DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Integrating Genetic and Conventional Risk Factors for Improving Coronary Heart Disease Risk Prediction

by

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Supervisor

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CHAPTER ONE

Introduction

1.1 Background

Coronary heart disease (CHD) is one of the leading causes of death and morbidity as well as one of the leading causes of premature disability among adult men and women globally [1-3]. In 2019, CHD was responsible for 197 million prevalent cases, 182 million DALYs, and 9.14 million fatalities (almost 16% of global mortality). CHD was expected to affect 244.1 million individuals in 2020, with males (141.0 million) outnumbering females (103.1 million), while mortality rates from CHD were 112.37 per 100,000. These numbers increased steadily by 2021 and reached almost 185 million DALYs and 9.44 million deaths [4-6]. The WHO estimates that by 2030, the number of annual deaths caused by CVD (mainly from CHD and stroke) will reach nearly 23.6 million [7-8]. Although the incidence and death rate of CVD (mainly CHD) has decreased in many European countries, CHD remains a major source of morbidity and mortality in central and eastern European nations [9-11]. CHD is also the leading cause of death in Hungary, with a mortality rate of more than 350 per 100,000 people in 2016, and 32,102 deaths (almost 24.5%) expected in 2020[10-14].

CHD also known as ischemic heart disease or coronary artery disease, is caused by atherosclerosis. It is a condition of narrow or blocked-off main arteries supplying the myocardium with oxygen and nutrients, consequently, impairing the heart function due to insufficient blood flow [3,15-18]. It is a silent disease that essentially begins with no symptoms, followed by the progression of lesion occurs over years [19]. Manifestations of this disease include angina pectoris, myocardial infarction, and sudden cardiac death [20]. Angina pectoris and myocardial infarction are the most common types of CHD. Angina pectoris (the CHD symptom) is caused by insufficient heart blood flow and presents as chest pain, pressure, and discomfort. A myocardial infarction, often known as a heart attack, occurs when the heart receives insufficient blood flow, resulting in the death of heart muscles due to a lack of oxygen (blockage in the main arteries) [21].

CHD development is a result of the complex interaction between modifiable and non-modifiable risk factors. Modifiable risk factors for CHD include dyslipidaemia (described as; elevated TC, high LDL-C, high TG, and low HDL-C), high blood pressure (SBP and DBP

abnormalities), hyperglycaemia (elevation of blood glucose), smoking, stress, and a sedentary lifestyle (unhealthy diet, and physical inactivity). Non-modifiable risk factors include age, gender, and genetic heritability [22-36].

For a better understanding of CHD pathophysiology, and clinical features it is important to demonstrate the cellular concept, structure of blood vessels, and implication of atherosclerosis in the large arteries. Initially, an artery consists of three distinguished layers including: tunica adventitia (the outermost layer of connective tissue that supplies the middle layer with blood), tunica media (the middle layer of smooth muscle), and tunica intima (the innermost layer composed of endothelial cells) [19, 31]. In the beginning, endothelium dysfunction which is the fundamental process of atherosclerosis in the main arteries injured (due to interaction between risk factors), which allows the migration of inflammatory cells that lead to the release of cytokines, and lipids. Consequently, foam cell and plaque formation occur and develop over time in the innermost layer of the main artery (tunica intima), thus decreasing blood flow (causing the lesion to become more fibrous and accumulate calcium mineral) and narrowing the arteries. The build-up of lipids can be chronic when plaque grows slowly over time or can be acute when plaque forms suddenly [16, 19, 31].

It is well established that genetic risk plays a pivotal role in etiology and contributes to individual susceptibility. Many risk factors of CHD are influenced by genes (40%-60%). Genome-wide association studies (GWAS) has successfully identified and validated several loci robustly associated with CHD among different populations [20,30-36]. Modern technology powered by GWAS helps in identifying the single nucleotides polymorphism (SNPs) that are implicated in the high-risk populations propensity for CHD. This technology is also utilized to establish an appropriate intervention by estimating the alleles frequencies and determining the effect size of genetic risk scores (both weighted and unweighted) [36].

Over the past few decades, considerable advances in the clinical diagnosis and curative procedure of CHD have been achieved; however, no significant reduction in the morbidity and mortality from CHD has occurred [37]. The disease burden is increasing steadily, and the DALYs and YLL are also growing substantially. The morbidity and mortality of CHD vary among countries, populations, and specific ethnic minorities, and these variations might be due to large inequalities in socioeconomic status and education, and possibly due to differences in genetic susceptibility [29-30, 38].

A wide range of preventive interventions for CHD is available for high-risk individuals through effective medication and comprehensive modification of an aforementioned risk factors, however, a comprehensive and precise assessment of CHD risk is needed to identify those high-risk subgroups [22, 24,29-30, 39-40]. Both types of interventions can be significantly improved by accurate risk assessment [28]. Accurate risk identification enables medical professionals to intervene in risk factor management prior to the onset of disease or critical conditions, thereby improving the patient's quality of life, resulting in a more cost-effective treatment strategy [37, 41-43]. Risk assessment of CHD requires thoroughness, completeness, and accuracy in obtaining information and measurements for identifying subgroups with elevated risk and predicting the timing of disease onset [41-43].

Early identification of those individuals at high risk, considering both genetic and environmental risk factors for future CHD, is crucial for health promotion and prevention strategy. Such identification can lead to reduced mortality and morbidity and improved cost-effectiveness [44-45].

Prediction models play a pivotal role in assessing the risk associated with CHD among population groups, including Framingham, SCORE, QRISK, and ASSIGN models [23,28-29, 40]. These prediction models are generally classified as developmental model, validation, or combination of the two [45]. Adoption of such models can assist in identifying high-risk CHD individuals generally as well in Hungary for early intervention.

1.2 Questions for Research

1.2.1 Questions for Conducting a Systematic Review in the general populations

1.2.1.1 What is the precise model for predicting the risk of CHD in the general populations?

1.2.1.2 Which biomarkers should be incorporated beside the conventional risk factors (CRFs) for CHD risk predictions?

1.2.1.3 Will risk prediction be significantly improved by genetic information?

1.2.2 Questions for CHD/AMI Risk Prediction in Hungarian Populations

1.2.2.1 Dose systematic coronary risk evaluation (SCORE) appropriate risk prediction model (alone or in combination with genetic risk score (GRS)) for CHD/AMI disease in the populations of Hungary?

1.2.2.2 Will the integration of (GRS and CRFs) help in assessing CHD/AMI risk in the Hungarian populations?

1.3 Research Objectives

1.3.1 Objective for Conducting a Systematic Review of the Literature on CHD Risk Prediction Modelling Studies

1.3.1.1 To summarize genetic and CRFs modelling studies for predicting CHD risk in the general populations.

1.3.1.2 To explore and identify the "optimal" risk prediction model (incorporating biomarkers or GRS in addition to CRFs) for CHD risk by assessing its performance.

1.3.1.3 To evaluate the potential improvement in risk prediction by incorporating genetic information into the models.

1.3.2 **Obje*ctives Regarding CHD/AMI Risk Assessment and Intervention in the Hungarian Populations***

1.3.2.1 To compare the sociodemographic characteristics, lifestyle factors, and clinical risk factors associated with CHD/AMI risk in Hungarian (Roma and general) populations.

1.3.2.2 To estimate and compare the allele frequencies of GRSs (based on 30 selected SNPs) associated with CHD/AMI in the Hungarian Roma and the general populations.

1.3.2.3 To calculate and compare the genetic risk scores (both weighted and unweighted) and thus genetic load in these Hungarian populations.

1.3.2.4 To assess and compare the role of the modifiable and non-modifiable risk factors in the development of CHD/AMI in the Hungarian populations.

1.3.2.5 To develop new models (by integrating GRS and CRFs) for predicting CHD/AMI risk among the Hungarian populations and assess their performance.

1.4 Research Hypothesis

1.4.1 Hypothesis for Conducting a Systematic Review in the general populations

1.4.1.1 Adding GRSs to CRFs-based model would improve the ability of these models to predict CHD events in the general populations.

1.4.2 Hypothesis for CHD/AMI Risk Prediction in Hungarian Populations

1.4.2.1 Hungarian Roma have more genetic variation and environmental risk factors for CHD than the general populations.

1.4.2.2 Including GRSs into the CRFs-based SCORE model would increase model capacity to predict CHD events in the Hungarian Roma population.

CHAPTER TWO

Literature Review

2.1 CHD Burden among the Hungarian Populations

CHD and stroke are the two leading causes of death in Hungary, accounting for one-third of all fatalities [13-14]. Despite the fact that the CVD burden has steadily decreased in European countries over the last few decades, the rising prevalence of CHD in Hungary is creating public health concerns. Hungary has the highest CHD risk among European Union members, (almost 40% of adults in Hungary have a chronic disease) [13-14]. Half of all deaths in Hungary are attributed to lifestyle risk factors [13-14]. A large number of risk factors, including poor diet (24% of all deaths), smoking (21% of all deaths), obesity (26.5%), and physical inactivity (2% of all deaths), are more prevalent in Hungary than other European countries [13-14]. Hungary has a lower life expectancy than the majority of European countries, including gender disparities (women (79.3) live over 7 years longer than men (72.5)), and socioeconomic status, which are reflected in educational gaps and living standards [13-14]. Socioeconomic deprivation is more common among the Hungarian Roma population than the general, and it is associated with health inequalities (chronic diseases) and life expectancy [46-50]. It is commonly understood that genetics, physical environment, and access to and usage of the health care system, as well as social environment, all influence an individual's health state [17-18]. CHD burden increases by social deprivation among countries, groups, and specific ethnic minorities, these disparities could be related to inequalities in socioeconomic status and education, as well as genetic vulnerability [46-47]. The Hungarian Roma minority is a vulnerable and disadvantaged ethnic group in Hungary, according to the country's ethnic background; the majority of them are severely impoverished, live in inadequate housing facilities, and are below the poverty line [48-50]. The poor health of Roma population is well documented, Roma population has relatively limited access to healthcare units as a result most Roma CHD patients have a worse cardiometabolic profile at the entry of care, which is characterized by a high risk of premature death [51]. The Roma population is exposed to risk factors for CHD, including smoking, obesity, metabolic syndrome, diabetes mellitus, high triglyceride levels, and low HDL-C concentrations [52-53]. As there is no data on CHD risk prediction for the Roma population is available, no previous study assessed genetic

background of CHD/AMI risk and no model focused on the integration of CRFs and GRS, an accurate estimation of CHD risk is therefore required. After identifying people who live with higher (genetic) risk we can provide them specific advice for more effective prevention.

2.2 Risk Factors for CHD

In general, the development of the CHD is the result of a complex combination of risk factors (predictors/prognosis). The vast majority of which are linked to lifestyle and human activities [17-18, 29, 40]. Despite advances in our understanding of the etiology of atherosclerosis and advancements in preventative efforts (diagnostic technology, therapy, and smoking regulations), CHD remains the major cause of premature adult's mortality and morbidity worldwide [1-14]. There is still much we do not know about the specific trigger mechanism; however, a wide range of risk factors have been discovered, the majority of which are too general to identify the primary beginning process of atherogenesis [23]. Risk factors such as hypertension, hyperlipidemia, diabetes mellitus and obesity, which are thought to be the main causes of CHD development, are also increasing significantly [4,9-11, 54, 55]. Public health practices and clinical practitioners rely on these predictors to assess a patient's risk of developing CHD over the course of a given time period because there is no particular time when CHD emerges, and risk factors are frequently inadequately treated even in high-risk individuals [29-30]. The common predictors used in CHD risk prediction models were listed below;

2.2.1 Age

Many chronic diseases including CHD, are significantly connected to increasing age [17-18, 54-60]. Although age standardized rates for prevalent cases, DALYs, and deaths due to CHD have decreased, the global prevalence of CHD is increasing due to populations growth, aging, as well as the health care system [54-61]. The incidence of CHD rises dramatically with age in both men and women [56-58, 61-64], and the prevalence of CHD rises with age in men in all age group compared to women until menopause, at the advanced age, women outnumber men, and the absolute number of female patients increases [60-61]. By the end of the century, it is expected that more than 30% of the populations in Europe would be over the age of 65, which could have an impact on the prevalence of disease, the cost of healthcare [55, 65]. It is widely accepted that the risk of dying from CHD increases considerably with age [57, 62, 66]. When

integrating with CRFs in multivariable regression models, age is an independent risk factor for developing CHD, therefore, age was included in all models constructed for CHD risk prediction [64]. This predictor, together with other risk factors, is used to assess an individual's risk of future CHD in various risk prediction scores which may be used to indicate the intensity and duration of exposure [64].

2.2.2 Sex

The most effective predictor for determining who is most likely to develop CHD is sex [63]. This predictor was included in all CHD risk prediction models developed for the general populations [18, 29, 54]. Sex variation exist in CHD pathogenesis, clinical manifestation, responsiveness to treatment, and outcome. These variations may be due to differences in risk factors, comorbidities that influence CHD presentation, and underlying biological differences (gene, and sex hormone) [18, 57, 62-64, 67]. CHD are more prevalent in males than in females [62]. In both sexes, CHD increases with age, but males get CHD at a younger age and have a higher predisposition to develop CHD than females [56, 61, 64, 67]. Females present with MI at a later age and have a higher burden of comorbidities than males, and they have a higher mortality rate after MI [64, 68]. It is expected that females born in ESC member countries will live 80.8 years and males 74.8 years [55]. Previous study found that males have a 2 to 5 times higher prevalence of CHD than females among middle-aged persons, and this sex ratio varies between populations [4]. Throughout adulthood, males had a greater CHD death rate than females, but the magnitude of the difference varied by age. The males-to- females CHD death rate ratio was 4-5 throughout middle age (30-64 years) and 2 beyond that (65-89 years) [62].

2.2.3 High Blood Pressure

High blood pressure is the single most useful predictor for identifying people who are at high risk of developing CHD [18], often known as hypertension, is defined as a rate of raised systolic blood pressure of 140 mm Hg or higher and/or a diastolic pressure of 90 mm Hg or higher that is regarded to be above normal norms [69]. Among adults in ESC member countries was 25%, prevalence of hypertension was lower in females compared to males [55]. Several studies have shown that untreated hypertension is a major contributor to CHD, and the second leading cause of deaths worldwide [18, 27, 55, 62, 69]. The development of atherosclerosis is accelerated by hypertension (induces endothelial dysfunction), especially when it is combined with

hyperlipidemia [18]. Many investigators indicated that systolic hypertension (SBP) is actually more important as a predictor for CHD than DBP [18, 27, 54-55]. All CHD risk prediction models created for the general populations incorporated (SBP) predictor [54].

2.2.4 High Blood Cholesterol Level

High blood cholesterol (LDL-C and TG) is a substantial risk factor for CHD death, particularly in young adulthood and middle-aged males [70]. Hypercholesterolemia (>8.0 mmol/L) is frequently inherited, with heterozygous involvement [54-55]. It is also a significant predictor of premature CHD morbidity [55, 71]. Low-density lipoprotein cholesterol (LDL-C) is the most important risk factor for CHD and the primary focus for treatment [72]. This association is still considered to be etiologically significant, along with age, sex, smoking status, systolic blood pressure, and HDL cholesterol, as a crucial element of cardiovascular risk prediction models that are frequently used in clinical practice to determine a person's risk of CHD and to direct clinical decision-making regarding the start of statin therapy and other lipid-level regulating medications [70]. Low-density lipoprotein cholesterol (LDL-C) is the most critical factor in the occurrence of atherosclerotic CHD and is the main target for preventing it [45-55].

2.2.5 Smoking

Smoking is a primary cause of morbidity and premature mortality in avoidable diseases such as CHD [73], accounting for 25% of CHD deaths under 65 years of age and causing sudden deaths in males under 50 years of age [17-18]. There is no such thing as a safe level of smoking; light smokers who consume one cigarette per day have approximately a 50% increased chance of developing CHD [55]. Passive smokers are also at risk, and significant secondhand smoke exposure is associated with a similar relative risk of CVD as low active exposure [55, 74-75]. A cross ESC member nations, more than 20 % of adult’s smoke on a daily basis, with the prevalence ranging between males (28.3%) and females (14.8%) [55]. Smoking contributes significantly to premature CHD and promotes atherosclerosis by increasing oxidation of low-density lipoprotein (LDL-C) and causing coronary endothelial vasodilation damage [76]. Smoking interacts synergistically with other risk factors such as hypertension and increased blood cholesterol [17-18]. Smokers who stop can reduce their risk of CHD by 39% in 5 years,

but the effect of quitting in lowering CHD risk takes at least five to ten years, and possibly 25 years, after quitting [54-55].

2.2.6 Diet

It's widely accepted that dietary factors contribute significantly to CHD risk, dietary fat (which is associated with all cause and CHD mortality), sugar (more than is recommended for a healthy diet), a higher intake of dietary sodium (which is associated with increased blood pressure level), and a low intake of fruit and vegetables are all major risk factors for CHD. Diet control can dramatically reduce the number of deaths caused by CHD [55].

2.2.7 Physical Inactivity

Physical activity has been demonstrated to reduce death and CHD risk in middle age by lowering blood pressure, losing weight, increasing insulin sensitivity, and lowering cholesterol [55].

2.2.8 Obesity

Obesity affects more than one in every three females and one in every four males in European member countries, with similar frequency in high- and low-income countries [38]. Over the last 35 years, the prevalence of overweight and obesity has more than doubled, and it continues to climb in both industrialized and developing countries (with an average of 60% adults) [38,55]. Obesity with a body mass index (BMI) of 25 km/m^2 is a risk factor for CHD (BMI 25 km/m^2 led to 2.0 million deaths), with the lowest all-cause mortality reported at BMI (20 to 25 km/m^2), however, BMI of 20 km/m^2 increased all-cause mortality [38]. BMI is a decent predictor of CVD risk (mostly CHD), especially at higher levels, although there is strong evidence that visceral adiposity and liver fat are major risk factors at all levels of BMI. This helps to explain why the CHD risk profile in the overweight differs based on the location of adipose deposition. Some argue that, in addition to BMI reduction, waist circumference reduction as a proxy for visceral fat reduction should be a more essential objective for preventing CHD [38].

2.2.9 Diabetes Mellitus

Diabetes mellitus (type 1, and 2), defined as a chronic hyperglycaemic state characterized by a lack in the synthesis or action of the insulin hormone (insulin resistance or intolerance), which

regulates glucose, lipid, and amino acid metabolism, is a key risk factor for CHD [17-18]. A fasting blood glucose level of 70 to 99 mg/dL (3.9 to 6 mmol/L) is considered low or normal. Higher values could suggest pre-diabetes or diabetes, with prediabetes ranging from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) and diabetes ranging from >125mg/dL or higher [18]. People with DM have 2 to 4-fold higher risk of developing CHD compared with non-diabetic people in both sexes for all age groups [77]. Incidence of CHD was found to be higher among diabetic patients, and about 75% of deaths in people with diabetes [78-79]. Diabetes was discovered to work synergistically with other variables such as obesity, smoking, hyperlipidaemia, and hypertension in hastening the atherosclerosis process [80].

2.1.10 Genetic Risk Predictors for CHD

Despite the value of CRFs and the utility of risk estimate models, many high- and low-risk individuals are misclassified as low and high risk by CRF algorithms, resulting in overuse or underuse of preventative methods for predicting CHD in the general populations [29-30]. CHD risk classification for primary prevention based solely on CRFs appears inefficient [30, 81, 89]. The GRS computed from recently discovered genetic variants could provide a potential solution in cardiovascular primary prevention. Studies shown that genotyping the population with a microarray containing these genetic risk variants, and genetic risk stratification based on the GRS is superior to that of conventional risk factors in detecting those at high risk and who would benefit most from statin therapy [26, 90-92]. The identification of genetic risk variants might lead the development of a novel therapy like the discovery of PCSK9 has led to the development of a novel treatment for high plasma LDL-C. Risk variants in the genome does not change over an individual's lifetime, it does not vary with time, and it can be easily detected by a single blood test, and a single variant can exert multiple influences [26, 90-92]. It is now universally accepted that CHD risk is known to be modified by the interaction of both multiple genetics and environmental components [32, 93]. GWAS has so far identified a hundred loci associated with many CVD risk and traits [94]. Out of these, more than 97 single-nucleotide polymorphisms (SNPs) have been associated with CHD risk and myocardial infarction [90-92, 95-99]. SNPs on chromosome 9p21 have been consistency associated with CHD [30], variant at 3q22, and 6p24, 6q23, 6q26 and 12q24, also found to be a risk factors for developing CHD [96]. Individual risk of CHD prognosis is determined by hereditary and lifestyle factors. GRS-based risk classification, followed by lifestyle adjustments (e.g., physical

activity, nutrition) or statin treatment, was found to be associated with a significant 40% to 50% reduction in cardiac events in the high-genetic-risk group [54-55, 100-102]. Previous study indicated that, incorporation of GRSs based stratification for primary and secondary preventions have several advantages over CRFs because it is independent of age and can be determined at birth or anytime thereafter [100]. GRS is constructed from a list of common genetic variations associated with CAD on a risk-weighted basis. Each risk variant's weight is multiplied by the number of variants at that site (0 for absent risk, 1 for heterozygous (moderate risk), or 2 for homozygous (high risk), and the final score is simply the sum of the weighted dosage for each risk variant included in the GRS [100]. Formerly, the association between some SNPs (e.g., gene encoding for ion-channel subunits, and in coronary blood flow regulation), and coronary microvascular function independently from CHD were defined; specifically, the role of adenosine triphosphate-sensitive potassium channels (ATP), which are the end effectors of several regulatory mechanisms for coronary flow reserves [101].

2.2.11 Other Predictors

Several studies have indicated that CRFs are insufficient for identifying patients at high risk of CHD [30, 81], a novel biomarker (present in the blood) was then added to models besides the CRFs in order to contribute to CHD improvement in models performance which includes beside the genetic marker for quantifying the added value of genetic biomarkers and family history of premature CHD (both plasma cholesterol concentration and hypertension are heritable) [30, 81-84], coronary artery calcium [85-86], C-reactive protein [30, 87], fibrinogen [30, 88] and homocysteine, lipoprotein, cystatin c, and apolipoproteins [30].

2.3 Concept of Prognostic Models for CHD Risk

Prognostic models are used to estimate the probability of developing a particular outcome in the future with the aim of assisting clinicians in disease prediction and enhancing informed decision-making with the patients [37,41-45 102-105]. These models in general, use two types of performance measures: discrimination and calibration [102, 105]. Prognostic models are more likely to be reliable and useful in practice when they are developed using a large, high-quality data set, based on a study protocol with a sound statistical analysis plan (logistic regression model or Cox proportional hazard model), evaluated a long-term outcome (10 years' incidence of CHD, with some patients censored), and externally validated by using independent

data sets [102-105]. Despite the importance of predicting future CHD among initially healthy adults, the predictive accuracy of the models often seemed disappointing because most individuals who eventually suffered a CHD event were previously at average risk rather than high risk [81,89]. In observational studies, data from the cohort (retrospective and prospective), nested case-control, or case-cohort studies are recommended for prognostic modelling studies (developmental and/or validation) [45, 81,105].

Several prognostic models, such the Framingham, SCORE, QRISK, QRISK2, and ASSIGN models [29-30,54, 84], have been created in the recent decades to assess the risk of developing the CVD outlined below;

2.3.1 Framingham

The Framingham heart study was the first and most widely used risk prediction model for CHD, created by Framingham, a town in Massachusetts, USA in 1968 [27]. This model was built for predicting 10 years' risk of CHD, using three generations of residents includes 1968-1971, 1971-1975, and 1984-1987[29]. Members of the generations were age (30-75) years, the second generation was an offspring cohort made up of children of the original cohort and their spouses [29, 45, 106]. The original Framingham model included age, sex, LDL-C, HDL cholesterol levels, blood pressure level, hypertension medication, smoking, and diabetes mellitus [45]. This study discovered important risk variables that predispose to the development of CHD, which may help in classify patients and prescribe statin therapy for those with high risk to develop CHD [103]. Framingham risk score functions have overestimated the CHD risk in some populations (British and European natives), leading to a concern that it may not be appropriate for other populations [97, 107-108].

2.3.2 Systematic Coronary Risk Evaluation (SCORE), SCORE2, and SCORE-OP

The SCORE, SCORE2, and SCORE2-OP models are a risk models developed by the ESC for use in clinical cardiovascular risk management in European clinical practice, these models recommended for high and low risk regions of Europe including Hungary [9, 51, 109], SCORE model is based on data from 117,098 males and 88,080 females who participated in 12 European cohort studies between the ages of 40 and 65, and it estimates the 10-year risk of overall CVD death at baseline in (1972-1991) [29, 65]. This risk assessment estimates fatal CVD events over a ten-year period based on integrated CRFs such as sex, age, TC, or TC/HDL-

C ratio, SBP, and smoking status [29, 54, 109]. SCORE provides calibrated risk estimation for total CVD events for low, moderate, and high-risk populations. The validity of this risk functions was analyzed with the area under the ROC curve (discrimination) and the Hosmer-Lemeshow test (calibration), respectively, SCORE is also overestimated the CHD risk in some populations (Spanish) [110]. SCORE2 risk prediction algorithm is a revised version of SCORE, developed to estimate the 10-year risk of first-onset CVD in the European population [9], in individuals without prior CVD or diabetes mellitus in the age range (40-69) years, using data from 45 cohorts in 13 countries (677, 684 individuals, 30,121 CVD events). This model included age, smoking status, SBP, total- and HDL-cholesterol predictors, and it is recommended for apparently healthy people <70 years of age without a history of CVD, DM, CKD, genetic, lipid, or blood pressure disorders to estimate the 10-year fatal or nonfatal CVD, however patients with stablished CVD and or DM, CKD are to be considered at high or very high CVD risk [9, 111]. The competing risk adjusted SCORE2-Older Persons (SCORE2-OP) risk model is recommended in apparently healthy people aged 70 years or older. It was developed to estimate the 5- and 10-year risk of CVD in older adults in four geographical risk regions, with the models including age, smoking status, diabetes, systolic blood pressure, and total- and high-density lipoprotein cholesterol [9, 112]. In this model, the 10 years CVD risk were classified as; low to moderate (<2.5%), high (2.5-7.5%), and very high (≥7.5%) based on age categories (<50, 50-69, and ≥70). CVD risk is higher (≥7.5%) in apparently healthy people aged <50 years and having SBP (140 to 130 mmHG), but very high (≥10%) if people aged (50-69), with SBP (<140 to 130 mmHG), and LDL-C (<2.6 mmol/L, or <100mg/dL) level, and greater (≥15%) in people aged ≥70 years old [9].

2.3.3 QRISK 1, and QRISK2

QRISK is a CVD risk prediction model developed using data (QRESEARCH) collected from general practice databases in the United Kingdom between 1993 and 2008 [29, 54]. This risk stratification method included 1.28 million participants in QRISK1, and 2.29 million in QRISK2, aged 30 to 74, calculated a 10-year risk of CVD [54-55]. The CRFs included in QRISK1 were gender, age, TC to HDL-C ratio, SBP, smoking status, and diabetes, as well as indices of social deprivation, family history of CVD, BMI, ethnicity, chronic conditions, and antihypertensive medication [54, 113], however, QRISK2 includes age, gender, ethnicity, deprivation, SBP, BMI (height, weight), TC to HDL-C ratio, smoking, family history of CHD,

antihypertensive medication, and some of the medical condition variables such as DM, CKD, atrial fibrillation, and rheumatoid arthritis [54, 114].

2.3.4 ASSIGN

ASSIGN is a CVD risk prediction developed using the Scottish Heart Health Extended Cohort Study from the general populations in Scotland between 1984 and 1987. It included 6,540 males and 6,757 females, ages 30 to 75 years, it estimates the 10-year risk of overall CVD event [54]. ASSIGN uses conventional risk factors including sex, age, TC, HDL-C, SBP, smoking, and DM, measures of area based index for social deprivation, and family history of CVD predictors [29, 54, 115].

2.3.5 PROCAM

PROCAM is a CVD risk prediction developed (in German city of Munster) using health employee's databases (1978-1995) [29, 54]. It is based on data from 18,460 males and 8,515 females, ages 20 to 75 years who participated in two separate scores calculate 10-year risks of major coronary events and cerebral ischemic events. The CRFs includes age, sex, LDL-C, DM, smoking and SBP. This model developed using Weibull methods, which allow extension of risk estimation to females and broader age range [54]. PROCAM functions has miscalibrated for some European populations [29].

2.3.6 Pooled Cohort Studies Equations

Pooled Cohort Studies Equations is a CVD risk prediction model based on data from four Pooled prospective studies: ARIC, CHS, CARDIA, and Framingham (original and offspring studies), with populations baselines from 1987-1989 (ARIC), 1990 and 1992-1993 (CHS), 1985-1986 (CARDIA), 1968-1971, 1971-1975, and 1984-1987 (Framingham)[45,54,72,107]. It comprised 11,240 white females, 9,098 white males, 2,641 African-American females, and 1,647 African-American males between the ages of 20 and 79 to estimate a 10-year risk of a CVD incident. Predictors of lifetime risk Age, gender, race (white or other/African American), total cholesterol, HDL-C, SBP, antihypertensive medication, diabetes, and smoking are all CRFs [45, 54, 72, 107].

2.3.7 CUORE

CUORE is a CVD risk prediction model that was developed between the 1980s and 1990s, and it includes 7,520 males and 13,127 females aged 35-69 [54]. It calculates the 10-year risk of developing an event (MI or stroke) based on CRF factors such as age, gender, SBP, total cholesterol, HDL-C, antihypertensive treatment, and smoking habit [54].

2.3.8 Globorisk

Globorisk CVD risk prediction model based on 8 pooled prospective studies includes Atherosclerosis Risk in Communities, Cardiovascular Health Study, Framingham Heart Study original cohort and offspring cohort, Honolulu Program, Multiple Risk Factor Intervention Trial, Puerto Rico Heart Health Program, and Women's Health Initiative Clinical Trial, included populations from 8 prospective studies from North America (1948-1993), included 33,323 males and 16,806 females, aged 40-80 years [54].

Most of the existing models are based on the Framingham model [54]. Different markers were then added to this model as a response to deficiencies in improving performance, such as coronary artery calcification scores, C-reactive protein, fibrinogen, homocysteine, and apolipoprotein [29-30,81-88]. Previous studies found that all three models based on the Framingham score; the Framingham Adult Treatment Panel (ATP) III model, the Framingham Wilson model, and pooled cohort equations (PCE) provide an incomplete prognosis of CHD events [107]. However, two problems remain: first, there is no consensus about the most suitable and optimal model for predicting CHD in the general populations, and second, it is not clear which biomarkers or genetic markers associated with events should be incorporated into the risk model in addition to conventional factors [29].

For any novel CHD risk factor to be useful in a clinical setting, it must significantly enhance event prognosis based on easily measurable CRFs such as age, cholesterol level, blood pressure, or body mass index; thus, any such factor(s) must have a major impact on risk [43-44].

