Proprotein Convertase Subtilisin/Kexin Type 9 Gene Variants in Familial Hypercholesterolemia: A Systematic Review and Meta-Analysis

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Keywords: PCSK9 gene, familial hypercholesterolemia, mutation, meta-analysis

Abstract:

Proprotein Convertase Subtilisin Kexin type 9 (PCSK9), comprises 12 exons, encoded for an enzyme which plays a critical role in the regulation of circulating low density lipoprotein. The gain-of-function (GOF) mutations aggravate the degradation of LDL receptors, resulting in familial hypercholesterolemia (FH), while loss-of-function (LOF) mutations lead to higher levels of the LDL receptors, lower the levels of LDL cholesterol, and preventing from cardiovascular diseases. It is noted that, previous publications related to the mutations of PCSK9 were not always unification. Therefore, this study aims to present the spectrum and distribution of PCSK9 gene mutations by a meta-analysis. A systematic literature analysis was conducted based on previous studies published by using different keywords. The weighted average frequency of PCSK9 mutation was calculated and accessed by MedCalc®. A total of 32 cohort studies, that included 19,725 familial hypercholesterolemia blood samples, were enrolled in the current study. The analysis results indicated that, based on the random-effect model, the weighted prevalence of PCSK9 mutation was 5.67% (95%CI = 3.68?8.05, p < 0.0001). The prevalence of PCSK9 GOF mutations was 3.57% (95%CI = 1.76?5.97, p < 0.0001) and PCSK9 LOF mutations was 6.05% (95%CI = 3.35?9.47, p < 0.0001). Additionally, the first and the second exon were identified as the hot spot of mutation occurred in PCSK9. Both GOF and LOF mutations have a higher proportion in Asia and Africa compared with other regions. The GOF PCSK9 p.(Glu32Lys) and LOF PCSK9 p.(Leu21dup/tri) were dominant in the Asia region with the proportion as 6.58% (95%CI = 5.77?7.47, p = 0.62) and 16.20% (95%CI = 6.91?28.44, p = 0.0022), respectively. This systematic analysis provided scientific evidence to suggest the mutation of PCSK9 was related to the metabolism of lipoprotein and atherosclerotic cardiovascular disease.

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Proprotein Convertase Subtilisin/Kexin Type 9 Gene Variants in Familial Hypercholesterolemia: A Systematic Review and Meta-Analysis

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Abstract: *Proprotein Convertase Subtilisin Kexin type 9* (*PCSK9*), comprises 12 exons, encoded for an enzyme which plays a critical role in the regulation of circulating low density lipoprotein. The gain-of-function (GOF) mutations aggravate the degradation of LDL receptors, resulting in familial hypercholesterolemia (FH), while loss-of-function (LOF) mutations lead to higher levels of the LDL receptors, lower the levels of LDL cholesterol, and preventing from cardiovascular diseases. It is noted that, previous publications related to the mutations of *PCSK9* were not always unification. Therefore, this study aims to present the spectrum and distribution of *PCSK9* gene mutations by a meta-analysis. A systematic literature analysis was conducted based on previous studies published by using different keywords. The weighted average frequency of *PCSK9* mutation was calculated and accessed by MedCalc®. A total of 32 cohort studies, that included 19,725 familial hypercholesterolemia blood samples, were enrolled in the current study. The analysis results indicated that, based on the random-effect model, the weighted prevalence of *PCSK9* mutation was 5.67% (95%CI = 3.68–8.05, $p < 0.0001$). The prevalence of *PCSK9* GOF mutations was 3.57% (95%CI = 1.76–5.97, $p < 0.0001$) and *PCSK9* LOF mutations was 6.05% (95%CI = 3.35–9.47, $p < 0.0001$). Additionally, the first and the second exon were identified as the hot spot of mutation occurred in *PCSK9*. Both GOF and LOF mutations have a higher proportion in Asia and Africa compared with other regions. The GOF *PCSK9* p.(Glu32Lys) and LOF *PCSK9* p.(Leu21dup/tri) were dominant in the Asia region with the proportion as 6.58% (95%CI = 5.77–7.47, $p = 0.62$) and 16.20% (95%CI = 6.91–28.44, $p = 0.0022$), respectively. This systematic analysis provided scientific evidence to suggest the mutation of *PCSK9* was related to the metabolism of lipoprotein and atherosclerotic cardiovascular disease.

Keywords: *PCSK9* gene; familial hypercholesterolemia; mutation; meta-analysis

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1. Introduction

Familial hypercholesterolemia (FH; OMIM#143890), also known as Familial Hypercholesterolemia type 2 or Fredrickson Class 2A Hyperlipidemia, is a common dominant disorder of cholesterol metabolism characterized by elevated level of serum cholesterol [1]. The pathogenesis of FH have been reported to be significantly linked to the genetic alterations, which occurred on many identified genes, including *Low Density Lipoprotein Receptor* (*LDLR*), *Apolipoprotein B* (*ApoB*), *Low density lipoprotein receptor adaptor protein 1* (*LDLRAP1*), *proprotein convertase subtilisin/kexin type 9* (*PCSK9*), etc. [2].

