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GENETICS OF LEFT VENTRICULAR HYPERTROPHY

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GENETICS OF LEFT VENTRICULAR HYPERTROPHY

A Dissertation

by

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GENETICS OF LEFT VENTRICULAR HYPERTROPHY

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"That which does not kill us makes us stronger."

Friedrich Nietzsche (1844 - 1900)

To John Bruce Cantrell ("Uncle Bruce");

And to Lidia I Rivera, my mother;

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SOMMARIO

La pressione alta aumenta il carico di lavoro cardiaco e causa l'ipertrofia ventricolare sinistra (LVH). L'ipertrofia ventricolare sinistra è un importante fattore di rischio per la morbilità e la mortalità cardiovascolari. L'ipertrofia ventricolare sinistra è caratterizzata principalmente da due diversi fenotipi: (1) un ventricolo sinistro ingrandito a causa dell'aumento dello spessore della parete del muscolo cardiaco e (2) un ventricolo sinistro ingrandito a causa dell'aumento della dilatazione della parete del muscolo cardiaco. I meccanismi molecolari e patologici con i quali si verificanoentrambi i fenotipi sono sconosciuti. Inoltre, entrambe le varianti fenotipiche dell'ipertrofia ventricolare sinistra sono determinate da cambiamenti quantitativi e qualitivi nell'espressione genica di cellule miocardiche che si traducono in alterazioni strutturali ed emodinamiche nel miocardio. Pertanto, la genetica gioca un ruolo importante nello sviluppo dell'ipertrofia ventricolare sinistra.

In questo lavoro, abbiamo effettuato uno studio di associazione genome-wide al fine di indagare sulla genetica dell' ipertrofia ventricolare sinistra (LVH) e dell'indice di massa ventricolare sinistra (LVMI). Abbiamo valutato questi tratti in una popolazione di studio di 1,212 soggetti di origine europea bianca e 2,5 milioni di polimorfismi nucleotidici (SNPs).

I risultati di questa indagine hanno dato 19 varianti significative ($P < 5x10^{-7}$), tra cui due varianti sul cromosoma 1 nei locus genici di *C1orf106* (rs6427864, $P = 1.21 \times 10^{-7}$), e del gene *MCOLN2* (rs1030932, $P = 2.61x10^{-7}$), due varianti localizzate vicine al gene *IGBP5* (rs13389579, $P = 1.33x10^{-9}$) e al gene *SP140* (rs4972945, $P = 2x10^{-9}$), uno sul cromosoma 3 vicino *ZNF717* (rs686591, $P = 3.93x10^{-9}$), tre sul cromosoma 4 in *VEGFG* (rs4557213, $P = 9.52x10^{-11}$), in *GABRB1* (rs728294, $P = 1.28x10^{-8}$), e in *ADH1C* (rs283410, $P = 2.36x10^{-8}$), due sul cromosoma 6 nei pressi di un pseudogene piruvato chinasi (rs93992718, $P = 1.15x10^{-8}$) e *HLA-DRA* (rs6911419, $P = 2.69x10^{-7}$), uno sul cromosoma 7 in *VSTM2A* (rs1403237, $P = 4.90x10^{-10}$), uno sul cromosoma 8 vicino *KCNU1* (rs7464912 , $P = 5.97x10^{-8}$), uno sul cromosoma 9 in *ABL1* (rs10556171, $P = 1.67x10^{-7}$), tre sul cromosoma 11 tra *HEPHL1* e *PANX1* (rs4753538, $P = 4.19x10^{-10}$), in *ARHGEF12* (rs11217837, $P = 5.54 \times 10^{-8}$),

in *SLC35C1* (rs7130656, P = 8.71x10^{-8}) e due sul cromosoma 12 vicino *KLRA1* (rs11053849, P = 9.43x10^{-8} e rs10845156, P = 1.06x10^{-7}).

I risultati genome-wide dell'esame LVMI hanno mostrato varianti significative; tuttavia al suggestivo valore di P <1x10^{-5} due regioni potenzialmente suscettibili di 97.6 Kb nel gene *SYT14* e 3.4 kb nel gene *GAS1* sono stati identificati per associazione con LVMI.

In conclusione, abbiamo identificato 19 regioni suscettibili ad ospitare varianti comuni che sono state associate con l'ipertrofia ventricolare sinistra e 2 regioni potenzialmente associate con LVMI. Ulteriori studi funzionali genetici sono necessari per caratterizzare la rilevanza biologica nell'ipertrofia indotta da sovraccarico di pressione.

ABSTRACT

High blood pressure makes the heart work harder and promotes enlargement of the left ventricle, left ventricular hypertrophy (LVH), which is an important risk factor for cardiovascular disease and death.

LVH is characterized for the most part by two different sets of observable characteristics (genetic phenotypes): an enlarged left ventricle (1) due to increasing wall thickness and (2) due to increasing wall dilation. The molecular and pathological mechanisms by which either phenotype occurs is unknown. We do know, however, that both phenotypic variations of LVH are determined by quantitative and qualitive changes in the genetic expression of cardiac cells that result in structural alterations in the muscular tissue that affect the blood flow within the heart.

Genetics, the study of heredity and the variation of inherited characteristics, therefore play a prominent role in the development of LVH. A genome-wide association (GWA) study to investigate the genetics of LVH and left ventricular mass index (LVMI) in a cross-sectional-study of 1,212 subjects of white European ancestry and 2.5 million nucleotide polymorphisms (SNPs) yielded a total of 19 genome-wide significant ($P < 5x10^{-7}$) variants. The GWA revealed no genome-wide significant variants; however, at suggestive *P* value $< 1x10^{-5}$ were found two potentially susceptible regions of 97.6 Kb in the *SYT14* gene and 3.4 kb in the *GAS1* gene for association with LVMI.

Nineteen (19) susceptible regions harboring common variants associated with LVH and 2 potential regions associated with LVMI were found. Further functional genetic studies (relating to a variable quantity whose value depends on one or more other variables) are required to characterize the biological relevance of these findings to high blood pressure associated with enlargement of the left ventricle.