2.4 Estimating A Model Performance

There are several ways to evaluate prediction model performance, including R^2 statistic and Brier score (Kaplan Meier estimator) to indicate over all model performance (traditional methods for binary ad survival outcome), discrimination ability using sensitivity, specificity,

and the AUC (or ROC) curve, or concordance (c) statistic and calibration measures via Hosmer-Lemeshow "goodness of fit" [102, 104, 116-118], as described below;

2.4.1 Discrimination

Discrimination assesses the model's ability to distinguish between people who develop CHD events (at high risk) and those who do not (at low risk) [116]. It is determined by the distribution of patient characteristics in the populations where the model is applied, which includes variables such as age, gender, clinical, and genetic data [104]. Several measurements can be used to assess its including; 1. The concordance (c) statistic for survival (the most often used performance metric) is a rank order statistic related to the D statistic that reflects generalized linear regression models' discriminative capabilities, 2. The AUC or ROC curve for a binary outcome, which plots the sensitivity (true positive rate) against 1- (false positive rate) for consecutive cutoffs for the probability of CHD outcome. If the model predicted a higher probability for patients with CHD than those who do not, the c statistic or (ROC) value is 1.0 (the more accurate the prediction model); however, if the c statistic or (ROC) value is equal to 0.50, the model cannot discriminate between high risk and low risk individuals [104]. Even though physicians should not utilize a model that fails to differentiate between high risk and low risk people, discrimination alone is insufficient to assess a model's prediction capability [104].

2.4.2 Calibration

Calibration (goodness or fit) refers to how well the model forecast matches the overall observed event rates, or how well the observed outcomes and predictions accord [104, 116]. It is recommended to report calibration performance [102]. A good calibration should be present in a useful model [103- 104, 116-118]. It may be effective in some patients but not in others. A poorly calibrated model will either underestimate or overestimate the disease outcome [103-104, 117]. When the average predicted risk is higher than the overall event rate, the algorithm overestimates risk in general, and overestimates risk in individuals at high risk by more than 20% (a high disease incidence) [104, 118]. Underestimation happens when the observed event rate exceeds the average anticipated risk [103-104, 117]. Even if they overstated risk by more than 20% in high-risk people, this model would still be clinically valuable. The calibration intercept and calibration slope can be used to detect poor calibration [118]. The Hosmer-

Lemeshow goodness-of-fit test is the most commonly used calibration test, and it was extended for survival data by Grnnesby and Borgan test (for cox proportional hazards regression model, which suggested separating the data into groups based on the risk score defined by Kaplan-Meier) and Nam and D'Agostino test (for survival model utilizing a counting process approach) [117].

2.4.3 Other Performance Metrics

The likelihood function is a statistical method used to evaluate how well a model fits the data; it indicates how much the likelihood increases by the novel marker (molecular, genetic, imaging), and it measures using the likelihood ratio test, the Wald test in nested models, or log likelihood for binary outcomes in nonparametric models and machine learning. Other likelihood-based measures, such as the Akaike Information Criterion (AIC) or the Bayes Information Criterion (BIC) can be used. These are particularly valuable when non-nested models are used [116, 119]. The easiest technique to compare a model with or without a new marker is to validate it in completely independent, external data [116]. Reclassification table is used to show how many subjects are reclassified by adding a marker to a model (the increase or the extension in c statistic by adding a new predictor). The net reclassification improvement computes the proportion moving up or down in risk strata in cases and non-cases separately. The overall NRI is a sum of improvement in each set [116, 119]. Integrated discrimination improvement is defined as the difference between the discrimination slopes of two models, one with and one without the new variable [120].

2.5 Prevention of CHD

Strong prediction for incidents of CHD by identifying high-risk subgroups (family history of premature CHD or familial hypercholesteremic can prevent CHD) [55]. The American Heart Association developed seven criteria for a healthier life including exercise, maintain a healthy weight, educate yourself on cholesterol, abstain from smoking or using smokeless tobacco, eat a heart-healthy diet, maintain a healthy blood pressure, and educate yourself on blood sugar and diabetes [4]. Exercise is commonly acknowledged to have a good impact on the majority of health outcomes, including CVD. Even at highly intense levels of exercise, the risk of death and morbidity is negligible, and in the vast majority of cases, the benefits outweigh the risks

[4, 55]. The single most cost-effective CVD prevention strategy is quitting smoking, and some benefits can be noticed as soon as a few months have passed [4, 55].

CHAPTER THREE

Methodology

3.1 Methodology for Conducting a Systematic Review for Predicting CHD/AMI Risk in the General Populations

3.1.1 Definition and Objectives

Recognizing the need for larger studies, we first performed a systematic literature review. It's a process of identifying and collecting available studies related to CHD risk factors [102]. Our objectives were to provide an overview of multivariable prognostic models developed to predict the risk of CHD in the general populations (genetic and conventional risk factors), explore and identify the optimal models by evaluating how well they performed in estimating CHD risk, and provide researchers with prognostic models by outlining the optimal combination of predictors, including conventional and genetic risk scores, and biomarkers covariates.

3.1.2 Protocol and Registration

The protocol was registered in PROSPERO (ID: CRD42021234224). We conducted a systematic review based on the PRISMA guidelines by following the recently published Cochrane Prognosis Methods Group guidelines [121], by using the Checklist for Critical Appraisal and Data Extraction for the Systematic Review of Prediction Modelling Studies (CHARMS) statement for assessing the quality of the prognostic modelling studies [105]. The Genetic Risk Prediction Studies (GRIPS) Statement was used to assess genetic prognostic modelling studies [122]. We performed the search of using items of the PICO framework including P(opulation) for subjects (people) free of coronary heart disease, I(ntervention) for developmental prediction models, C(omparator) for validation prediction models, and O(utcome) for the incidence of CHD within a specified time interval. The developmental prediction model (internal or external) seeks to derive a prognostic model by selecting relevant predictors and statistically combining them into a multivariable model (logistic regression, Cox proportional hazards methods), and then quantifying the model's predictive power (discrimination, calibration, and classification), validation modelling studies (with or without

updating) aim to assess and compare the predictive performance of an existing prediction model using new participant data that weren't utilized to develop a prediction model [45].

3.1.3 Information Sources, and Search

An intensive systematic search was conducted utilizing five databases including Embase, PubMed, Cochrane, Web of Science, and Scopus. We applied a human filter on 30 November 2019 to identify original articles of the developmental and/or validation of prognostic models describing the combination of conventional and genomic risk factors for incident CHD. We searched the databases using the following key search terms: *("validation" OR "prediction" OR "predict" OR "risk" OR "prognosis") AND ("ROC" OR "area under the curve" OR "c-statistic" OR "c statistic" OR "discrimination" OR "discriminate") AND ("coronary heart disease" OR "CHD" OR "coronary disease").*

3.1.4 Eligibility Criteria

We included all original articles describing the estimation of risk associated with CHD morbidity or mortality in individuals in developmental and/or validation modelling studies (internal/external) where the models' performance for predicting CHD in the general populations (performance measures regarding calibration, discrimination, andreclassification) were available. Two study designs were included: (nested) case-control and cohort studies. Articles describing clinical models with intervention (treatment) and studies describing the prediction models of CHD in individuals with certain health conditions, such as HIV, HBV, congenital heart disease, kidney failure, diabetes mellitus, hypertension and cancer were excluded.

3.1.5 Study Selection

Initially, two reviewers independently screened the titles and abstracts of all studies identified according to the keywords and inclusion criteria, and then duplicates were removed. After consensus, full-text articles were then obtained and examined for quality. If there was any disagreement regarding the article's inclusion, a third person evaluation was performed to reach a consensus. We compared the work of the reviewers using the Epi Info7 program developed by the Centre for Disease Control and Prevention to minimize bias. The Preferred Reporting

Items for Systematic Review and Meta-Analysis (PRISMA) flow chart summarizes the selection process (Figure 3.1).

According to our objectives, we selected all articles describing the prognostic modelling studies for CHD in the general populations (where subjects are free from CHD), in cohort design (n= 66) and nested case-control studies (n=6), these prospective studies allow the optimal documentation of the predictors and outcomes. Case-control studies are not suitable due to recommendations; therefore, they were excluded from this study, leaving both logistic regression and proportional hazard analyses to describe the effect of the categorical predictors. The details of the selection process were described in Figure 3.1. below;

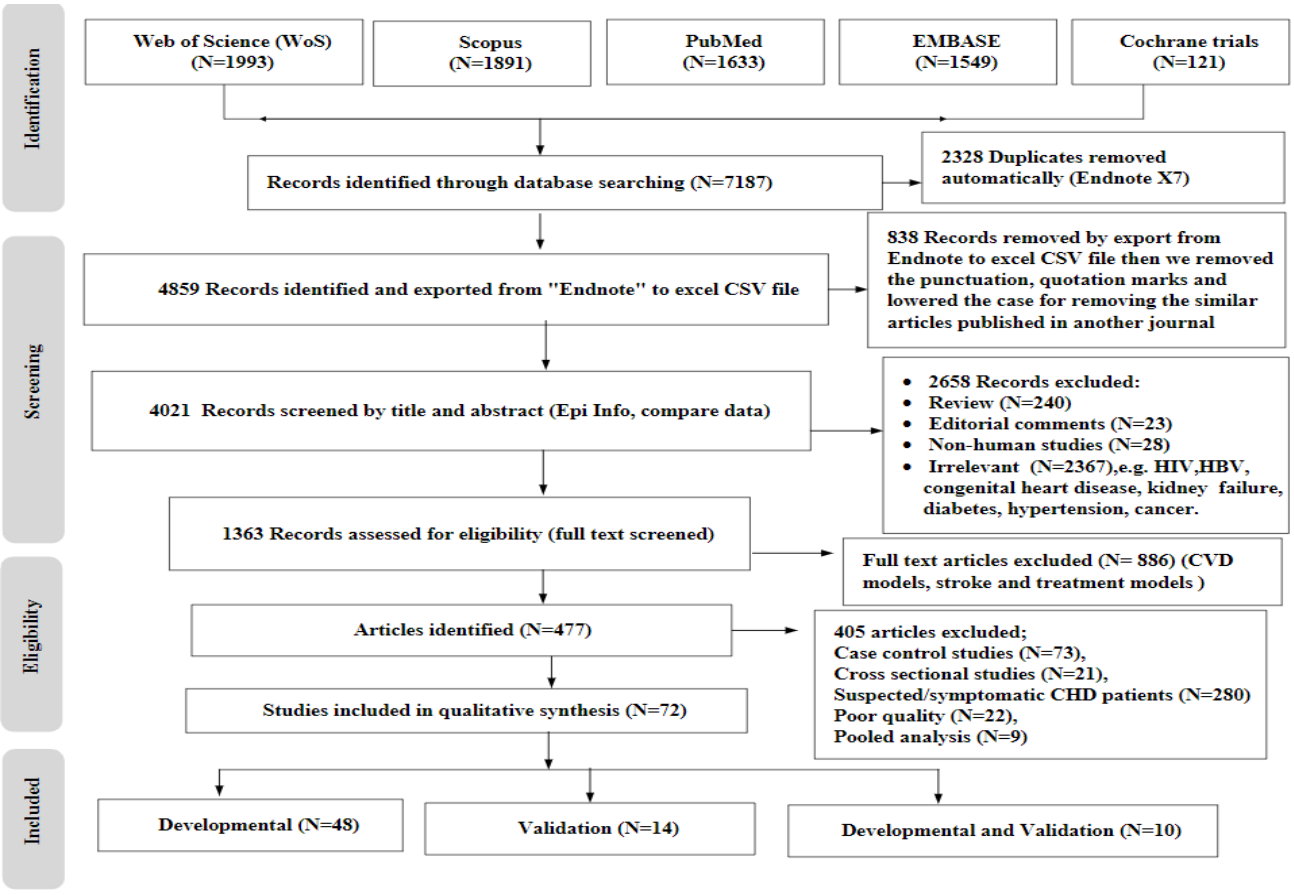


Figure 3. 1 PRISMA Flow chart of the selection process of CHD risk prognostic models.

3.1.6 Data Collection Process

The two reviewers extracted the articles independently. The list of extracted items was based on the CHARMS and GRIPS statements for reviewing the prognostic modelling of conventional and genetic studies [105, 121-122]. The full list of extracted articles [27, 94,123-

192] is available (see references list of the included studies in the Appendix table 12.1, and 12.2). We categorized the eligible full-text articles into three groups: (1) *Developmental studies* are such models commonly aim to identify important predictors by selection, combining them into a multivariable model, and then developing a final model and quantifying the predictive performance and validating the model internally using forms such as bootstrapping or cross-validation; (2) *Validation studies* with or without updating a model, aim at assessing and comparing the predictive performance of an existing prognostic model using new participants' data that were not used to develop the prognostic model; and possibly adjust or update the model in case of poor performance based on the validation data; and (3) *Developmental studies with external validation* in independent data [89, 105].

3.1.7 Risk of Bias Across Studies

The two reviewers separately assessed the quality of the included articles. Based on our objectives, the strengthening of the report of observational studies in epidemiology (STORBE) guideline was utilized to assess the epidemiological quality of the included studies [193].

3.2 CHD/AMI Risk Assessment and Intervention in the Hungarian Populations

3.2.1 Study Design and Setting

Second, an observational cross-sectional study was conducted to predict CHD/AMI risk in Hungarian Roma and general populations. We compared the prevalence of CHD/AMI in both populations based on the collected data to determine which group is more likely to develop CHD/AMI, by relating CHD/AMI (as dependent variable), associated with an independent variable of interest (age, sex, HTC-Med, HTN-Med, smoking, DM, and GRS/wGRS. We used preliminary data (on the health condition of Hungarian general and Roma adults living in segregated colonies) obtained in a complex health survey conducted by the MTA-DE Public Health Research Group that began in 2018, with the recruitment of 1000 participants from the Hungarian general (500), and Roma (500) randomly selected, aged 18 years or older [194]. The study included 558 participants (who provided complete genetic and phenotypic data). After completing the questionnaire, all participants were invited to a medical evaluation and blood sample collection (for genetic analysis, routine laboratory examination, and lipid profile) by their primary health care provider. Notably, Northeast Hungary is the geographical region

where most of the Roma Hungarians live in segregated colonies (Hajdú-Bihar and Szabolcs-Szatmár-Bereg counties) [194].

3.2.2 Participants and procedure

Overall, 558 study subjects were enrolled from two Hungarian populations: the general population (n=279) and Roma individuals (n=279). They were selected randomly in the framework of a complex comparative health survey by MTA-DE Public Health Research Group in 2018, this group are focused mainly on the investigation of the health (genetic and environmental factors for CVD (smoking), diabetes and colorectal cancer) of Roma population [194]. Initially, 92 segregated colonies were identified in a complex health survey; of these, 25 colonies were chosen at random using certified household lists from general practitioners (GPs). Following that, 20 families were chosen from each colony, and one individual aged 18 years and older from each household was interviewed face-to-face at the respondent's home by Roma University students who were supervised by public health coordinators. Participants who were interviewed were also invited for a physical examination and blood sample collection. The respondents' self-declaration indicated their Roma ethnicity [194]. Hungarian general subjects were drown from the same source population [194]. Data was collected through paper questionnaires, physical examinations, and laboratory testing. Blood samples for DNA extraction (for genetic analysis) were also drawn from all the subjects. The details of the sample and data collection have been previously described [194]. Subjects with complete genotype and phenotype data were included in this study. The details of the sample and data collection have been previously described [194]. The Hungarian Scientific Council on Health Research committee approved the protocol (61327-2017/EKU). All participants provided written consent before their participation.

3.2.3 Variables, Data Sources, and Measurements

In general, demographic information (background variables on demography, socioeconomic and health status), physical examination (height, weight, WC, blood pressure measurement), and blood sample collection for genetic analysis and laboratory investigation data were collected.

CHD and AMI was confirmed when participants answered “yes” to one of the following questions: “Did you have CHD or AMI during the last 12 months?”, “Have you been

diagnosed with CHD or AMI by a medical doctor?", or "Have you received hospital treatment for CHD or AMI?".

Blood pressure (SBP, and DBP) was measured during the physical investigation. Hypertension identified when participants answered "yes" to one of the following questions: "Did you have HTN during the last 12 months?", "Have you been diagnosed with HTN by a medical doctor?" or "Have you received a hospital treatment for HTN?". In addition to that, elevated hypertension was defined if the average SBP ≥140 mmHg or a DBP ≥ 90 mmHg based on the Fifth Joint National Committee Guideline (JNC-V) [195] (see table 12.10). Thus, in case of measured raised blood pressure or hypertension in the history we considered that the subject has hypertension.

The lipid profiles data (HDL-C, LDL-C, and TG) were determined in the laboratory (mmol/l), however, they were not employed in models' construction. HDL-C, TC and LDL-C were all found to be protective factors. The protective characteristics in the lipid profiles could be due to the low prevalence of CHD/AMI, or small enough sample size, or possibly due to the confounding effect. In addition to that an elevated TC was confirmed when participants answered "yes" to one of the following questions: "Did you have a high cholesterol level during the last 12 months?", "Have you been diagnosed with a high cholesterol level by a doctor?" or "Have you received a hospital treatment for a high cholesterol level?"

Diabetes mellitus (DM) was measured by laboratory examination (glucose level mmol/l). In addition to that DM was confirmed when participants answered "yes" to one of the following questions: "Did you have a DM during the last 12 months?", "Have you been diagnosed with DM by the medical doctor?", or "Have you received a hospital treatment for DM?".

Stroke was confirmed when participants answered "yes" to one of the following questions "Did you have a stroke during the last 10 months?", "Have you been diagnosed with a stroke by a medical doctor?", or "Have you received a hospital treatment for stroke?".

Chronic kidney disease (CKD) was confirmed when participants answered "yes" to one of the following questions: "Did you have CKD during the last 12 months?", "Have you been diagnosed with CKD by a medical doctor?", or "Have you received hospital treatment for CKD?"

Smoking status (yes/no) as lifestyle risk factors was confirmed when participants answered "yes" to the following question: "Are you a current smoker?". Other environmental and lifestyles risk factors including air pollution, noise and neighbourhood characteristics, physical activity, alcohol consumption were not a part of this study, however, it was investigated by the MTA-DE Public Health Research group.

3.2.4 SNPs Selection Procedure and Genotyping

Genetic variants (30 SNPs) were selected based on on their robust association with CHD/AMI risk in GWAS and had been extensively investigated in a systematic literature search of previous investigations [196-201]. In general, twenty-five SNPs that were significantly associated with CHD at a genome-wide level in prior analyses (see the details in Mega et al.) were selected [196]. Furthermore, an additional three variants were added based on a paper by Tikkanen et al. [197], and two SNPs were selected from Schenkert et al. [198] and Teslovich et al. [199]. These publications that had several overlapping SNPs were the most cited GWASs on CHD. All SNPs were successfully genotyped by the Mutation Analysis Facility, Clinical Research Centre, Karolinska University Hospital (Sweden).

3.2.5 Weighted and Unweighted GRS Constructions

SNPs were coded based on the number of risk alleles as follows: zero was assigned in the absence of the risk allele, subjects homozygous for the risk allele were coded as 2, and the single risk allele was coded as 1. The unweighted GRS was calculated by simply counting the number of risk alleles present for each SNP for every study subject, while the weighted GRS was computed by multiplying the risk allele score (0, 1, 2) carried for each SNP by the published effect size measure (ln of odds ratio) [100, 202-203].

3.2.6 Statistical Analysis and Software Were Utilized

First, DNA samples, clinical data and the questionnaire responses were matched in order to link the data and avoid duplicate subjects. Individuals with any missing genotype and phenotype values or individuals who did not specify their gender were excluded before the statistical analyses to minimize the possibility of systematic errors. Data quality control guidelines were applied based on a previous publication [204-205].

Based on the normality distribution, the difference between the baseline demographic and clinical data were compared using Pearson's chi-square (for categorical variables) and two-sided students t-tests (for continuous variables). The difference in means of the genetic risk scores (GRS, wGRS) between the two populations was examined using two-sided students t-tests. Additional analyses were performed with the goal of estimating and comparing the allele frequencies of 30 selected SNPs linked to CHD/AMI risk, as well as to calculate and compare the genetic load (weighted and unweighted GRSs). The allele frequencies of 30 SNPs associated with CHD in the Hungarian general and Hungarian Roma populations were analysed using Plink 1.07 software. The force-specific allele technique was used to ensure that the affected risk alleles were assigned to be first before running the commands in the allele frequency comparison analyses [206]. The HWE test in Plink 1.07 was used to exclude any SNPs that failed to be in HWE [207]. LD calculation was performed by using Haploview 4.2 software to examine whether there was any correlation between these SNPs [208] (see Figure 4.5). Bonferroni correction was performed (αnew = (α old/n) (p-value 0.05/29= 0.002) [209].

Multivariable logistic regression analyses were conducted by using Stata 13 software to assess the possible interaction between the CHD/AMI (binary dependent variable categorised into 0 for absent of CHD/AMI, and 1 for presence of CHD/AMI) when integrated with CRFs includes age, sex, HTC-Med, HTN-Med, smoking, DM, ethnicity, and smoking as independent variables (categorical or continuous variables). We created different models using the CRFs variables (age, sex, HTC-Med, HTN-Med, Smoking) suggested by the SCORE model [54, 210]. In addition, we updated the model by adding some potential explanatory predictors, such as genetic risk scores (wGRS and GRS divided into tertiles for low-, intermediate-, and high-risk subgroups) and diabetes mellitus (DM) [96, 131]. HDL-C, TG, and LDL-C were not included in the final derived model (there was no reasonable association between these predictors and CHD risk (see Appendix tables 12.8 and 12.9).

Ethnicity were also included in the statistical models, as shown in Table 3.2. To evaluate the potential value of the integration of CRFs and genetic risk score (GRS and wGRS) in risk prediction, the model's performance (discrimination, calibration) was assessed. First, the area under the receiving operating characteristic curve of models with and without the GRSs was computed. Second, the calibration by the Hosmer- Lemeshow goodness of fit test was

measured. Marginal plot analysis was also used to predict the interaction between the genetic risk score (GRS, wGRS), age, and sex for CHD/AMI risk prediction.

Table 3. 1 Single nucleotide polymorphisms (SNPs) and relevant weights associated with CHD.

No	SNPs	Nearest gene	Region/ Band	RA	RAF			Weight	References
					1	2	3	OR	
1	rs646776	SORT1	1p13.3	T	0.77	0.79	0.81	1.19	[196, 197, 201]
2	rs17114036	PPAP2B	1p32.2	A	0.92	0.89	0.91	1.17	[196, 197, 198]
3	rs11206510	PCSK9	1p32.3	T	0.81	0.82	0.81	1.15	[26, 196, 198]
4	rs17465637	MIA3	1q41	C	0.75	0.75	0.74	1.14	[196, 197, 198]
5	rs6725887	WDR12	2q33.1	C	0.13	0.14		1.17	[196, 200]
6	rs2306374	MRAS	3q22.3	C	0.10	0.18		1.12	[197, 198]
7	rs9818870	MRAS	3q22.3	T	0.15	0.17		1.15	[196, 201]
8	rs17609940	ANKS1A	6p21.31	G	0.79	0.81	0.75	1.07	[196, 197, 198]
9	rs9349379	PHACTR1	6p24.1	G	0.43			1.12	[196]
10	rs12526453	PHACTR1	6p24.1	C	0.67			1.10	[198]
11	rs12190287	TCF21	6q23.2	C	0.63	0.62		1.08	[196, 198]
12	rs3798220	LPA	6q25.3	C	0.01			1.47	[196]
13	rs10455872	LPA	6q25.3	G	0.07			1.70	[196]
14	rs11556924	ZC3HCL1	7q32.2	C	0.64	0.67	0.62	1.09	[196, 197, 198]
15	rs4977574	CDKN2B	9p21.3	G	0.55	0.43	0.46	1.29	[196, 197, 198]
16	rs579459	ABO	9q34.2	C	0.22	0.21		1.10	[197, 198]
17	rs635634	ABO		T	0.20			2.05	[199]
18	rs1746048	CXCL12	10q11.21	C	0.86	0.84		1.17	[195, 196]
19	rs12413409	CYP17A1	10q24.32	G	0.90	0.92	0.89	1.12	[196, 197, 198]
20	rs964184	APOA5	11q23.3	G	0.13	0.14	0.13	1.13	[196, 197, 198]
21	rs2259816	HNF1A	12q24	T	0.35	0.37		1.08	[196, 201]
22	rs3184504	SH2B3	12q24.12	T	0.48	0.44		1.13	[196, 198]
23	rs4773144	COL4A1	13q34	G	0.41	0.40	0.44	1.07	[196, 197, 198]
24	rs2895811	HHIPL1	14q32.2	C	0.45	0.42	0.43	1.07	[196, 197, 198]
25	rs3825807	ADAMTS7	15q25.1	A	0.57	0.65		1.08	[196, 197]
26	rs12936587	RASD1	17p11.2	G	0.53	0.65	0.56	1.07	[196, 197, 198]
27	rs216172	SMG6	17p13.3	C	0.64	0.35	0.37	1.07	[196, 197, 198]
28	rs46522	UBE2Z	17q21.32	T	0.48	0.55	0.53	1.06	[196, 197, 198]
29	rs1122608	LDLR	19p13.2	G	0.77	0.79		1.15	[196, 197]
30	rs9982601	KCNE2	21q22.11	T	0.13			1.20	[196]

Table 3. 2 Multivariable logistic regression analysis for CHD/AMI risk prediction models

No	Models	Explanatory variables
1	SCORE based models + Ethnicity	Age, Sex, HTC-Med, HTN-Med, Smoking (CRFs)
		CRFs + Ethnicity*
2	Genetic based models only	GRS per tertiles
		wGRS per tertiles
3	SCORE based models + Genetic models + Ethnicity	CRFs + GRS + Ethnicity
		CRFs + wGRS + Ethnicity
4	SCORE based models upgraded*	CRFs + DM
		CRFs + DM + Ethnicity
5	SCORE based models + Genetic models + Ethnicity upgraded*	CRFs + DM + GRS +Ethnicity
		CRFs + DM + wGRS +Ethnicity

Note: * *The Hungarian population was set as the reference. Outcome variable: CHD/AMI. * Upgraded by adding DM as an explanatory variable. HTC-Med: High total cholesterol level/or Taking cholesterol-lowering therapy; HTN-Med: Elevated blood pressure and/or Taking blood pressure lowering therapy*

CHAPTER FOUR

Results

4.1 Results of the Systematic Review for CHD/AMI Risk Prediction in General Population

4.1.1 Study Selection, and Characteristic

The search strategy of our systematic review identified 7187 potential articles; 2328 duplicates were removed automatically by Endnote X7 software, and 838 articles were removed after exporting the Endnote file to the CSV file (to create a new output style, removing the punctuation, lowering the case, and sorting the file). A total of 2658 articles were excluded based on title and abstract not being related to conventional or/and genetic risk modelling of CHD (reviews, editorial comments, and nonhuman and irrelevant studies) or prognostic modelling studies with subjects having comorbidities (HIV, HBV, diabetes mellitus, congenital heart disease, Chagas heart disease, and kidney failure). In total, 477 full texts were included after the exclusion of 405 other studies, such as Suspected patients (symptomatic patients who present with onset chest pain) of CHD or CVD or stroke (n=280), case-control studies (n=73), cross-sectional studies (n=21), case report (n=2), treatment (n=1), poor-quality studies with no follow up (n=6) or blinded comparison (n=1), pooled analyses (meta-analysis studies) (n=9), and no study design information (n=12) (see Table 4.1 for details). Finally, 72 eligible articles were included in this review (Figure 4.1) [27, 94,123-193].

Table 4. 1 The numbers of full-text articles identified and the reason of exclusion during the investigation of eligibility.

No	Study design	Identified	Included	Not included	Exclusion reason
1	Longitudinal (Cohort)	346	66	280	Suspected patient/CVD and stroke
2	Case control	73	0	73	Potential bias
3	Nested case control	7	6	1	Intervention
4	Cross sectional	21	0	21	Diagnostic models
5	Case cohort study	6	0	6	Poor quality (No follow up).
6	Case report	2	0	2	Short follow up
7	Blinded comparison	1	0	1	Poor quality
8	Pooled analysis	9	0	9	No value of use it
9	No information	12	0	12	Poor quality
Total		477	72	405	

Note: Table 4.1 above summarized the eligible articles (n=477) identified and included for the full text screening process, these articles covered both healthy subjects and a population with existing chronic disease conditions such as CVD (suspected patients with one or more symptoms) and stroke, and used various study designs (cohort, case control, nested case control, cross sectional, pooled analysis, and others). Based on our objectives and the PICO framework, we included a cohort study (n=66) and nested case control study (n=6), which were recommended for predicting CHD risk among the general populations (healthy subjects with no previous symptoms).

We identified (n = 48) articles concerning the developmental CHD risk prognostic models; 14 articles described the external validation of the models, and 10 articles described the combinations of developmental and external validation.

The number of validation modelling studies (including genetic risk models) was zero in 1997 and 14 in 2019, while the number of developmental and validation modelling studies was zero in 1997 and 10 in 2019. The developmental modelling studies increased over the period of time from 1997 (n=1) to 2019 (n=48), while the number of external (validation only) and developmental validation modelling studies declined (n=24) (Figure 4.1).

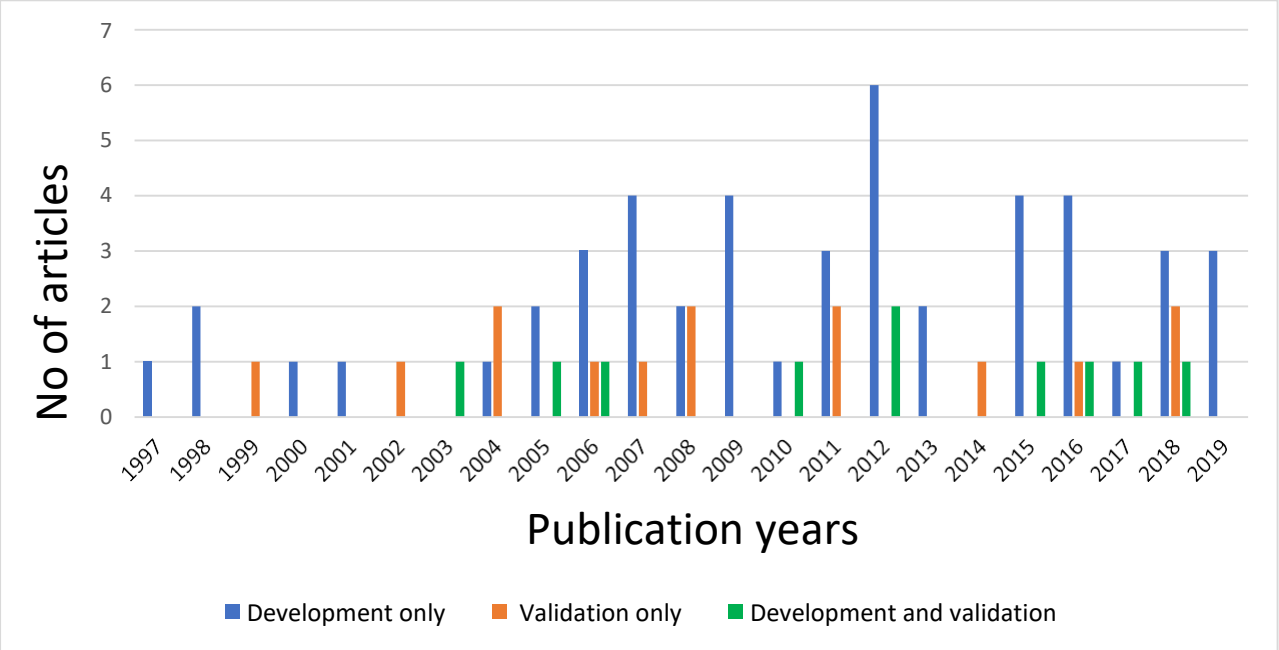


Figure 4. 1 Numbers of publications on prognostic models included per year.