Up to date, more over 10,000 genetic variants of those genes have been identified and reported in several public databases, such as Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/), LOVD (https://www.lovd.nl/), etc. In summary, three major monogenic causes of FH, including *LDLR*, *ApoB* and *PCSK9*, have been reported. Notably, approximately, 85–90%, and 1–12% of patients with FH have been associated with mutations of *LDLR* and

ApoB, respectively. 2–4% gain-of function *PCSK9* mutations were identified in the patients with FH [3].

The *proprotein convertase subtilisin/kexin type 9* (*PCSK9*), also known as *Neural Apoptosis Regulated Convertase1* (*NARC1*), located at 1p32.3. *PCSK9* consists of 12 exons, encoding for the proprotein convertase subtilisin/kexin type 9 of 692 amino acids, which belongs to the proprotein convertase of the subtilase family and is predominantly expressed in the kidneys, liver, cerebellum and small intestine [4]. The PCSK9 protein plays an important role in the LDL metabolism. PCSK9 functions as a chaperone to mediate the degradation of LDL receptors by interacting with the extracellular domain as well as leading to the degradation of lysosome, resulting in leading to high LDL-C level in plasma [4].

Two types of mutations of the variants of *PCSK9* gene: Gain-of-function mutation (GOF) and Loss-of-function (LOF) have been identified [4,5]. Concerning to the GOF mutations in *PCSK9*, several variants, including p.(Ser127Arg), p.(Asp129Asn) (in prodomain); p.(Arg215His), p.(Phe216Leu), p.(Arg128Ser) (in catalytic domain); p.(Arg469Trp), p.(Arg469Trp) (in C-terminal), etc., have been reported. These variants lead to decrease the number of LDL receptors at the cell surface, resulting familial hypercholesterolemia as well as increasing the risk of cardiovascular disease (CVD). The LOF mutations, such as p.(Arg46Leu), p.(Gly106Arg), p.(Tyr142*) (in prodomain); p.(Leu253Phe) (in catalytic domain); p.(Ala443Thr), p.(Cys679*) (in C-terminal), etc., have been reported to be associated with lower cholesterol levels. As the result, it leads to the reduction of CVD via the LDLR degradation [6].

It is noted that there is a challenge to clarify pathogenicity assessment of FH variants to gain more accurately assesses of CVD risk in FH populations within various phenotypes [7]. To date, most studies of genetic analysis for FH were unclear in the identification of the functional effect of variants on FH patients due to the small sample size or lack of functional analysis assessments. Even though numerous variants of *PCSK9* and have been reported in the database of ClinVar, LOVD, there are still lots of controversies and its' pathogenicity assessment remains unclear. For instance, the p.(Val4Ile) variant is still the subject of conflicting interpretations of pathogenicity with likely benign or uncertain significance interpretation on the Clinvar database. However, in the study of Hori et al., p.(Val4Ile) was reported to be pathogenic. The p.(Val4Ile) variant plays a key role in the increase of plasma LDL-C levels, resulting in an increased risk of coronary atherosclerosis in Japanese FH patients [8].

More one matter of concern is that heterogeneity still existed among previous studies. The heterogeneity may arise from different characteristic of inputs, such as age, sex, severity, ethnicity, etc. This could be the significant heterogeneity. Thus, this significant heterogeneity could affect the associated conclusion of the studies. As much, there are still much to discuss, therefore, in current study, the meta-analysis are needed to be performed to consider how to handle the heterogeneity to tease out the important relevant information as the systematic reviews to guide the decision-making.

2. Materials and Methods

2.1. Search Strategy, Inclusion and Exclusion Criteria of Literature

The current meta-analysis was performed according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). For literature research, separation or combination of following keywords: familial hypercholesterolemia", "proprotein convertase subtilisin/kexin type 9 (PCSK9)", "mutation", were applied to retrieve related published articles (updated on August, 2019). Additional studies were also identified via the references listed in the articles.

The studies were eligible when they met all following criteria: (1) The article was limited to studies written in English; (2) cohort study design; (3) provided the data about the identification of *PCSK9* gene mutation, correlated with FH; (4) provided that data about the frequency of *PCSK9* mutation.

Exclusion criteria include: (1) the non-English articles, congress abstracts, editorials, letters, books, reviews, systematic reviews, in vitro and in vivo studies, functional studies

and pharmacogenomic studies were eliminated; (2) other genes/mutations involved in FH were excluded; (3) articles with insufficient data were not included in current study.

2.2. Data Extraction and Statistical Analysis

The relevant data of each eligible study were independently retrieved by two authors. In the case of disagreement, it will be resolved through their discussion within the third author. The following information, including the Author's last name, year of publication, the country where the study was performed, sample type, experimental methods to assess and identify the mutations, number of patients/families; clinical significance of the variants; nucleotide and amino acid change, were retrieved. Notably, nomenclature for the description of sequence variants was applied to all genetic entries according to the Human Genetic Variation Society (HGVS) recommendations (http://varnomen.hgvs.org/). Entries with unknown or uncorrected name were removed and/or corrected to ensure the adherence to HGVS. The functional effect of each variant was classified as GOF or LOF based on the database of ClinVar and LOVD.

