they analyzed five genes. Therefore, the results of each gene analysis was incomplete. Wang et al. (36) conducted a study regarding the association of *ENPP1* K121Q gene polymorphism with the risk of T2DM in Chinese population. They showed that the Q allele of *ENPP1* K121Q gene was significantly associated with the risk of T2DM in Chinese population (OR 95% CI = 1.50 [1.39–1.62] p < 0.001). However, they only studied the Chinese population, and several references on Chinese population were difficult to find because most of them published their studies in China National Knowledge Infrastructure (CNKI). Ruoqi et al. (37) conducted a study regarding the correlation of *ENPP1* K121Q gene polymorphism with the risk of obesity in European population. They showed that the *ENPP1* K121Q gene polymorphism correlated with the risk of obesity (OR 95% CI = 1.25 [1.04–1.52] p = 0.021). McAteer et al. (38) conducted a study on the association between *ENPP1* K121Q gene polymorphism and the risk of T2DM in European population. They showed that the Q allele of *ENPP1* K121Q gene increased the risk of T2DM (OR 95% CI = 1.38 [1.10–1.74] p = 0.005). However, they only studied the European population. Therefore, a study with a larger population scale is required to determine more precise association. Tang et al. (39) conducted a study regarding the association of *ENPP1* K121Q gene polymorphism with the risk of T2DM in different populations. They found that the Q allele of the *ENPP1* K121Q gene may contribute to the susceptibility to T2DM in Caucasians and Asians (OR 95% CI = 1.29 [1.16–1.44] p < 0.001). However, there were several error calculations. Summary of meta-analysis studies on the association between *ENPP1* K121Q gene polymorphism and the risk for T2DM in different areas is described in Table 3. Our study reported the correlation of *ENPP1* K121Q gene polymorphism with the risk of T2DM in different populations. Furthermore, in the subgroup analysis, the *ENPP1* K121Q gene polymorphism was associated with the risk of T2DM in three genetic models of the Europe continent subgroup (K vs. Q p = 0.019; Q vs. K p = 0.019; QQ vs. KK + KQ p = 0.005), two genetic models of the small sample subgroup (KK vs. KQ + QQ p = 0.029; KQ vs. KK + QQ p = 0.045), and three genetic models of the large sample subgroup (K vs. Q p = 0.033; Q vs. K p = 0.033; QQ vs. KK + KQ p = 0.043). The *ENPP1* K121Q gene polymorphism had no significant association with the risk of T2DM in two genetic models of the Europe continent subgroup (KK vs. KQ + QQ p = 0.095; KQ vs. KK + QQ p = 0.453), all genetic models of the America continent subgroup (K vs. Q p = 0.276; KK vs. KQ + QQ p = 0.263; KQ vs. KK + QQ p = 0.217; Q vs. K p = 0.276; QQ vs. KK + KQ p = 752), all genetic models of the Africa continent subgroup (K vs. Q p = 0.264; KK vs. KQ + QQ p = 0.346; KQ vs. KK + QQ p = 0.472; Q vs. K p = 0.264; QQ vs. KK + KQ p = 804), three genetic models of the small sample subgroup (K vs. Q p = 0.053; Q vs. K p = 0.053; QQ vs. KK + KQ p = 0.693), and two genetic models of the large sample subgroup (KK vs. KQ + QQ p = 0.061; KQ vs. KK + QQ p = 0.136). However, these results should be interpreted with caution because the relatively small sample size or multiple testing could drive false positive findings.

The results of the study also showed the tendency of the Q allele of *ENPP1* K121Q gene to correlate with an increased risk of T2DM, while the K allele of *ENPP1* K121Q gene correlated with a reduced risk of T2DM. The mechanism underlying these results is complex and difficult to explain. However, it is alleged that the mechanism involving ENPP1 and insulin resistance has an important role in the process. Although the primary factors causing T2DM are unknown, it is clear that insulin resistance plays a major role in T2DM development (40,41). The mechanism of insulin resistance is a complex, involving several genes including *ENPP1*. The role of ENPP1 in T2DM is associated with impaired insulin signaling. ENPP1 binds to IR molecule, thereby inhibiting insulin-induced conformational change (42) that leads to IR autophosphorylation and tyrosine kinase activation (25,43). Inhibition

TABLE 3. Summary of meta-analysis studies regarding the association between the *ENPP1* K121Q gene polymorphism and the risk of T2DM in different areas

Author & Year	Cases/controls	Population	OR [95% CI]	*p*	Comments
McAteer *et al.* 2008	15801/26241	Europe	1.38 [1.10-1.74]	0.005	Only European population, need larger sample size
Wang *et al.* 2010	539/404	China	1.50 [1.39-1.62]	0.001	The Chinese references were difficult to find
Ruoqi *et al.* 2011	11372/12952	Europe	1.25 [1.04-1.52]	0.021	Only European population, need larger sample size
Li 2012	6362/5493	China	1.29 [1.09-1.53]	0.003	The Chinese references were difficult to find
Jing *et al.* 2012	NA	China	1.41 [1.13-1.76]	0.003	The Chinese references were difficult to find
Tang *et al.* 2014	24348/32613	Mix	1.29 [1.16-1.44]	0.001	There were several error calculations
Our results	17717/28022	Europe, America, Africa	1.11 [1.02-1.22]	0.014	

OR: Odds ratio, CI: Confidence interval, *p*: Significance, NA: Not available

of this conformational change is caused by protein interaction. The change in the exon 4 of *ENPP1* gene which leads to a lysine (K) to glutamine (Q) substitution in codon 121 (K121Q) might influence protein-protein interactions. Furthermore, the Q allele of *ENPP1* K121Q gene has been shown to influence ENPP1 protein function by inhibiting insulin receptor function and insulin signaling (44). This is because the 121Q variant binds to IR with higher affinity than the 121K variant (25) and is more potent in inhibiting IR autophosphorylation (43). This might explain the results of this study which indicated that the Q allele of *ENPP1* K121Q gene correlated with an increased risk of T2DM.

There were several limitations in the meta-analysis. First, this analysis was primarily based on unadjusted effect estimates. Therefore, the potential covariates including age, gender, body mass index, environmental factors such as smoking, and level of blood glucose, which might influence the effect estimates, were not controlled for. Second, the possibility of a false negative remains due to the small size of the studies even when combined. Thus, further studies with larger sample size are required to investigate the associations.

CONCLUSION

In summary, this meta-analysis suggested that the *ENPP1* K121Q gene polymorphism was associated with decreased and increased risk of T2DM in the American, European, and African populations. Further studies considering gene-environment interactions should be conducted to investigate the associations between the *ENPP1* K121Q gene polymorphism and the risk of T2DM.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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