4.1.2 Developmental Risk Prognostic Models for CHD

4.1.2.1 Frequency of Models, Study Designs, and Study Populations

In general, 58 articles (developmental plus developmental validation) described more than 157 different models (based on Framingham models plus the novel models created by the researchers) (Figure 4.2, and 4.3). Most of the prognostic models (*n* = 68, 94%) were developed using data from cohort studies. Most of the models originated in the United States and Canada (*n* = 32, 45%) or Europe (*n* = 26, 36%); few studies originated from Asia (*n* = 14, 19%), and no developmental modelling studies originated from African countries. Framingham risk models developed for the US population were used multiple times to derive a novel model for different populations and countries. Table 4.2 describes the list of published risk prediction models for predicting CHD in general populations that have been developed and validated over the past decades. Framingham models which developed by Wilson and/or D'Agostino was used in 30 articles, and Framingham Adult Treatment Panel III (2001 and 2002) was used in 9 articles. The SCORE risk-estimation model developed for the European population was used seven times, PROCAM was used five times and QRISK2 was used two times (see the other less-frequently used models in Table 4.2).

The description of study populations based on PICOTS was summarized in Appendix Table 12.1, however, the population characteristic, eligibility, selection criteria (inclusion and exclusion), race/ethnicity, geographical region, sample collection technique, settings, recruitment method, periods of recruitment, length of follow-up, and methods of data collection of the reviewed models were described in Appendix1 Table 12.2. In general, there was variation between the study populations regarding the age groups: seven models (10%) were developed for people with ages ranging from 30 to 74 years, and eight (11%) models were developed for the subjects with ages between 45 to 64 years, while the majority (*n* = 57, 79%) of the models used several different age groups.

Most of the models (*n* =53, 74%) targeted the general population (males and females), few models (*n* =17, 23%) were developed for males, and only two (3%) models were available for females. Regarding the inclusion and exclusion criteria in most studies (*n* =47, 71%), the researchers stated that participants with a history of coronary heart diseases (including a history of unstable angina or acute myocardial infarction, recorded ECG, stroke, heart attack), other diseases such as cancer (*n* =6, 9%), diabetes mellitus (*n* =10, 15%), or chronic medical conditions were excluded. participants who were taking lipid-lowering medication or aspirin were also excluded from several studies. Additionally, few studies (*n*=7, 5%) excluded participants because of race/ethnicity status, and one model had no information. In the modelling studies with genetic parameters, the investigators explicitly stated that they excluded study participants with no genotypic data (*n* = 11, 15%).

Table 4. 2 A list of the published risk prediction models for CHD in the general population that have been developed and validated over the past decades.

No	Name of the Models	Frequency of the Models (n)		
		Developmental	**Validation**	**Total**
1	Framingham-Wilson-D'Agostino, 1998	11	9	20
2	SCORE 2003	5	2	**7**
3	Framingham-ATP III, 2002	6	0	6
4	Framingham-Anderson, 1991	2	3	5
5	Framingham-Kannel, 1979	2	3	5
6	Framingham-Wilson, 1998	4	1	5
7	Framingham-ATP III, 2001	1	2	3
8	PROCAM-Assmann, 2002	3	1	4
9	Framingham-D'Agostino, 2008	3	0	3
10	QRISK2-Hippisley-Cox, 2008	0	2	2
11	PROCAM-Assmann, 2007	0	1	1
12	Framingham-Splansky, 2007	0	1	1
13	Framingham-Kannel, 1959	0	1	1
14	Framingham-Kannel, 1986	1	0	1
15	Framingham-Polak, 2011	1	0	1
16	Framingham-Wilson, 1991	1	0	1
17	Framingham-Wang, 2006	1	0	1
18	Framingham-Wilson, 2005	0	1	1
19	Framingham-Ridker, 2002	1	0	1
20	Framingham-Franklin	1	1	2
21	Framingham-ARIC, 2003	1	0	1
22	Framingham-Rotterdam	2	0	2
23	Framingham-MESA, 2002	1	1	2
24	Framingham-Lee, 2016	1	0	1
25	Framingham (not specified)	19	4	23
Total		67	33	100

4.1.2.2 CHD Definition and Outcomes

We observed a wide variety of fundamental definitions of CHD disease as well as CHD outcomes among the general populations. We specified the fundamental definitions of CHD disease according on Damen et al 2019 [107]. There were almost 20 distinct classifications for the definition of CHD, however, the international classification definitions codes of CHD were reported in (n=28, 39.9%) models, but it showed heterogeneity, which described as ninth and

tenth revision (codes 410-414) (n=18, 25%), hospitalization or death with any of the following primary diagnoses: acute MI and unstable angina and surgical codes(n= 2, 2.8%), 10th revision codes 121 (n=5, 7%), 9th edition (ICD-9) codes (410-414) or ICD-10 codes (I20-I25) (n= 2, 2.8%), and ICD-8 (n=2, 2.8%) (see Table 4.3 for other CHD definitions). The CHD outcomes as endpoints were also showed considerable heterogeneity; the majority (*n* = 42, 58%) of the prognostic models characterized CHD disease as an occurrence of CHD with no categorization, whereas some models (*n* = 27, 38%) specified the endpoints of CHD as (fatal or nonfatal) myocardial infarction, stable or unstable angina, percutaneous coronary revascularization or bypass grafting, or death due to CHD. There were more than eight classifications for the outcomes of CHD. Other outcomes were identified, such as fatal/nonfatal CVD events (*n* = 1, 1%), and three models (n = 3, 4%) with no information (Table 4.4).

4.1.2.3 Time Span, Follow Up and Duration in the Prognostic Modelling Studies

Prognostic models follow participants over a period of time and record whether a specific outcome occurs after the prognostic time origins, this period start at time zero and goes through the end of follow up that specified in the study. Duration involved in prediction models should be described accurately in the study. Between the zero-time and the end of the follow-up researcher should do at least one more data collection for accurate result [45]. Follow-up time period in the reviewed prognostic models ranged between 3-30 years, 4 models (6%) predicted the incidence of CHD for less than 5 years, 33 models (56%) predicted CHD outcomes for 5-10 years, a longer (>10-15 years) follow-up was described in 29 models (40%), and the length of follow-up was longer than 15 years in a few models (n = 6, 8%).

Table 4. 3 Coronary heart diseases definitions before the studies were enrolled as described in the models reviewed.

No	Outcome definition	Frequency	%
1	International Classification of Diseases, Ninth Revision, codes 410 to 414 or International Classification of Diseases, Tenth Revision, codes I22 to I25.	16	22.22
2	International Classification of Diseases 10th Revision (acute myocardial infarction, code I21).	2	2.78
3	Hospitalization or death with any of the following primary diagnoses: acute MI and unstable angina (ICD-10: I20.0, I21, I22; ICD-9: 410, 411B; ICD-8: 410, 411 and surgical codes: FNG02, FNG05, FNC, FND, FNE).	2	2.78
4	'Hard' CHD events, comprising acute myocardial infarction, sudden death and other coronary deaths, non-fatal CHD events, defined according to the International Classification of Diseases 10th Revision (acute myocardial infarction, code I21).	3	4.17
5	International Classification of Diseases, 9th edition (ICD-9) codes 410-414 or ICD-10 codes I20-I25 were present on the death certificate. Non-fatal CHD included first non-fatal MI or first definite angina. Non-fatal MI was defined following MONICA criteria18 based on study electrocardiograms (ECGs), hospital acute ECGs and cardiac enzymes. Incident angina was defined based on clinical records and nitrate medication use, excluding cases based solely on self-reported data without clinical verification and Study participants with definite angina at baseline.	2	2.78
6	International Classification of Diseases (ICD)-9 codes consistent with non-fatal or fatal AMI (410.x), angina pectoris (411.1, 413.x), CHD (414.x), coronary revascularization procedures (CPT4 codes 33510, 33511, 33512, 33513, 33514, 33515, 33516, 33517, 33518, 33519, 33521, 33522, 33523, 33530, 33533, 33534, 33535, 33536, 92980, 92981, 92982, 92984, 92995, 92996) or CHD death (ICD-9 codes 410-414 or ICD-10 codes I20-I25).	1	1.39
7	Acute myocardial infarction (MI), old (recognized and unrecognized) MI, angina pectoris, and CHD death. The International Classification of Disease (ICD-8) codes for diseases of the circulatory system	2	2.78
8	Acute myocardial infarction (MI), silent MI, sudden cardiac death within 1 hour after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty.	1	1.39
9	Acute myocardial infarction (MI), silent MI or undergoing coronary surgery.	3	4.17
10	Acute MI, coronary death, hospitalization for angina, or coronary revascularization (angioplasty of coronary arteries and coronary artery bypass graft surgery).	1	1.39
11	Myocardial infarction or coronary death, CHD death was defined as the absence of non-atherosclerotic cause of death and1 or both of the following: chest pain within 72 hours of death or history of chronic ischemic heart disease in the absence of valvar heart disease or non-ischemic cardiomyopathy.	1	1.39
12	Myocardial infarction, fatal CHD, or cardiac procedure.	2	2.78
13	Sudden coronary death, fatal acute myocardial infarction, and nonfatal acute myocardial infarction.	1	1.39
14	Myocardial infarction as the presence of 2 of the following 3 factors: (1) prolonged chest pain prompting hospital admission, (2) diagnostic evolutionary ECG changes, and (3)	1	1.39

	elevation of serum creatine kinase to twice the upper limits of normal or a positive serum creatine kinase MB fraction.		
15	Myocardial infarction, resuscitated cardiac arrest, definite angina (symptoms of typical chest pain and physician diagnosis of angina followed by coronary artery bypass grafting and percutaneous transluminal coronary angioplasty (PTCA), evidence of ischemia by stress tests or resting electrocardiogram, or ≥70% obstruction on coronary angiography), and probable angina.	1	1.39
16	Nonfatal myocardial infarction or coronary death (corresponding to ‘‘hard’’ events, as defined in the current FRS, and hospitalization for angina or revascularization (coronary angioplasty or surgery).	1	1.39
17	Fatal or non-fatal myocardial infarction, stable or unstable angina, percutaneous coronary revascularization, or bypass grafting.	11	15.28
18	Fatal or non-fatal CHD event, which included definite and possible acute MI or coronary death, unstable angina pectoris, revascularization, and unclassifiable fatal events.	5	6.94
19	first-ever acute myocardial infarction (MI), silent MI, sudden cardiac death within 1 hour after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty.	1	1.39
20	Definite or probable hospitalized myocardial infarction or a definite CHD death.	2	2.78
21	Angina pectoris, coronary insufficiency, myocardial infarction, and death due to CHD.	1	1.39
22	Definite or probable myocardial infarction, silent myocardial infarction (indicated by electrocardiogram), definite CHD death, or coronary revascularization).	4	5.56
23	Definite nonfatal or fatal myocardial infarction or death due to CHD. Definite and possible fatal CHD were coded by using the definitions applied within the Cardiovascular Health Study.	1	1.39
24	Stable or unstable angina or coronary revascularization procedures (coronary bypass or percutaneous intervention), or death because of CHD, defined according to the International Classification of Diseases-Ninth Revision and International Classification of Diseases-Tenth Revision codes used in event ascertainment.	2	2.78
25	Recognized or unrecognized MI, angina pectoris, coronary insufficiency, or CHD death.	2	2.78
26	IHD death, clinical non-fatal (definite acute) MI and electrocardiographic MI, as previously described. A major IHD event was defined as one or more of the three possible outcomes described above.	1	1.39
27	No reported	2	2.78
Total		72	100

Table 4. 4 Definition of CHD events after the studies were completed based on the models reviewed.

No	Outcome category	No	%
1	CHD incident	42	58
2	Fatal and non-fatal myocardial infraction	12	17
3	Acute CHD events, coronary artery revascularization procedures, and silent myocardial infarctions.	2	3
4	Definite or probable MI's, CHD deaths, coronary revascularizations, silent [ECG-confirmed] MI's)/major or minor ECG abnormalities	2	3
5	CHD deaths, non-fatal MI, angina-driven revascularizations, resuscitated cardiac arrests	7	10
6	Death of CHD	1	1
7	CHD events included cases of surgery for angina pectoris	2	3
8	Fatal and nonfatal CVD	1	1
9	No reported	3	4
Total		72	100

Note: Table 4.4 described the types of CHD events (single or combined endpoints) that occurred after the investigation were completed.

4.1.2.4 The Candidate Predictors

Figures 4.2 included an overview of the predictors that were included based on the systematic review. These predictors in general categorized into different factors subgroups such as demographic and anthropometric, genetic, biomarker, comorbidities, reproductive, behavioral, metabolic syndromes, and psychological factors. Figure 4.3 include types of predictors (conventional, genetic and genomic and biomarker) risk prediction models as well as the general procedures for deriving a novel model for CHD risk prediction, in order to improve the accuracy of prognostic models, and to discover the most widely used predictors. Figure 4.4 depicts the set of predictors used in the reviewed prognostic models. In general, more than 237 different predictors were included. The major categories of the predictors used were conventional risk factors (number of predictors ranged between 7-20), genetic risk variables (ranged between 1-153 SNPs) and biomarker variables (ranged between 1-141). Age and smoking as predictors for CHD were used in all the studies, total cholesterol level was reported in 67 (93%) models, HDL cholesterol level was used in 62 (86 %) models, diabetes mellitus and systolic blood pressure were used in 63 (87%) models, sex was included as a predictor in 57 (79%) models. Most of the models (n = 46, 63%) included a set of similar predictors, such

as age, sex, smoking, total cholesterol, blood pressure, BMI, blood cholesterol/HDL cholesterol level, and diabetes mellitus. Other prognostic models included several different variables, such as hypertension (*n* = 25, 35%), family history of CHD and LDL-C (*n* = 27, 38%), triglycerides (*n* = 29, 40%), genetic risk score (*n* = 17, 23 %), C-reactive protein (*n* = 12, 16%), apolipoprotein B (*n* = 8, 11%), and coronary artery calcification (*n* = 6, 8 %). Treatment as a predictor for CHD was included in a few studies (*n* = 6, 8%), described as the use of antihypertensive/antidiabetic and lipid-lowering medications (see figure 4.4 for more detail).

Figure 4. 2 The main categories of CHD predictors as reported by the models.

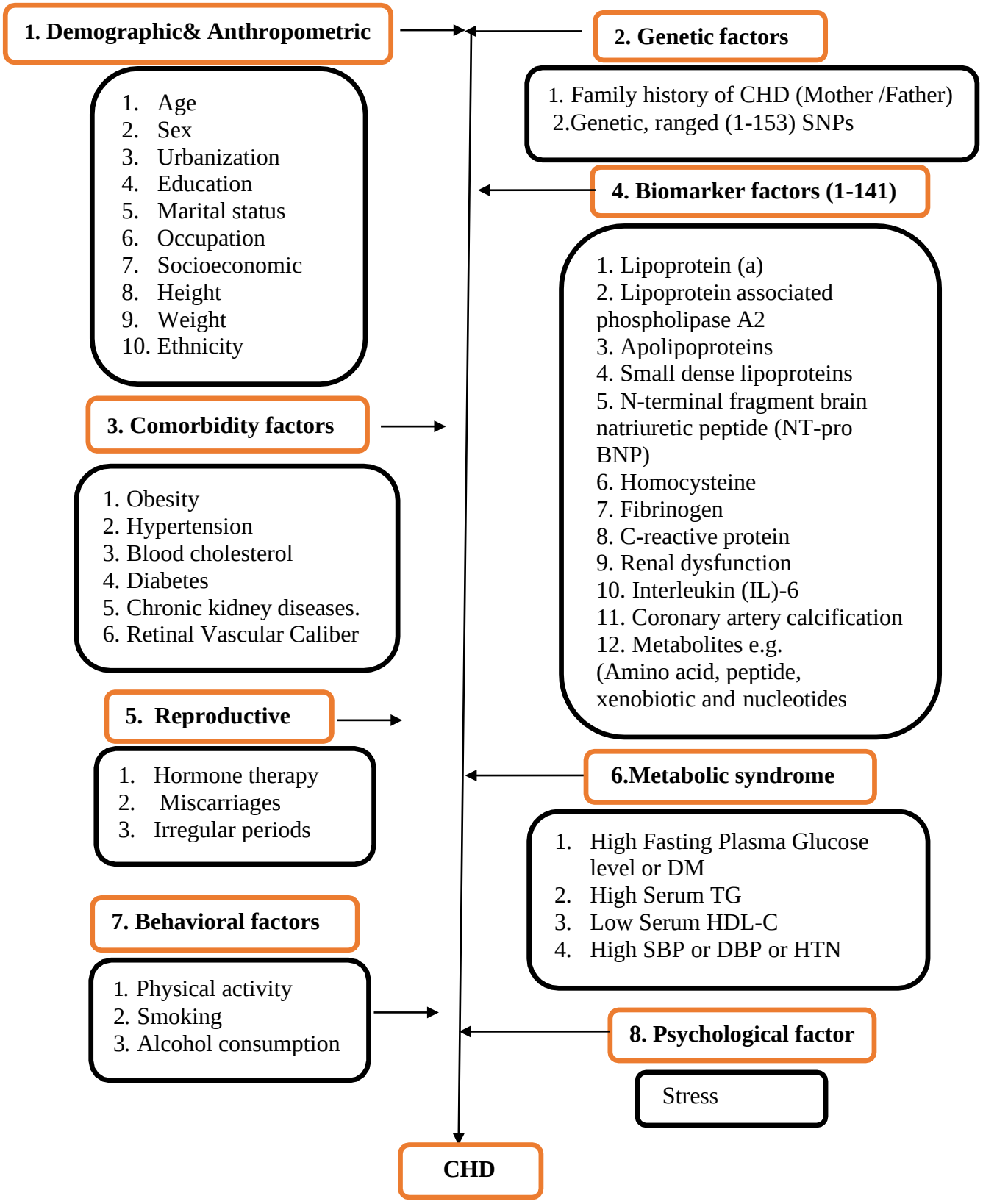
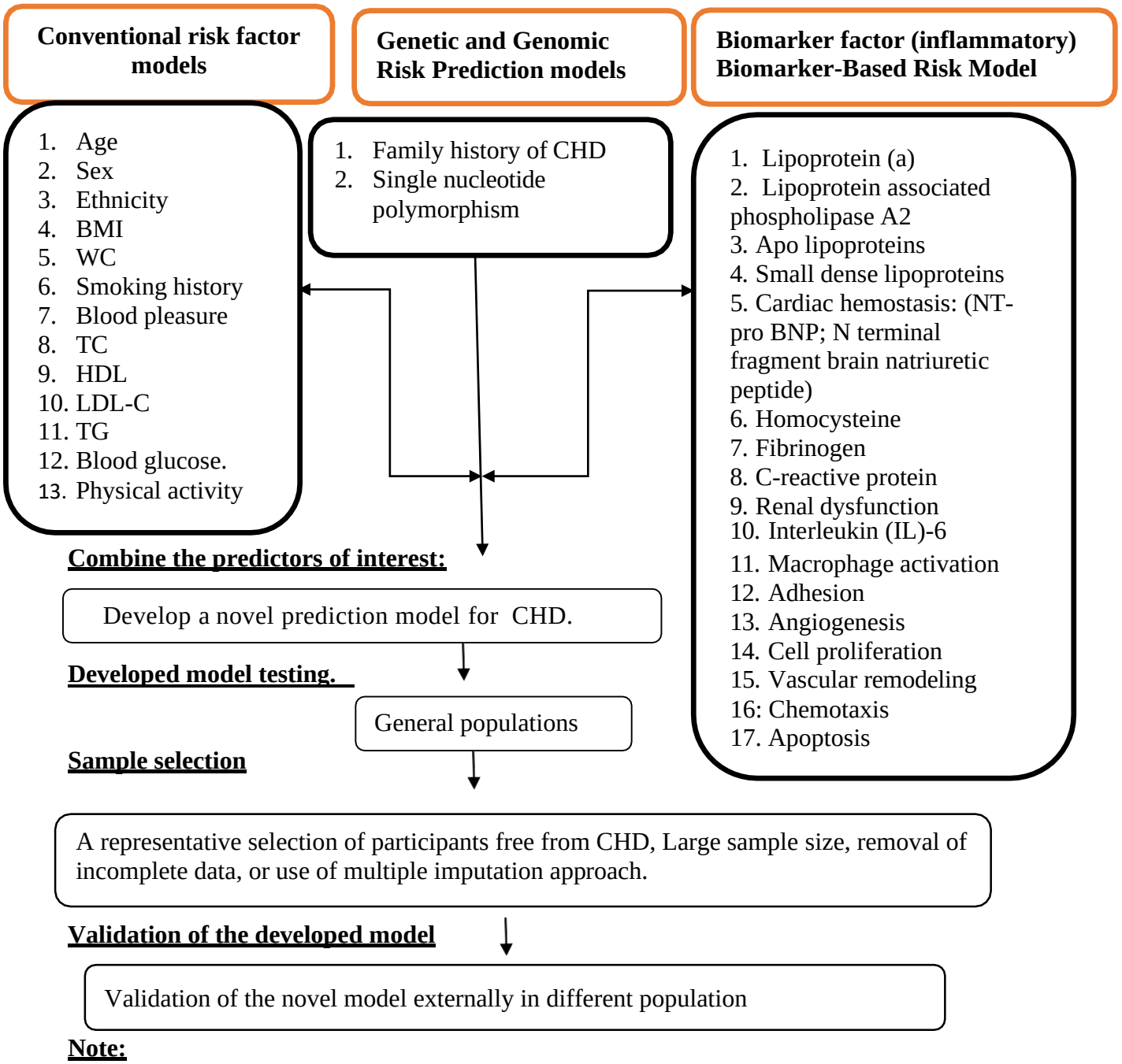


Figure 4. 3 General procedures of developing a novel model.



Note:

The figure above described the general steps for developing a novel model which includes specifying and combining the predictors of interest (based on the Framingham, SCORE, QRISK 1 or 2, PROCAM models), selecting the predictors (have a plausible relationship with the outcome) to develop a novel model for a specific population/country, using a large sample size (to limit overfitting, or use shrinkage or penalization methods), validating the developed model internally (using cross validation or bootstrapping methods, and avoiding randomly splitting sampling) or externally in other populations/countries, determining whether this model is optimal (valid and accurate) by measuring the model performance (improvement).

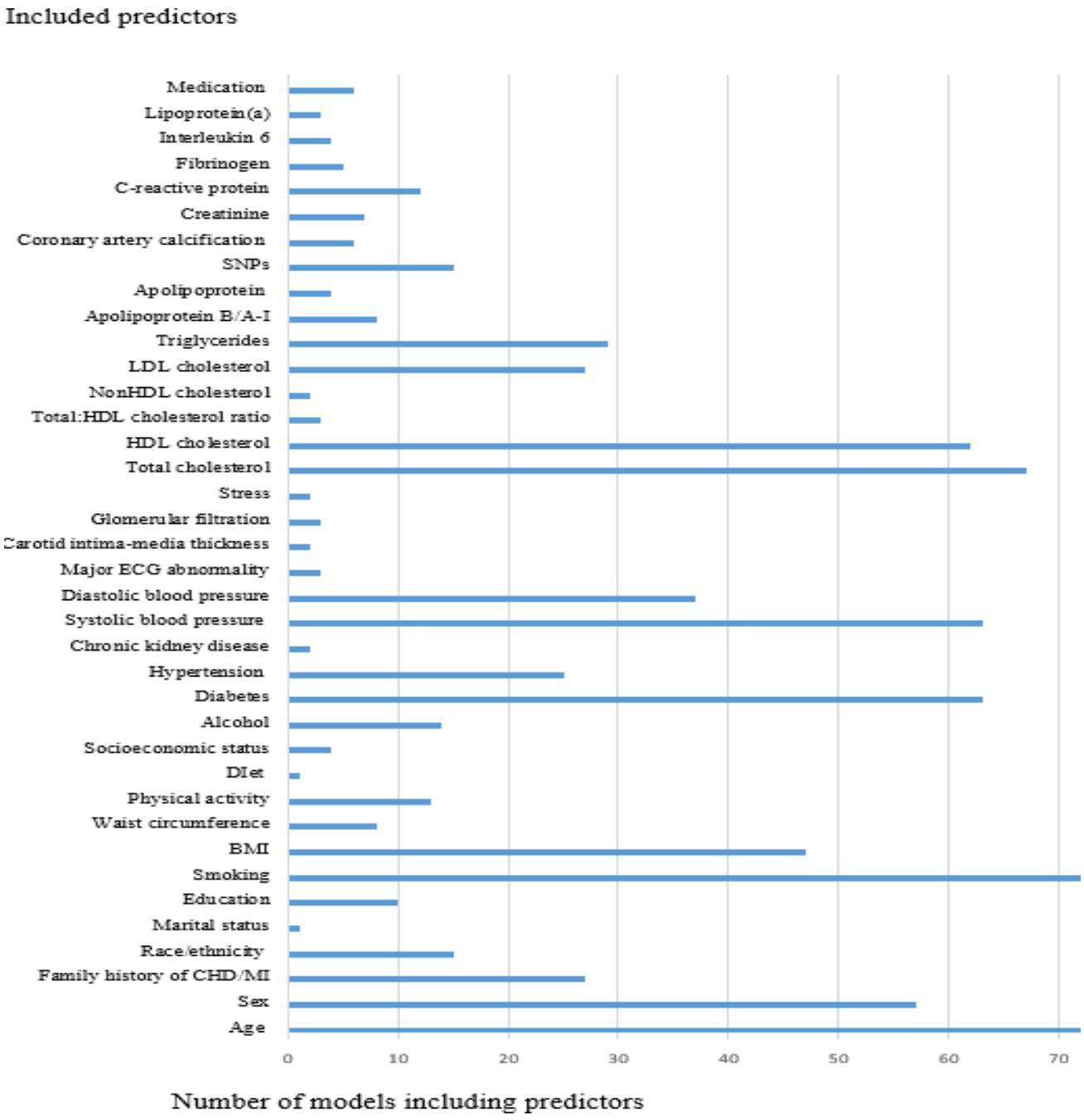


Figure 4. 4 CHD predictors as stated in the models under review. Several novel predictors were added to the Framingham model for predicting CHD events. Framingham predictors were used the most commonly (age, sex, smoking, SBP, TC, HDLC, and diabetes) compared to other predictors.

4.1.2.5 Sample Size and Number of Outcomes

It is beneficial to utilize a large sample size when creating a risk prediction model in order to prevent overfitting (underestimate the probability of CHD in low-risk group or overestimate it in high-risk group thus not generalizable model) [209]. According to the evaluated articles, the number of participants used to develop and validate the prognostic modelling studies were ranged between 112 and 268,315 (median 4,651); almost half of the models (*n* = 50, 69%) recruited their participants from multiple centres, while (*n* = 16, 22 %) recruited subjects from one centre, (*n* = 2, 3%) recruited from two centres, and (n=4, 6%) models did not describe the recruitment method. Regarding the study setting (primary or secondary health care centres), (n=45, 62%) models described that they selected the participants from primary health care centres, (n=4, 6%) models selected the participants from secondary health care centres, and (n=23, 32%) models described that they selected the participants from the communities by using a random selective sampling procedure. The number of outcomes that occurred during the follow-up period (CHD events) ranged between 56 and 203,666 (median 467), and (*n* =2, 3%) models did not report the number of outcomes.

4.1.2.6 Missing Data

Missing data for developed and validated models should be kept to a minimum, if its present in the model, researcher should avoid leaving out individuals with incomplete data, if they do, they should explain the strategy used to deal with the missing data (multiple imputation) [209]. As a result, we identified the number of participants with any missing value as well as whether the subjects were censored, or whether migration happened to the participants during the follow-up were described in (n=27, 37%) models. Methods for handling the missing data were described for several models. Seventeen models explained that they excluded the participants with missing data before starting the analysis, whereas four models reported that they used the imputation resampling technique, and only one reported that they repeated the measurement. Genetic modelling studies reported that they excluded the participants if they missed information related to genetic data, ECG, and C-reactive protein, blood pressure, total serum cholesterol, fasting serum glucose, smoking status, and body mass index.

4.1.2.7 Modelling Method

In relation to the evaluation of the model type (statistical analysis technique) used to create a prognostic model; (n=47, 65%) models used Cox proportional hazards to develop the prognostic model for CHD; logistic regression was used in (n=17, 24%) studies. Furthermore, conditional logistic regression and lifetime survival analysis were described in some models (*n* = 6, 8%) (Table 4.5).

Table 4. 5 Modelling method used to develop prediction models.

No	Modelling method	Frequency	%
1	Cox proportional hazards regression/ Weibull	47	65
2	Logistic regression/ stepwise	17	24
3	Conditional logistic regression	4	5
4	Lifetime survival analysis	2	3
7	No report	2	3
Total		72	100

4.1.2.8 Models' Assumptions and Normality Distribution

Model assumptions (linearity and distributional) underpin statistical approaches. One of the most important factors to consider when evaluating prediction accuracy utilizing discrimination and calibration measurements (to provide an accurate estimation of patents prognosis), both of which should be unbiasedly confirmed using bootstrapping or cross validation [101, 104, 117]. As results, the distributional assumptions about the residuals as well as whether the researchers selected the right predictors in their models, (n=18,) models reported how they checked the assumption of the normality distribution using linear regression, seven models used Schoenfeld residuals to verify the proportional hazard assumption, and two models reported that they fit the models by Grambsch and Therneau (Table 4.6).

The methods used for selection of the best predictors during multivariable modelling were a backward approach in (n=3, 4%) models, forward selection in 3 (4%) different models, Bayes information criterion (BIC) in (n=9, 13%) models, Akaike information criterion in (n=5, 7%) models, likelihood ratio test (LR) in (n=12, 17%) models, and Shrinkage or penalized estimation in (n=3, 4%) models (Table 4.6).

Table 4. 6 Method for selection of predictors in models included.

No	Predictor's selection	Study types		
		Developed (n)	Validation(n)	Total
1	Imputation	5	1	6
2	Backward approach	3	0	3
3	Forward approach	2	1	3
4	Bayes information criterion (BIC)	6	3	9
5	Akaike information criterion	2	3	5
6	Grambsch and Therneau statistic	2	0	2
7	Schoenfeld test	5	2	7
8	Likelihood ratio test (LR)	9	3	12
9	Shrinkage/penalization	1	2	3
Total		35	15	50

4.1.2.9 Assessing Models Performance of the Reviewed Studies to Explore the Optimal Risk Prediction Model

Models' performance can be assessed usually by using different methods which included discrimination, calibration, and reclassification measures. When evaluating the models performance, discrimination and calibration are both crucial factors. Discrimination measured by AUC ROC curve, or concordance (or c) statistic; calibration measured by calibration slope, survival analysis (Hosmer-Lemeshow goodness-of-fit test), Grønnesby and Borgan test. Reclassification is measured by net reclassification improvement (NRI). The net reclassification index is a measure for evaluating the improvement in prediction performance gained by adding a marker to a set of baselines predictors [102, 210, 211].