All data was analyzed by using Medcalc® (version 19.1.7; https://www.medcalc.org/). The proportion of *PCSK9* mutation was calculated in patients with FH. The statistical heterogeneity among included studies was estimated based on the Cochran's Q and I^2 tests. The cut-off point: $p = 0.10$ for the Q test and I^2 were used to test the heterogeneity between studies. The scale of I^2 value is classified as following: $I^2 < 25\%$: no heterogeneity, $25\% \leq I^2 \leq 50\%$: moderate heterogeneity, and $I^2 > 50\%$: strong heterogeneity. The random-effects model was applied if the heterogeneity among studies existed ($p < 0.10$ for Q test, $I^2 > 50\%$) [9–11]. Finally, the weighted average frequency of *PCSK9* mutation was identified.

3. Results

3.1. The Characteristics of Eligible Studies

After exclusion of studies that did not meet the inclusion criteria, finally, 32 eligible articles, including 19,725 individuals from 16 different countries, were enrolled in our systematic analysis (Table 1). We noticed that most *PCSK9* molecular research was conducted in developed countries (29 of 32 studies, accounting for 90.63%). The studies carried out on Asian populations, European countries and North American countries were 43.75% (14 of 32 studies), 40.63% (13 of 32 studies) and 9.36% (9.38%), respectively. Notably, different molecular methods, including Sanger sequencing (16 of 32 studies, accounting for 50%), NGS and exome sequencing (10 of 32 studies, accounting for 31.25%), etc., have been used for the identification of *PCSK9* gene mutations.

3.2. Meta-Analysis: The Proportion of PCSK9 Mutation in FH Patients

In the meta-analysis, the heterogeneity among included studies was significant for Q test (Q = 1070.12, $p < 0.0001$, $I^2 = 97.10\%$, 95%CI for $I^2 = 96.53$–97.58) (Figure 1). Thus, the random-effect model was employed to evaluate the proportion of *PCSK9* mutation in FH patients. According to Table 1, the prevalence of *PCSK9* mutation, calculated based on thirty-two eligible studies included 19,725 individuals, was 5.67% (95%CI = 3.68–8.05).

Subgroup analysis was performed according to the region, shown in Table 2. Europe (13 studies; $n = 8547$), North America (three studies; $n = 6910$) and Oceania (one study; $n = 57$) studies tended to report lower prevalence estimates than our overall prevalence estimate, while Asia (14 studies; $n = 4056$) and African (two studies; $n = 155$) studies reported a greater *PCSK9* mutation prevalence than our estimate. However, due to the limitation of the small sample size, the analysis of African and Oceanian population subgroup in the current study should be interpreted cautiously.

Table 1. *PCSK9* mutation proportion of unrelated FH patients group.

Study, Year	Region	Method	Sample Size	Proportion (%)	95% CI	Weight (%)
Hori et al., 2019 [8]	Asia	Sequencing	650	8.46	6.44–10.87	3.55
Lee et al., 2019 [12]	Asia	Sequencing	19	36.84	16.30–61.64	2.05
Sánchez-Hernández et al., 2019 [13]	Europe	NGS	70	4.29	0.89–12.02	2.98
Cao et al., 2018 [14]	Asia	NGS	105	1.91	0.23–6.71	3.17
Kim et al., 2018 [15]	Asia	NGS	283	0.71	0.09–2.53	3.45
Raal et al., 2018 [16]	Africa	NGS	141	2.84	0.78–7.10	3.28
Tada et al., 2018 [17]	Asia	NGS	500	7.20	5.09–9.83	3.53
Tada et al., 2017 [18]	Asia	Sequencing	636	5.82	4.13–7.93	3.55
Xiang et al., 2017 [19]	Asia	Sequencing	219	1.37	0.28–3.95	3.40
Zhu et al., 2017 [20]	Asia	NGS	8	12.50	0.32–52.65	1.33
Abul-Husn et al., 2016 [21]	North America	NGS	6015	0.12	0.05–0.24	3.63
Medeiros et al., 2016 [22]	Europe	Sequencing	220	0.46	0.01–2.51	3.40
Ohta et al., 2016 [23]	Asia	Sequencing	224	12.95	8.85–18.06	3.40
Tada et al., 2016 [24]	Asia	Sequencing	240	17.50	12.91–22.91	3.42
Wang et al., 2016 [25]	North America	NGS	313	1.28	0.35–3.24	3.47
Mabuchi et al., 2014 [26]	Asia	Invader assay	1055	5.88	4.54–7.47	3.59
Maglio et al., 2014 [27]	Europe	NGS	77	1.30	0.03–7.03	3.03
Saavedra et al., 2014 [28]	North America	RFLP	582	3.09	1.84–4.84	3.54
Ahmed et al., 2013 [29]	Asia	HRM, RFLP	11	18.18	2.28–51.78	1.58
Vandrovcova et al., 2013 [30]	Europe	NGS	168	0.60	0.02–3.27	3.33
Abifadel et al., 2012 [31]	Europe	Sequencing	75	5.33	1.47–13.10	3.02
Palacios et al., 2012 [32]	Europe	Microarray	5430	0.02	0.0005–0.10	3.63
Noguchi et al., 2010 [33]	Asia	SSCP, RFLP	55	40.00	27.02–54.09	2.85
Strøm et al., 2010 [34]	Europe	Sequencing	1130	2.66	1.80–3.80	3.59
Abifadel et al., 2009 [35]	Asia	Sequencing	51	29.41	17.49–43.83	2.80
Homer et al., 2008 [36]	(*)	Sequencing	71	5.63	1.56–13.80	2.99
Tosi et al., 2007 [37]	Europe	Sequencing	32	25.00	11.46–43.41	2.47
Berge et al., 2006 [38]	Europe	Sequencing	475	0.63	0.13–1.84	3.52
Evans & Beil, 2006 [39]	Europe	RFLP	506	9.49	7.08–12.38	3.53
Allard et al., 2005 [40]	Europe	Sequencing	130	3.08	0.85–7.69	3.25
Sun et al., 2005 [41]	Europe	Sequencing	25	8.00	0.98–26.03	2.28
Leren et al., 2004 [42]	Europe	Sequencing	209	1.44	0.30–4.14	3.39
Total (random effects)			**19,725**	**5.67**	**3.68–8.05**	**100**
Heterogeneity: Chi^2 = 1070.12; df = 31 (p < 0.0001); I^2 = 97.10%						