INTRODUCTION

Blood pressure

Blood pressure (BP) measured in millimeters of mercury (mmHg) is defined as forced exerted by the blood against unit area of the vessel wall. In hemodymic terms, blood pressure is described as:

$$BP \cong CO \times SVR \qquad \text{mmHg}$$

BP is generated by the left ventricle ejecting oxygenated blood through the aortic valve into the aorta and into the systemic vasculature, which acts as a resistance to cardiac output. These series of events are actually described by the following equation:

$$CO = SV \times HR \qquad \text{liters/minute,}$$

where CO, cardiac output; SV, stroke volume; and HR, heart rate;

Ejection of the blood into the aorta is characterized by two pressure measures occurring at different instants of time, the highest pressure called systolic pressure ($P_{systolic}$) and the lowest pressure called diastolic pressure ($P_{diastolic}$), as shown in Figure 1. The difference between these two pressures is called pulse pressure (PP), defined as

$$PP = P_{systolic} - P_{diastolic} \qquad \text{mmHg,}$$

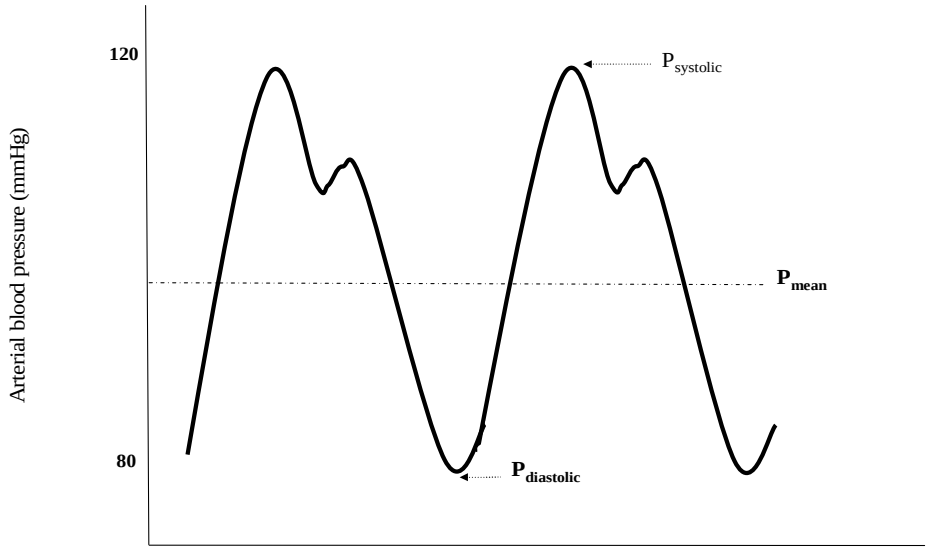


Figure 1. Arterial blood pressure wave

The mean pressure is estimated by the mean arterial pressure (MAP), defined as

$$MAP \cong 1/3 \times (P_{systolic} - 2 \times P_{diastolic}) \quad \text{mmHg}$$

As blood flows down from the aorta through the systemic vasculature, changes in the diameters of the arterial bed, which serve to regulate blood perfusion into the organs, are encountered. These changes in arterial vessel diameters are known as systemic vascular resistance. Thus, the factors that determine the actual mean arterial pressure are cardiac output (CO), systemic vascular resistance (SVR), and the central venous pressure (CVP), as given in the following equation:

$$MAP = (CO \times SVR) + CVP \quad \text{mmHg}$$

From this equation, it is easily to see that a change in the mean arterial pressure is dependent on a change in any of the determinants on the right side of the equation. (Note that since CVP ranges from 3 - 8 mmHg, for practical MAP calculation CVP is considered 0 mmHg). A graphical representation of the mean arterial pressure within the systemic circulation is illustrated in Figure 2.

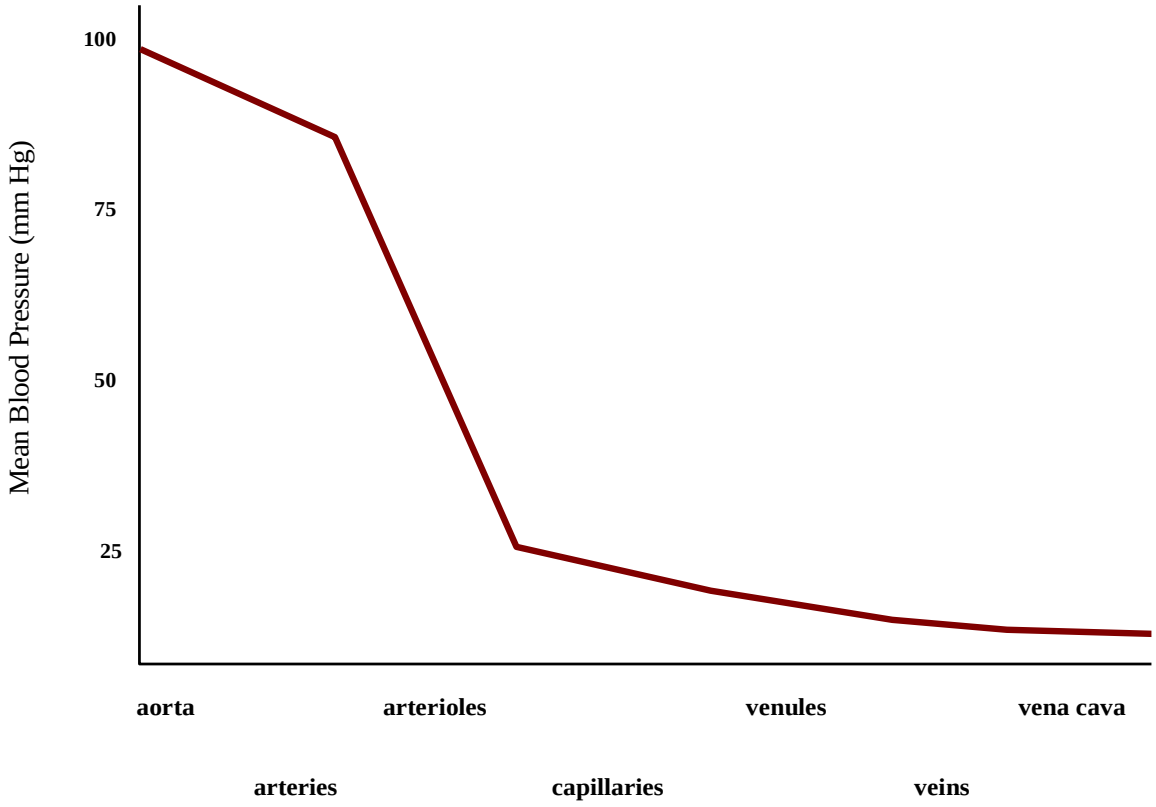


Figure 2. MAP within the systemic circulation

Hypertension

Hypertension, or high blood pressure (elevated arterial blood pressure), defined as systolic BP ≥140 mmHg and/or diastolic BP ≥ 90 mmHg, is the most common risk factor of cardiovascular disease. It is the major cause of heart attacks (cardiovascular mortality), stroke, and end-stage renal disease.

Hypertension is a quantitative disease wherein the distribution of blood pressure is continuous, becoming skewed at high levels. Frequently it is classified clinically by three mechanisms: by its severity (i.e., blood pressure elevation), by its underlying cause (i.e., primary or essential hypertension vs. secondary hypertension), and by its prevalence in an aging population.

Prevalence of hypertension

Hypertension is highly prevalent everywhere, affecting more than 600 million people and accounting for 6 % of adult deaths worldwide[1]. In Italy, hypertension is a common medical disorder, with 37.7% of the population suffering from the disease[2].