The study of a statistical prognostic model's performance revealed significant heterogeneity, and discrimination measures of predictive performance were given for (n=72, 100%) models (See Table 4.7, and Appendix Table 12.3). The concordance index (Harrell's C statistic) or ROC curve was utilized by the majority of the models (n = 57, 79%); The D statistics were employed in seven (10%) of the models; (n=6, 8%) models reported lifetime CHD risks, but only one model characterized the log rank. Calibration measurements were provided in 29 (45%) of the models, Calibration slope and intercept were assessed in (n=3, 4%) models, and calibration plots were presented in (n=2, 3%) models, Hosmer-Lemeshow was employed to examine the variation between observed and predicted rates in (n=20, 28%) models, and

Grønnesby and Borgan was used to test the goodness of fit of 5 (7%) models. Classification measurements were employed in (n=54, 75%) models to evaluate risk predictions, (n=24, 33%) models applied sensitivity and specificity measurements, net reclassification improvement (NRI) was reported in (n=28, 39%) investigation, in (n=16, 22%) models, integrated discrimination improvement (IDI) was utilized to quantify how close prognostics were to the actual outcome, and clinical NRI was calculated in (n=3, 4%) studies to assess the improvement between the basic and extended models. In addition, Kaplan-Meier survival curves were employed in (n=16, 22%) models. In total, 45 (63%) of the 72 models were developmental (internally verified), with most models (n=18, 25%) employing a random split of the dataset, bootstrapping (n=21, 29.2%), or cross-validation (n=4, 6%), and only a few models (n=6, 8%) using multiple imputation to impute the missing values on all predictors (see Appendix Table 12.3 for more details|).

4.1.3 Validation Risk Prognostic Models for CHD

Model validations were performed on only 24 models: (n=10, 16%) studies were subjected to developmental validation, whereas 14 were subjected to external validation investigations (See Figure 3.1, 4.2). However, the ten models had been developed and validated in the same study using different populations. Three of the 10 models stated genetic risk scores, while the remaining 14 models were internally validated. Framingham models were the most commonly used by researchers: Framingham which Wilson and D'Agostino developed in 1998 was reported in 33 models, Adult Treatment Panel III Framingham 2002/2001 has been described in 9 models, five different investigations reported on the Framingham models suggested by Anderson in 1991 and Kannel in 1979. Other models used to predict CHD development that were identified in our analysis were SCORE (2003), which was reported in seven models. PROCAM (created by Assmann in 2002) was mentioned in three separate papers. QRISK2 (2008) was utilized in two models, whereas the reference models used in 20 other experiments were not specified (see Table 4.2).

The qualifying criteria utilized for the subjects engaged in the external validation modeling studies (particularly the developmental validation kinds) varied significantly. The models were developed and validated using different age groups. The majority of the models assessed discrimination ability solely using C-statistics, while just a few models described the calibration measure (n = 5, 7%).

Table 4. 7 The performance measures reported for the developed and validated models.

No	Discrimination Measures	Developmental	Validation	Total
1	C statistic/AUC	54	9	63
2	D statistic	2	1	3
3	Log rank	0	1	1
4	Lifetime risks for CHD	3	2	5
No	**Calibration Measures**	**Developmental**	**Validation**	**Total**
1	Calibration slope and intercept	0	3	3
2	Calibration plot	0	2	2
3	Hosmer-Lemeshow test	11	9	20
4	Grønnesby-Borgan χ^2 test	4	1	5
No	**Classification measures**	**Developmental**	**Validation**	**Total**
1	Sensitivity, specificity	14	10	24
2	Predictive value	5	2	7
3	Net reclassification improvement (NRI)	19	9	28
4	Integrated discrimination improvement (IDI)	10	6	16
5	Clinical NRI	0	3	3
No	**Others**	**Developmental**	**Validation**	**Total**
1	R^2	2	0	2
2	Kaplan-Meier estimates	11	5	16
3	Bootstrap resampling	16	5	21
4	Cross-validation	2	2	4

4.1.4 Genetic Risk Prognostic Models for CHD

The genetic risk modelling studies identified in this review were used to determine whether the including genetic factors in addition to conventional risk factors based on the Framingham score, improved the CHD risk prognosis. The majority of the models (*n* =16, 22.22%), were developed in healthy populations in longitudinal cohort studies while one study employed a nested case-control strategy (See Figure 4.2, and Appendix Table 12.1).

The majority of the genetic modelling research originated in Europe (*n* = 11, 15.28%) and the United States (*n* = 5, 6.94%), with only one model was from Asia (China). The European modeling studies included four studies from the United Kingdom, two models from Sweden and one model each from Norway, Spain, Switzerland, the Netherlands, and Scotland. there was no model for predicting CHD risk in Africa. Most of the models (*n* = 11, 15.28%) were developed with Caucasian (white non-Hispanic) populations.

The genetic models were recruited using healthy participants from multiple centres (*n* = 12, 16.67%). The recruitment periods were ranged from 1987 to 2007; seven models recruited participants between 1987 and 2001, five models recruited people between 2003 to 2007, and two models provided no information. In terms of age, the majority of the models (n=11, 15.28%) were developed for adults aged 45-75 years, three models for people aged 25-64 years, and two models had no information.

The majority of the investigations used healthy Caucasian populations of both sexes with complete genetic data. The authors excluded all subjects with missing genotype data, those with prevalent CHD or stroke, and those with no follow-up data. The number of participants ranged from 840 to 51,954, with a total of 183 to 3,217 events.

The models examined incorporated genetic risk scores with varying numbers of SNPs. There were a total of 230 SNPs reported in the articles. It ranged from 1 to 156 SNPs per article: four models used a small number of SNPs (1-19), while thirteen models used a large number of GRSs ranging from 38 to 156 SNPs. rs17114036 (gene *PLPP3*) has been reported in 12 articles; rs1122608 (gene *SMARCA4*) and rs3184504 (gene *SH2B3*) in 7 articles; rs9818870 (gene *MRAS*), rs67258870 (gene *DHRSX*), and rs501120 (unknown gene) in 6 articles; and rs7692387 (gene *GUCY1A1*), rs12413409 (gene *CNNM2*), rs9515203 (gene *COL4A2*), rs11556924 (gene *ZC3HC1*), rs11206510 (unknown gene), rs273909 (gene *SLC22A4*, and gene *MIR3936HG*), rs12190287 (gene *TCF21*), rs2048327 (gene *SLC22A3*), rs12526453 (gene *PHACTR1*), rs4252120 (gene *PLG*), rs2505083 (gene *JCAD*), rs974819 (unknown gene), and rs9982601 (unknown gene) in 5 articles. Other SNPs were utilized less frequently (see Table 3.8). These SNPs were discovered to be associated with well-known phenotypic traits or biomarkers such as systolic blood pressure, total cholesterol, LDL-C, HDL-C, apolipoprotein-B, lipoprotein (a), plasma C-reactive protein, health behavioral factor (smoking). Family history of premature CHD was also investigated because plasma cholesterol concentration and hypertension are both heritable risk factors for CHD. The majority of the SNPs chosen (*n*=9) were discovered in genome-wide association studies and the CARDIoGRAMplusC4D (*n*= 4) investigation. Based on a literature analysis, one model stated that SNPs were incorporated.

Heterogeneity was found in the genotyping methods used for the analysis, which included TaqMan technology, and Illumina MetaboChip both of which reported in three different models, Affymetrix GeneChip (*n*= 2), custom-designed Affymetrix Axiom arrays and genome-

wide arrays (*n* = 1), and MassARRAY (*n*= 1), as well as the other models had no information reported.

In the models reviewed, the majority (n = 12) weighted the GRS by multiplying the participants' allele score (1, 0, 1) by the SNP beta coefficient, while two models reported that they weighted the GRS by multiplying the number of risk alleles by the 'combined beta' of the CARDIoGRAMplusC4D meta-analysis and summing the products.

In the constructed models, the GRS variables were classified as tertiles, quartiles, and quantiles. Most models (n = 11) used the quartile as low GRS (quartile 1), intermediate (quartiles 2 and 3), and high (quartile 4) risk categories; tertiles were described in (n = 5) models as low GRS (tertile 1), intermediate (tertile 2), and high (tertile 3) risk categories; and only one model used quantiles as low GRS (quintile 1), intermediate GRS (quintiles 2 to 4) risk categories.

The models' follow-up period ranged from 5 to 19.4 years. Seven models projected CHD outcomes in >10-15 years, six models indicated a follow-up duration of 5-10 years, two models predicted a follow-up period of less than 5 years, and two models predicted a follow-up period of more than 15 years. The majority of the models (n = 12) reported collecting data using questionnaires, physical examinations, and laboratory diagnoses. Bootstrapping was detailed for n = 5 models, and genotype imputation was performed in five models.

The Framingham risk function developed by Wilson et al.1998 [27] was used in the majority of the studies (*n* = 6) to predict the ten-year risk for CHD, the Framingham Adult Treatment Panel III was utilized in three models, and some models did not specify which Framingham model was used.

Genetic modeling study performance was reported in (n = 16) models, discrimination measures using the concordance index (Harrell's C statistic) or area under the receiver operating characteristic curve were reported in (n= 13) models, calibration measures were reported in (n= 10) models, Hosmer-Lemeshow goodness of fit was reported in (n = 5) models, and Grnnesby and Borgan were reported in (n= 2) models. Classification measurements were provided in (n=10) models, and calibration measures were reported in (n=10) models using the net reclassification improvement (NRI).

Table 4. 8 SNPs included in the genetics modelling studies.

No	SNPs' ID	Nearest gene	Frequency
1	rs7412	APOE	1
2	rs429358	APOE	1
3	rs11591147	PCSK9	1
4	rs10757274	CDKN2B-AS1	4
5	rs599839	PSRC1	3
6	rs10455872	LPA	3
7	rs17465637	MIA3	4
8	rs9818870	MRAS	6
9	rs1746048	unknown	4
10	rs328	LPL	1
11	rs7025486	DAB2IP	1
12	rs1801177	LPL	1
13	rs3798220	LPA	4
14	rs662799	APOA5	1
15	rs708272	CETP	1
16	rs4341	ACE	1
17	rs1042031	APOB	1
18	rs1799983	NOS3	1
19	rs17228212	SMAD3	1
20	rs7692387	GUCY1A1	5
21	rs17114036	PLPP3	12
22	rs12413409	CNNM2	5
23	rs1122608	SMARCA4	7
24	rs9515203	COL4A2	5
25	rs9369640	PHACTR1	2
26	rs11556924	ZC3HC1	5
27	rs602633	unknown	3
28	rs1412444	LIPA	1
29	rs4845625	IL6R	4
30	rs11206510	unknown	5
31	rs17464857	TAF1A, TAF1A-AS	1
32	rs67258870	DHRSX	6
33	rs515135	unknown	3
34	rs2252641	TEX41, LOC 100505498	3
35	rs1561198	VAMP5	4
36	rs6544713	ABCG8	4
37	rs1878406	unknown	3
38	rs273909	SLC22A4, MIR3936HG	5
39	rs12190287	TCF21	5
40	rs2048327	SLC22A3	5
41	rs12526453	PHACTR1	5
42	rs10947789	KCNK5	4
43	rs4252120	PLG	5
48	rs2954029	unknown	4
49	rs1333049	unknown	4
50	rs3217992	CDKN2B, CDKN2B-AS1	4
51	rs579459	unknown	4
52	rs2505083	JCAD	5
53	rs501120	unknown	6
54	rs2047009	unknown	4
55	rs2246833	LIPA	3
56	rs974819	unknown	5
57	rs9326246	unknown	2
58	rs3184504	SH2B3	7
59	rs9319428	FLT1	4
60	rs7173743	unknown	4
61	rs12936587	unknown	5
62	rs2281727	SMG6	3
63	rs15563	UBE2Z	2
64	rs2075650	TOMM40	2
65	rs445925	APOC1	3
66	rs9982601	unknown	5
67	rs10507391	ALOX5AP	1
68	rs17222842	unknown	1
69	rs9315050	ALOX5AP	1
70	rs17216473	ALOX5AP	1
71	rs3008621	MIA3	1
72	rs646776	CELSR2	3
73	rs3127599	LPAL2	1
74	rs7767084	LPA	1
75	rs10755578	LPA	2
76	rs2259816	HNF1A	3
77	rs6922269	MTHFD1L	1
78	rs3900940	MYH15	2
79	rs1010	VAMP8	2
80	rs7439293	PALLD	2
81	rs2298566	SNX19	2
82	rs10797416	SKI	1
83	rs1490738	PKN2-AS1	1
84	rs4268379	SARS1	1
85	rs12127701	MYBPHL	1
86	rs7515901	MYBPHL	1
87	rs11806316	unknown	1
88	rs11204666	ADAMTSL4-AS1	1
89	rs12125501	NME7	1
90	rs6700559	DDX59-AS1	1

44	rs12205331	ANKS1A	2
45	rs2023938	HDAC9	4
46	rs12539895	COG5	1
47	rs264	LPL	3
95	rs10495907	unknown	1
96	rs816889	RND3	1
97	rs2351524	NBEAL1	1
98	rs2571445	TNS1	1
99	rs4566357	COL4A4	1
100	rs11718455	unknown	1
101	rs11710224	LRRC2	1
102	rs7642590	MAP4	1
103	rs11916151	unknown	1
104	rs1393786	PPP2R3A	1
105	rs2306374	MRAS	2
106	rs4301033	LINC01214, LOC10798641	1
107	rs17655141	unknown	1
108	rs17083481	unknown	1
109	rs17087335	NOA1	1
110	rs7356185	USP53	1
111	rs1429141	unknown	1
112	rs4469055	unknown	1
113	rs6841581	EDNRA	1
114	rs4690974	unknown	1
115	rs2736100	TERT	1
116	rs10051876	unknown	1
117	rs246600	ARHGAP26	1
118	rs2294461	LY86, LY86AS1	1
119	rs9472428	PHACTR1,LOC107984015	1
120	rs883947	PHACTR1	1
121	rs13211739	PHACTR1	1
122	rs1321309	unknown	1
123	rs3778448	KCNK5	1
124	rs4613862	LINC02542	1
125	rs17062853	TARID	1
126	rs12663498	PLEKHG1	1
127	rs6926458	LPA	1
128	rs1247351	LOC102724087	1
129	rs972158	SNX10	1
130	rs217	JAZF1	1
131	rs1167800	HIP1	1
132	rs2395858	COG5	1
133	rs4591971	unknown	1

91	rs2292096	CAMSAP2	1
92	rs2820315	LMOD1	1
93	rs16986953	unknown	1
94	rs7561273	MFSD2B	1
144	rs7074064	BMPR1A	1
145	rs11203042	LIPA	1
146	rs11191447	AS3MT, BORCS7-ASMT	1
147	rs12765878	STN1	1
148	rs93139	SWAP70	1
149	rs7116641	HSD17B12	1
150	rs12801636	PCNX3	1
151	rs590121	SERPINH1	1
152	rs606452	SERPINH1	1
153	rs683800	DCPS	1
154	rs4762911	unknown	1
155	rs4149033	SLCO1B1	1
156	rs2681472	ATP2B1	1
157	rs6490029	CUX2	1
158	rs3809274	unknown	1
159	rs17630235	TRAFD1	1
160	rs2891403	RPH3A	1
161	rs2244608	HNF1A	1
162	rs11057841	SCARB1	1
163	rs9316753	unknown	1
164	rs10507753	unknown	1
165	rs11617955	COL4A1	1
166	rs7139492	COL4A1	1
167	rs12873154	COL4A1	1
168	rs4773144	COL4A1, COL4A2	3
169	rs11619057	COL4A2	1
170	rs9515201	COL4A2	1
171	rs2895811	HHIPL1	4
172	rs2146238	CYP46A1	1
173	rs6494488	LOC107984737	1
174	rs2487928	JCAD	1
175	rs11072794	LOC105370913, LOC112268142	1
176	rs7181240	unknown	1
177	rs2880765	AKAP13	1
178	rs17514846	FURIN	3
179	rs2521501	FES	1
180	rs7496815	unknown	1
181	rs4299203	DRC3	1
182	rs2071167	UBT, LOC101926967	1

134	rs10237377	PARP12	1
135	rs6984210	BMP1	1
136	rs17485781	NUGGC	1
137	rs10962774	unknown	1
138	rs16905599	CDKN2B-AS1	1
139	rs10965228	CDKN2B-AS1	1
140	rs495828	unknown	1
141	rs11238956	unknown	1
142	rs17155842	unknown	1
143	rs3748242	ANXA11	1
193	rs2832227	MAP3K7CL	1
194	rs1034565	ARVCF	1
195	rs9608859	unknown	1
196	rs17609940	ANKS1A	2
197	rs10953541	BCAP29, DUS4L-BCAP29	2
198	rs964184	ZPR1	1
199	rs10757274	CDKN2BAS1	1
200	rs2383206	CDKN2B-AS1	1
201	rs17011666	MIA3	1
202	rs3825807	ADAMTS7	2
203	rs4380028	unknown	1
204	rs2228671	LDLR	1
205	rs7278204	unknown	1
206	rs20455	KIF6, LOC 1079865 94	2
207	rs11016076	MKI67	1
208	rs2213948	unknown	1
209	rs2296436	HPS1	1
210	rs428785	ADAMTS1	1
211	rs402007	ADAMTS1	1
183	rs16948048	ZNF652, LOC102724596	1
184	rs4793721	CA10	1
185	rs2070783	PECAM1	1
186	rs4410190	unknown	1
187	rs892115	SPC24	1
188	rs17318596	DMAC2	1
189	rs2288911	APOC2, APOC4-APOC2	1
190	rs8111989	CKM	1
191	rs6088638	ACSS2	1
192	rs867186	PROCR, MMP24-AS1-EDEM2	1
212	rs4994	ADRB3	1
213	rs12102203	DMXL2	1
214	rs1122955	ZNF132	1
215	rs1799963	F2	1
216	rs2961135	OR2A25, LOC105375548	1
217	rs89962	KRT5	1
218	rs10822891	CTNNA3	1
219	rs4796603	HAP1	1
220	rs1800437	GIPR	1
221	rs3749817	FSTL4	1
222	rs8089	THBS2, LOC 101929523	1
223	rs4977574	CDKN2B-AS1	1
224	rs216172	SMG6	1
225	rs46522	UBE2Z	1
226	rs318090	UBE2Z	1
227	rs2028900	MAT2A, STUDYICL	1
228	rs4299376	ABCG8, LOC 102725159	1
229	rs11984041	HDAC9	1

4.1.5 Optimal Risk Prediction Models for CHD Risk by Assessing Models Performance

Only two validation models compared performance ability in different populations, according to our review. The first model was a genetic risk modeling study based on the Framingham risk score that assessed performance using discrimination, calibration, and reclassification in three different populations: the ARIC (Atherosclerosis Risk in Communities) study, the Framingham Offspring Study, and the Rotterdam Study (Netherlands) [132]. This work revealed conflicting

results regarding model performance: the discrimination ability and reclassification showed significant improvement in the developed model but not in the validated models. The second study investigated whether adding coronary artery calcification as a measure predicting CHD risk could improve model performance [171], this model developed using three different groups form the Multi-Ethnic Study of Atherosclerosis, Heinz Nixdorf Recall Study, and Dallas Heart Study. The performance of these models was tested using discrimination and calibration, and the study found that adding coronary artery calcification to conventional risk factors resulted in considerable increases in risk prediction. There was also evidence of very good discrimination and calibration.

4.2 Integration of Genetic and Conventional Risk Factors for Predicting CHD/AMI Risk Among the Hungarian Populations

4.2.1 Descriptive Characteristics of the Participants

The overall characteristics of the enrolled participants were shown in Table 4.10; 279 from the Hungarian Roma population and 279 from the Hungarian general population were included in the current study. The mean (SD) age of the Hungarian Roma and general populations were 42.73± 12.99 and 44.14 ±12.12 years respectively, with the Roma population being younger. The findings revealed a significant difference between Hungarian Roma and the general population in terms of sex distribution, height, weight, educational levels, job activities, and family members, with Hungarian Roma having a lower proportion of male individuals, lower education, lower economic activities, and having a large family's members. The prevalence rates of CHD, AMI, and stroke were lower among both groups (Roma and general), with no statistically significant difference between them, but were considerable higher among Roma. The results also indicated that SBP and glucose levels were likewise much higher in the general population, despite the fact that Roma had significantly higher prevalence of HTN-Med and HTC-Med use and significantly lower HDL-C levels than the general population. Furthermore, Roma was more likely to be a current smoker and were likely to be exposed to chronic diseases such as DM, HTN, and CKD (see Table 4.9).

Table 4.9 Characteristics of the Hungarian general and Hungarian Roma populations.

	Characteristics	Hungarian General (n= 279)	Hungarian Roma (n=279)	p-value
1	Age (years, mean ± SD); 18-36 Age-group (%) 37- 49 Age-group (%) 50-77 Age-group (%)	` 44.14 ± 12.12 27.60 35.48 36.92	42.73 ± 12.99 34.77 30.82 34.41	0.177
2	Sex male/female (%)	43.73/56.27	23.3/76.7	**<0.001**
3	Height cm (mean ± SD)	168.97±9.58	161.09±9.41	**<0.001**
4	Weight kg (mean ± SD)	77.41±0.99	71.04±10.27	**<0.001**
5	Education levels; Primary Education (%) Secondary Education (%) High Education (%)	 46.95 36.92 16.13	 97.13 2.51 0.36	**<0.001**
6	Economic Activity; Employed full/part time or Family Support (%) Unemployed/ Disable or Full-time study (%) Childcare benefit or household or other (%)	 78.85 16.13 5.02	 59.14 26.16 14.70	**<0.001**
7	Family Member; Small (1-3) (%) Moderate (4-6) (%) Large (7-9) (%)	 81.36 16.85 1.79	 44.44 48.39 4.48	**<0.001**
8	BMI kg/m^2 (mean ± SD)	27.11 ± 5.49	27.29 ± 6.72	0.725
9	SBP mmHg (mean ± SD)	126.94 ± 15.23	123.57 ± 17.73	**0.017**
10	DBP mmHg (mean ± SD)	78.91± 8.71	79.97± 10.26	0.189
11	TC mmol/l (mean ± SD)	4.95 ±1.14	4. 90 ± 1.07	0.563
12	HDL-C mmol/l (mean ± SD)	70.11 ± 33.15	61.00 ± 30.98	**0.001**
13	LDL-C mmol/l (mean ± SD)	3.07 ± 1.00	3.15 ± 0.94	0.328
14	TG mmol/l (mean ± SD)	1.58 ± 1.09	1.57± 0.95	0.884
15	Glucose mmol/l (mean ± SD)	5.375 ±2.06	4.986 ±1.357	**0.004**
16	CHD yes (%)	2.87	3.94	0.484
17	AMI yes (%)	1.08	3.23	0.080
18	Stroke yes (%)	1.43	3.23	0.161
19	DM yes (%)	6.09	12.54	**0.009**
20	HTN yes (%)	28.32	34.05	0.144
21	CKD yes (%)	0.72	4.3	**0.007**
22	HTN-Med yes (%)	4.3	15.41	**<0.001**
23	HTC-Med yes (%)	7.89	14.34	**0.015**
24	Smoking currently yes (%)	32.97	69.95	**<0.001**

Table 4.10 The observed and expected frequencies of Hardy Weinberg Equation for CHD variants among Hungarian general and Hungarian Roma population.

No	SNPs ID	TEST	A1	A2	GENO	O(HET)	E(HET)	p-value
1	rs11206510	Total	T	C	405/132/21	0.237	0.263	0.023
		Roma	T	C	219/50/10	0.179	0.219	0.005
		Hungarian	T	C	186/82/11	0.294	0.303	0.558
2	rs17114036	Total	A	G	473/79/6	0.142	0.150	0.248
		Roma	A	G	236/41/2	0.147	0.148	0.694
		Hungarian	A	G	237/38/4	0.136	0.151	0.101
4	rs646776	Total	T	C	364/171/23	0.307	0.313	0.590
		Roma	T	C	191/77/11	0.276	0.292	0.410
		Hungarian	T	C	173/94/12	0.337	0.334	1
3	rs17465637	Total	C	A	255/247/56	0.443	0.436	0.772
		Roma	C	A	122/121/36	0.434	0.453	0.509
		Hungarian	C	A	133/126/20	0.452	0.418	0.200
5	rs6725887	Total	C	T	6/91/461	0.163	0.168	0.453
		Roma	C	T	2/43/234	0.154	0.154	1
		Hungarian	C	T	4/48/227	0.172	0.181	0.499
6	rs2306374	Total	C	T	12/94/452	0.169	0.189	0.022
		Roma	C	T	4/29/246	0.104	0.124	0.023
		Hungarian	C	T	8/65/206	0.233	0.248	0.331
7	rs9818870	Total	T	C	12/92/454	0.1649	0.186	0.011
		Roma	T	C	4/29/246	0.1039	0.124	0.023
		Hungarian	T	C	8/63/208	0.2258	0.243	0.221
8	rs9349379	Total	G	A	116/273/169	0.4892	0.496	0.798
		Roma	G	A	65/135/79	0.484	0.499	0.632
		Hungarian	G	A	51/138/90	0.495	0.490	1
9	rs12526453	Total	G	C	62/257/239	0.461	0.450	0.638
		Roma	G	C	31/127/121	0.455	0.448	0.894
		Hungarian	G	C	31/130/118	0.466	0.451	0.691
10	rs17609940	Total	G	C	470/84/4	0.151	0.151	0.782
		Roma	G	C	252/27/0	0.097	0.092	1
		Hungarian	G	C	218/57/4	0.204	0.206	0.776
11	rs12190287	Total	C	G	201/267/90	0.479	0.480	0.930
		Roma	C	G	83/142/54	0.509	0.495	0.717
		Hungarian	C	G	118/125/36	0.448	0.457	0.793
12	rs3798220	Total	C	T	0/8/550	0.014	0.014	1
		Roma	C	T	0/1/278	0.004	0.004	1
		Hungarian	C	T	0/7/272	0.025	0.025	1
13	rs10455872	Total	G	A	1/41/516	0.073	0.074	0.569
		Roma	G	A	0/11/268	0.039	0.039	1
		Hungarian	G	A	1/30/248	0.108	0.108	1
14	rs11556924	Total	C	T	261/241/52	0.439	0.430	0.694
		Roma	C	T	137/120/22	0.430	0.415	0.665
		Hungarian	C	T	124/125/30	0.448	0.443	1
15	rs4977574	Total	G	A	165/260/133	0.466	0.498	0.12
		Roma	G	A	92/134/53	0.480	0.480	0.716

		Hungarian	G	A	73/126/80	0.452	0.500	0.119
16	rs579459	Total	C	T	43/206/309	0.369	0.386	0.324
		Roma	C	T	19/97/164	0.348	0.363	0.509
		Hungarian	C	T	25/109/145	0.391	0.408	0.466
17	rs635634	Total	T	C	36/183/340	0.328	0.351	0.146
		Roma	T	C	10/87/182	0.312	0.31	1
		Hungarian	T	C	25/96/158	0.344	0.386	0.087
18	rs1746048	Total	C	T	362/174/22	0.3118	0.314	0.893
		Roma	C	T	177/88/14	0.3154	0.329	0.469
		Hungarian	C	T	185/86/8	0.3082	0.299	0.692
19	**rs12413409**	Total	G	A	399/130/29	0.233	0.280	**<0.001***
		Roma	G	A	189/69/21	0.2473	0.3187	**<0.001***
		Hungarian	G	A	210/61/8	0.219	0.2379	0.203
20	rs964184	Total	G	C	19/151/388	0.271	0.281	0.367
		Roma	G	C	12/84/183	0.301	0.312	0.565
		Hungarian	G	C	7/67/205	0.2401	0.248	0.627
21	rs3184504	Total	T	C	113/273/172	0.489	0.494	0.798
		Roma	T	C	33/138/108	0.495	0.464	0.303
		Hungarian	T	C	80/135/64	0.484	0.498	0.632
22	rs2259816	Total	T	G	94/272/192	0.488	0.485	0.931
		Roma	T	G	66/136/77	0.488	0.499	0.719
		Hungarian	T	G	28/136/115	0.488	0.451	0.232
23	rs4773144	Total	G	A	110/243/205	0.4355	0.486	0.015
		Roma	G	A	57/117/105	0.419	0.485	0.026
		Hungarian	G	A	53/126/100	0.452	0.486	0.267
24	rs2895811	Total	C	T	86/250/222	0.448	0.470	0.28
		Roma	C	T	37/118/124	0.423	0.451	0.291
		Hungarian	C	T	49/132/98	0.473	0.485	0.711
25	rs3825807	Total	A	G	167/276/115	0.495	0.496	1
		Roma	A	G	70/141/68	0.505	0.5	0.905
		Hungarian	A	G	97/135/47	0.484	0.484	1
26	rs216172	Total	C	G	49/242/267	0.434	0.424	0.618
		Roma	C	G	21/117/141	0.419	0.408	0.769
		Hungarian	C	G	28/125/126	0.448	0.438	0.785
26	rs12936587	Total	G	A	259/223/76	0.400	0.446	0.014
		Roma	G	A	150/108/21	0.387	0.393	0.763
		Hungarian	G	A	109/115/55	0.412	0.481	0.018
28	rs46522	Total	T	C	176/273/109	0.489	0.493	0.864
		Roma	T	C	90/139/50	0.498	0.490	0.808
		Hungarian	T	C	86/134/59	0.480	0.495	0.629
29	rs1122608	Total	G	T	315/201/42	0.360	0.380	0.221
		Roma	G	T	153/96/30	0.344	0.403	0.017
		Hungarian	G	T	162/105/12	0.376	0.356	0.401
30	rs9982601	Total	T	C	10/110/438	0.197	0.206	0.304
		Roma	T	C	3/42/234	0.151	0.157	0.439
		Hungarian	T	C	7/68/204	0.244	0.251	0.633

Note: Bold values indicate those allele frequencies that remain significant after the Bonferroni correction

Table 4. 11 Alleles frequencies of CHD risk in Hungarian General and Roma Hungarian populations.