Note: (*), samples of FH patients from Oceania and Africa.

Table 2. The geographical analysis of *PCSK9* mutation in FH.

Region	GOF (%)	95%CI	p Value	LOF (%)	95%CI	p Value
Asia	6.26	4.45–8.36	<0.0001	22.14	18.04–26.70	0.14
Africa	7.14	N/A	N/A	2.24	0.54–5.95	0.17
Europe	1.84	0.29–4.70	<0.0001	2.55	0.97–4.85	<0.0001
North America	0.30	0.002–1.11	0.06	1.56	0.03–5.37	0.0015
Oceania	1.75	N/A	N/A	N/A	N/A	N/A

Note: N/A, not available data.

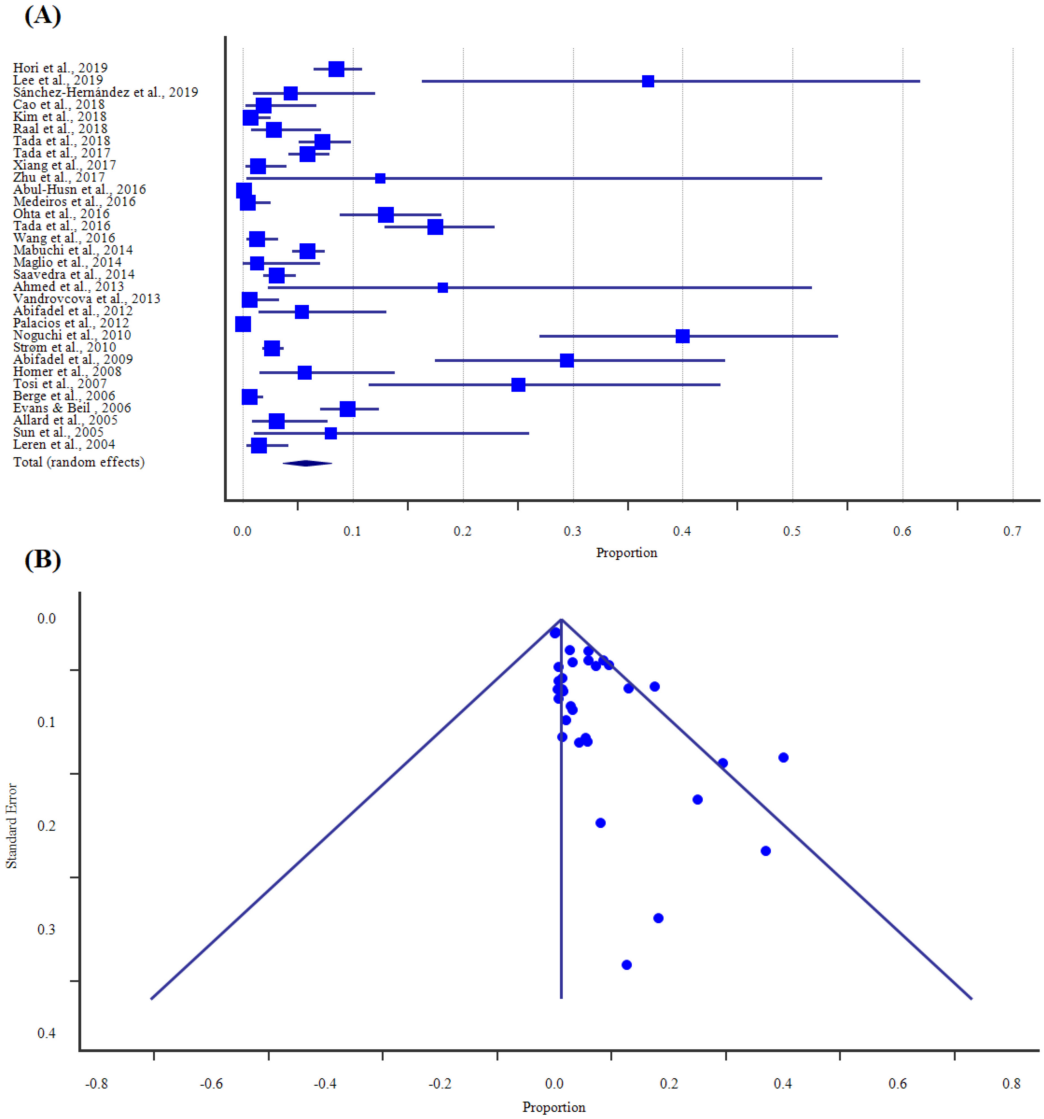

Figure 1. Forest plots (**A**) and funnel plots (**B**) of *PCSK9* mutation associated with FH.