Unlike tropical and infectious diseases where prevalence and incidence are well documented by epidemiologic and demographic studies, in hypertension these epidemiological an demographic measures are inconsistent across geographical regions, mostly because of variations attributed to differences in economic development and social and cultural determinants at the local level[3]. Other determinants, such as lack of standardized measurement, variability in drug treatment, confounding effects of aging, and intrinsic (genetic) factors for the disease, also contribute to changes in hypertension in various populations.

Hypertension and aging

The structural changes of the heart are inevitable as cardiac cells age and contractile forces debilitate, causing apoptosis and necrosis to occur. The mechanisms that regulate blood pressure work differently in the young and middle-aged (age 20–59 years) and in the old (age ≥ 60 years), resulting in various cardiac structural changes and the flow of blood (hemodynamic changes) within the heart[4, 5].

The hemodynamics of blood pressure are not linearly correlated with increasing age. While elevated systolic blood pressure is a persistent risk factor for cardiovascular disease in subjects younger than 50 years of age, diastolic blood pressure is a stronger predictor, and

in subjects older than 60 years of age pulse pressure becomes a better predictor since diastolic blood pressure is inversely associated with cardiovascular disease in older persons. In the geriatric population, hypertensive subjects, especially those whose arteries fail to distend in response to applied pressure (due to hardening of the arteries, or arteriosclerosis), systolic blood pressure tends to increase (with little or no effect on diastolic blood pressure).

In all persons, with the exception of the obese, the pattern of blood flow in the heart, while maintaining normal cardiac output, changes under high blood pressure, increasing total peripheral resistance.

Hypertension and other risk factors

Risk factors, such as obesity, a sedentary lifestyle, the excessive consumption of alcohol, excessive use of salt, and low potassium intake, profoundly affect to the manner of development (pathogenesis) of hypertension; however, how these risk factors are involved in the molecular changes of the heart has yet to be determined.

Molecular and cellular biology of hypertension

The molecular and cellular biology of high blood pressure requires an understanding of phenotypes, polygenes, and common variants. A phenotype consists of the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment. A polygene is a gene whose individual effect on a phenotype is too small to be observed, but which can act together with others to produce observable variation. A common variant is a common allele that is unchanged across human populations. Common variants can be classified either as a disease-causing allele (also known as causal variant) or non-causal allele whose function is not yet determined. A causal variant is found in the coding or regulatory sequence of a gene or between genes, and it has an effect on protein structure and function. A non-causal variant is found in the non-coding region of a gene or between genes, and it has no effect in the pathway of the disease.

Like most common diseases, hypertension is a complex, polygenic disease with a number of causes, or sets of causes (etiologies), interacting to produce complex phenotypes.

In a given population, it is believed (1) that 40-50 % of variance in blood pressure is from genetic and environmental factors, (2) that 30-35 % is from heritable genetic factors[6-8], and (3) that 10 % is from Mendelian forms of hypertension[9-14]. As for the Mendelian forms of hypertension, although many studies have been done that have identified mutations underlying various monogenetic (descended from a single pair of ancestors) forms of hypertension, these are rare in the general population, thus leaving 90 % of the genetic variants still undetermined.

The disordered physiological processes (pathophysiological) associated with high blood pressure during the systolic phase, diastolic phase, or both, are still unknown.

The multifactorial etiology of hypertension results from multiple gene interactions working together or independently at many different cellular levels. Such interactions are known as gene-gene interactions, and because of environmental influences that are likely to induce modification resulting from external rather than genetic influences, these epigenetic changes affecting DNA transcripts are known as gene-environment interactions.

Advanced genetic technology has advanced our understanding of the genes involved in regulating blood pressure. But to date what thus technology has revealed is only a part of the wide range of genetic architecture involved in the study of high blood pressure.

Diseases caused by high blood pressure

Of the diseases caused or greatly augmented by high blood pressure, three in particular are notable:

- **Stroke:** Hypertension is the most important modifiable risk factor of stroke and stroke mortality in adults. Hypertension is characterized by microaneursyms, lipohyalinosis, and fibroid necrosis of the penetrating arteries that supply oxygenated blood to various parts of the brain, including the basal ganglia, the cerebral deep

white matter, and the pons[15]. Thus, stroke (also known as brain attack) is a neurological deficit caused by disrupt disturbance of blood flow in the brain. Stroke is the leading cause of death and disability in western societies. There are two types of stroke, ischemic and hemorrhagic. And in hypertensive subjects, ischemic stroke accounts for 80% of all strokes while the remaining percent is caused by the hemorrhagic type[16]. Severe hypertension is a common feature of subarachnoid hemorrhage[17].

- **End-stage renal disease:** Hypertension and diabetes mellitus are the two leading causes of end-stage renal disease (ESRD). Hypertension is in fact both a consequence and a cause of ESRD[18]. End-stage renal disease also known as chronic renal function (CRF) is defined as complete or almost-complete failure of the kidneys to function. The main function of the kidney is to remove waste and excess water from the body, while maintaining stable homeostatic functions.

- **Chronic heart failure:** Hypertension is an important cause of chronic heart failure. Chronic heart failure (CHF) often called heart failure (HF) is defined as a pathophysiological state in which the heart fails to pump blood and support physiological circulation. Mean survival after diagnosis of CHF without treatment is 1.4 years in men and 2.5 years in women as reported by the Framingham study[19].

  Heart failure is a complex disease that involves many pathological mechanisms that lead to malfunction of cardiac muscle and impaired physiology. Currently, six pathological causes can lead to heart failure:

  1. Abnormality of the myocardium due to
     a. *Myocardial infarction (MI)*, an irreversible condition that causes the death of cardiac muscle (necrosis of heart cells) because of lack of blood supply;

b. *Left bundle-branch block (LBBB)*, a condition that causes uncoordinated contraction because of delay or obstruction of cardiac impulses propagating from the bundle of His to the left bundle branch;

c. *Cardiotoxicity*, a condition that impairs electrophysiological and mechanical cardiac functions caused by alcoholism, adverse effects of chemotherapy, and drug toxicity, developing eventually into cardiomyopathy;

d. *Cardiomyopathy*, a condition characterized by injury of heart muscle making the heart larger and weaker. This condition is manifested in three forms: dilated, hypertrophic, and restrictive;

e. *Hypertrophy*, a condition where enlargement of cardiomyocytes (thickening of the myocardium) occurs to support cardiac function as to meet body tissue demands. Hypertrophic causes decrease the size of the heart chambers, including the right and left ventricles;

2. External work overload, a condition resulting from long-standing or severe hypertension. As demonstrated by the Framingham Heart study, hypertension accounts for 65 – 85 % of heart failure cases. Heart failure can develop in hypertensive patients with either reduced left ventricular systolic function or with preserved systolic function and diastolic relaxation abnormalities;

3. Valve abnormalities, a condition involving the dysfunction of one or more valves of the heart (i.e., the tricuspid or pulmonary valve on the right-side of the heart, or the mitral or aortic valve on the left-side of the heart)

4. Arrhythmias, a condition where the heart rate is irregular due to problems in the heart's electrical conduction system;

5. Presence of pericardial abnormalities or a pericardial effusion (tamponade)

6. Congenital deformities of the heart, a condition caused by developmental cardiac malformations during embryogenesis or later in life. It is of heterogeneous etiology associated with mechanical and/or electrical

dysfunction of the myocardium[20]. Genes and environmental influences account for 10% of cases of congenital heart disease; however, understanding of probable genetic links is increasing.