No	ASSAY ID	CHR	RA	Roma	General	OA	p-value
1	rs11206510	1	T	0.875	0.814	C	0.005
2	rs17114036	1	A	0.919	0.918	G	0.913
3	rs646776	1	T	0.823	0.789	C	0.151
4	rs17465637	1	C	0.654	0.703	A	0.084
5	rs6725887	2	C	0.084	0.100	T	0.352
6	rs2306374	3	C	0.066	**0.145**	T	**<0.001**
7	rs9818870	3	T	0.066	**0.142**	C	**<0.001**
8	rs9349379	6	G	0.475	0.430	A	0.133
9	rs12526453	6	G	0.339	0.344	C	0.850
10	rs17609940	6	G	**0.952**	0.884	C	**<0.001**
11	rs12190287	6	C	0.552	**0.647**	G	**0.001**
12	rs3798220	6	C	0.002	0.013	T	0.033
13	rs10455872	6	G	0.020	**0.057**	A	**0.001**
14	rs11556924	7	C	0.706	0.669	T	0.175
15	rs4977574	9	G	0.570	0.488	A	0.006
16	rs579459	9	C	0.238	0.285	T	0.077
17	rs635634	9	T	0.192	0.262	C	0.005
18	rs1746048	10	C	0.792	0.817	T	0.291
19	rs12413409	10	G	0.801	0.862	A	0.007
20	rs964184	11	G	0.194	0.145	C	0.031
21	rs3184504	12	T	0.366	**0.529**	C	**<0.001**
22	rs2259816	12	T	**0.480**	0.344	G	**<0.001**
23	rs4773144	13	G	0.414	0.416	A	0.952
24	rs2895811	14	C	0.344	0.412	T	0.019
25	rs3825807	15	A	0.504	0.590	G	0.004
26	rs216172	17	C	0.285	0.324	G	0.153
27	rs12936587	17	G	**0.731**	0.597	A	**<0.001**
28	rs46522	17	T	0.572	0.548	C	0.433
29	rs1122608	19	G	0.720	0.769	T	0.064
30	rs9982601	21	T	0.086	**0.147**	C	**0.002**

4.2.2 Frequencies and associations of the individual genetic variants associated with CHD risk

Following the HWE test, one SNP (rs12413409) in the Hungarian Roma community showed deviation and was thus eliminated from further GRS computation (see Table 4.10).

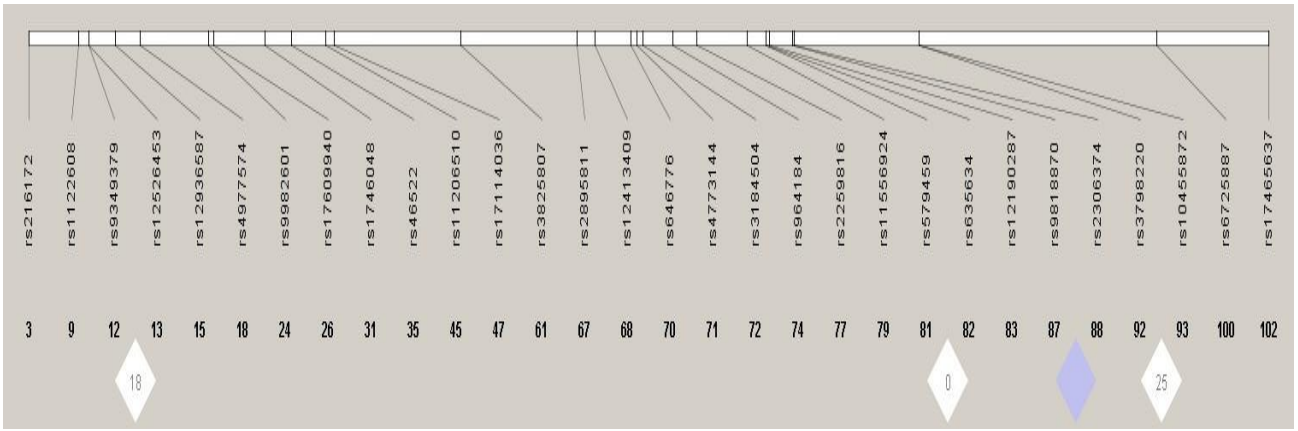
Table 4.11 display the allele frequencies of 30 SNPs which are associated with CHD risk in the Hungarian (general and Roma) populations. These finding were consistent with previously published evidence (see Table 3.1).

Even after the Bonferroni adjustment, it was shown that nine SNPs had statistically differing prevalence in the two research populations: Six SNPs were more prevalent in the Hungarian general population, including rs2306374 (gene MRAS), rs9818870 (gene MRAS), rs12190287 (gene TCF21), rs10455872 (gene LPA), rs3184504 (gene SH2B3), and rs9982601 (gene KCNE2), while three SNPs were more frequently among Roma individuals including, rs17609940 (gene ANKS1A), rs2259816 (gene HNF1A), and rs12936587 (gene RASD1), (Table 4.12).

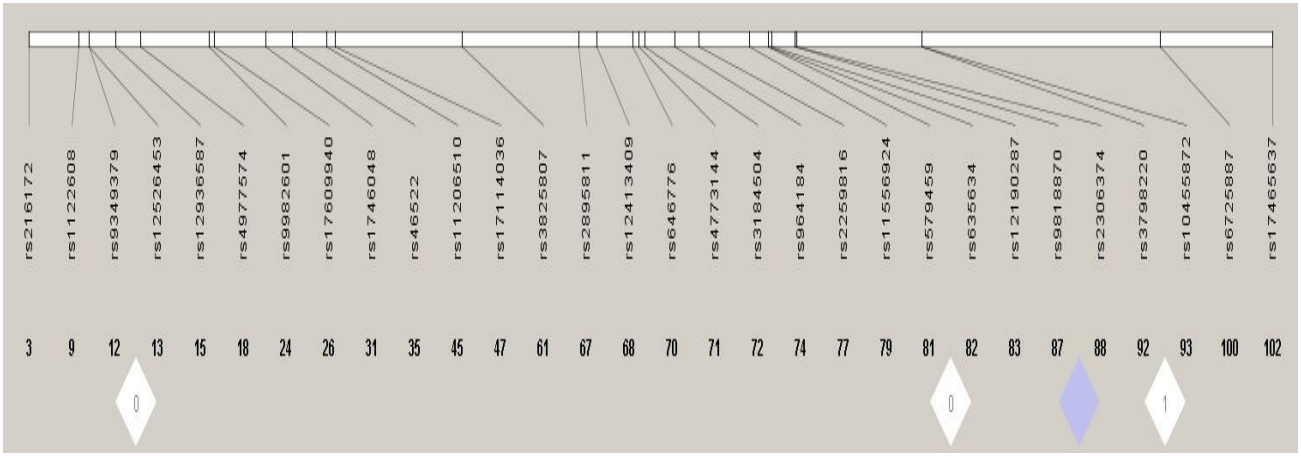
The connection based on genotyped alleles counts for predicted linkage disequilibrium was studied for the 30 SNPs predisposed to CHD in Hungarian general (n=279), and Roma Hungarian populations (n=279), with Hungarian general were described in Figure A, and Roma Hungarian populations is depicted in Figure B (see Figure 4.5; A and B). The nonrandom association between the alleles in the 30 CHD loci was measured using an alternative LD color scheme standard (D′)/LOD. The results revealed no correlation between the SNPs.

The mean GRS and wGRS were greater in the general population than in the Roma community (27.27 3.43 vs. 26.68 3.51, p value=0.046 and 3.52 0.68 vs. 3.33 0.62, p value=0.001, respectively) (see Figure 4.6 A and B).

Figure 4. 5 Haploview LD results in the study populations.

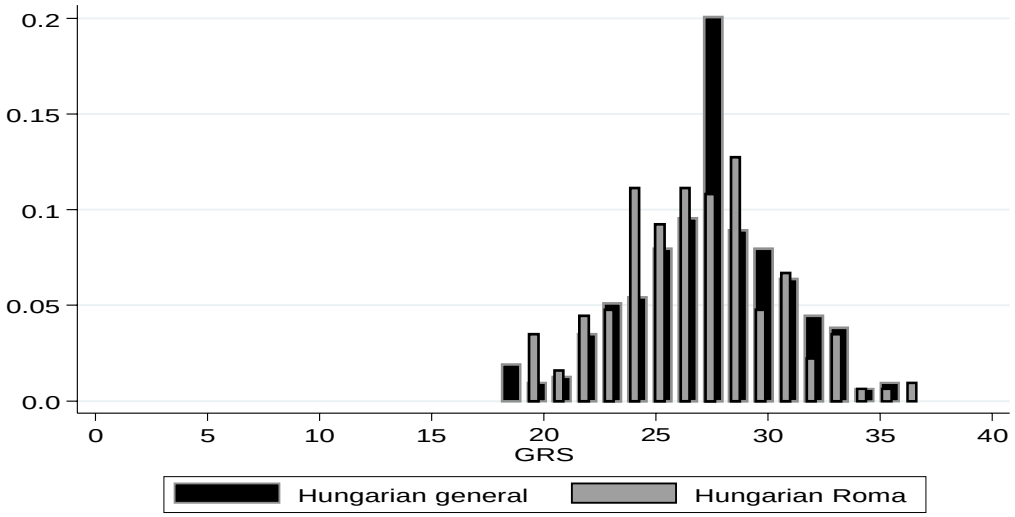


A. Haploview LD displayed the 30 SNPs for CHD risk among the Hungarian general populations.

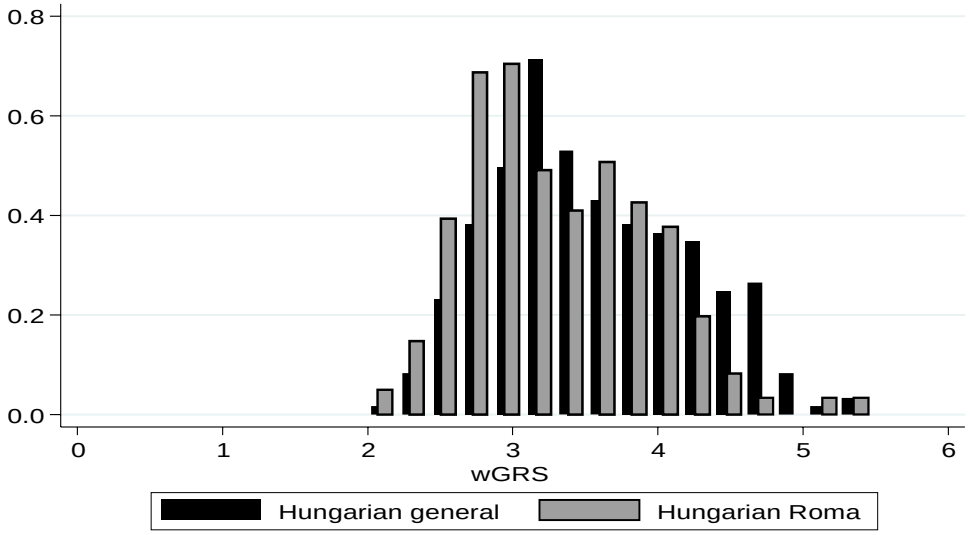


B. Haploview LD displayed the 30 SNPs for CHD risk among the Roma Hungarian Populations.

Figure 4. 6 Distribution of GRS, and wGRS among the Hungarian populations



A: The unweighted genetic risk score distribution among the Hungarian general and Hungarian Roma populations (p-value = 0.046).



B: Distribution of the weighted genetic risk score in study populations (p-value = 0.001).

4.2.3 Multivariable regression analyses for CHD/AMI

The odds ratio of CRFs according to SCORE-based models for CHD/AMI risk prediction revealed that age and elevated cholesterol or therapy for high cholesterol level were associated significantly with CHD/AMI risk in the Hungarian general population ((OR = 1.18, p-value = 0.046, 95% CI 1.00-1.17) and (OR= 4.90, p-value = 0.032, 95% CI 0.24-0.22)), respectively,

while HTN-Med showed a significant association with CHD/AMI risk only among the Roma population (OR = 7.85, p-value = 0.001, 95% CI 2.41-25.55) (Table 4.12).

When we combined the study population and used ethnicity as a possible predictor: age (OR = 1.05, p-value = 0.030, 95% CI 1.01-1.10), HTC (OR = 3.55, p-value = 0.007, 95% CI 1.42-8.90), and HTN-Med (OR = 4.79, p-value = 0.001, 95% CI, 1.90-12.07) were associated significantly with CHD/AMI in the Hungarian populations (Table 4.13). Although ethnicity did not prove to be a significant predictor, Roma seems to have a higher risk of developing CHD/AMI independently from conventional risk factors.

Table 4. 12 Odds ratio associated with CRFs in the model for predicting CHD/AMI risk in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.18	0.046*	1.00-1.17	1.03	0.317	0.97-1.09
Sex (Male)	1.62	0.480	0.42-6.16	2.34	0.149	0.74-7.40
HTC-Med	4.90	0.032*	0.24-0.22	3.00	0.078	0.89-10.16
HTN-Med	1.37	0.781	1.15-20.85	7.85	0.001*	2.41-25.55
Smoking	0.92	0.905	0.15-12.78	1.26	0.704	0.38-4.23

*Note:*Statistically significant (p-value<0.05)*

Table 4. 13 Odds ratio associated with CRFs in model for predicting CHD/AMI risk in the combined populations.

CHD/AMI	OR	p-value	95% CI
Age	1.05	0.030*	1.01-1.10
Sex (Male)	1.92	0.139	0.81-4.54
Ethnicity **	1.47	0.410	0.59-3.67
HTC-Med	3.55	0.007*	1.42-8.90
HTN-Med	4.79	0.001*	1.90-12.07
Smoking	1.06	0.901	0.43-2.63

Note*:**Hungarian general population was a reference population. *Statistically significant (p-value<0.05*

Although no significant association was observed between CHD/AMI and GRS/wGRS in these models, the ORs revealed a proportionally increased risk in the second and third tertiles in the general population (Tables 4.14, and 4.15).

Table 4. 14 Odds ratio of the GRS based model for CHD/AMI in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
GRS -T2	1.97	0.550	0.22-17.99	0.37	0.125	0.11-1.32
GRS -T3	2.83	0.348	0.32-24.82	0.67	0.508	0.20-2.02

Note:GRS tertiles; GRS T1 (18–24) was set as reference, GRS T2 (25–28), GRS T3 (29–37).

Table 4. 15 Odds ratio of the wGRS based model for CHD/AMI in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
wGRS -T2	1.11	0.908	0.18-6.84	0.93	0.899	0.28-3.02
wGRS -T3	1.80	0.490	0.34-9.54	0.78	0.699	0.22-2.76

Note:wGRS tertiles; wGRS T1 (1.964–3.047) was set as reference, wGRS T2 (3.049–3.686), wGRS T3 (3.692–5.509).

The addition of GRS and wGRS tertiles to CRFs revealed that age, HTC-Med, and HTN-Med were significantly associated with CHD/AMI in the combined population independent of the effect of ethnicity (Tables 4.16, and 4.17). The results of the analyses for each group are shown in the Table 4.18-4.19. Although ethnicity was not shown to be a significant and independent risk factor by itself, the results suggest that the Roma population has a higher risk of developing AMI/CHD.

Table 4. 16 Odds ratio of CRFs plus ethnicity and GRS for CHD/AMI risk prediction in the study populations.

CHD/AMI	OR	p-value	95% CI
Age	1.05	0.040*	1.00-1.09
Sex (Male)	2.06	0.114	0.84-5.02
Ethnicity **	1.47	0.411	0.59-3.71
HTC-Med	3.86	0.005*	1.51-9.86
HTN-Med	4.67	0.001*	1.82-2.01
Smoking	1.03	0.957	0.41-2.57
GRS-T2	0.53	0.261	0.17-1.61
GRS-T3	1.00	0.993	0.34-2.93

Note:***Hungarian general population was a reference population.*Statistically significant (p-value<0.05), and GRS-T1 were set as references.*

Table 4. 17 Ethnicity and wGRS for predicting CHD/AMI risk in the study populations.

CHD/AMI	OR	p-value	95% CI
Age	1.05	0.029*	1.01-1.10
Sex (Male)	1.88	0.161	0.78-4.54
Ethnicity **	1.47	0.413	0.59-3.66
HTC-Med	3.52	0.007*	1.41-8.83
HTN-Med	4.91	0.001*	1.92-12.54
Smoking	1.06	0.903	0.43-2.63
wGRS-T2	1.17	0.779	0.39-3.48
wGRS-T3	1.07	0.904	0.37-3.07

Note*:**Hungarian general population was a reference population. *Statistically significant (p-value<0.05), and wGRS-T1 were set as references.*

Table 4. 18 Odds ratio of CRFs and GRS for predicting CHD/AMI risk in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.08	**0.045**	1.00-1.17	1.02	0.529	0.96-1.08
Sex	1.50	0.560	0.38-5.88	2.75	0.103	0.82-9.30
HTC -Med	5.79	**0.022**	1.29-26.04	3.45	0.055	0.97-12.19
HTN-Med	1.13	0.916	0.11-11.50	8.14	**0.001**	2.39-27.69
Smoking	0.93	0.927	0.22-4.02	1.23	0.740	0.36-4.18
GRS-T2	1.03	0.982	0.10-10.73	0.33	0.133	0.08-139
GRS-T3	2.19	0.505	0.22-21.76	0.75	0.695	0.18-3.09

Table 4. 19 Odds ratio of CRFs and wGRS for predicting CHD/AMI risk in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.08	**0.050**	1.00-1.17	1.03	0.303	0.97-1.09
Sex	1.66	0.465	0.43-6.46	2.51	0.138	0.74-8.49
HTC-Med	4.82	**0.036**	1.11-20.87	2.95	0.063	0.87-10.02
HTN-Med	1.27	0.837	0.13-12.48	**7.80**	**0.001**	2.39-25.42
Smoking	0.93	0.918	0.21-3.95	1.21	0.754	0.36-4.07
wGRS-T2	0.90	0.916	0.13-6.33	1.12	0.865	0.29-4.31
wGRS-T3	1.29	0.775	0.22-7.43	0.73	0.680	0.16-3.25

By including DM as a reasonable new predictor to the updated SCORE-based genetic models (GRS and wGRS included), age, HTC-Med, and HTN-Med remained significant risk factors for developing CHD/AMI (for more details, see Table 4.20-4.22). However, the GRSs still did not show a significant association with the CHD/AMI (see Table 4.23-4.25).

Table 4. 20 Odds ratio of CRFs with DM for CHD/AMI risk prediction among the study populations.

CHD/AMI	OR	p-value	95% CI
Age	1.05	0.046*	1.00-1.09
Sex (Male)	1.94	0.134	0.82-4.62
Ethnicity**	1.56	0.346	0.62-3.93
HTC-Med	3.32	0.011*	1.32-8.39
DM	1.68	0.323	0.60-4.68
HTN-Med	4.41	0.002*	1.71-11.34
Smoking	1.05	0.911	0.42-2.62

Note:***Hungarian general population was a reference population. *Statistically significant (p-value<0.05).*

Table 4. 21 Odds ratio of CRFs plus ethnicity, DM and GRS for CHD/AMI risk prediction model among the study populations.

CHD/AMI	OR	p-value	95% CI
Age	1.04	0.059	1.00-1.09
Sex (Male)	2.06	0.115	0.84-5.06
Ethnicity**	1.55	0.359	0.61-3.92
HTC-Med	3.61	0.008*	1.40-9.29
DM	1.57	0.395	055-4.46
HTN-Med	4.10	0.002*	1.68-11.46
Smoking	1.03	0.958	0.41-2.58
GRS-T2	0.56	0.311	0.18-1.72
GRS-T3	1.03	0.958	0.35-3.06

Note:**Hungarian general population was a reference population.*Statistically significant (p-value<0.05), and GRS-T1 were set as references.*

Table 4. 22 Odds ratio of CRFs plus ethnicity, DM and wGRS for CHD/AMI risk prediction model among the study populations.

CHD/AMI	OR	p-value	95% CI
Age	1.05	0.044*	1.00-1.10
Sex (Male)	1.89	0.161	0.78-4.59
Ethnicity**	1.56	0.347	0.62-3.93
HTC-Med	3.28	0.012*	1.30-8.28
DM	1.71	0.306	0.61-4.78
HTN-Med	4.56	0.002*	1.75-11.91
Smoking	1.05	0.914	0.42-2.62
wGRS-T2	1.24	0.701	0.41-3.75
wGRS-T3	1.10	0.857	0.38-3.22

Note: **Hungarian general population was a reference population.*Statistically significant (p-value<0.05), and wGRS-T1 were set as references.*

Table 4. 23 Odds ratio of the CHD/AMI risk prediction model based on the CRFs plus DM in separate the study groups.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.07	0.096	0.99-1.16	1.03	0.325	0.97-1.09
Sex	1.43	0.611	0.36-5.57	2.34	0.149	0.74-7.41
HTC-Med	5.25	**0.027**	1.20-22.89	3.00	0.081	0.87-10.33
DM	4.34	0.081	0.83-22.58	0.99	0.988	0.26-3.76
HTN_Med	1.13	0.914	0.12-10.97	7.86	**0.001**	2.39-25.90
Smoking	1.15	0.859	0.26-5.11	1.26	0.705	0.38-4.23

Table 4. 24 Odds ratio of CRFs plus DM and GRS for CHD/AMI risk prediction model among the study populations

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.07	0.095	0.99-1.16	1.03	0.325	0.97-1.09
Sex	1.34	0.678	0.34-5.32	2.40	0.147	0.84-7.83
HTC-Med	5.42	**0.024**	1.25-23.57	3.02	0.080	0.88-10.37
DM	4.23	0.087	0.81-22.08	0.98	0.973	0.23-3.74
HTN_Med	1.00	0.998	0.10-10.28	7.71	**0.001**	2.31-25.74
Smoking	1.18	0.831	0.26-5.29	1.24	0.733	0.36-4.20
GRS	1.07	0.591	0.847-1.34	0.98	0.839	0.83-1.17

Table 4. 25 Odds ratio of CRFs plus DM and wGRS for CHD/AMI risk prediction model among the study populations

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.07	0.098	0.99-1.16	1.09	0.340	0.97-1.09
Sex	1.42	0.612	0.36-5.57	2.55	0.126	0.77-8.44
HTC-Med	5.24	**0.028**	1.20-22.89	3.03	0.079	0.88-10.49
DM	4.32	0.084	0.82-22.75	0.97	0.961	0.25-3.70
HTN-Med	1.13	0.916	0.12-10.98	7.54	**0.001**	2.26-25.17
Smoking	1.15	0.859	0.265.11	1.23	0.739	0.37-4.11
wGRS	1.02	0.967	0.35-2.99	0.76	0.593	0.28-2.09

Several models were developed (Framingham, Basic SCORE, and PROCAM) utilizing the data from the lipids profiles of (HDL-C, LDL-C, and TG), but these studies did not provide useful information. Furthermore, due to confounding, it was not useful to include these predictors for CHD/AMI risk prediction in the Hungarian populations. HDL-C was show to have a significant association with CHD/AMI in the Hungarian general population (OR=0.95, p value 0.009, 95% C (0.91-0.99)) in Framingham model, with no statistically significant association of HDL-C predictor among Roma, however, HTN-Med revealed statistically significant (OR=8.16, p value 0.001, 95% C (2.40-27.67)) (see Table 12.4). TC was likewise associated with CHD/AMI risk in the Hungarian general population (OR=0.47, p value 0.028, 95% C (0.24-0.92)). TG was not associated with CHD/AMI in all population in the included model in SCORE basic model, with no statistically significant association among Roma (Table 12.5), and PROCAM risk model indicated similar results (see tables 12.4-12.10 analyses for additional details).

4.2.4 ROC curve analyses

The model performances (discrimination, calibration, and risk classification) are described in Tables 4.26 and 4.27. The AUC curve estimates LROC for CHD/AMI risk prediction when integrating the CRF basic, CRFs with GRS, and CRFs with wGRS models, which were 0.8149, 0.8346, and 0.8160 in the Hungarian general population and 0.8616, 0.8549, and 0.8674 in the Roma population, respectively. When DM was added to the models, minimal improvement occurred in the AUC values in the general population, and no significant improvement was observed among Roma individuals (Table 4.26). Considerable improvements in the AUC value were observed when the CRF basic model with DM was combined with GRS in the Hungarian general population (from 0.8299 to 0.8400). For the Roma population, the CRF basic model (without DM) showed the greatest improvements in the AUC value (0.8616 to 0.8674) compared to the CRFS+wGRS model. In the combined populations (Table 4.27), the CRF basic model with DM and ethnicity showed the greatest AUC value (LROC=0.8525) compared to the basic model (without DM). The results of the Hosmer-Lemeshow tests indicated that all the models had a good fit (p value≥0.11).

Table 4. 26 Models' performances for CHD/AMI risk based on the CRFs and GRS/wGRS in the Hungarian populations.

Models	**Hungarian general**					**Hungarian Roma**				
	SENS	SPEC	CLASS	CALIB	LROC	SENS	SPEC	CLASS	CALIB	LROC
1. CHD/AMI models based updated SCORE (HTC-Med,and HTN-Med)										
CRFs basic	60.00	82.90	82.08	0.9601	0.8149	81.25	76.81	77.06	0.2193	**0.8616**
CRFs+GRS	70.00	85.50	84.95	0.9241	**0.8346**	81.25	77.57	77.78	0.7087	0.8549
CRFs+wGRS	60.00	84.01	83.15	0.9349	**0.8160**	81.25	77.57	77.78	0.3803	**0.8674**
2. CHD/AMI models based on the updated SCORE plus DM										
CRFs+DM	60.00	86.99	86.02	0.2818	0.8299	81.25	77.19	77.42	0.2298	**0.8611**
CRFs+DM+ GRS	60.00	86.99	86.02	0.6587	**0.8400**	81.25	77.57	77.78	0.5061	0.8534
CRFs+DM+ wGRS	60.00	86.62	85.66	0.9496	**0.8333**	81.25	77.19	77.42	0.3807	**0.8670**

Note: The bold highlight indicated that the LROC is showing improvement after adding Ethnicity (E), GRS, and DM to the basic model.

Table 4. 27 Model's performance for CHD/AMI risk based on the CRFs and GRS in the combined populations using ethnicity predictor.

Models	Hungarian general				
	SENS	SPEC	CLASS	CALIB	LROC
1. CHD/AMI models based updated SCORE (HTC-Med, and HTN-Med)					
CRFs+E	76.92	81.02	80.82	0.7843	0.8479
CRFs+GRS+E	80.77	81.77	81.18	0.1082	**0.8490**
CRFs+wGRS+E	76.92	81.02	80.82	0.7922	0.8456
2. CHD/AMI models based on the updated SCORE and DM					
CRFs+DM+E	80.77	80.83	80.82	0.5329	**0.8525**
CRFs++DM+GRS+E	80.77	80.83	80.82	0.4737	0.8518
CRFs+DM+wGRS+E	80.77	81.20	81.18	0.7842	0.8497

Note:*The bold highlight indicated that the LROC is showing improvement after adding Ethnicity (E), GRS, and DM to the basic model.*

4.2.5 Marginal Plots Analyses

In the Hungarian general population, CHD/AMI risk was low among males (margin= 0.001, p= 0.707; 95% CI: -0,001-0.001) and females (margin= 0.006, p= 0.516; 95% CI: -0,013-0.026) subjects between 18 and 45 years of age. The risk of the trait increased after the age of 46 years among females (margin= 0.028, p value= 0.048, 95% CI 0.000-0.056) and after 54 years among male subjects (margin= 0.065, p value= 0.039, 95% CI 0.003-0.126); thus, female subjects were predicted to develop CHD/AMI earlier than male subjects. For the Roma population, the marginal plot shows that CHD/AMI risk is low for younger subjects (males and females between 18-40 years of age; margin= 0.03, p= 0.397; 95% CI: -0,043-0.109 and margin= 0.003, p= 0.387; 95% CI: -0,004-0.011, respectively). The risk starts to significantly increase at the age of 41 years for both males and females (male subjects: margin= 0.080, p value= 0.047, 95% CI: 0.001-0.159; female subjects: margin= 0.024, p value= 0.045, 95% CI 0.000-0.048 for age 41 years) (see Figure 4.7).

The prediction of CHD/AMI risk in the combined population revealed that the risk is higher for the Roma population. The risk becomes significant at 34 years of age in the Roma group (margin= 0.022, p value= 0.046, 95% CI: 0.000-0.043), while for the general population, the risk becomes significant at the age of 44 (margin= 0.020, p value= 0.046, 95% CI: 0.000-0.041) (see Figure 4.8).

The prediction of the marginal plot revealed that CHD/AMI risk interacted by age and gender, the younger population is at low risk of developing the disease (18-40) years, adult's male of both Hungarian general and Roma are more likely to express CHD/AMI than adult's female subjects; males of Hungarian general population expected to develop CHD/AMI risk in ages (54-80) years however, Roma male develop CHD/AMI risk earlier in ages (41-60) years. Female subjects of the Hungarian Roma also have a greater risk of developing CHD/AMI compared to female subjects of the Hungarian general, the risk among Roma female begins at ages 41-70 years, and at 47-58 years among the females of the Hungarian general population.

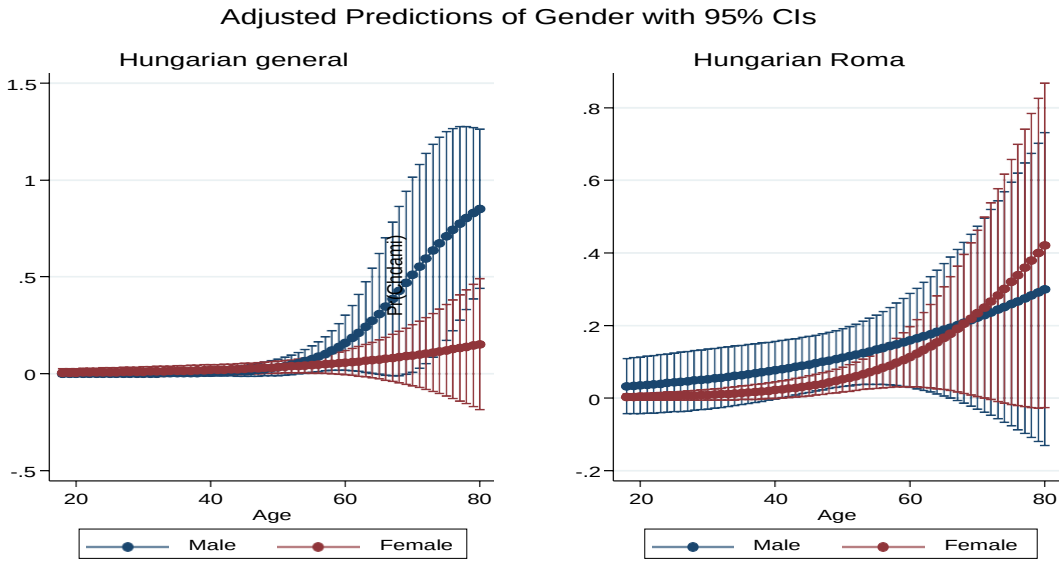


Figure 4. 7 The marginal plot interaction of the combined CHD and AMI based on age and adjusted gender in the Hungarian (general and Roma) populations.