3.3. The Frequency of PCSK9 GOF and LOF Variants

A total of 55 variants were reported from FH patients, including 52 missenses (94.55%), two synonymous substitutions (3.64%) and one in-frame insertion (1.82%). According to the Clinvar, LOVD database and article remarks, 19 variants were identified as GOF, 13 variants were identified as LOF, 20 VUS and three variants were benign (Table 3).

The frequency of the *PCSK9* GOF and LOF variants were calculated. Based on the random-effect model, our results revealed that the proportion of *PCSK9* GOF and LOF mutation were 3.57% (95%CI = 1.76–5.97, $p < 0.0001$) and 6.05% (95%CI = 3.35–9.47, $p < 0.0001$), respectively.

According to the Asian population, the frequency of the variants was determined (Table 4). The most common GOF and LOF variants were p.(Glu32Lys) and p.(Leu21dup/tri) with the dominant proportion compare to other variants have been observed in Asian: 6.58% and 16.20%, respectively. According to the European population, the frequency of the variants was determined (Table 4). The most common GOF and LOF variants were p.(Asp374Tyr) and p.(Val474Ile) with the dominant proportion compare to other variants have been observed in European countries: 1.44%, and 18.75%, respectively. In the North American population, the most common GOF were p.(His417Gln) and p.(Arg469Trp) with the frequencies of 0.32% of each, and no variants of LOF were identified.

Table 3. List of 55 PCSK9 (gene and protein) variations and their characteristics.

Location	Chromosome Position (GRCh38)	Amino Acid Change	Nucleotide Change	Annotation	LOVD	Clinvar	Article Remark	References
Exon 1	Chr1:55039847	p.(Val4Ile)	c.10G > A	Missense	Pathogenic	Conflicting	GOF	[8,14,23]
	Chr1:55039880-55039902	p.(Leu21dup/tri)	c.61_63dup/triCTG	In-frame insertion	N/A	N/A	LOF	[8,13,20,23,27,31,33,35,37]
	Chr1:55039931	p.(Glu32Lys)	c.94G > A	Missense	Pathogenic	Conflicting	GOF	[8,17,18,23,24,26,33]
	Chr1:55039940	p.(Asp35Tyr)	c.103G > T	Missense	N/A	VUS	GOF	[31]
	Chr1:55039974	p.(Arg46Leu)	c.137G > T	Missense	Likely benign	Likely benign	LOF	[16,28,34,38]
	Chr1:55039995	p.(Ala53Val)	c.158C > T	Missense	Benign	Benign	LOF	[5,8,23,33]
	Chr1:55040022	p.(Ala62Asp)	c.185C > A	Missense	N/A	GOF	GOF	[22]
Exon 2	Chr1:55043888	p.(Glu85Lys)	c.253G > A	Missense	N/A	VUS	VUS	[8,15]
	Chr1:55043902	p.(Ser89=)	c.267G > A	Synonymous substitution	N/A	Likely benign	Likely benign	[16]
	Chr1:55043912	p.(Arg93Cys)	c.277C > T	Missense	Pathogenic	Conflicting	LOF	[5,8,12,23]
	Chr1:55043922	p.(Arg96Leu)	c.287G > T	Missense	N/A	N/A	GOF	[19]
	Chr1:55043948	p.(Arg105Trp)	c.313C > T	Missense	N/A	VUS	GOF	[19]
	Chr1:55043949	p.(Arg105Gln)	c.314G > A	Missense	VUS	VUS	LOF	[5,29]
	Chr1:55043951	p.(Gly106Arg)	c.316G > A	Missense	N/A	N/A	LOF	[5,38]
	Chr1:55043958	p.(Leu108Arg)	c.323T > G	Missense	N/A	GOF	GOF	[31]
	Chr1:55044016	p.(Ser127Arg)	c.381T > A	Missense	N/A	GOF	GOF	[5,31,36]
	Chr1:55044020	p.(Asp129Asn)	c.385G > A	Missense	Pathogenic	Conflicting	GOF	[5,8,30]
	Chr1:55044021	p.(Asp129Gly)	c.386A > G	Missense	N/A	Conflicting	GOF	[5,36]
	Chr1:55044031	p.(Glu132Asp)	c.396G > C	Missense	N/A	N/A	VUS	[8]
Exon 3	Chr1:55046587	p.(Pro155Leu)	c.464C > T	Missense	N/A	VUS	VUS	[29]
	Chr1:55046594	p.(Asn157Lys)	c.471C > A	Missense	N/A	VUS	LOF	[5,38,42]
	Chr1:55046626	p.(Ala168Glu)	c.503C > A	Missense	N/A	VUS	VUS	[36]
	Chr1:55046626	p.(Ala168Val)	c.503C > T	Missense	N/A	N/A	VUS	[8]
	Chr1:55046640	p.(Pro173Ser)	c.517C > T	Missense	N/A	VUS	Benign	[12]
Exon 4	Chr1:55052381	p.(Pro209Leu)	c.626C > T	Missense	N/A	N/A	VUS	[14]
	Chr1:55052398	p.(Arg215His)	c.644G > A	Missense	Pathogenic	Conflicting	GOF	[8,14,21]
	Chr1:55052408	p.(Arg218Ser)	c.654A > T	Missense	N/A	VUS	GOF	[5,40]