Heart failure results from a combination of two of more causes of heart disease that occur asynchronously, simultaneously, or in sequence at different points in life. This makes it difficult to define the cause of heart failure to a single form of heart disease.

For example, long-standing or severe hypertension may lead to structural heart disease and cardiac failure through at least two pathways: (1) the development of left ventricular hypertrophy and/or left ventricular dilation accompanied by abnormal contractility (systolic) or relaxation (diastolic), and (2) myocardial infarction resulting in a wall motion abnormality. Either pathway is a sequence of events that lead to impaired cardiac function resulting in failure of the heart to pump blood to meet the needs of the body.

Left ventricular hypertrophy

Left ventricular hypertrophy (LVH) is a common manifestation of hypertension that is life threatening if left untreated. LVH manifests itself in increased left ventricular mass (LVM) and is a powerful predictor of morbidity and mortality. Subjects with untreated LVH are at increased risks for coronary heart disease, stroke, congestive heart failure, and sudden death.

LVH occurs either from a response to long pressure overload that increases the left ventricular (LV) wall thickness (concentric LVH, as in hypertrophic cardiomyopathy) or from a response to chronic volume overload that promotes LV dilation (eccentric LVH, as in dilation cardiomyopathy). In both responses, myocardial muscle mass increases due to enlargement of cardiomyocytes and changes in individual myofibrils.

Left ventricular concentric hypertrophy is described by an increase in the left ventricular wall thickness in response to long-standing pressure overload to normalize left

ventricle wall tension. Under this condition, the weight of the heart increases disproportionately, increasing the overall cardiac size. By Laplace's law, this phenomenon can be described as:

Wall tension = (Pressure x LV radius) / (LV wall thickness),

where LV, left ventricle. The left ventricular wall thickness may exceed 2 cm and the heart weight may exceed 500 g.

Concentric hypertrophy tends to occur because of hypertension or aortic stenosis and it is associated with normal or reduced left ventricular end-diastolic volume (LVEDV). With time, the thickened left ventricular wall shifts to stiffness leading to dysfunctional diastolic filling.

LVH caused by long-standing or severe hypertension is an independent predictor of mortality and a well-known established precursor of heart failure, myocardial infarction, and stroke. Population studies indicate that each 50 g/m^2 increase in left ventricular mass (LVM) correlates to a factor increase of 1.7 in sudden death[21]; However, a much recent study reported that every 39 g/m^2 increase in LVM confers to a 40% increase in risk of cardiovascular events, particularly in hypertensive subjects[22].

Progression from LVH to heart failure is associated with LVH (either eccentric, concentric, or a mixture of the two), ischemia, increase fibrosis and ventricular stiffness, necrosis, apoptosis, and systolic ventricular failure.

Diagnosis of left ventricular hypertrophy

Left ventricular hypertrophy (LVH) diagnosis can be performed by: electrocardiogram, echocardiogram, and cardiac magnetic resonance imaging.

ECG-defined LVH is a low-cost and easy diagnostic test commonly perform during periodic examinations; however, its sensitivity and specificity are < 50 % and >90 %, respectively. ECG testing captures a wide variety of voltage abnormalities, which may not be necessarily correlated with LVH, thus making the assessment for LVH unreliable for persons

who do not meet all the ECG-LVH criteria. The general ECG features for assessing presence of LVH include:

- ≥ QRS amplitude (voltage criteria)
- Prolongation of the depolarization (delayed intrinsicoid deflection)
- Widened QRS / T angle (signal pattern criteria)
- Leftward shift in frontal plate QRS axis (ST-T abnormality criteria)
- Evidence of atrial enlargement (LAE)

ECG-LVH characterized by voltage alone underestimates the risk of adverse cardiovascular outcomes of left ventricular hypertrophy with or without repolarization abnormalities. Moreover, the exact mechanism of the voltage increase is not clear.

Echo-defined LVH is a much more sophisticated and accurate diagnostic test; however, it is more costly than ECG. Echo-LVH measures the geometric and blood flow patterns in the heart using ultrasound technology. Specifically, it offers the opportunity to calculate true left ventricular volumes, to estimate LV overall size and performance, and to evaluate systolic and diastolic functions in time. This is an improved method for assessing presence of LVH with sensitivity of 70 – 90 %.

Approximately 20% of heart failure cases are attributed to ECG-LVH diagnoses, whereas 60 – 70 % to Echo-LVH.

MIR-LVH is an optimum method for evaluating cardiac chamber and vessel anatomy as well as for determining functions and structures of the cardiovascular system using high quality imaging techniques. This diagnostic tool is an expensive modality for assessment of LVH and it is not always available in clinical settings and to every patient, making its usage limited.

Molecular mechanisms of left ventricular hypertrophy

The molecular mechanisms involved in the pathogenesis of LVH, which include increasing muscular, vascular, and collagenous components of the myocardium, are not known.

LVH is a multifactorial process influenced by a complex interplay of genetic, environmental, hemodynamic, and neurohumoral factors as well as demographic factors (e.g., age, gender, race), comorbid diseases (e.g., obesity, diabetes mellitus, coronary artery disease), and coincident pharmacologic therapies[23].

LVH is associated with structural and molecular mechanisms far distinct from physiological left ventricle hypertrophy and compensatory cardiac remodeling[24].

Under pressure overload conditions, both neurohumoral and mechanical stimuli activate various protein processes leading to the activation of several signaling molecules (e.g., calcium dependent proteins, protein kinases, and intracrine growth factors [25, 26]) and to the involvement of many transcription factors [27, 28].

Monogenic versus polygenic phenotypes of LVH

Monogenic phenotypes of left ventricular hypertrophy result from genetic mutations of Mendelian forms (i.e., rare mutation of single genes), including a variety of missense mutations encoding sarcomeric proteins (e.g., beta-myosin heavy chain, myosin-binding protein C, troponin T and I, titin, and alpha-actin, to name a few), mutations encoding nonsarcomeric proteins (e.g., *PRKAG2, LAMP-2*), and mutations encoding mitochondrial proteins [29].

Polygenic phenotypes of left ventricular hypertrophy results from a combination of multiple genes and environmental factors. However, it is very likely that each of the genes involved in the development of LVH provides a small contribution (i.e., genetic additive effect) to the overall phenotype. Such additive effects are either amplified or masqueraded when combined with environmental factors.

Heritability of LVH

Genetic factors account for up to 60% of variance of LV mass[30-36], and the remaining 40% is explained by conventional factors, such as age, gender, blood pressure, and body mass index.

Family history of LVH

Family history of cardiac hypertrophy is an important index for quantifying familial susceptibility to the disease, in particular for monogenic forms of LVH. For polygenic forms of LVH as the one induced by hypertension, family history of high blood pressure precedes familiarity of LVH since it carriers a 2-fold risk in first-degree relatives.