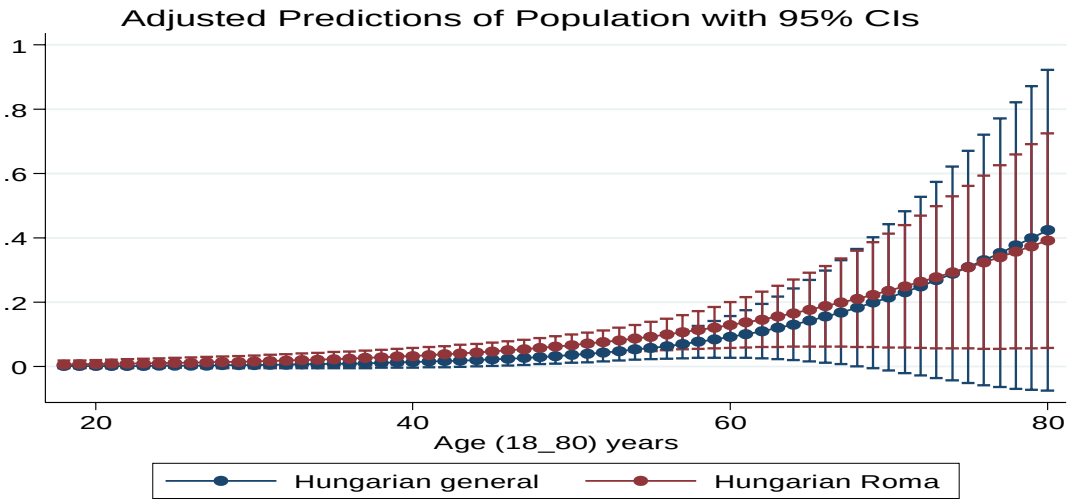


Figure 4. 8. Marginal plot of the combined CHD/AMI based on age and population interaction.

CHAPTER FIVE

Discussion

The significant information from the systematic literature review of CHD risk prediction modelling studies among general populations, and the main finding from the original study of CHD/AMI risk prediction among the Hungarian population (the cross-sectional study) were both discussed in this chapter.

5.1 Discussion of the main finding of systematic literature review of CHD risk prediction modelling studies among general populations

Our comprehensive assessment of the literature reveals that a number of prognostic models have been developed for estimating the risk of CHD in the general healthy population, and the basic foundation for the predictors is the Framingham model. The Framingham model is the most widely used model for predicting CHD risk in the general population, despite the fact that it overestimated and underestimated the CHD risk in various populations. There is still no consensus about the best model(s). By include models with genetic risk components in addition to conventional models, our review offers some fresh and organized knowledge concerning CHD prognostic modelling studies.

The trend of CHD mortality and morbidity has reportedly continued to rise over time, especially in low- and middle-income countries, despite an increase in the number of publications in the medical literature on clinical prognostic modelling studies combining multiple predictors (CRFs, GRSs, and biomarkers) for identifying high subgroups at increased risk of CHD, the GWAS has facilitated a better understanding of the causal risk factors (by linked more than 97 SNPs) to CHD risk. However, translation of the acquired knowledge is lacking, and research among various populations does not support the use of genetic data in health and public health practice, it has been shown previously that data based on the use of genetic risk for calculating CHD is limited [214].

It was expected that incorporating a genetic risk score into conventional risk-factor-based models would increase their ability to predict the onset of CHD in the general population. We discovered that, with the exception of one notable model [134], the majority of the genetic modelling studies had not yet been externally validated in various populations, despite the fact that, the majority of these studies have indicated improvements in performance (discrimination

and calibration ability) [99-101,121,128-134,197]. Demonstrating the good performance of the generated models is not sufficient, improvements must have confirmed in different populations to ensure the generalizability. It has been previously demonstrated that models should not be recommended for clinical use until external validity is established, because prediction model performance in new patients is frequently lower than in the development population [116, 215, 216].

Healthcare providers and policymakers feel that the prevention by identifying high risk subgroup and assisting them in making lifestyle changes prior to the onset of CHD will have a greater impact on reducing CHD mortality and disability than treatment, and this requires robust reduction of risk factors through the accurate estimate of the population at risk [215]. Clinicians are also willing to identify appropriate guidelines for making quick decisions in everyday medical practice (about allocating health care resources and lowering costs) in order to improve patient outcomes as much as possible [217]. They can achieve improved outcomes while lowering total healthcare expenditures by using precise prognostic and predictive model(s). Before beginning to develop a risk prognostic model, it is critical to consider whether a new model is required. One recommended solution would be the validation of the existing models instead of constructing several new models. It is difficult to translate and disseminate the results of the recognized model into clinical practice, and no prognostic application is suitable for adoption in normal daily practice.

Our review identified variance in model geographical location: most models were developed and validated in populations from the United States of America, Canada, and European. According to WHO, low- and middle-income countries account for more than three-quarters of all CHD deaths [218]. Our review confirmed that no prognostic modelling studies for CHD originated in developing countries, for example, when we performed the search for this review, no prognostic model for people from Africa had yet been developed. Only a few studies from Asia (China, Turkey, Thailand, Japan, and Korea) were conducted.

The majority of participants were recruited from primary care and community settings, although the selection methods was not fully documented. Most of the studies did not specify which sampling approach was utilized, and only a few indicated the random method of the participant's selection. Sampling techniques are an important strategy for obtaining representative target populations; researchers should be more precise in this stage in order to avoid selection bias and deliver relevant information to physicians. Although few research

reported consecutive participant's selection, but in some other studies, participants were selected nonconsecutively and thus increased the risk of bias due to selective sampling, it has been already demonstrated that, if the participants in the sample are not well defied or representative, such data necessarily contain selection biases in their collection, and model building must take such difficulties into account [212].

Although age is the most common risk factor considered in CHD risk prediction and affects the two sexes for developing CHD, there was great variation between the developed and validated models in terms of the participants age groups; the validation studies were performed in people outside the age range of the developmental studies, and most of the developed models predicted the risk in the elderly population compared with the validated models, which may affect the number of CHD outcomes and may influence the model performance, it was previously indicated that an incorrect interpretations of basic epidemiological statistics such as age-incidence curves and hazard ratios may occur from failing to account for unobserved individual variation (frailty) [213, 219]. Researchers should specify the age group by sex because females have a lower risk of developing CHD than males [220].

Although disparities of CHD risk depend on factors such as age, gender, and geographical location of populations, only a few researchers are focusing on females and middle- and low-income countries possibly due to data availability, population needs, or priorities. Priorities for studies in developing countries (Africa and Asia) are different from Europe or America. Developing regions of the world must focus on the leading causes of death such as malaria, HIV and AIDS, dengue, and tuberculosis [221]. Developmental validation studies should compare diseases in the same age group in both models developed and validated.

The method of outcome determination should be accurate to provide proper patient risk stratification and to support personal clinical decision making with the goal of improving patient outcomes and quality of care [222]. Incorrect outcome assessment and measurement of predictors may inflate the predictive accuracy of the predictors and that of the final prognostic model [121]. With respect to CHD definitions reported in the models reviewed, most models defined the incidence of coronary heart disease as fatal or nonfatal myocardial infarction, and over 80 different definitions for the disease outcomes were identified. In addition, most outcomes were not fully defined, and the International Classification of Diseases (ICD) codes for CHD were described in only a few models. Different outcome definitions and measurement methods may lead to differences in study results and are a source of heterogeneity across

studies, and thus risk of bias [121]. CHD definition and outcomes must be readily accessible, precisely stated, and have a low measurement errors rate in order to be valuable. Overall, heterogeneity of definitions of CHD outcome identified in this review may affect the discrimination ability of the models (overestimate/underestimate): models fail to discriminate between case and non-case subjects and thus may influence the predictive accuracy of the final prognostic model [223-224]. A standard consistent definition of CHD and outcome will increase transparency in reporting the predicted outcomes and may improve the quality of research. Similar to outcome predictors, several candidate predictors were reported in the models identified. Most models had the same common predictors, such as age, sex, smoking, blood pressure, and total cholesterol levels. Many novel predictors, such as genetic risk scores, biomarkers (coronary artery classification and C-reactive protein), and others (e.g., creatinine, fibrinogen, and interleukin 6) have been described in a few models. Most of the novel and newly developed models show good performance in predicting CHD, but the strategies of how the predictors were selected (backward or forward stepwise regression) and which approaches were used (plot or multiple linear regression) are still questionable in most studies reviewed. Regarding the models' performance, most of the models measured discrimination ability with less commonly used calibration and classification measures. Therefore, the performance of these models has not been fully examined, and discrimination alone can be insensitive and less useful in evaluating risk prediction of future events. It will be valuable if it is used for comparing the fit of predictive models using the calibration statistic and reclassification improvement, it has been previously reported that a prediction model will constantly require reporting discrimination and calibration. If the prediction model is to be utilized to make clinical decisions. Other performance indicators may be required in specialized situations [225]. It is essential to assess the goodness of fit and to validate the model to ensure predictive performance. The use of good strategies for model selection in addition to adequate performance and goodness-of-fit measures is needed in developing accurate predictions[226]. It is important to ensure that the model is well-calibrated if the prognostic value is close to the true value of disease outcomes. Model calibration was assessed via Hosmer-Lemeshow goodness of fit with other measures, such as adjusted R square, cross-validation, and Akaike's and the Bayesian information criterion for small numbers of predictors [227]. If there are many predictors, then forward stepwise regression can be used [226].

Our review assessed prognostic modelling studies in community-based settings, aiming to summarize the available evidence about the optimal model in predicting CHD events in healthy populations and to explore whether the inclusion of the genetic risk score in conventional risk factors improves the ability of these models. MESA risk score with coronary artery calcification, which is described by McClelland et al. (2015) seems to be an optimal model for predicting CHD risk in the general population [173]. This model is a conventional modelling study including age, gender, CAC, ethnicity, DM, smoking, family history of heart attack, TC, HDLC, SBP, and treatment (hypertension and lipid lowering) predictors. We would recommend this model be used in low-income countries (it is less expensive compared to GRS models). Genetic risk score (unweighted and weighted) in the model of Brautbar et al. (2012) might help in predicting CHD risk when integrated to Framingham [134], despite the fact that the improvement only occurred in one population and failed to persist in the other included populations.

These findings can help clinicians and decision makers improve the quality of interventions and improve the health of the population at risk. Future validation studies for genetic modelling studies are needed to ensure the quality and transparency of the developed model. Methodological assessment of genetic models is required. Most of the genetic risk scores incorporated into the conventional risk factors improved the discrimination and reclassification ability in the derivation models. Most genetic modelling studies were developed using only Caucasian populations; thus, the generalizability of the existing prognostic models is questionable. Genetic modelling studies might be used to target the prevention of CHD if the individual's genetic risk is comprehensively evaluated. An accurate assessment of an individual's risk is fundamental to future efforts in personalized medicine for the primary prevention and proper management of CHD.

Even though, genetic analysis is expensive for assessing SNPs associated to CHD/AMI risk, an individual's genetic risk is thoroughly examined by a comprehensive collection of genetic association data the result may be utilized to target primary prevention of CHD. But unfortunately, most of the genetic variation were not fully examined, nor validated in different populations other than Caucasian. Understanding an individual's genetic risk through genetic modeling studies could be beneficial in targeting interventions for preventing coronary heart disease at an early stage. The costs associated with genetic assessments can vary widely. Factors influencing costs include the type of genetic testing, the number of genes analyzed, and

the technologies used. Generally, genetic testing has become more accessible and affordable over time, but it still may incur expenses. Implementing genetic assessments on a population level is a complex task. Challenges include cost, logistics, ethical considerations, and the need for a robust infrastructure to handle and interpret large-scale genetic data. Additionally, the practicality and ethical implications of population-wide genetic screening should be carefully considered. The use of genetic information in targeting primary prevention has potential public health relevance. Identifying individuals at higher genetic risk for CHD could allow for more targeted public health interventions, education, and resources. However, the ethical and social implications of using genetic information on a large scale need careful consideration. The approach aligns with the principles of preventive medicine by emphasizing the importance of understanding and mitigating risk factors before the onset of disease.

Future validation studies should include genetic application in different geographic locations, and fully independent validation by independent investigators using alternative measurements of these risk factors in different population settings may improve the prognosis of the disease. Incomplete reporting of information in both conventional and genetic modelling studies was observed regarding the following methodologies: sampling technique, subject selection criteria, categories and blinding, genetic information (selection, coding), construction of the final models, classification measures, duration of follow-up, and missing values of the participants and technique used for handling this issue (bootstrapping, cross validation, and resampling method). Therefore, simply excluding the participants with missing values from the analysis reduces the effective sample size and may also lead to inaccurate estimates of the predictor outcome associations and the predictive performance of the final model [43]. The performance of a predictive model is overestimated when simply determined on the sample of subjects that were used to construct the model, and statistical techniques such as shrinkage and bootstrapping are available to attempt to reduce over-optimism at the model-building stage [89, 225]. Comprehensive and valid information on conventional and genetic models is needed. Researchers should enhance the quality of their reports by describing and highlighting this important information.

To ensure the generalizability of the prognostic model and the ability of the model to predict CHD in populations with different characteristics, an external validation study is needed to evaluate the model's performance and to avoid overfitting in prognostic modelling studies. The

shrinkage and penalization method should also be applied to reduce overfitting by readjusting the regression coefficients [43-44].

Our review reveals that only one conventional model was considered a good prognostic model for CHD in the general population (decision analysis) and applicable for use in clinical practice (classification marker for CHD risk prognosis) [173]. One genetic modelling study (Brautbar et al., 2012), was externally validated in three different populations and performed decision analysis but had limitations regarding classification improvement in the comparator model [134].

For conventional and genetic modelling studies, the identified previous models might be considered a good and optimal prognostic for CHD risk in the general population (decision analysis) and applicable for use in clinical practice (classification marker for CHD risk prognosis). However, the model of Brautbar et al. (2012) was externally validated in three different populations and performed decision analysis, but this model had limitations regarding the discrimination and classification improvement that occurred in the developmental group without significant improvement in the comparator groups [134].

There are several reasons why the performance of a prognostic model needs to be evaluated before its results can be used; most of the models fail to satisfy certain statistical notions of correctness (statistically invalid), fail to be useful in a clinical setting, or have invalid prognostic information. Furthermore, the same model might fail according to one clinical criterion and pass according to another. There were two definitions of the validation prognostic modelling study. First, a statistically validated model passes all appropriate statistical checks, including the goodness of fit on the original data set and unbiased prognosis on a new data set. Second, a clinically validated model performs satisfactorily on a new data set according to context-dependent statistical criteria [205].

5.2 Discussion of CHD/AMI risk prediction among the Hungarian population

The Hungarian cross-sectional study's main finding indicated a significant difference between populations in terms of gender distribution, height, weight, education levels, economic activities, family member, SBP, HDL-C, and glucose level. Roma had lower educational levels, a higher proportion of unemployed subjects and a bigger family size than the general population. Despite the fact that the frequency of CHD, AMI, and stroke was lower and non-significant among the included participants, clinical risk variables such as DM, CKD, HTN-

Med, HTC-Med were greater among Roma. Although Smoking and decreased HDL-C levels were higher among Roma, SBP and glucose levels were significantly higher in the Hungarian general population. These findings revealed that the Roma population is at high risk for cardiometabolic illnesses, and our findings were consistent with previously Roma research. [48-53, 230-234].

When we investigated the effect size of the 30 SNPs chosen for predicting CHD/AMI risk in Hungarian (general and Roma) populations (for real CHD/AMI risk susceptibility loci using PLINK). The result show that, six SNPs, including rs2306374 (gene MRAS), rs9818870 (gene MRAS), rs12190287 (gene TCF21), rs10455872 (gene LPA), rs3184504 (gene SH2B3), and rs9982601 (gene KCNE2), showed strong association with CHD/AMI risk after Bonferroni correction in the Hungarian general population, and three SNPs, rs17609940 (gene ANKS1A), rs2259816 (gene HNF1A), and rs12936587 (gene RASD1), were shown to be significant associated with CHD/AMI risk among the Roma.

Our findings also indicated that the general population's mean weighted GRS and GRS were greater. Despite the fact that the vast majority of the chosen SNPs were not shown to be independently linked to CHD/AMI, it appears that the general population is more likely to acquire CHD/AMI. Before looking at how factors like allele frequency can be inherited beside the CRFs for CHD/AMI risk, we ran HWE to discover any deviations of markers that failed to be at HWE (which may cause genotyping errors). One SNP (rs12413409) indicated that was not in HWE was eliminated, leaving 29 SNPs integrated with CRFs to determine the weighted and unweighted GRSs burden for CHD/AMI.

Based on prior research, age is an important non-modifiable risk factor for CHD/AMI in multivariable analytic prediction models. We used this risk factor to predict CHD/AMI in Hungarian populations. The fundamental reason for this is because CHD is a condition caused by a multitude of risk factors, the majority of which are thought to be age related. In the multivariable regression model, age was related with the development of CHD/AMI, particularly in the general population, this result was like some previous reports on the risk factors of CHD [1, 235]. Furthermore, the marginal plot analyses revealed that the risk of CHD/AMI is obviously low among the younger populations in general; however, both populations (general and Roma) were susceptible to premature onset of CHD/AMI (before age 55 years), with the risk expected to begin earlier among Roma individuals.

In this investigation, there was no significant association between male subjects and CHD/AMI risk, this conclusion may be due to of the small fraction of Hungarian males who participated in this study. However, the marginal plot predictions revealed that the risk of CHD/AMI increased among male subjects of the Hungarian (general and Roma) population compared to female subjects. However, Roma (males and females) individuals tend to develop CHD/AMI earlier than the Hungarian general population. According to previous studies, male sex is also an independent predictor of CHD (particularly AMI mortality) and plays a role in increasing CHD risk [18, 57, 62-64], despite the fact that the mortality rate of CHD is remain greater among females than males [5, 13-14, 23, 229, 236-241].

Hyperlipidemias (elevated total cholesterol or lipid-lowering medication) was a major risk factor for CHD/AMI in the general population, and hypertension (elevated blood pressure or antihypertensive medication) showed a significant association with the trait only in the Roma population. Despite widespread agreement on the importance of lowering blood cholesterol levels, particularly LDL-C [45-55], in lowering the risk of CHD, this predictor was not employed in derived mode. In several models, including Framingham, SCORE (basic and updated), and PROCAM, LDL-C, HDL-C, and TG were found to be underestimating CHD/AMI risk in Hungarian populations, possibly due to the low prevalence of CHD/AMI among included subjects, or due to the complex interaction with other predictors. There have been no previous studies that predict CHD/AMI using increased total cholesterol rather than LDL-C, our study contrasted with these previous studies. Other research indicated, hypertension, dyslipidemias, diabetes, obesity, and smoking account for more than 90% of the population's attributable risk of acute myocardial infarction (AMI) [55]. DM was found to have no association with CHD risk in all models, probably due to its low prevalence, or interaction with other variables. DM had a substantial correlation with CHD/AMI in both (general and Roma) populations in a bivariate analysis (Appendix 12.9). Our study was consistent with previous study that indicated that SCORE was less precise for estimating risk in patients with DM [241].

Smoking is known to be the strongest contributor to CHD/AMI in all available models, such as the Framingham, SCORE, QRISK1, QRISK2, and PROCAM models [17-18, 23, 27, 29, 234, 241-243], all of which have been developed and validated to predict CHD risk in general populations. Herein, smoking was found to be a nonsignificant predictor of an elevated risk of

CHD in the Hungarian populations, which could be owing to selection bias or interaction as a confounding factor with other predictors. One reason for this could be the presence of other highly correlated predictor variables in the model that have a stronger association with CHD. For example, high blood pressure, high cholesterol levels were also well-established risk factors for CVD and may have a stronger association with the outcome variable in the multiple regression model. In this case, the effect of smoking may be overshadowed by the effects of these other variables, thus making smoking a nonsignificant factor.

Based on previous studies [96, 129, 154, 149, 165, 244, 245], the combination of the CRFs (based on SCORE variables) and genetic components (GRS or wGRS) might improve the model performance and predict AMI/CHD better than CRFs alone. Although GRS/wGRS were not significantly associated with CHD/AMI risk in study populations, the utility of genetic factors is still questionable in risk prediction. We examined the predictive ability of the combined models where CRFs (basic model) and genetic risk scores were integrated. A basic model that included DM showed good discrimination improvement compared to the model without DM. In addition, the models with GRS integration showed the greatest discrimination improvement in general. The highest improvement for the Hungarian general occurred when we added the GRS to DM however the greatest improvement in the Hungarian Roma occurred when we added the wGRS to the basic SCORE.

5.3 Conclusions

Although the GWAS has improved our understanding of CHD etiology by identifying and validating genetic variants associated with complex human traits, the use of genetic architecture for clinical assessment of CHD is still uncertain, as the majority of the SNPs' functions for CHD risk prediction are unknown or are poorly understood. McClelland et al. (2015) provided an optimal model for predicting CHD in the general population by incorporating CAC marker to the CRFs basic model, this model can predict CHD risk in different populations.

This study can help clinicians and decision makers to improve the quality of interventions using CAC predictor in CRFs for CHD/AMI risk prediction and can help in improving the health of the population at risk. Future genetic modeling validation studies are required to assure the quality and transparency of the created models. Genetic models must be evaluated methodologically. Because most genetic models for CHD include Caucasian populations, there

is no generalizability of the available genetic prognostic models. If an individual's genetic risk is thoroughly examined by a comprehensive collection of genetic association data, including numerous studies using graphical displays and extensive textual material. Genetic modelling studies may be utilized to targeted primary prevention of CHD. An accurate assessment of an individual's risk is fundamental to future efforts in personalized medicine.

We attempted to develop a genetic scoring model with a careful selection of CHD/AMI-associated SNPs that could provide a somewhat valid estimate (made possible by the chosen method) of the genetic load in our study populations; however, due to the small number of CHD/AMI patients in the study groups, the GRSs were not significantly associated with the trait in the regression models, but the predictive accuracy of the models with a genetic component was remarkable.

Aging, elevated total cholesterol (in the general population), and hypertension (in the Roma) may all interact to raise the chance of developing CHD/AMI. In the future, verifying the genetic risk prediction model with independent datasets or cohorts to examine its accuracy, dependability, and generalizability would aid in determining the model's resilience and external validity. It would also be useful to assess the clinical usability and possible impact of the genetic risk prediction model in clinical settings. This could include conducting prospective studies or implementation trials to evaluate the efficacy of our approach in clinical practice, as well as its potential for directing individualized risk assessment, prevention, and treatment options. However, using genetic risk prediction models has the potential to enhance health outcomes by providing individualized risk estimates and assisting in early diagnosis and management. However, careful assessment and discussion of these models' limitations and problems is required for their effective adoption in clinical practice.

5.4 Strengths and Limitations of This Study

The systematic review's strengths are that the search was done in several databases, including Embase, PubMed, Cochrane, Web of science, and Scopus with a solely human filter. In addition to that one reviewer extracted the data, which was then thoroughly reviewed by another, and the individual study characteristics were provided. Quality assessment was performed in duplicate using CHARM, and GRIPS statements and because of the heterogeneity

and the huge number of different predictors identified, this data were unsuitable for conducting meta-analysis which may limit the scope of this investigation.

A single point data collection was likely performed for the cross-sectional study in order to identify the risk factors of CHD/AMI, and understand the role of the modifiable and nonmodifiable risk factors in developing CHD/AMI, then described the features among the general and Roma populations using genetics (weighted and unweighted GRSs) and conventional risk factors. The weighted and unweighted GRSs were developed based on careful selection of 30 SNPs for CHD/AMI risk prediction; however, due to the small number of CHD/AMI patients in the study groups, the GRSs were not significantly associated with the trait in the regression models. Likely, the predictive accuracy of the models with a genetic component showed a remarkable AUC improvement. Age, and medication of both high total cholesterol and hypertension all increased the risk for CHDAMI, indicating a synergistic interaction between these predictors. Our findings show that SCORE risk prediction dose not estimated the actual risk of CHD/AMI as many of the risk factors like male sex, SBP, DBP, LDL-C, HDL-C, smoking, DM, and GRSs (GRS, and wGRS), some predictors were not incorporated. Other limitations of this study include the lack of prospective or retrospective follow-up, making it unsuitable for predicting CHD/AMI, the small sample size, selection, and recall bias, and small number of CHD/AMI events. In the future, verifying the genetic risk prediction model accuracy, reliability, and generalizability with independent datasets and cohorts could aid in identifying the model's resilience and external validity.

CHAPTER SIX

Novelty

6.1 Our systematic review identified CAC as the most effective marker for predicting CHD risk. We also outlined the ideal model for investigating CHD prognostic modeling studies in general populations. This represents the initial comprehensive analysis of studies exploring genetic and traditional risk factors for CHD risk prediction, employing the CHARM and GRIPS statements.

6.2 In this study, the GRSs (weighted and unweighted) and the allele frequencies of the 30 SNPs associated with CHD/AMI in Hungarian populations were calculated and compared for the first time.

6.3 This study is contemporary and pioneering as no genetic risk prediction model for the Roma population has been developed, no studies combining genetic and traditional risk factor modeling have been undertaken, and no assessments of model performance (discrimination, calibration, and risk classification) have been conducted.

6.4 This study is the inaugural examination of CHD/AMI risk prediction to explicitly address the condition of premature onset (before the age of 55 years) in both general and Roma populations.

CHAPTER SEVEN

Summary

CHD is recognized as a major cause of illness and mortality, as well as disability, in central Europe, developing countries, and some developed countries. Despite this, the incidence and death rate of CVD (mostly from CHD) has been reduced in several countries as a result of successful technologies developed by GWASs (which led to the identification of genetic variations (SNPs) that may have been implicated in CHD formation). Preventive intervention is also available through effective medication of lifestyle risk factors and comprehensive medication with statins. In many nations, successful legislation for a significant killer (smoking) has also been developed and implemented. Identifying high-risk individuals who may develop CHD risk remains challenging; a complete and precise assessment of CHD risk is required to identify those high-risk groupings. Obesity, diabetes, and financial hardship are just a few of the risk factors for coronary heart disease that are on the rise throughout countries, organizations, and communities. The bulk of these factors have been proven to work synergistically with other factors such as obesity, smoking, hyperlipidaemia, and hypertension to accelerate the atherosclerosis process. Early identification of persons at high risk of developing CHD is crucial for health promotion and prevention initiatives, taking into consideration both hereditary and environmental risk factors. This method of identification has the potential to reduce mortality and morbidity while increasing cost-effectiveness. Treatment can benefit from a well-structured strategy for identifying persons at moderate risk of CHD before symptoms develop. Framingham, SCORE, QRISK, and ASSIGN prediction models are crucial in assessing the risk of CHD among population groups. Our systematic review identified CAC as the most effective marker for predicting CHD risk. We also outlined the ideal model for investigating CHD prognostic modeling studies in general populations. This represents the initial comprehensive analysis of studies exploring genetic and traditional risk factors for CHD risk prediction, employing the CHARM and GRIPS statements. In this study, the GRSs (weighted and unweighted) and the allele frequencies of the 30 SNPs associated with CHD/AMI in Hungarian populations were calculated and compared for the first time. The majority of these models, including Hungary, overstated and underestimated the frequency of CHD in the general population. However, several models have been developed in an attempt

to assess the risk of CHD using individual risk factors. We developed a novel model for CHD risk prediction across Hungarian communities by hypothesizing that Hungarian Roma had greater genetic diversity and environmental risk factors for CHD than the overall population. According to our data, Hungarian general has a larger burden of GRS (weighted and unweighted) than the Roma community, which may predispose them to CHD development. Hungarian Roma, on the other hand, are more vulnerable to environmental risk factors that may interact synergistically to accelerate CHD, such as low socioeconomic deprivation including educational gap, lower economic activities, and living in a large family member, having low HDL-C levels, being more smokers, and having a higher rate of diabetes, hypertension (stage 2 of SBP and DBP), chronic kidney disease, and stroke. When CRFs and GRSs were integrated in multivariable models, age, medication of elevated total cholesterol and hypertension were found to be strongly and independently associated with CHD/AMI, other predictors overstated CHD/AMI risk in Hungarian populations, probably due to the low prevalence of CHD/AMI in the sample. This study is contemporary and pioneering as no genetic risk prediction model for the Roma population has been developed, no studies combining genetic and traditional risk factor modeling have been undertaken, and no assessments of model performance (discrimination, calibration, and risk classification) have been conducted. This study is the inaugural examination of CHD/AMI risk prediction to explicitly address the condition of premature onset (before the age of 55 years) in both general and Roma populations. We urged the Hungarian Roma population to address their modifiable risk factors in order to protect themselves from this hazardous disease because it is asymptomatic, and the majority of CHD patients are unaware that they are at risk. Subjects with proven risk factors for premature CHD/AMI or familial hypercholesteraemic should be prioritized for prompt intervention and therapy.

CHAPTER EIGHT

Recommendations

8.1 Application of the optimal model for predicting CHD in the general population by incorporating CAC marker to the CRFs basic model, this model can predict CHD risk in different populations including Hungarians (Roma, general). Clinicians should use CAC biomarkers in addition to the lipid levels and comorbidities to predict CHDAMI risk in suspected patients.

8.2 The Hungarian Roma population should improve their modifiable risk factors by adopting a healthy diet in order to increase the lower HDL-C, lowering their cholesterol levels (LDL-C), controlling HTN, DM and stopping smoking. To avoid complications and CHDAMI development, hypertensive patients should consult their doctor on a regular schedule, physical activity can help in lowering the blood pressure, and increasing insulin sensitivity, and lowering the LDL-C.

8.3 Targeted screening to identify members of the Roma and general Hungarian populations who have a family history of familial hypercholesteremic disease or CHD/AMI in order to attempt lifestyle modification or medication as a preventive measure.

8.4 Validation of this models in a large group of Hungarian populations will help in confirming the results and provide more information about CHDAMI risk.

Activate health education programs for Roma communities to improve their knowledge about CHD/AMI risk.

CHAPTER NINE

List of Abbreviations

AA	African American
A1	Minor allele code
A2	Major allele code
AIDS	Acquired Immunodeficiency Syndrome
AMI	Acute Myocardial Infarction
ARIC	Atherosclerosis Risk In Communities
AS	Asian
ATP	Adult Treatment Panel
AUC	Area Under the Receiver Operating Characteristics
BMI	Body Mass Index
BP	Blood Pressure
CAC	Coronary Artery Calcification
CALIB	Calibration
CARDIA	Coronary Artery Risk Development In Young Adults
CCA	Common Carotid Artery
CHD	Coronary Heart Disease
CHD/AMI	Coronary Heart Disease and Acute Myocardial Infarction
CHR	Chromosome
CHS	Cardiovascular Health Study
CKD	Chronic Kidney Disease
CLASS	Classification
CRFs	Conventional Risk Factors
CRP	C-Reactive Protein
CVD	Cardiovascular Diseases
DALYs	Disability Adjusted Life Years
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
E	Ethnicity
EBCT	Electron-Beam Computed Tomography
ECG	Electrocardiography
E (HET)	Expected heterozygosity
EUR	European
ESC	European Society of Cardiology
FH	Family History
FRS	Framingham
GENO	Genotype counts
GRSs	Genetic Risk Scores
GRS	Unweighted Genetic Risk Score
GWAS	Genome Wide Association Studies
HBV	Hepatitis B Virus
HDL-C	High Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Viruses

HTC-Med	High total cholesterol level/or taking cholesterol-lowering therapy
HTN	Hypertension
HTN-Med	Hypertensive Medication
hs Troponin	high-sensitivity Troponin
HWE	Hardy-Weinberg Equilibrium
ICD	International Classification of Diseases
IDI	Integrated Discrimination Improvement
JNC-V	Joint National Committee
LAT	Latinos
LD	Linkage Disequilibrium
LDL-C	Low Density Lipoprotein Cholesterol
LROC	Logit ROC measures
MetS	Metabolic Syndrome
MI	Myocardial Infarction
NCEP	National Cholesterol Education Program
NRI	Net Reclassification Improvement
OA	Another Allele
O(HET)	Observed Heterozygosity
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
RA	Risk Allele
RA(E/O)	Expected to Observed ratios (corrected to uncorrected estimate ratios)
ROC	Receiver Operating Characteristics
SBP	Systolic Blood Pressure
SCORE	Systematic Coronary Risk Evaluation
SD	Standard Deviation
SENS	Sensitivity
SNPs	Single Nucleotide Polymorphisms
SNPs ID	SNPs identifier
SPEC	Specificity
TEST	Pearson's $\chi 2$ goodness-of-fit test
TC	Total Cholesterol
TG	Triglyceride
WC	Waist Circumference
WHO	World Health Organization
wGRS	Weighted Genetic Risk Score
YLL	Years of Life Lost.