Table 3. *Cont.*

Location	Chromosome Position (GRCh38)	Amino Acid Change	Nucleotide Change	Annotation	LOVD	Clinvar	Article Remark	References
Exon 5	Chr1:55052701	p.(Arg237Trp)	c.709C > T	Missense	VUS	Conflicting	LOF	[22,25,36,38]
	Chr1:1:55052779	p.(Gly263Ser)	c.787G > A	Missense	N/A	Conflicting	LOF	[8,23,33]
	Chr1:55052783	p.(Thr264Ile)	c.791C > T	Missense	N/A	Conflicting	VUS	[8,23]
Exon 7	Chr1:55057404	p.(Arg357His)	c.1070G > A	Missense	VUS	VUS	GOF	[5,40]
	Chr1:55057454	p.(Asp374Asn)	c.1120G > A	Missense	N/A	VUS	VUS	[21]
	Chr1:55057454	p.(Asp374His)	c.1120G > C	Missense	N/A	GOF	GOF	[5,22]
	Chr1:55057454	p.(Asp374Tyr)	c.1120G > T	Missense	Pathogenic	GOF	GOF	[5,32,41,42]
Exon 8	Chr1:55058106	p.(His417Gln)	c.1251C > A	Missense	N/A	Conflicting	GOF	[5,25]
	Chr1:55058125	p.(Ile424Val)	c.1270A > G	Missense	N/A	Likely benign	VUS	[8,23]
	Chr1:55058182	p.(Ala443Thr)	c.1327G > A	Missense	Benign	Conflicting	LOF	[5,40]
Exon 9	Chr1:55058524	p.(Val460=)	c.1380A > G	Synonymous substitution	Benign	Benign	VUS	[37]
	Chr1:55058543	p.(Pro467Ala)	c.1399C > G	Missense	Pathogenic	Conflicting	GOF	[22]
	Chr1:55058549	p.(Arg469Trp)	c.1405C > T	Missense	Pathogenic	Conflicting	GOF	[21,25,40]
	Chr1:55058564	p.(Val474Ile)	c.1420G > A	Missense	Benign	Likely benign	LOF	[5,33,37]
	Chr1:55058576	p.(Ala478Thr)	c.1432G > A	Missense	N/A	Conflicting	VUS	[8]
	Chr1:55058630	p.(Arg496Trp)	c.1486C > T	Missense	Pathogenic	Conflicting	GOF	[8,21,23]
	Chr1:55058639	p.(Arg499Cys)	c.1495C > T	Missense	N/A	VUS	VUS	[15]
	Chr1:55058640	p.(Arg499His)	c.1496G > A	Missense	N/A	VUS	VUS	[13]
Exon 10	Chr1:1:55059492	p.(Gly504Trp)	c.1510G > T	Missense	N/A	VUS	VUS	[8,23]
	Chr1:55059519	p.(Asn513Asp)	c.1537A > G	Missense	N/A	VUS	VUS	[25]
Exon 11	Chr1:55061485	p.(Ala598Thr)	c.1792G > A	Missense	VUS	VUS	LOF	[12]
Exon 12	Chr1:55063391	p.(Gly629Asp)	c.1886G > A	Missense	N/A	N/A	VUS	[8]
	Chr1:55063435	p.(Val644Ile)	c.1930G > A	Missense	N/A	N/A	VUS	[8]
	Chr1: 55063450	p.(Ala649Thr)	c.1945G > A	Missense	N/A	N/A	VUS	[8]
	Chr1:55063459	p.(Asn652Asp)	c.1954A > G	Missense	N/A	VUS	Benign	[12]
	Chr1:55063509	p.(Ser668Arg)	c.2004C > A	Missense	N/A	Conflicting	LOF	[8,23,33]
	Chr1:55063514	p.(Gly670Lys)	c.2009G > A	Missense	Benign	Likely benign	VUS	[33,39]
	Chr1: 55063550	p.(Arg682Gln)	c.2045G > A	Missense	N/A	N/A	VUS	[8]

Table 4. Frequency of *PCSK9* variants in Asian, European and American population.