Genetics of LVH

An increasing number of single nucleotide polymorphisms in genes that contribute to the development of cardiac remodeling and variation of LV mass have been identified, such angiotensin converting enzyme (*ACE)*[37-39], peroxisome proliferator-activated receptor alpha *(PPARA)*[40], guanine nucleotide binding G protein beta polypeptide 3 *(GNB3)*[41], and cytochrome P450 family 11 subfamily B polypeptide (*CYP11B2)*[42]. Other genetic loci that have been association with LVH include cardiotrophin-1 (*CT-1*)[43], ryanodine receptor 1 (*RYR1*)[44], and neural cell adhesion molecule 1 (*NCAM1*)[45].

Fundamental concepts

Genetics

From studies in human genetic architecture, it is known that on average, any two individuals share more than 99.5 % of their DNA sequences. The remarkable diversity of humans is encoded in less than 0.5 % of DNA, approximately 15 million base pairs. It is within this range that variations in disease predisposition and response to environmental factors reside.

Moreover, DNA variations are determined by two common forms, single-nucleotide polymorphisms (SNPs) and copy number variations (CNVs), both of which can occur anywhere in the genome. In particular, SNPs may occur throughout the genome (e.g., in exons, introns, intergenic, or regulatory regions) at a frequency of approximately one nucleotide in every stretch of about 1000 base pairs. However, only 1 % of SNPs occur in coding regions, and therefore variations of these coding sequences could alter the gene product and susceptibility to a phenotypic difference or to a disease.

SNPs serve both as a physical landmark within the genome and as a genetic marker whose transmission can be followed in a family pedigree. Also, because of their prevalence and nonrandom distribution throughout the genome, SNPs can be used in linkage analysis for identifying haplotypes associated with disease.

CNVs, on the other hand, are genetic variations consisting of large contiguous stretches of DNA, ranging from 1000 to millions of base pairs. CNVs alike SNPs, but in complex rearrangements of genomic material and with multiple alleles, can be present or missing in a given population, thereby causing genotypic and phenotypic differences among human populations.

From monogenic studies, it is known that mutations caused by single base pair substitutions or insertion/deletions (frameshift) can lead to dramatic changes on the organism. Mutations that occur in exonic regions alter all or part of the amino acid sequence, thus causing detrimental effects on the organism. However, there are more variations outside the exonic regions than within; therefore, there is a greater need for investigating these regions and the role that they play in the onset of a disease. Additionally, thoughtful investigation of such gene products as encoded proteins, transcription factors, promoter regions, and microRNAs, as well as associated regulatory functions, is also necessary to complete the genetic spectrum of the disease.

Phenotype

Studying well-defined phenotypes (or traits) of complex diseases can make the outcome of the study more homogenous, as it focuses on a specific pathophysiological pathway. Defining a phenotype, enable us to study the genetic epidemiology of complex disease as a discrete or quantitative trait. However, defining a well-definite phenotype for a disease is a challenging task, since there is phenotypic heterogeneity, that is, for the same disease (or trait), different features are manifested among related individuals or within a homogenous population.

Genetic epidemiology

Genetic epidemiology is the study of genes, environmental factors, and their joint effects concerning the pathogenesis of a disease within and across populations.

Genetic epidemiology was defined by Morton NE, 1982 as “a science which deals with the etiology, distribution, and control of disease in groups of relatives and with inherited causes of disease in populations”.

Studying genetic epidemiology of a complex disease is a challenging task, since many genes and environmental factors play a role and it is difficult to quantify all factors accounting for the disease. On the other hand, studying plausible factors that are related to the disease, although it only accounts for a small risk, broadens our knowledge about the course of the development of disease.

Working together with genotypes and phenotypes in a population, it is possible to study the genetic epidemiology of a disease as it is illustrated in Figure 3

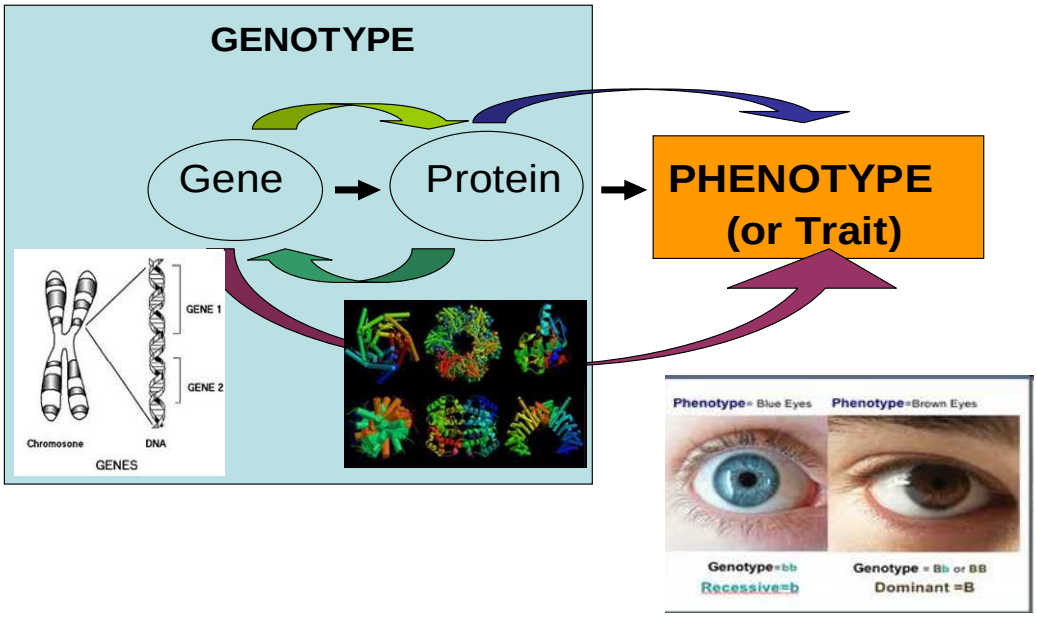


Figure 3. Genetic epidemiology framework

Gene discovery approaches

The objective of gene discovery testing is being able to predict the phenotype (or trait) based on genotypic and environmental information. Genetic discovery testing is an approach to (1) provide new knowledge regarding the etiology and molecular mechanisms in the pathogenesis of a disease, (2) contribute to advanced development of novel treatments (e.g., personalized medicine), (3) identify people at risk, and (4) implement early diagnosis and prevention treatment measures.

To pursue unconventional gene discovery for complex diseases, genome-wide association studies (GWAS) (examination of thousands of SNPs across the genome) or candidate gene studies (examination of certain number of SNPs at a genomic location of interest) need to be conducted in a population, consisting of a large number of individuals with and without the disease. Particularly, a population study can be based on a cross-sectional or case-control study design. Having a large sample size, guarantees enough statistical power to detect genetic variants (particularly common variants with allele frequency greater than 10%) associated with the trait or disease of interest.