CHAPTER TEN

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Candidate: Nayla Mohamed Gomaa Nasr
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List of publications related to the dissertation

1. **Nasr, N. M. G.**, Soltész, B., Sándor, J., Ádány, R., Fiatal, S.: Comparison of Genetic Susceptibility to Coronary Heart Disease in the Hungarian Populations: Risk Prediction Models for Coronary Heart Disease.
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2. **Nasr, N. M. G.**, Soltész, B., Sándor, J., Ádány, R., Fiatal, S.: Prognostic Modelling Studies of Coronary Heart Disease: A Systematic Review of Conventional and Genetic Risk Factor Studies.
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List of other publications

3. Dahlia, D., Artanti, K. D., Hargono, A., Martini, S., **Nasr, N. M. G.**, Li, C. Y.: Death risk among COVID-19 patients with diabetes mellitus.
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DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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CHAPTER ELEVEN

Keywords

Coronary heart disease, Systematic review, Prognostic modelling studies, Genetic risk factors, Conventional risk factors, Developmental models, Validation models, Coronary artery calcification, SCORE, Hungarian Populations, Discrimination, Calibration.

CHAPTER TWELVE

Thesis Appendix

Table 12. 1 PICOTS elements of the articles reviewed.

No	Authors	Countries	Population (Free from CHD)	Sample	Intervention and Comparator	Outcome (Incident of CHD)	Times Follow-Up	Study Design
1	Iribarren et al., 2016 [128].	Spanain	GERA, white non-Hispanic	51,954	GRS + CRFs and CRFs	1864; 1077 males/787 females	5.9	Cohort
2	Hughes et al., 2012 [129].	UK	MORGAM, Caucasian	4818	GRS + CRFs and CRFs	1736; 632 cases, 1361 non-cases	18	Case-cohort
3	Talmud et al., 2008 [130].	UK	NPHSII, Caucasian	2742 Male	GRS + CRFs and CRFs	270 males	15	Cohort
4	Humphries et al., 2006 [131].	UK	NPHSII, Caucasian	2057 Male	GRS + CRFs and CRFs	183 males	10.8	Cohort
5	Beaney et al., 2017 [132].	UK	NPHSII, Caucasian	2075 Male	GRS + CRFs and CRFs	284 males	13.5	Cohort
6	Antiochos et al., 2016 [133].	Switzerland	Colaus, Caucasian	4283	GRS + CRFs and CRFs		5.6	Cohort
7	Brautbar et al., 2012 [134].	USA	Non-Hispanic white	1.8542 2.2068 3.2339	GRS + CRFs and CRFs in 1. ARIC, 2.Rotterdam, 3. FRS	1.1110 2.2068 3.2339	1.18 2.10 3.10	Cohort
8	Chien et al., 2007 [135].	China	Chin-Shan	3568	Derived, CRF-based	122(79 males/ 43 females)	13.6	Cohort
9	Simmons et al., 2008 [136].	UK	Norfolk	10,295	Derived, CRF-based	430 males, 250 females	8.5	Cohort
10	Macleod et al., 2006 [137].	UK	Scottish men	5191	Derived, CRF-based	203 deaths, 200 hospitalized	15	Cohort
11	Nishimura et al., 2014 [94].	Japan	Japanese urban (Suita)	5521	Derived, CRF-based	213	11.8	Cohort
12	Ingelsson et al., 2007 [138].	Boston	Framingham, Massachusetts	3322	Derived, CRF-based	291,198 males	15	Cohort
13	Cao et al., 2017 [139].	USA	MESA	4679	Derived, CRF-based	150 MI, 24 resuscitated cardiac arrest, 70 deaths, and	12.5	Cohort

						132 definite anginas		
14	Nambi et al., 2009 [127].	Houston	ARIC	13145	Derived, CRF-based	1812	15.1	Cohort
15	Cushman et al., 2005 [126].au	Boston	CHD	3971	Derived, CRF-based	547 MI or deaths	10	Cohort
16	Cooper et al., 2005 [140].	UK	NPHSII, Caucasian	3052 Male	Derived, CRF-based on PROCAM	110 males (PROCAM), and 109 males (Framingham)	10.8	Cohort
17	Orford et al., 2002 [141].	Boston	Normative aging study	1393	Derived, CRF-based	206 CHD	10	Cohort
18	Jee et al., 2014 [142].	Korea	Korean Heart Study	268,315	Derived, CRFs (Framingham)	2596 (1903 nonfatal, 693 fatal)	11.6	Cohort
19	Merry et al., 2011 [143].	Netherlands	Dutch CAREMA	21,148	Derived, CRFs (SCORE)	783	10.9	Cohort
20	Khalili et al., 2011 [144].	Iran	Urban	5101	Derived, CRFs	387 (169 Females)	9.3	Cohort
21	Auer et al., 2012 [125].	Switzerland	Health ABC study	2192	Derived, CRFs Framingham	351 (96 CHD, 101 MIs, 154 anginas)	8	Cohort
22	Taylor et al., 2001 [145].	USA	US Army	630	Derived, CRFs Framingham	No information	5	Cohort
23	Rana et al., 2009 [124].	UK	EPIC Norfolk	25,663	Derived, CRFs	No information	6	Nested case-control
24	Parikh et al., 2016 [146].	USA	Women's health initiative	27,982	Derived, CRFs	4607	12	Cohort
25	De Vries et al., 2015 [147].	Netherlands	Rotterdam, Ommoorod	5899	GRS + CRFs and CRFs	904 (460 MIs)	10	Cohort
26	Paynter et al., 2011 [148].	Boston	ARIC	12,834	Derived, CRFs	No information	3	Cohort
27	Morrison et al.,2007 [149].	Houston	ARIC	15,792	GRS + CRFs and CRFs	1452	13	Cohort
28	Folsom et al., 2006 [150].	USA	ARIC	15,792	Derived, CRFs	No information	5	Nested case-control
29	Detrano et al., 1999 [151].	USA	South Bay Heart	1196	Derived, CRFs	17 deaths and 29 nonfatal MIs, 4 fatal	3.5	Cohort
30	Tada et al., 2016 [152].	Sweden	Malmo diet and cancer	23,595	GRS + CRFs and CRFs	2213	14.4	Cohort

31	Aekplakorn et al., 2007 [153].	Thailand	Electricity employees	2536	Derived, CRFs	66	17	Cohort
32	Bolton et al., 2013 [154].	Scotland	Edinburgh artery study	840	GRS + CRFs and CRFs	319	15	Cohort
33	Lloyd et al., 2004 [155].	USA	Framingham	6216	Derived, CRFs	93 CHD and 1363 died free of CHD.	10	Cohort
34	Empana et al., 2003 [156].	France	PRIME, Men (Belfast, and France)	1.2399 2.7359	CRFs (Framingham, and PROCAM)		5	Cohort
35	Rodondi et al., 2012 [157].	USA	Health ABC study	2193	CRFs (Framingham older and recalibrated)	351	8	Cohort
36	McGeechan et al., 2008 [158].	USA	ARIC	9155	Derived, CRFs	700	8.8	Cohort
37	Onat et al., 1997 [159].	Turkey	TARF study	2259	Derived, CRFs	55 deaths, 69 nonfatal coronaries	5	Cohort
38	Wilson et al., 1998 [27].	USA	Framingham	5345	Derived, CRFs	383 males and 227 females	12	Cohort
39	Mainous et al., 2008 [160].	USA	ARIC	9307	Derived, CRFs	299 males and 131 females	6	Cohort
40	Pyorala et al., 1998 [161].	Finland	Helsinki policemen	970	Derived, CRFs	164 males, major CHD event	22	Cohort
41	Marshal et al., 1994 [162].	USA	Angiographically controlled study	848	GRS + CRFs and CRFs	No information	3.2	Cohort
42	Bye et al., 2016 [163].	Norway	HUNT studies	212	miRS + CRFs and CRFs	No information	10	Nested case-control
43	Kavousi et al., 2012 [164].	Netherlands	Rotterdam	5933	Derived, CRFs	347: (190 nonfatal MIs, and 157 CHD deaths)	6.8	Cohort
44	Ganna et al., 2013 [163].	Sweden	Swedish	10,612	GRS + CRFs and CRFs	781	4.3	Cohort
45	Cooper et al., 2000 [164].	UK	NPHS-II	928	Derived, CRFs	104: (71 acute CHD events, 15 anginas, 18 new major Q waves)	7.8	Nested case-control
46	Arima et al., 2007 [123].	Japan	Japanese	2589	Derived, CRFs	129	14	Cohort

47	Brautbar et al., 2009 [167].	USA	ARIC	9998	9p21 + CRFs and CRFs	1349	13.5	Cohort
48	St-Pierre et al., 2006 [168].	Canada	Quebec cardiovascular study men	2072	Derived, CRFs	230 deaths or nonfatal MIs	13	Cohort
49	Ryoo et al., 2016 [169].	Korea	Korean men	23,918	Derived, CRFs	5763	5	Cohort
50	Yarnell et al., 2004 [170].	UK	Caerphilly collaborative study	4860	Derived, CRFs	525	10	Cohort
51	Everage et al., 2009 [171].	USA	CARDIA	1362	Derived, CRFs	No information.	15	Cohort
52	Iribarren et al., 2015 [172].	USA	ADVANCE	1135	Derived, CRFs	164	11.3	Cohort
53	McClelland et al., 2015 [173].	USA	1. MESA 2. Heinz 3. Dallas	1.6726 2.3692 3.1080	Derived, CRFs	1.422 2.274 3.58	1.10.2 2.10.4 3.9.3	Cohort
54	Liu et al., 2004 [174].	China	1. CMCS 2. Framingham	1.30121 2.5251	Derived, CRFs	1.192, and 273 Hard 2.625, and 293 deaths.	12	Cohort
55	Brant et al., 2010 [175].	USA	1. USA (BLSA) 2. Europe (VHD and PP)	1.1966 2.150667	Derived, CRFs	2457	7.5	Cohort
56	Onat et al., 2010 [176].	Turkey	Turkish	2232	Derived, CRFs	302	7.6	Case-cohort
57	Cross et al., 2012 [177].	USA	1. CHDRA 2. MESA	1.1084 2.6814	Derived, CRFs	179	5, and 5	Case-cohort
58	Hadaegh et al., 2012 [178].	Iran	Tehran lipid and glucose study	2568	Derived, CRFs	127	9.3	Cohort
59	Kang et al., 2012 [179].	Korea	The third Korean national survey	5271	Derived, CRFs	100	13.7	Cohort
60	Kivimaki et al., 2011 [180].	UK	Whitehall	5533	Derived, CRFs	160 deaths and nonfatal MIs	11.3	Cohort
61	Gander et. al., 2015 [179].	USA	Aerobic centre	29,854	Derived, CRFs	499	12	Cohort
62	Arad et al., 2004 [181].	USA	Francis heart study	4613	Derived, CRFs	119	4.3	Cohort
63	Pischon et al., 2005 [183].	USA	Health professionals' study	18,225	Derived, CRFs	266	6	Nested case-control
64	Polak et al., 2015 [184].	USA	MESA	6606	Derived, CRFs	484: (209) angina	11.2	Cohort

65	Cavus et al., 2019 [185].	Germany	BiomarCaRE	10,741	Derived, CRFs	2166	9.2	Case-cohort
66	Subirana et al., 2018 [186].	Spain	REGICOR	638	Derived, CRFs	105	6.1	Case-cohort
67	Hindy et al., 2018 [187].	Sweden	Malmö diet and cancer	24,443	GRS + CRFs and CRFs	3217	19.4	Cohort
68	Chien et al., 2018 [188].	Taiwan	Taiwanese	3559	Derived, CRFs	63	9.7	Cohort
69	Iribarren et al., 2018 [189].	USA	GERA	11,242	Derived, CRFs	450	8.7	Meta-analysis.
70	Can et al., 2019 [190].	Turkey	TARF	3203	Derived, CRFs	573	9.93	Cohort
71	Wang et al., 2019 [191].	USA	ARIC	3598	Derived, CRFs	633	30	Cohort
72	Thomsen et al., 2002 [192].	Denmark	Danish	1.4757 2.2562	Derived, CRFs	1.311 2.113	10	Cohort

Table 12. 2 Description of the study populations, settings, locations, periods of recruitment, length of follow-up and method of data collection of the reviewed models.

No	Populations
1	The Genetic Epidemiology Resource in Adult Health and Aging (GERA) is a cohort of (males and females) free of CHD at baseline (2007-2008), chosen at random from Kaiser Permanente of Northern California, 51,954. White non-Hispanic participants aged 30 to 74 years old were tracked for a maximum of 5.9 years. All participants completed a self-administered questionnaire that included medical history, ancestry, and health behaviors.[128]
2	The MORGAM case-cohort study included (4,818) healthy men at baseline, white Caucasian, randomly selected from nine prospective European cohorts, aged 25-64 years, followed up for a median of 18 years, participants used to examine the associations between CHD and risk scores based on genetic variants representing 13 genomic regions, baseline examination of this cohort took place in 1997.[129]
3	The second Northwick Park heart study (NPHSII) is a cohort of 2,742 white Caucasian, middle-aged men (50-64 years) recruited (2005) from nine medical practices in the United Kingdom. A questionnaire was used to assess family history of CHD. Participants with genetic data were tracked for 15 years for the occurrence of CHD.[130]
4	This study comprised 2,057 participants from the second Northwick Park heart study (NPHSII), middle-aged males (50-64 years), Caucasian men with complete trait data (and full genotype data), recruited in 2005.[131]
5	The QRISK2 and NPHSII prospective studies were compared. The QRISK2 was used to assess CHD risk using conventional risk factors (CRFs), whereas the Northwick Park Heart Study (NPHSII) was utilized to evaluate the performance of a 19 single nucleotide polymorphism (SNP) gene score (GS) for CHD. The NPHSII trial included 2,775 healthy UK men aged (50-64) recruited from nine general practices over a period of 13.5 years.[132]
6	The CoLaus study is an ongoing prospective survey that investigates the biological and genetic determinants of cardiovascular disease in the Lausanne, Switzerland, population. The study relies on personal interviews, physical examinations, and laboratory testing, and the baseline investigation was conducted in 2003-2006, with a first follow-up in 2009-2012.[133]
7	Three separate prospective studies (ARIC, Rotterdam, and Framingham Offspring) were compared. In total, 8,542 ARIC participants, aged 45-64 years, non-Hispanic whites followed for a maximum of 18 years, 2,068 Rotterdam; all inhabitants of Ommoord, a district of Rotterdam in the Netherlands, aged under 65 years information from baseline (1990-1993) until January 1, 2007, and 2,339 Framingham Offspring participants, aged 45-64 years, followed for a maximum of 18 years, 2,068 Rotterdam; and 2,339 Framingham Offspring participants, aged Studies that were free of CHD at the outset and had genetic data were followed from study admission at exam 1 (1971-1975) for the first occurrence of CHD (incident CHD), with data obtained using a questionnaire and a clinical examination.[134]
8	Chin-Shan Community Cardiovascular Cohort Study of 3,568 (males and females), homogeneous selected from Chinese ethnicity, living in the Chin-Shan township, aged 35 years, with blood lipid data and free from CVD, 13.6 years' follow-up, the baseline study was carried out between 1990 and 2005.[135]
9	EPIC-Norfolk is a prospective cohort research in which 10,295 males and females aged 40-79 years were recruited from general practices in the Norfolk region of England between 1993 and 1998, with an 8.5-year follow-up.[136]
10	This study is based on 5,191 men who were recruited between 1970 and 1973 from 27 workplaces in the west of Scotland, aged 35-64, with a 15-year follow-up.[137]
11	A total of 5,521 Suita study participants, a Japanese urban population of males and females, aged 30-79 years, free of CHD at baseline in 1989-2004, were compared with the original Framingham Score over a median follow-up duration of 11.8 years.[94]

12	The Framingham Offspring Study included 3,322 participants, aged 30-74 years, from the city of Framingham, Massachusetts (males and females), who underwent a routine medical history, a physical examination that included blood pressure measurement and anthropometry, and blood sampling during the fourth examination cycle (1987-1991).[138]
13	The study population was drawn from the Multi-Ethnic Study of Atherosclerosis (MESA), with (4,679) males and females, Caucasian, African-American, Hispanic, or Chinese-American participants, aged 45 to 84 years and free of clinically apparent CVD recruited between 2000 and 2002 from (6) U.S. communities, and 12.5 years of follow-up.[139]
14	A total of 13,145 people were followed for the development of clinical CHD in the Atherosclerosis Risk in Communities (ARIC) cohort study from the United States, aged 45-64 years, Caucasian non-Hispanic whites followed for a maximum of 15.1 years, recruitment year 1987-1989.[127]
15	3,971 Boston males and females, blacks and whites, participated in the Cardiovascular Health Study (CHS). Participants were invited and chosen at random, and were recruited between 1989-1990 or 1992-1993, with a 10-year follow-up.[126]
16	The Second Northwick Park Heart Study (NPHS-II) included 3,052 healthy Caucasian UK men aged 50 to 64 who were monitored for a median of 10.8 years for CHD events. The study examined the PROCAM and Framingham risk algorithms' ability to predict CHD occurrences in the NPHS-II group, which was recruited in 2004.[140]
17	The Veterans Administration established the prospective study of aging known as the Normative Aging Study in 1961. It included 1,393 white males from a single geographic area (Boston, Massachusetts), aged 30-74, and had a 10-year follow-up.[141]
18	Korean Heart Study (KHS) population, 26,8315 people (males and females), non-randomly selected, who voluntarily underwent private health check-ups in 18 sites located in South Korea's capital and six provinces between 1996 and 2004, aged 70-79 years, 11.6 years' follow-up.[142]
19	The Dutch Cardiovascular Registry Maastricht (CAREMA) study population of the Netherlands, 21,148 participants (males and females) were recruited at random from the Maastricht region in 1987-1997, aged 20-59 years, with a 10.9-year follow-up.[143]
20	A total of 5,101 participants were recruited from the Tehran Lipid and Glucose Study, a prospective population-based study conducted on a representative sample of district-13 of Tehran (Iranian urban population males and females), aged 30 and free of CHD at baseline, between 1999 and 2001, with a 9.3-year follow-up.[144]
21	Participants were from the Health, Aging, and Body Composition research (Health ABC Study), a population-based cohort research of 2,192 community-dwelling males and females aged 70-79 years recruited in 1997-1998 and followed for an average of 8 years.[125]
22	630 active-duty US Army men stationed within the National Capital Area of the Walter Reed Health Care System, aged 39-45 years, free of CHD, 5 years follow-up, recruited in 1998-1999, were included.[145]
23	25,663 European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study participants, residents of Norfolk, UK, who completed a baseline questionnaire survey and attended a clinic visit were randomly selected to participate in a nested case-control study (males and females), aged 45-79 years, recruited in 1993-1997, 6 years' follow-up.[124]
24	The Women's Health Initiative (WHI) research had 72,982 individuals in the prospective cohort analysis: the median follow-up was 12 years, they were free of CHD, and they were recruited in 1991-1993.[146]
25	A total of 5,899 Rotterdam cohort study participants, aged ≥55 years, subjects were recruited to participate from Ommoord, a district of Rotterdam in the Netherlands. From 1990 to 1993, the baseline examination lasted 12.8 years of follow-up.[147]
26	The ARIC project is a prospective investigation of atherosclerosis, including 5,533 and 7,301 participants (males and females respectively) chosen from four towns in the United States, aged 45-64 years, during a three-year period. Prior to the baseline, there was a period of follow-up and recruiting (1986-1989).[148]

27	The ARIC is a prospective investigation of atherosclerosis and its clinical sequelae, which included 15,792 US participants aged 45-64 years, recruited in (1986-1989), individuals were genotyped for 116 single nucleotide polymorphisms associated with CHD in multiple case-control studies, 1,452 CHD cases defined as participants with either definite or probable myocardial infarction, and 13,907 participants were followed for incident CHD for a median of 13 years between the baseline.[149]
28	A total of 15,792 participants from the Atherosclerosis Risk in Communities (ARIC) case-control studies, aged 45-64 years, were sampled from four U.S. communities in (1987-1989) to investigate the association of 19 new risk indicators with incident CHD.[150]
29	A total of 1,196 participants (males and females) were recruited from the South Bay Heart Watch prospective study, which was designed in 1990-1992. They received mail letters inviting them to participate in this study, and subjects who agreed to return for testing and be followed up for an additional 3 years were included. The participants' mean age was 66 years, 3.5 years. Follow-up.[151]
30	23,595 Malmo Diet and Cancer Study participants (prospective, population-based study), Malmo, Sweden citizens, males and females invited to join, for a median follow-up of 14.4 years, recruitment in 1991-1996, aged 46-73 for males and 45-73 for females.[152]
31	A total of 2,536 male employees from Thailand's Electricity Generating Authority participated in this cohort study, which covered 17 years of follow-up with participants aged 35-59 years at baseline.[153]
32	The Edinburgh Artery Study (EAS) is a prospective study from Edinburgh, Scotland, in which 840 males and females were randomly selected to see if a panel of SNPs systematically selected from genome-wide association studies (GWAS) could improve risk prediction of coronary heart disease (CHD), participants aged 54-75, with a 15-year follow-up. Clinical evaluations were conducted between 1987 and 1988, and comprehensive follow-up was provided until June 2003.[154]
33	The Framingham Heart Study (Massachusetts, USA) included 6,216 participants (males and females). All subjects were examined from 1971 to 1996 who were free of CHD, stratified into age and gender-specific tertiles of Framingham risk score, and lifetime risk for CHD was estimated, aged 40-94, 10 years follow-up.[155]
34	PRIME stands for Prospective Epidemiological Study of Myocardial Infarction, and it was recruited in centers in Belfast (Northern Ireland), Lille, Strasbourg, and Toulouse (France), as well as a Coordinating Centre in Paris. This study was compared with the Framingham and PROCAM risk functions to see if they were applicable to men in Belfast and France. In general, the PRIME trial included men recruited in Belfast (2,399) and France (7,359) who were 50-59 years old, free of CHD at baseline (1991-1993), and tracked for CHD occurrences over a 5-year period. Two validation studies of the original Framingham and Framingham Offspring cohorts consisted of 2,489 men aged 30-74 years who were free of any cardiovascular disease at the time of their examination from 1971-1974 within 10 years and PROCAM risk function study estimates the risk function of CHD and myocardial infraction within 10 years, it was developed from a sample of men, It consisted of 5,389 men aged 35-65 years who were free of any cardiovascular disease at baseline bet.[156]
35	The Health, Aging, and Body Composition Study (Health ABC Study) included 2,193 males and females from a community-dwelling population aged 70-79 during the study enrolment period in 1997-1998. Participants were chosen at random from a white and all black population, with an 8-year follow-up.[157]
36	A total of 9,155 participants (males and females) without diabetes were chosen from the Atherosclerosis Risk in Communities (ARIC), 4 US communities, to investigate whether quantitative retinal vascular caliber is associated with an increased risk of incident coronary heart disease, aged 45-64 years, enrolment period 1987-1989, and a mean of 8.8 years of follow up.[158]
37	A total of 2,259 subjects (males and females) from the Turkish middle-aged adult population (TARF study) took part in this study; the study is based on a longitudinal follow-up of a survey conducted initially in 1990 in all geographic regions of Turkey; they were chosen at random, aged 35-84, between 1990 and 1995, with a 5-year follow-up.[159]

38	The Framingham Cohort Studies included 2,489 males and 2,856 females aged 30-74 at baseline, with a 12-year follow-up in 1971-1974. Participants took part in either the 11th Framingham cohort test or the first Framingham Offspring Study examination.[27]
39	The Framingham risk score was generated for members in the ARIC cohort, 3,901 malesand 5,406 females aged 45-64 years old at enrolment (1986-1989 through 2001), at baseline (visit 3) and (3 - 6) years before.[160]
40	This study is based on a cohort of 970 males aged 34 to 64 who were free of CHD, other cardiovascular disease, and diabetes during the second examination of the Helsinki Policemen Study in 1971-1972, with a 22-year follow-up.[161]
41	848 males (30-64) and females (35-69) years old from Utah, USA (Caucasian living) were included to assess the distribution of apolipoprotien genetic polymorphism (n=12), free of myocardial infraction illness. Subjects having unstable angina and a myocardial infarction during the previous 6 months were eliminated, with a 10-year follow-up.[162]
42	The HUNT studies were conducted in three waves in Nord-Trndelag County, Norway, HUNT1 (1984-1986), HUNT2 (1995-1997), and HUNT3 (2006-2008), with participants attending HUNT2 included in a prospective nested case-control design to assess the utility of circulating microRNAs (miRs) to predict future fatal acute myocardial infarction (AMI) in healthy participants as endpoint (I21, ICD-10), 112 cases Participants aged 40-70 years in the derivation cohort and 100 apparently healthy males (n = 56) and females (n = 44) in a separate validation cohort with a 10-year follow-up.[163]
43	The study was embedded within the Rotterdam Study, a prospective population-based cohort of people aged 55 or older in Rotterdam, the Netherlands, to see if newer risk markers for CHD risk prediction and stratification improve Framingham risk score (FRS) predictions, 5,933 participants (males and females), recruitment in (1997-1999) and (2000-2001), 6.8 years follow-up.[164]
44	The study included 10,612 Swedish participants (males and females) from five separate sub-studies with DNA and data on cardiovascular risk factors that were conducted within the registry (SATSA, OCTO-Twin, GENDER, HARMONY, and TwinGene). The study aimed to compare several multilocus genetic risk scores (MGRS) and CHD in 1886 -2000 &1920 -1924, aged 44-80 years, with a 4.3 year follow-up.[165]
45	The Second Northwick Park Heart Study (NPHS-II) included 11,153 healthy UK men aged (50-61 years) who were free of myocardial infarction at recruitment in 1989 and were monitored for a median of 7.8 years for CHD occurrences.[166]
46	2,589 Hisayama study participants (males and females) aged 40 years or older were participated in this study from 1988 to 2002, with a 14-year follow-up.[123]
47	9,998 participants in the Atherosclerosis Risk in Communities (ARIC) cohort study from the United States, Whites (males and females), for whom the 9p21 genotype and conventional risk factor information was available, aged 45-64, recruited in (1987-1989).[167]
48	A total of 2,072 healthy Canadian men from the Québec Cardiovascular Study were enrolled, free of CHD at the time of enrolment and followed for 13 years, aged 35-64 years from 1990-1991, and in 1998, participants were contacted by mail and requested to participate in this study.[168]
49	23,918 healthy Korean men were followed until 2010 in a cohort made up of participants who had visited the Health Promotion Centre at Kangbuk Samsung Hospital for a medical check-up in 2005, were over the age of 30, and had been followed for 5 years.[168]
50	Two UK populations, Caerphilly and Speedwell, from two neighbouring health centres, comprising 4,860 men, were tested for indications of IHD between 1979 and 1983. Over a ten-year period, men were monitored and validated coronary events were documented.[170]
51	The Coronary Artery Risk Development in Young Adults (CARDIA) research recruited participants in four U.S. cities. It is a prospective cohort research in which 571 American Black males and 791 females aged 33-45 years were recruited in (1985-1986), with a 15-year follow-up period.[171]

52	A total of 1,135 healthy males and females, aged 33 to 69 years, were selected as participants in the ADVANCE (Atherosclerotic Disease, VAscular FunctioN, and GenetiC Epidemiology) study at Kaiser Permanente Northern California and Stanford University. All participants in this study were free of CVD at baseline and attended comprehensive baseline clinic visits in 2002-2003, with an 11.3-year follow-up.[172]
53	The Multi-Ethnic Study of Atherosclerosis (MESA), the Heinz Nixdorf Recall Study (HNR), and the Dallas Heart Study (DHS) were all compared. In total, 6,726 MESA participants (males and females), aged 45-84, who identified as white, African-American, Hispanic, or Chinese were recruited from six U.S. communities from 2000 to 2002, they were free of clinical heart disease at baseline and followed for ten years, the study validated in two independent longitudinal cohort studies included, the Heinz Nixdorf Recall Study, 3,692 Caucasians, participants from three neighbouring communities. The Dallas Heart Study is a multi-ethnic, population-based random sample of Dallas County, Texas, with 1,080 participants aged 45-65, Caucasians, African Americans, and Hispanics, who were followed for a median of 9.3 years from 2000 to 2002.[173]
54	At baseline, the CMCS cohort contained 30 121 Chinese individuals aged 35-64 years. From 1992 to 2002, participants (males and females) were recruited from 11 provinces and followed up on for new CHD occurrences. The Framingham Heart Study included 5,251 white US residents of Framingham, Massachusetts, who were 30-74 years old at baseline in 1971-1974 and were followed up on for 12 years.[174]
55	The Baltimore Longitudinal Study of Aging (BLSA) recruited 152,633 people (males and females) from two community-dwelling cohort studies in the United States and Europe. people were Caucasian, healthy, well-educated, middle- to upper-middle-class, aged 30-74, with a mean follow-up of 7.5 years.[175]
56	The TARF Study is a 7.6-year longitudinal population-based cohort study (Turkey), with 2,232 individuals (males and females), 30-74 middle-aged adults free of CHD at baseline, recruited from randomly selected towns in 2002-2003.[176]
57	PMRP and MESA, 1,084 participants (males and females), initially CHD-free, randomly selected from Marshfield Clinic Personalized Medicine Research Project (PMRP), a population-based sample repository collected, aged 45-84, recruited in 2002-2004, 5 year follow-up, and Multi-Ethnic Study of Atherosclerosis (MESA), 623 participants (males and females), aged 40-80, self-identified as White, African-American, Hispanic, or Chinese, 5.4 year follow-up.[177]
58	The Tehran Lipid and Glucose Study (TLGS) is a prospective ongoing study aimed at determining risk variables. The study population comprised of 2,568 females aged 30 years, free of CHD symptoms at study entry 1999-2001, with a median follow-up of 9.3 years.[178]
59	This study comprised 5,271 healthy Koreans (males and females) from the third Korea National Health and Nutrition Examination Survey, aged 20-78, recruited in 2005, and followed for 13.7 years.[179]
60	5,533 adults (men and women) from the prospective Whitehall II cohort study in the United Kingdom were chosen using a stratified, multistage probability sampling design to see if adding information on job strain to the Framingham model improves its predictive power in a low-risk working population, aged 35-55, adults were ascertained in Phases 1 (1985-88), 2 (1989-90), and 3 (1991-93), who were CHD free at baseline, 11.3 year follow-up.[180]
61	A total of 29,854 males from the Aerobics Centre Longitudinal Study were enrolled to assess the association of cardiorespiratory fitness (CRF) with risk of coronary heart disease, aged 30-74 years, with a 12-year follow-up.[181]
62	The St. Francis Heart Study, New York population, USA, included 4,903 people (males and females) aged 50-70 years with no history, symptoms, or indicators of ASCVD between July 1996 and March 1999, with a 4.3 year follow-up.[182]
63	18,225 participants in the Health Professionals Follow-up Study were chosen at random to participate in a nested case-control study among males aged 40 to 75 years who were free of documented cardiovascular disease at the time of blood collection and were followed for 6 years between 1993 and 1995.[183]
64	Whites, African Americans, Hispanics, and Chinese participants in the Multi-Ethnic Study of Atherosclerosis (MESA), aged 45-84 years, 11.2 years of follow-up, recruiting in 2000-2002.[184]

65	This study included 10,741 individuals (males and females) from 6 European cohorts to evaluate the association between circulating (141) metabolites and incident CHD. They were randomly selected to participate in a case-cohort study, had no history of myocardial infarction, stroke, heart failure, or atrial fibrillation, and had 9.2 years of follow-up.[185]
66	A total of 743 REGICOR cohort study participants, aged 35-74 years, with 6.1 years of follow-up, were included.[186]
67	The MDCS (Malmö Diet and Cancer Study) recruited 24,443 participants from southern Sweden to study if the genetic prediction of CHD varied depending on smoking behavior, with a 19.4-year follow-up.[187]
68	In 2002, 3,559 males and females from Taiwan's 2002 Triple High Survey were chosen at random for a 9.7-year follow-up.[188]
69	A total of 11,242 people from the GERA cohort study were randomly selected to investigate the clinical utility of two previously validated multi-locus genetic risk scores (GRSs) in Europeans aged 30-79 years, with 8.7 years of follow-up.[189]
70	3,203 people were chosen at random from the TARF cohort study of the Turkish population, with a 9.93-year follow-up and enrolment between 2002-2003 and 2007-2008.[190]
71	ARIC cohort research (Atherosclerosis Risk in Communities) included 3,598 people to detect CHD by variations in the blood metabolome, 30 years of follow-up, aged 45-64 years from four US communities, Metabolomics measurements in 1997, 2010, and 2014.[191]
72	Data from two population studies, Glostrup (n=4,757) and Framingham Heart studies (n=2,562), were used to assess three different levels of cross validation of a CHD risk score, participants (males and females), aged 30-70, free of myocardial infraction disease, and a ten-year follow-up.[192]

Table 12.3 Discrimination, calibration, and risk classification as described in the reviewed models.