ASIAN	**GAIN-OF-FUNCTION**					
	Location (GRCh38)	**Nucleotide Change**	**Amino Acid Change**	**Proportion (%)**	**95%CI**	***p* Value**
	Chr1:55039847	c.10G > A	p.(Val4Ile)	3.63	0.31–10.35	0.02
	Chr1:55039931	c.94G > A	p.(Glu32Lys)	6.58	5.77–7.44	0.62
	Chr1:55043922	c.287G > T	p.(Arg96Leu)	0.46	N/A	N/A
	Chr1:55043948	c.313C > T	p.(Arg105Trp)	0.46	N/A	N/A
	Chr1:55044020	c.385G > A	p.(Asp129Asn)	0.15	N/A	N/A
	Chr1:55052398	c.644G > A	p.(Arg215His)	0.33	0.05–1.05	0.18
	Chr1:55058630	c.1486C > T	p.(Arg496Trp)	0.68	0.25–1.48	0.95
	LOSS-OF-FUNCTION					
	Chr1:55039902-55039903	c.63_64insCTG	p.(Leu21dup/tri)	16.20	6.91–28.44	0.0022
	Chr1:55039995	c.158C > T	p.(Ala53Val)	5.63	1.34–12.61	0.09
	Chr1:55043912	c.277C > T	p.(Arg93Cys)	10.66	3.28–56.19	0.0001
	Chr1:55043948	c.314G > A	p.(Arg105Gln)	9.09	N/A	N/A
	Chr1:55052698	c.787G > A	p.(Gly263Ser)	2.01	0.71–4.41	0.24
	Chr1:55058564	c.1420G > A	p.(Val474Ile)	7.27	N/A	N/A
	Chr1:55061485	c.1792G > A	p.(Ala598Thr)	5.26	N/A	N/A
	Chr1:55063509	c.2004C > A	p.(Ser668Arg)	0.93	0.17–2.89	0.28
EUROPEAN	**GAIN-OF-FUNCTION**					
	Chr1:55039940	c.103G > T	p.(Asp35Tyr)	1.33	N/A	N/A
	Chr1:55043958	c.323T > G	p.(Leu108Arg)	1.33	N/A	N/A
	Chr1:55044016	c.381T > A	p.(Ser127Arg)	1.33	N/A	N/A
	Chr1:55044020	c.385G > A	p.(Asp129Asn)	0.60	N/A	N/A
	Chr1:55052408	c.654A > T	p.(Arg218Ser)	0.77	N/A	N/A
	Chr1:55057404	c.1070G > A	p.(Arg357His)	0.77	N/A	N/A
	Chr1:55057454	c.1120G > T	p.(Asp374Tyr)	1.44	0.001–5.84	0.0001
	Chr1:55058549	c.1405C > T	p.(Arg469Trp)	0.77	N/A	N/A
	LOSS-OF-FUNCTION					
	Chr1:55039902-55039903	c.63_64insCTG	p.(Leu21dup/tri)	4.17	0.10–9.40	0.03
	Chr1:55039974	c.137G > T	p.(Arg46Leu)	2.11	0.36–5.25	0.0009
	Chr1:55046594	c.471C > A	p.(Asn157Lys)	0.41	0.07–1.02	0.50
	Chr1:55052701	c.709C > T	p.(Arg237Trp)	0.45	N/A	N/A
	Chr1:55058182	c.1327G > A	p.(Ala443Thr)	0.77	N/A	N/A
	Chr1:55058564	c.1420G > A	p.(Val474Ile)	18.75	N/A	N/A
AMERICAN	**GAIN-OF-FUNCTION**					
	Chr1:55052398	c.644G > A	p.(Arg215His)	0.02	N/A	N/A
	Chr1:55058106	c.1251C > A	p.(His417Gln)	0.32	N/A	N/A
	Chr1:55058549	c.1405C > T	p.(Arg469Trp)	0.32	N/A	N/A
	Chr1:55058630	c.1486C > T	p.(Arg496Trp)	0.1	N/A	N/A

Note: N/A, not available data.

3.4. PCSK9 Mutation Proportion In Exon

Proprotein Convertase Subtilisin Kexin type 9 (*PCSK9*) comprises 12 exons. Overall, *PCSK9* mutation frequencies in each exon were significantly different (Table 5). We identified both GOF and LOF variants in exons 1, 2 and 9; while no functional variant was recorded on exons 6 and 10. Additionally, exons 3, 4, 5, 7, 8, 11 and 12 have at least one GOF or LOF variant. Notably, the first and the second exon of the *Proprotein Convertase Subtilisin Kexin type 9* gene was noticed to have higher frequencies in both GOF and LOF mutations than most other exons.

Table 5. The distribution analysis of functional variants on twelve exons.

Exon	GOF (%)	95%CI	*p* Value	LOF (%)	95%CI	*p* Value
1	6.46	4.89–8.23	0.0007	6.54	3.36–10.67	<0.0001
2	1.13	0.29–2.48	0.04	10.91	0.05–41.07	<0.0001
3	N/A	N/A	N/A	0.41	0.08–1.23	0.50
4	0.28	0.01–0.91	0.02	N/A	N/A	N/A
5	N/A	N/A	N/A	1.02	0.45–1.96	0.10
6	N/A	N/A	N/A	N/A	N/A	N/A
7	1.78	0.15–5.16	<0.0001	N/A	N/A	N/A
8	0.32	N/A	N/A	0.77	N/A	N/A
9	0.42	0.11–0.94	0.03	12.69	3.80–25.77	0.12
10	N/A	N/A	N/A	N/A	N/A	N/A
11	N/A	N/A	N/A	5.26	N/A	N/A
12	N/A	N/A	N/A	0.93	0.17–2.89	0.28

Note: N/A, not available data.

4. Discussion

The active form of PCSK9 combines with the EGF-A domain of LDL receptor. The intracellular and extracellular pathway of PCSK9 facilitates the transport of LDLR to lysosomes. While, the GOF variant enhanced the LDL degradation activities of PCSK9, therefore, increased plasma LDL levels, the LOF increases the number of LDL receptors [4]. The discovery of LOF *PCSK9* variants has opened the way to a better understanding PCSK9 function while reinforcing the notion of PCSK9 as a therapeutic target [5].