GWAS and candidate gene studies are powerful methods of identifying genetic variants that are associated with an increased risk of developing the disease. Such variants themselves may be causative or may be in linkage disequilibrium (LD) with other genetic variants that are located in the vicinity and of which are responsible for the increased risk.

In a population, genotypes of individuals are compared (usually under and additive model) as illustrated in Figure 4.

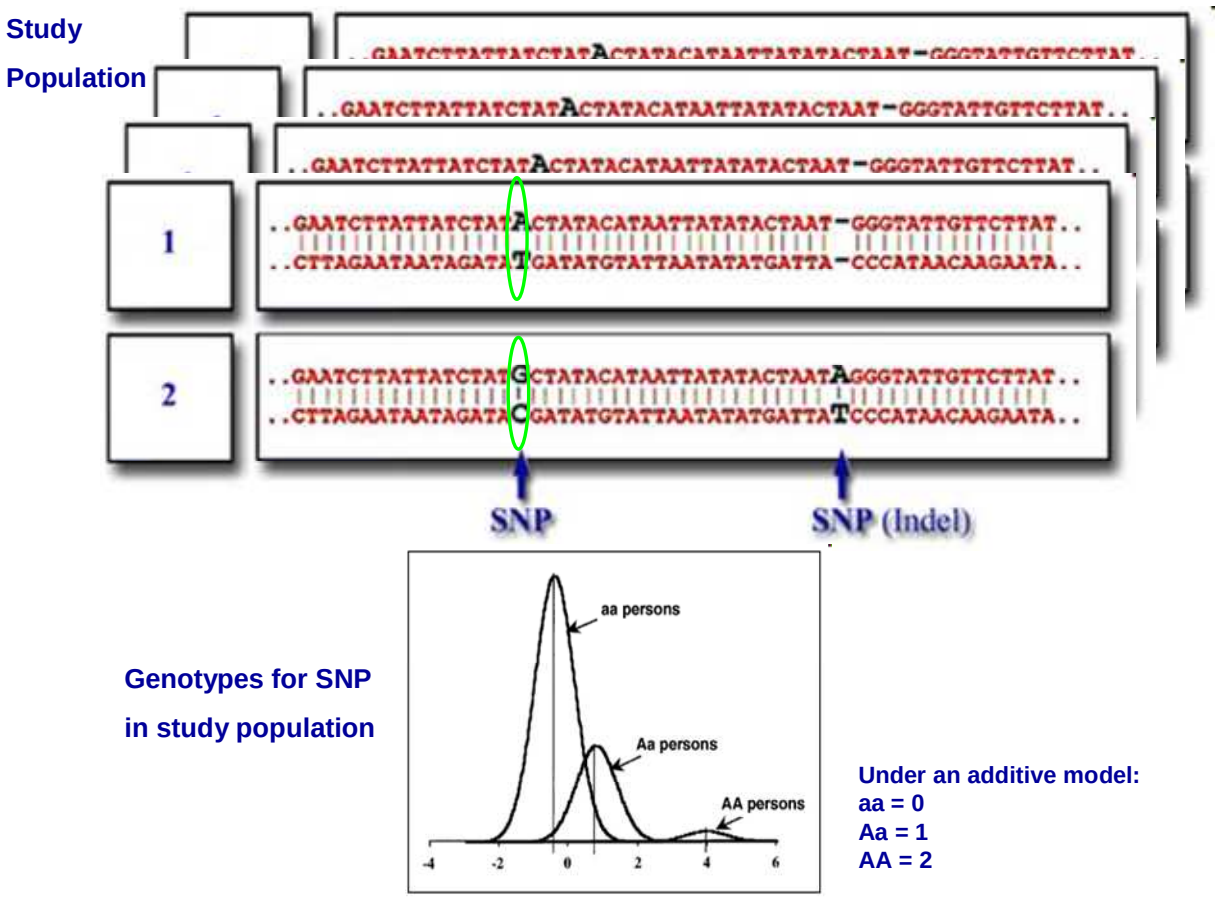


Figure 4. Association analysis of a SNP in a population

Currently, there are various genetic approaches, including

- **Genome-wide linkage analysis**

  A genome-wide linkage approach examines the whole genome in a hypothesis-free approach with a limited number of genetic markers (about few hundreds) in populations of related individuals (based on family-based study disease) to identify regions of the genome that contain genes possibly predisposing to the disease or a trait of interest.

  GWA linkage analysis is based on the principle that loci that are close together, segregate together more often than loci that are further apart, as chromosomal recombination will occur more frequent between distance loci with a

probability of 0.5. The rationale of linkage is that deviation from this probability indicates linkage between the disease and the genetic locus.

This technique has been successful at the identification of the genetic variation underlying single-gene (monogenic/Mendelian) disorders, but not very successful for multifactorial diseases or traits.

The advantage of linkage studies is that they are powerful for traits explained by rare variants with large effects.

- **Candidate gene analysis**

A candidate gene analysis examines variants in a particular gene whose function is suspected to be involved in the pathogenesis of the disease of interest. Therefore, a hypothesis regarding the gene involvement in the development of the disease is adopted prior to analysis. Candidate gene analysis is performed in a population-based setting for association of one or more variants in the gene with the trait and/or phenotype of interest. In a candidate gene analysis, one or more genes can be analyzed given a priori hypothesis.

The disadvantage of this approach is that suffers from lack of replication in independent large-scale studies.

- **Genome-wide association analysis**

A genome-wide association (GWA) analysis examines dense maps of single nucleotide polymorphism (SNP) makers in a hypothesis-free manner, that is, without making any assumption of genes involved in the pathogenesis of the disease (or trait) of interest.

The feasibility of performing a genome-wide association analysis have been due to the availability of rapid advancement in the field of array genotyping technology and of the completion of the reference sequence of the human genome project and the International HapMap project.

GWA examines the full genome (determined by the number of markers according to the genotyping technology) with the aim of detecting genetic variants in multifactorial diseases and related-traits by comparing genoytpes of thousands of individuals in a a population or across populations.

AIM OF THE PROJECT

Genetic architecture, nucleotide, and polymorphism are terms use in genetics. Genetic architecture is the complex genetic structure of something, in the present case heart tissue manifesting enlargement of the left ventricle. Nucleotides form the basic structural unit of nucleic acids, such as DNA. And polymorphism refers to the presence of genetic variation within a population, upon which natural selection can operate.

The goal of this project was to determine the genetic architecture of the left ventricular hypertrophy (LVH) and left ventricular mass (LVM) in a cross-sectional-study of 1,212 subjects of white European ancestry (Italian) and 2.5 million nucleotide polymorphisms (SNPs), performing a genome-wide association analysis on these traits.