No	Authors	Predictors (Genetics and Biomarkers)	Improvement of the Model's Performance		
			Discrimination	**Calibration (Goodness-of-Fit Test)**	**Risk Classification**
	Iribarren et al., 2016 [128].	FRS + 4 constructed GRSs; (GRS-8, GRS-12, GRS-36, and GRS-51) plus	ΔC-statistic: 1. GRS-8b = (0.008), 2. GRS-36 = (0.008), 3. GRS-12 = (0.007), 4. GRS-51 = (0.009), p = <0.001 for all models	Hosmer-Lemeshow chi-square >0.20 in all GRS models	NRI: (5%) GRS-8 and GRS-12 and GRS-36, and (4%) for GRS-51.
2	Hughes et al., 2012 [129].	2 GRS constructed; (GRS1) combined 11 SNPs + 2 haplotypes + FRS, GRS2 combined 11 SNPs plus 4 SNPs + FRS.	C index improvement 1.11%, p = 0.048, no significant discrimination improvement for GRS1 0.752, p = 0.11.	-	Yes (both score) NRI = 7.5%, p = 0.017 for GRS1, 6.5%, p = 0.044 for GRS2
3	Talmud et al., 2008 [130].	9p21.3 (rs10757274 + 10 models SNPs) + CRFs	AUC (rs10757274 + CRFs) = 0.64, p = 0.14. AUC for (CRFs + 10 models SNPs); addition of 1 SNP, p < 0.03, addition of 2 or more; p = < 0.001.	Hosmer-Lemeshow	Yes (event group to moderate risk 13.5% and 3.3%)
4	Humphries et al., 2006 [131].	12 genes + CRFs	AUC = 0.62, (0.58-0.66) [12.6% detection rate for a 5% false-positive rate (DR5)] (p = 0.001).	likelihood ratio	-
5	Beaney et al., 2017 [132].	GRS constructed; 19 SNP + QRISK2 compared to QRISK2 alone and 21 SNPs + QRISK2	AUC: 1. improvement for CRFs+19 SNPs = (0.68 vs. 0.70 p= 0.02), and CRFs+21 SNPs with no significant improvement in discrimination (p = 0.55)	1. 19 SNPs had good calibration (p= 0.17), and 21 SNPs were poorly calibrated (p = 0.03)	Yes; 19 SNPs, the NRI = (0.07, p=0.04), QRISK alone = (0.17), 21 SNPs no improvement in net reclassification (p= 0.10)
6	Antiochos et al., 2016 [133].	Parental history (PH) + GRS (153SNPs) + CRFs	(GRS + CRFs); C index improvement = 0.016, p = 0.048. GRS + CRFs + PH; C index improvement = 0.022, p = 0.006.	Hosmer-Lemeshow, 0.35 < p < 0.94	Yes, NRI was significant, IDI was significant
7	Brautbar et al., 2012 [134].	GRS (13SNPs) + CRFs in 3 groups	Yes, (CRFs + GRS); AUC Unweighted GRS: 1. ARIC: (0.742-0.749), 2. Rotterdam: (0.729-0.734), 3. Framingham: (0.773-0.775). Weighted GRS: 1. ARIC: (0.742 to 0.751), 2.	Grønnesby-Borgan for derived and based 1. ARIC: (p= 0.05), and (0.003). 2. Rotterdam: p > 0.4)	NRI: Unweighted GRS: 1. ARIC: 6.3%, 2. Rotterdam: 0.2%, 3. Framingham: 0.6% Weighted GRS: 1. ARIC: 7.3%,

			Rotterdam: (0.729-0.735), 3. Framingham: (0.773-0.784)	for both. Rottedam: $p > 0.4$) for both.	2. Rotterdam: 3.6%, 3. Framingham: 4.5%
8	Chien et al., 2007 [135].	CRFs + (ApoB,non-HDLC, LDLC)	Yes, AUC (ApoB = 0.63)	Hosmer-Lemeshow	-
9	Simmons et al., 2008 [136].	CRFs + glycated hemoglobin	AUC: 1. CRF-based = 0.72 for males, 0.80 for females. 2. new model = 0.73 and 0.80.	-	NRI was 3.4% male and 2.2 % female
10	Macleod et al., 2006 [137].	CRFs + psychosocial risk factors	AUC: 1 = 0.754, 2 = 0.745, 3 = 0.746, 4 = 0.746, 5 = 0.749	-	Recalibration coefficient based upon 20 iterations
11	Nishimura et al., 2014 [94].	CRFs + TC, LDLC + Suita with/without CKD	ROC for LDL Suita + CKD = (0.831), TC Suita + CKD = (0.835), Suita with/without CKD = (0.833 and 0.835)	Bayesian information criteria (BIC) and likelihood ratio.	NRI and IDI: markedly for TC (Suita) + CKD, NRI for TC Suita + CKD (46.8%, $p < 0.001$)
12	Ingelsson et al., 2007 [138].	CRFs + lipids (TC, LDLC, HDLC, ApoA, ApoB	C index of ApoA, ApoB model in both males and females (0.74, 0.76), $p > 0.70$	Likelihood ratio	Was not statistically significant
13	Cao et al., 2017 [139].	CRFs + ApoB (Roche, Kamiya, and Diazyme).	-	-	-
14	Nambi et al., 2009 [127].	CRFs + C-IMT + Plaque	AUC; CRFs = (0.674), others (0.690, and 0.686) in males, AUC = (0.759 to 0.762 and 0.770) females.	Grønnesby-Borgan, CRFs. Studyially good fits in females	NRI, clinical NRI, CRFs + CIMT + Plaque model was better
15	Cushman et al., 2005 [126].au	CRFs + CRP + CIMT	-	-	-
16	Cooper et al., 2005 [140].	CRFs + LDL, HDL (PROCAM, and FRS)	ROC = 0.6295 (PROCAM) and 0.6184 (FRS)	Hosmer-Lemeshow, 0.46 (PROCAM), and 0.47 (FRS) $p < 0.0001$ for both	-
17	Orford et al., 2002 [141].	CRFs (Framingham, and European)	C-statistic: 1. FRS (0.60), 2. EUR (0.58),	-	-
18	Jee et al., 2014 [142].	CRFs + LDL, HDL, TG (3 models)	AUC: 1. Male: (0.764, 0.758, and 0.757) 2. Female: (0.812, 0.809, and 0.815).	Hosmer-Lemeshow	NRI = (0.284, 0.185, and 0.109) in male, and (0.177, 0.160, and 0.207) in female.
19	Merry et al., 2011 [143].	CRFs (re-estimated SCORE) + Other factors (total, HDL) in 9 models	AUC: CAREMA (0.802), re-estimated SCORE high and low (0.789).	Calibration slope and intercept: CAREMA (1.00, 0.07%), re-estimated SCORE high and low (2.32,	NRI: CAREMA (28%), re-estimated SCORE high and low (28%), and (35%) respectively.

				3.29%), and (4.63, 4.19%).	
20	Khalili et al., 2011 [144].	CRFs + Psychosocial risk factors (Rose + ECG)	C-statistic: 1. Male (0.713) for Rose, and (0.717) for Rose + ECG, $p = 0.179$. 2. Female (0.770) for Rose, and (0.786) for Rose + ECG, $p = 0.09$.	-	Integrated discrimination improvement: 1. Male (4.01%) p (0.537). 2. Female (8.78%) p (0.309).
21	Auer et al., 2012 [125].	CRFs + ECG	C-index: 0.58	Hosmer-Lemeshow, calibration: $p = 0.01$, and $p = 0.03$	NRI: derived and based: 7.4% and 57%, IDI: 0.99% and 1.03%
22	Taylor et al., 2001 [145].	CRFs + CAC using EBCT	ROC = 0.62, $p < 0.001$, and 0.61, $p < 0.001$.	-	-
23	Rana et al., 2009 [124].	CRFs + CRP + myeloperoxidase in (8) models	C-statistic for derived and based = (0.65-0.54).	Hosmer-Lemeshow did not perform well.	NRI: derived and based on 27.4%, and 6.4%
24	Parikh et al., 2016 [146].	CRFs + reproductive factors	C-statistic for derived and based 1. Based (0.726) and derived (0.730)	-	IDI: 0.0013 ($p <$ 0.0001), and 0.002 ($p = 0.04$).
25	De Vries et al., 2015 [147].	GRS (152 SNPs) + CRFs in (3) models	C-statistic: 1.0.684, 2. 0.716, 3.0.716	-	NRI: derived and based: 1. 0.034-0.003, 2.0.01-0.022, 3.0.007-0.017
26	Paynter et al., 2011 [148].	CRFs + (SBP, HDL, and TC) in (3) models	AUC: 1. Male: 0.701, 0.704, and 0.704, 2. Female: 0.780, 0.785, and 0.785	Hosmer-Lemeshow, 1. Male: 12.4 (0.14), 12.3 (0.14), and 13.5 (0.10), 2. Female: 2.1 (0.98), 5.7 (0.68), 13.6 (0.09).	NRI: derived and based, 1. −1.1% (0.47), −0.2% (0.55), 2. 6.4% (0.008), 5.4 (0.016)
27	Morrison et al.,2007 [149].	GRS (116 SNPs) + CRFs.	AUC: 1. White (GRS + CRFs) = 0.766, and 0.764 for CRFs alone. 2. Black: (GRS + CRFs) = 0.769, and 0.758 for CRFs alone.	-	-
28	Folsom et al., 2006 [150].	CRFs + 19 novel risk markers included CRP	AUC: 1. Basic: (0.767-0.820) 2. Derived: (0.768-0.824)	-	-
29	Detrano et al., 1999 [151].	CRFs + EBCT Calcium score in (4) models	ROC: derived and based 1. Basic: 0.64 ± 0.05 2. FRS: 0.69 ± 0.05 3. Data derived: 0.68 ± 0.05 4. Derived + Ca: 0.71 ± 0.04	-	-
30	Tada et al., 2016 [152].	GRS (27, and 50 SNPs) + CRFs + FH in (4) models	C-statistic for derived and based (+GRS27, +GRS50, and +FH): 1. Established risk factors:	-	NRI: derived and based: 1. Established risk factors: 0.20% ($p <$

			(0.746, 0.748, 0.749, and 0.749) respectively; 2. Established risk factors + FH: (0.749, 0.751, 0.752 and NA), respectively.		0.001); 2. Established risk factors + FH + 27GRS = 0.15% (p < 0.001), 3. Established risk factors + FH + 50GRS = 0.17%, p < 0.0001
31	Aekplakorn et al., 2007 [153].	CRFs + BMI + WC, and waist-to-hip ratio, wais-to-height ratio in (4) models	AUC: 1.0.606, 2.0.627, 3.0.592, 4.0.651	-	-
32	Bolton et al., 2013 [154].	GRS (27 SNPs) + CRFs, (4) models for SNPs identified in GWAS and (2) models for SNPs identified in regression trees.	C-index: SNPs identified from (GWAS, and regression trees): (a) GWAS: 1. CHD: 0.761, 0.740, 0.671 and 0.741; 2. Fatal or nonfatal MI or coronary intervention: 0.717, 0.750, 0.718, and 0.753. (b) Regression trees: 1. CHD: 0.686 and 0.709 2. Fatal or nonfatal MI or coronary intervention: 0.694 and 0.718.	-	NRI plus IDI: (a) GWAS: 1. CHD: NRI = 54.4 for both models, IDI = 0.04 for both models, 2. Fatal or nonfatal MI or coronary intervention: NRI = 43.5 and 42.7, IDI = 0.05 for both models, (b) Regression trees: 1. CHD: NRI = 41.5, IDI = 0.04, 2. Fatal or nonfatal MI or coronary intervention: NRI = 42.9, IDI = 0.03
33	Lloyd et al., 2004 [155].	CRFs (Framingham) stratified into age and gender tertiles	Specific index ages: (40-80) per tertiles: 1. Males %: (31.2-16.1), (33.6-14.4), and (46.8-34.2); 2. Females %: (9.7 -11.), (15.1-19.6), and (25.9-24.9).	-	-
34	Empana et al., 2003 [156].	CRFs (Framingham and PROCAM) in Belfast and France	C-statistic in prime populations: 1. FRS: 0.66 in Belfast, and 0.68 in France. 2. PROCAM: 0.61 in Belfast, and 0.64 in France	1. Framingham: common ratios were 1.34 in Belfast and 2.35 in France; 2. PROCAM: 1.78 in Belfast, and 2.76 in France	-
35	Rodondi et al., 2012 [157].	CRFs (Framingham) directly and after recalibration	C-index: Framingham older: c-index = (0.583-0.606) in males and (0.577-0.598) in females	Hosmer-Lemeshow, and rift function (FRS, recalibrated FRS, and refit FRS) 1. Males: 16.27-16.11, and 4.89.; 2. Females:121.43, 22.73, and 7.96.	-

36	McGeechan et al., 2008 [158].	CRFs + retinal vascular	AUC: 1. Based: 0.695, 2. Derived: 0706	-	-
37	Onat et al., 1997 [159].	CRFs + (DBP)	-	-	-
38	Wilson et al., 1998 [27].	CRFs + TC (NCEP) + BP (JNC-V)	AUC: 1. Males: ? 2. Females: ?	-	-
39	Mainous et al., 2008 [160].	CRFs + (Framingham) at baseline visit and 3 and 6 years before	AUC: (baseline visit and 3 and 6 years before) 1. Males: 0.646, 0.667, and 0.649. 2. Females: 0.667, 0.677, and 0.709. 3. Total: 0.720, 0.727, and 0.730.	-	-
40	Pyorala et al., 1998 [161].	CRFs + OGTT + insulin measurements	AUC: (5, 10, 15, and 20) quantiles: 1. Age-adjusted: 3.29, 2.72, 2.14, and 1.61, 2. With AUC glucose: 2.36, 2.29, 1.76, and 1.32.	-	-
41	Marshal et al., 1994 [162].	CRFs + 12 SNPs (apolipoprotein)	Stringent criteria to classify patient (>60% stenosis), (<10% stenosis)	-	-
42	Bye et al., 2016 [163].	12 miRS + CRFs and CRFs	AUC: derived and baseline 1. 1. 1. Derived: 0.91, 2. FRS 0.72.	-	-
43	Kavousi et al., 2012 [164].	CRFs + CAC in (12) models	C-statistic: increased 0.05 by adding CAC score.	Roche Diagnostics, Mannheim, Germany.	NRI: 19.3% CAC score
44	Ganna et al., 2013 [165].	GRS (395 SNPs) + CRFs and CRFs in (4) models	C index: 1. Basic model: 0.702. 2. GRS (395 SNPs): Increment = 0.002, 3. CHD (46): Increment = 0.004., 4. polygene: Increment = 0.001	GrønnesbyBorgan: 0.96, 0.99, and 0.73	IDI: 0.2, 0.4, and 0.1
45	Cooper et al., 2000 [166].	CRFs + Hemostatic factors	AUC:1. Model a (0.54-0.77), 2. Model b (0.66-0.77)	-	-
46	Arima et al., 2007 [123].	CRFs + high sensitivity CRP	-	-	-
47	Brautbar et al.,	9p21 + CRFs	AUC: increased from 0.782 to 0.786.	Grønnesby-Borgan: 20.114, $p = 0.0172$	NRI: 1. CRFs + 9p21: 0.8% $p = 0.3$, clinical NRI = 6.2%, p value =

	2009 [167].				0.03. IDI: 0.002, p < 0.015, 2. CRFs: the clinical NRI 6.8%, IDI: 0.021.
48	St-Pierre et al., 2006 [168].	CRFs + ApoB (tertiles), LDLC in (3) models	AUC: Model 1 = 68.9%, Model 2:70.3%, p < 0.001 (Base + ApoB), Model 3: 70.7%, p < 0.001 (Base + LDLC)	-	-
49	Ryoo et al., 2016 [169].	CRFs + ApoB, ApoA, A/B ratio (Quintile)	-	-	-
50	Yarnell et al., 2004 [170].	CRFs + plasma lipid +3 haemostatic /inflammatory.	AUC: (lipid, haemostatic, and combined): 1. Whole cohort: 0.724, 0.728, and 0.737, 2. Men without CHD: 0.685, 0.693, and 0.707.	Hosmer-Lemeshow, 1. 6.37, p = 0.61, 10.46, p = 0.23.	-
51	Everage et al., 2009 [171].	CRFs + CAC score + Racial	Perceived racial discrimination score (respond to year 7, and 15 plus cumulative score): OR: 0.88, 0.82, and 0.90.	-	-
52	Iribarren et al., 2015 [172].	CRFs + cardiac troponin I	AUC: 1. With hs Troponin = 0.7008, 2. without hs Troponin = 0.6849.	-	NRI: 18% after adding hs Troponin to the models
53	McClelland et al., 2015 [173].	CRFs + CAC	C-statistic derived and basic models: 1. FRS: 0.750 (MESA), 0.720 (HNR), and 0.782 (DHS). 2. FRS + CAC: 0.800 (MESA), 0.779 (HNR), and 0.816 (DHS).	Hosmer-Lemeshow, p > 0.22 for each	Optimal model for CHD risk prediction: Discrimination slope: 1. FRS: 0.052 (MESA), 0.053 (HNR), and 0.046 (DHS).,2. FRS + CAC: 0.086 (MESA), 0.095 (HNR), and 0.078 (DHS).
54	Liu et al., 2004 [174].	CRFs (based and after recalibration.	C-statistic: 1. FRS: 0.705 (males), 0.742 (females). 2. CMCS: 0.736 (males), 0.759 (females).	Hosmer-Lemeshow, p < 0.01	No information -
55	Brant et al., 2010 [175].	CRFs + BP measurements per age and gender.	AUC: 1. PP models: 0.83-0.85, 2. BP models: 0.77-0.81	Hosmer-Lemeshow, 1. BLSA p = 0.75, male, p = 0.02 in VHM and PP in (female p = 0.01).	
56	Onat et al., 2010 [176].	CRFs + MetS + CRP per quintiles	AUC: 1. Derived models: 0.789 in males, and 0.806 in females., p < 0.001 for each.	No information -	Males and females in the highest quintiles were significantly and 20-27-fold more likely to develop

			2. Without CRP: 0.781 in men, and 0.803 in women. $p < 0.001$ for each, 3. FRS: 0.775 in males, and 0.783 in females. $p < 0.001$ for each.		CHD than those in the lowest quintiles.
57	Cross et al., 2012 [177].	CRFs + 19 biomarkers	AUC: 2. CHDRA = 0.72, Framingham = 0.73, p value = 0.70.	No information -	NRI:1. CHDRA: 25.7% with events, and 17% without events. A clinical NRI = 42.7%, $p < 0.001$. A clinical NRI = 42.7%, p value < 0.001.
58	Hadaegh et al., 2012 [178].	CRFs (Framingham) + Electrocardiogram	C-statistic derived and basic models: 1. reestimated FRS = 0.838, 2. FRS + electrocardiogram = 0.844, 3. combined = 0.843.	Hosmer-Lemeshow,	NRI: 1. Based = 6.0%, cut point -free NRI = 20.8%
59	Kang et al., 2012 [179].	CRFs (Framingham) + MetS	AUC: Males: FRS (0.767), and (0.677) for FRS + MetS, $p < 0.01$, Females: for FRS (0.777, and (0.733) for FRS + MetS, it was not significant.	-	-
60	Kivimaki et al., 2011 [180].	CRFs + job strain	C-statistic derived and basic models: 1. FRS = 0.7252. FRS + job strain = 0.726.	Hosmer-Lemeshow was not significant.	NRI: derived model = 0.7% and 1.0%
61	Gander et. al., 2015 [181].	CRFs + cardiorespiratory fitness (CRF)	AUC: derived and FRS = 0.80 for both $p = 0.97$	-	-
62	Arad et al., 2004 [182].	CRFs + CRP + CAC score	C-statistic: 1. Without CRP = 0.83, 2. Without CAC score = 0.80, 3. With CAC + CRP = 0085.	-	-
63	Pischon et al., 2005 [183].	CRFs + ApoB + non-HDL, LDL	-	Hosmer-Lemeshow	-
64	Polak et al., 2015 [184].	CRFs + CCA IMT	C-statistic: 1. Far-wall IMT + CRFs: Increment = 0.012, $p < 0.001$, mean IMT($p = 0.004$).	$R^2 = 0.31$, 0.26, and 0.22.	NRI: 6%, and 20%.
65	Cavus et al., 2019 [185].	CRFs + 141 metabolites	C-statistic: 1. 0.756, 2.0.755, 3 + 4. 0.754.	-	-

66	Subirana et al., 2018 [186].	CRFs + 9 biomarkers	C-index: 1. Without biomarker (%) = 1.74.3, 2. 81.3, and 3. 81.3%, 2. With biomarker (%) = 1.79.3, 2. 82.5, 3. 82.6	-	NRI: 33.7%
67	Hindy et al., 2018 [187].	CRFs + GRS (50 SNPs), and smoking	AUC: (never, former, and current smokers), 1. FRS:0.747, 0.742, and 0.740.; 2. Derived: 0.797, 0.749, 0.744.	-	IDI: 0.012, 0.006, and 0.004, $p < 0.0001$ for all.
68	Chien et al., 2018 [188].	CRFs + (FRS in Taiwanese in (8) models	AUC: ranged from 0.804-0.850 by estimates point, and 0.691-0.847 by predicted risks.	Hosmer-Lemeshow, p (0.10-0.79)	-
69	Iribarren et al., 2018 [189].	CRFs + CRFs + GRS (12 and 51 SNPs) in 3 population (AA, LAT, AS).	C-statistic: (GRS-12, GRS-51) 1. AA: 0.687, and 0.687(FRS), and 0.691, and 0.690 (FRS + GRS)., 2. LAT: 0.714, and 0.714(FRS), and 0.717, and 0.715 (FRS + GRS)., 3. AS: 0.745, and 0.745(FRS), and 0.747, and 0.750 (FRS + GRS).	Hosmer-Lemeshow, p values were not significant over all models.	NRI: overall; 10%, 7%, and 7%.
70	Can et al., 2019 [190].	CRFs + CRFs + BMI, WC, in (6) models	AUC: (unadjusted models) 1. Men: 0.572-0.600, 2. Female: 0.587-0.652, $p < 0.001$ for both.	-	-
71	Wang et al., 2019 [191].	CRFs + CRFs + (19) Metabolites	AUC: increased (0.724 to 0.740).	Cross-validation	NRI:0.522, $p < 0.001$, IDI: 0.038, $p = 0.002$
72	Thomsen et al., 2002 [192].	CRFs (Framingham+ Glostrup)	AUC: 1.FRS=0.77, 2. Glostrup = 0.75. AUC: 1. Glostrup=0.77, FRS= 0.76	Cross-validation	-

Note: The number refers to the table reference list (see pages 18-23 of the File).*

Table 12. 3 Odds ratio of CRFs based on Framingham risk score for predicting CHD/AMI among the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.13	**0.008**	1.03-1.23	1.05	0.103	0.99-1.12
Sex Male	0.52	0.427	0.10-2.61	2.11	0.216	0.65-6.92
TC	0.82	0.595	0.39-1.72	1.21	0.444	0.74-1.97
HDL-C	0.95	**0.009**	0.91-0.99	0.98	0.074	0.96-1.00
SBP	0.98	0.386	0.93-1.03	0.99	0.647	0.96-1.03
Smoking	1.53	0.607	0.30-7.83	0.81	0.738	0.23-2.79
DM	3.91	0.125	0.69-22.36	1.27	0.745	0.30-5.38
HTN-Med	1.64	0.690	0.14-18.76	8.16	**0.001**	2.40-27.67

Table 12. 4 Odds ratio of CRFs based on SCORE for predicting CHD/AMI among the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.12	**0.004**	1.04-1.21	1.08	**0.007**	1.02-1.14
Sex Male	1.34	0.666	0.35-5.15	2.11	0.173	0.72-6.17
TC	0.47	**0.028**	0.24-0.92	1.08	0.736	0.69-1.67
SBP	1.00	0.978	0.96-1.04	0.99	0.591	0.96-1.02
Smoking	1.02	0.975	0.24-4.44	1.12	0.848	0.36-3.41

Table 12. 5 Odds ratio of CRFs plus HDL-C, TG for CHD/AMI among the study popul tions

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.12	**0.016**	1.02-1.24	1.12	0.430	0.96-1.09
Sex Male	0.44	0.300	0.09-2.09	3.14	0.059	0.96-10.34
HDL-C	0.95	**0.005**	0.91-0.89	0.99	0.224	0.96-1.01
TG	1.09	0.798	0.58-2.04	0.83	0.619	0.40-1.72
DM	3.64	0.138	0.66-20.06	1.06	0.927	0.30-3.78
HTN-Med	1.00	0.999	0.20-5.01	29.26	**0.002**	3.29-260.11
Smoking	1.54	0.593	0.31-7.60	0.87	0.814	0.26-2.85

Table 12. 6 Odds ratio of CRFs based on PROCAM for CHD/AMI risk among the study populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.13	**0.007**	1.03-1.23	1.08	**0.011**	1.02-1.14
Sex Male	0.59	0.467	0.11-2.77	2.00	0.232	0.64-6.23
LDL-C	0.69	0.396	0.29-1.64	1.29	0.351	0.75-2.21
HDL-C	0.96	**0.008**	0.92-0.99	0.98	0.108	0.97-1.00
SBP	0.98	0.437	0.94-1.03	0.99	0.430	0.96-1.02
Smoking	1.43	0.657	0.29-7.03	0.83	0.739	0.26-2.70
DM	3.69	0.141	0.65-21.05	2.47	0.191	0.64-9.61

Table 12. 7 Odds ratio of CRFs and GRS for predicting CHD/AMI risk in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.08	**0.048**	1.01-1.17	1.03	0.319	0.97-1.09
Sex	1.49	0.567	0.38-5.84	2.40	0.146	0.74-7.83
HTC -Med	5.17	**0.027**	1.20-22.21	3.01	0.077	0.89-10.17
HTN-Med	1.18	0.887	0.12-12.06	7.69	**0.001**	2.33-25.42
Smoking	0.94	0.934	0.22-4.03	1.24	0.732	0.37-4.20
GRS	1.07	0.540	0.86-1.35	0.98	0.841	0.83-1.17

Table 12. 8 Odds ratio of CRFs and wGRS for predicting CHD/AMI risk in the Hungarian populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.08	**0.048**	1.00-1.17	1.03	0.335	0.97-1.09
Sex	1.61	0.488	0.42-6.18	2.55	0.126	0.77-8.44
HTC-Med	4.86	**0.032**	1.14-20.67	3.02	0.077	0.89-10.30
HTN-Med	1.35	0.795	0.14-12.74	**7.50**	**0.001**	2.27-24.76
Smoking	0.92	0.912	0.22-3.94	1.23	0.738	0.37-4.117
wGRS	1.13	0.824	0.40-3.16	0.76	0.595	0.28-2.09

Table 12. 9 Bivariate analyses of Cardio metabolic Risk factors for CHD/AMI among the study populations.

CHD/AMI	Hungarian general			Hungarian Roma		
	OR	p-value	95% CI	OR	p-value	95% CI
Age	1.10	**0.010**	1.02-1.19	1.08	**0.002**	1.03-1.13
Sex	1.30	0.685	0.37-4.59	2.75	0.054	0.98-7.70
TC	0.59	0.110	0.31-1.13	1.24	0.357	0.79-1.95
HDL-C	0.96	**0.004**	0.94-0.99	0.99	0.186	0.97-1.01
LDL-C	0.55	0.122	0.26-1.17	1.25	0.409	0.74-2.11
TG	1.47	**0.047**	1.00-2.15	1.38	0.152	0.89-2.14
DM	7.81	**0.006**	1.82-33.46	3.53	**0.028**	1.15-10.86
SBP	1.01	0.716	0.97-1.05	1.02	0.214	0.99-1.04
DBP	0.97	0.353	0.90-1.04	1.01	0.706	0.96-1.06
HTC-Med	9.30	**0.001**	2.40-35.95	5.42	**0.002**	1.89-15.54
HTN-Med	2.61	0.383	0.30-22.42	11.61	**>0.001**	3.96-34.06

Table 12. 10 Distribution of SBP and DBP based on the fifth Joint National Committee (JNC-V) categories in our study population.

JNC-V category		Hungarian General	Hungarian Roma	p-value
SBP (mm.Hg)				
Normal	< 130	27.24%	33.15%	<0.001
High normal	130-139	13.08%	8.60%	
Stage 1	140-159	8.78%	5.38%	
Stage 2	≥ 160	0.90%	2.87%	
DBP (mm.Hg)				
Normal	<85	36.56%	35.66%	0.030
High normal	85-89	7.35%	7.35%	
Stage 1	90-99	5.56%	4.30%	
Stage 2	≥100	0.54%	2.69%	

CHAPTER THIRTEEN

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