Briefly, our meta-analysis of 32 studies including 19,725 individuals found a *PCSK9* mutation prevalence in FH patients of 5.67% in the general population. In detail, the *PCSK9* GOF mutations which lead to reduced uptake low density lipoprotein cholesterol and, therefore, increased plasma LDL levels, affects 3.57% of FH patients. Nevertheless, we found a higher rate of *PCSK9* LOF mutation (approximately 6.05%) in FH patients. Loss-of-function mutations occur on *PCSK9* gene seem to distribute among and impact more widely FH patients, in particularly, Asian and African ones. In the study of Abifadel and co-workers, they concluded that *PCSK9* LOF carriers confer a selective advantage because they reduced susceptibility to severe parasitic infections through the restriction of cholesterol which is essential for parasite feeding. This in turn might interfere with the successful infection or life cycle of a parasite like the malaria parasite. Moreover, increased LDL receptor activity in the liver might reduce the exposure of peripheral tissues to infectious agents that circulate in association with lipoproteins [6].

We also found most of the genetic research on FH-related variants come from developed Asian and African countries, which suggests the need to fill the gap of studies on *PCSK9* gene in these areas. Thus far, much of the regional variation in *PCSK9* mutation proportion has been attributed to the presence of founder populations. Some variants have a higher frequency in one specific region due to the founder effect. For instance, p.(Glu32Lys) is a signature variant in Japanese FH patients. Likewise, p.(Leu21dup/tri)

is common in China, Japan and Lebanon, which may reflect immigration among these countries.

There was the considerable heterogeneity in those studies (I^2: 97.10%; 95%CI, 96.53–97.58), therefore, major asymmetry was present in Begg's funnel plot and the results of Egger's test suggested that publication bias may have been present ($p < 0.0001$). After determining the influence of some subgroups on the pooled proportion, we conclude that the heterogeneity between studies are thus more likely reflective of real differences in study populations, designs and outcome measurements. The I^2 index measures the extent of heterogeneity for choosing the best models for meta-analysis, therefore, meta-analysis performed throughout random effect model in this study.

We have calculated the proportion of *PCSK9* mutation on each exon to find the hotspot of functional variants on *PCSK9* gene. Interestingly, our investigation revealed a high frequency of GOF and LOF mutations on the first and the second exon of *PCSK9*. *PCSK9* comprises 12 exons, encoded the PCSK9 protein, with three domains: a N-terminal prodomain, a catalytic domain and a carboxyl-terminal domain. Among its exons, exon 1 encoded the peptide of 69 amino acids composed of the signal peptide (residue 1 to 30) and part of N-terminal prodomain (residue 31 to 69). While the rest of the N-terminal prodomain is encoded by exon 2 and exon 3 [4,43]. The N-terminal prodomain of *PCSK9* plays a key role as a modulator for its activities. In the study of Martin and co-workers, they reported that this prodomain is important for the activities of PCSK9 by releasing the catalytic domain [43]. We also found the report of Wicińsk and co-workers showed that the prodomain region in humans has a high frequent of mutations (34%) [4]. These findings support our result that exon 1 and 2 are the hotspots for functional variants.

Up to date, several treatments of FH have been developed based on PSCK9. For instance, two commercially available FDA approved monoclonal antibodies, alirocumab and evolocumad, function as inhibitors of PCSK9. Through the inhibition of PCSK9, it leads to the increased expression of LDL receptors, results in reduction of circulating LDL-C levels [3]. Therefore, all the investigation of the variants of *PSCK9* have also highlighting the possible opening of a wide panorama for beginning to implicating PCSK9 as the potential biomarker for FH therapy.

The present study has some limitations. The number of studies included in the meta-analysis is modest ($n = 32$). Firstly, most studies were carried out in Asian populations, European, limited to African and other populations. The heterogeneity still existed, due to the different patient selection criteria, and follow-up period. Secondly, it is not possible to clarify if the PCSK9 mutation is an early FH-causing aberration.

5. Conclusions

Cardiovascular disease remains the leading cause of death worldwide and, left untreated, nearly 85% of patients with FH are expected to suffer coronary events prior to old age. Thus, greater efforts should be made to explore region-specific frequencies of FH prevalence and more accurately characterize disease burden. Our meta-analysis found that the *PCSK9* variants proportion was 5.67%. While the GOF and LOF variants were found to affect 3.57% and 6.05% of FH patients, respectively, those variants are more prevalent in the Asian and African population. This study provides information about one of the most common genetic pathogen of familial hypercholesterolemia for diagnostic programs, improved management and future probable therapeutic strategies. With the recent advent of PCKS9 inhibitors, we recommend a genetic testing focus on the hotspots (the first and the second exon) of the *PCSK9* gene to identify individuals who stand to benefit from such therapy.

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Abbreviations

ApoB	Apolipoprotein B
CVD	Cardiovascular disease
EGF-A	Epidermal growth factor-like repeat A
FH	Familial hypercholesterolemia
GOF	Gain-of-function
HGVS	Human Genetic Variation Society
HRM	High Resolution Melt
LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein Cholesterol
LDLR	Low Density Lipoprotein Receptor
LDLRAP1	Low density lipoprotein receptor adaptor protein 1
LOF	Loss-of-function
LOVD	Leiden Open Variation Database
N/A	Not available data
NARC1	Neural Apoptosis Regulated Convertase 1
NGS	Next generation sequencing
PCSK9	Proprotein convertase subtilisin/kexin type 9
RFLP	Restriction fragment length polymorphism
SSCP	Single-strand conformation polymorphism
VUS	Variant of uncertain significance

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