MATERIAL AND METHODS

Study population

The population of this study was selected from the Campania Salute (CS) project, a network involving 12,000 outpatients, 23 hospital-based outpatient hypertensive clinics in different regional hospitals, and 60 randomly selected general practitioners allocated in the regional area, with the Hypertension Clinic of the Federico II University in Naples serving as the coordinating center. Further details of this cohort have been previously reported elsewhere[46]. Seven hundred and fifty-four (754) patients for whom echocardiography data was available were selected for the study. Additionally, 480 subjects with normal left ventricular (LV) mass and without cardiovascular disease were selected from the AVIS Blood Bank in Naples. The total final population of the study consisted of 1,212 subjects.

Participants were referred by their GPs to the coordinating center for evaluation of hypertension. Entry examination included ECG, blood and urine biochemistry, echocardiography, carotid ultrasound examination, and further examinations, if needed, with follow-up examination performed at least once a year. Prevalence of cardiovascular disease was defined as a history of previous myocardial infarction, angina, or procedures of coronary revascularization, stroke or transitory ischemic attack, congestive heart failure, or chronic kidney disease (with >grade 3 and GFR<30 ml/min per 1.73m^2) at the time of the first examination in the outpatient clinic.

The traditional risk factors for cardiovascular disease include: (1) diabetes mellitus, defined as treatment with insulin or oral hypoglycemic drugs, or as elevated (>126 mg/dL or 7.0 mmol/L) levels of fasting, non-stressed blood glucose on at least two separate occasions in conjunction with adhering to ongoing dietary measures to control glucose level; (2) smoking status; (3) family history of myocardial infarction before age 60 years; (4) systemic hypertension, defined as a >140 mm Hg systolic or a diastolic blood pressure ≥90 mm Hg and/or ongoing pharmacological treatment; (5) hypercholestolemia, defined as total cholesterol >200 mg/dL (or 5.17 mmol/L) and/or LDL-cholesterol > 100 mg/dL (or 2.58

mmol/L); (6) hypertrygliceridemia, defined as ≥150 mg/dL; chronic kidney disease, defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2; (7) metabolic syndrome, defined according to the NCEP ATP III criteria, i.e., if three or more of the following five criteria are met: waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level over 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women) and fasting blood sugar over 100 mg/dl.

Left ventricular mass was calculated using the Devereux equation[47] in accordance with the American Society of Echocardiography (ASE) criteria[48] defined as follows:

$$LV\ mass = 0.8 \times (1.04 \times [(IVS+LVDD+PWT)^3 - (LVDD)^3]) + 0.6 \quad grams,$$

where IVS is interventricular septal thickness in cm, LVDD is left ventricular diastolic diameter, and PWT is left ventricular posterior wall thickness in cm.

The LV mass was indexed by body surface area using normal limits from the Framingham Heart study (FHS). Left ventricular hypertrophy was defined by left ventricular mass index >116 g/m^2 in men and >104 g/m^2 in women as suggested in the FHS[21].

The study's protocol was approved by the Medical Ethics Review Board of the Federico II University in Naples. All participants provided informed consent.

Sample collection

During the regular routing examination, a blood sample (5 ml) was drawn from each participant enrolled in the study and was collected into EDTA tubes. All participants completed an interview that contained information about demographic and phenotypic data as aforementioned.

Genotyping

Genomic DNA from peripheral blood leukocytes was extracted using a commercially available kit (DNA Qiamp Midi kit (QIAGEN). This was performed at Scientific and Technological Park IRCCS Multimedica, Milan, Italy.

DNA samples were then processed according to standard Illumina protocol and were genotyped on Illumina SNP array.

The Italian study was genotyped in two stages. In the first stage, 727 subjects were genotyped on the Illumina Infinium™ II HumanHap300 Genotyping BeadChips v.2.0 (with 318,237 markers). In the second stage, 698 subjects were genotyped on the Illumina HumanOmni1-Quad BeadChip (with 1,140,419 markers)

Quality control for genotypes was determined using the Illumina-provided standard definition cluster-file for the HumanHap300 v.2.0 and the HummanOmni1-Quad products, respectively. Genotype sample quality control was accomplished by monitoring sample call rate, sex, heterozygote and homozygote frequencies, as well as other analogous parameters determined by the Bead Studio software. All steps of genotyping by Illumina platform were performed at Scientific and Technological Park IRCCS Multimedica, Milan, Italy.

Quality control (QC) filtering

In order to ensure robust association tests, a quality control framework was preformed prior of association analysis. This framework consisted of implementing filtering measures of call rates (by SNP and by sample), measures of SNP genotype distribution (Hardy Weinberg Equilibrium (HWE) test), measures of SNP allele frequencies, and measures of sample heterozygosities (in autosomes and sex chromosomes).

- ***Measures of call rates (by SNP and by sample):*** in the Italian study, a discovery phase with filtering thresholds, such as 97% genotyping call rate threshold, 97% call rate threshold, and >1% minor allele frequency (MAF) threshold were implemented.

- ***Measures of SNP genotype distribution (HWE test):*** To check for genetic deviation among genotypes HWE test filtering was performed.

  The Hardy-Weinberg law states that allele frequencies in one generation can be used to estimate what will happen to genotype proportions in the next generations as long as conditions must be met:

  a. the population is large and is randomly mating, and
  b. the population has no mutations and no migration,

  the allele frequencies will not be affected in the population. Henceforth, the genotypes will have equal fitness, i.e., there will be no selection.

  This can be mathematically expressed by considering a biallelic autosomal locus with alleles A and a, with define genotype frequencies in the population

$$P(aa) = p_{11}; \quad P(aA) = p_{12}; \quad P(AA) = p_{22}$$

$$p_{11} + p_{12} + p_{22} = 1$$

From this genotypes, allele frequencies P(a)=p and P(A)=q are calculated by

$$P(a) = p = p_{11} + 0.5 \times p_{12}$$

$$P(A) = q = p_{22} + 0.5 \times p_{12}$$

$$p + q = 1$$

Thus, to test that allele and genotype frequencies will not change in a population in successive generations, all SNPs in the control population should be tested for HWE tests with a given *P* value criterion. SNPs failing such test will be excluded from the analysis.

As comparing the HWE tests, the observed versus the expected, an inflation factor is calculated. This is commonly denoted as lambda (λ), which may indicate poor genotyping quality control and/or genetic stratification in the population.

In the Italian study, a HWE *P* value threshold < $1x10^{-6}$ was used.

The quality control of sample information was based on measures of sample mix-ups, false/cryptic relationships, and unexpected/mixed ancestry.

- ***Measures of heterozygosity:*** all female samples should have similar heterozygosity rates on the X-chromosome SNPs to the autosomes (1-22 chromosomes); all male samples should have no heterozygous X-chromosome SNPs and should be typed for the Y-chromosome SNPs. In the Italian samples, filtering checks for high heterozygosity (or high homozygosity) were performed. Subjects with either high heterozygosity or homozygosity were excluded from the Italian study.

- ***Measures of gender verification:*** all genotyped subjects were checked for gender verification by heterozygosity calculation of the X-chromosome SNPs; samples with low but non-zero X-chromosome heterozygosities, and typically with low call rate will be excluded from the analysis. On the other hand, samples with high call rate, and but low heterozygosity, their gender was corrected using the remaining X-chromosome SNPs for female or Y-chromosome SNPs for male gender verification, respectively.

- ***Measures of share segment analysis:*** in order to check relationships between samples, detect “odd” genotype distributions, or detect sample with different origins, Computation of the Identity-by-State (IBS) matrix between samples in each GWA dataset was performed.

For Italian study, all analysis within this filtering check was based on IBS matrix. Thus, comparison of segments (or alleles) using the identically by state (IBS) method, by means of checking whether the alleles look identical in the population were conducted. IBS alleles were treated mathematically in terms of population frequency rather than Mendelian probability of inheritance from the defined common ancestor.

- ***Measures of population stratification:*** in order to check for population admixture and stratification, principal components (PC) quality control was employed. This technique was used to detect pattern in relationships among individuals based on allele frequencies at small loci. The aim of using this approach was to infer a few underlying axes of variations (known as eigenvectors) that summarizes the allelic associations in the GWAS dataset. This approach was computationally efficient. Confounding by stratification was controlled by adjusting for genetic background and when testing for allelic association even at short-range.

  The difference between admixture and stratification is that admixture generates gametes that consist of a mosaic of segments inherited from each of the ancestral subpopulation, whereas stratification generates allelic associations that were independent of map distance. In either case, population admixture and stratification are often considered together because they usually occur together and because the two phenomena can be modeled with similar statistical methods.

  Ultimately, the goal of applying this methodology was to control for hidden population stratification as a confounder in genetic association studies, and to control for long-range association generated by admixture when undertaking fine mapping of a disease locus.

In summary, quality control (QC) filtering resulted in the first stage: 245,033 SNPs from the 320K array and 573 subjects, and in the second stage: 755,863 SNPs from the 1M Human-

DUO array and 639 subjects. QC genotyped data from both chips was then merged, totaling 857,445 SNPs and 1,212 subjects.

Imputation

In order to check for SNPs in the vicinity of the significant SNP associations, imputations to infer missing SNPs based on the genotypes of the typed SNPs as well as impute genotypes for SNPs not on the original panel were performed using build 36, release 22 HapMap CEU population as the reference (www.hapmap.org).

~2.5 million SNPs were attributed (imputed) using the maximum likelihood method implemented in MACH 1.0 software[49]. Genome-wide association analyses were performed using genotyped and imputed data.

Statistical analysis

Genome-wide association analysis of left ventricular hypertrophy

In the Italian study, genome-wide association analysis of left ventricular hypertrophy was performed using logistic regression analysis under an additive model with the ProbABEL[50] software for analyzing the genotyped and imputed SNPs. Logistic regression model was adjusted for sex, age, and age^2. A statistical significance threshold of *P* value < $5x10^{-7}$ used by the Wellcome Trust Case-Control study[51] was employed to identify genome-wide associated variants. Furthermore, a suggestive *P* value of < $1x10^{-5}$ was used for identifying potential candidate variants associated with LVH. The mathematical representation of this model is given as follows:

$$LVH \sim sex + age + age^2 + SNP,$$

where SNP genotypes under the additive models were coded as AA=0; Aa=1; and aa=2. Note that, “a” is the coded allele and “A” is the non-coded allele.

Genome-wide association analysis of left ventricular mass index

In the Italian study, genome-wide association analysis of left ventricular mass was performed using linear regression under an additive model using ProbABEL[50] software for analyzing all the genotyped and imputed SNPs. Linear regression model was adjusted for sex, age, and age^2. A statistical significance threshold of *P* value < $5x10^{-7}$ used by the Wellcome Trust Case-Control study[51] was employed to identify genome-wide associated variants. In addition, a suggestive *P* value of < $1x10^{-5}$ was used for identifying potential candidate variants associated with LV mass index.

$$LVMI \sim sex + age + age^2 + SNP,$$

where SNP genotypes under the additive models were coded as AA=0; Aa=1; and aa=2. Note that, "a" is the coded allele and "A" is the non-coded allele.

Genomic control

In order to correct for false-positives following GWA analyses of LVH and LVMI, a genomic control measure on the chi-square test statistic is computed. Thus, the chi-square test statistic for any candidate locus *c* is then corrected using the estimated inflation factor (lambda). For the additive model, an estimate of the inflation factor was calculated using the following mathematical expression:

$$estimated\ lambda = median((chisq_1)^2, (chisq_2)^2, (chisq_3)^2\ , \ldots., (chisq_N)^2) / 0.456,$$

where chisq, chi-square test statistic; N, number of SNPs tested for association;

The genomic controlled chi-square test statistic is defined as:

$$(chisq_{GC})^2 = (chisq_c)^2 / estimated\ lambda,$$

where $(chisq_{GC})^2$, genomic controlled chi-square test statistic; $(chisq_c)^2$, chi-square test statistic at locus *c*;

RESULTS

Study samples

Table 1 shows the descriptive data of the Italian study. The mean age was 48 ± 13 years. Out of 730 men and 482 women, 238 men (32.60 %) and 167 women (34.64 %) were diagnosed with left ventricular hypertrophy. The mean left ventricular mass index (LVMI) was 120.64 ± 19.23 g in men and 109.94 ± 16.87 g in women. The risk factor for hypertension was present in 754 subjects (62.21 %) and for smoking in 289 subjects (23.84 %). The mean SBP was 141.22 ± 22.24 mmHg, and mean DBP was 90.89 ± 13.39 mmHg. There not were significant differences of blood pressure traits among men and women. The mean BMI in men was 27.63 ± 3.34 kg/m^2 in men and 26.84 ± 4.79 kg/m^2 in women.

Table 1. Clinical characteristics of Italian study (N=1,212)

	Men	Women	Total
Age, mean (SD), years	47.86 (12.29)	49.06 (13.67)	48.33 (12.86)
Body mass index mean (SD), kg/m^2	27.63 (3.34)	26.84 (4.79)	27.32 (3.98)
Systolic BP mean (SD), mm Hg	141.22 (20.89)	141.21 (24.14)	141.22 (22.24)
Diastolic BP mean (SD), mm Hg	91.46 (13.22)	90.03 (13.63)	90.89 (13.39)
Hypertension, No. (%)	455 (62.32%)	299 (69.53%)	754 (62.21%)
Present smoking, No. (%)	187 (25.61%)	102 (21.16%)	289 (23.84%)
LVH, No. (%)	238 (32.60%)	167 (34.64%)	405 (33.42%)
LVM index mean (SD) , g/m^2	120.64 (19.23)	109.94 (16.87)	116.42 (19.06)

LV, left ventricular hypertrophy defined by LVMI >116 g/m^2 in men and >104 g/m^2 in women; LVM, left ventricular mass; Absolute value (percentage), No. (%); SD, standard deviation;

GWAS results of left ventricular hypertrophy (LVH)

The quantile-quantile (Q-Q) and Manhattan plots from the GWA results on LVH in the Italian study are illustrated in Figure 5 and Figure 6, respectively.

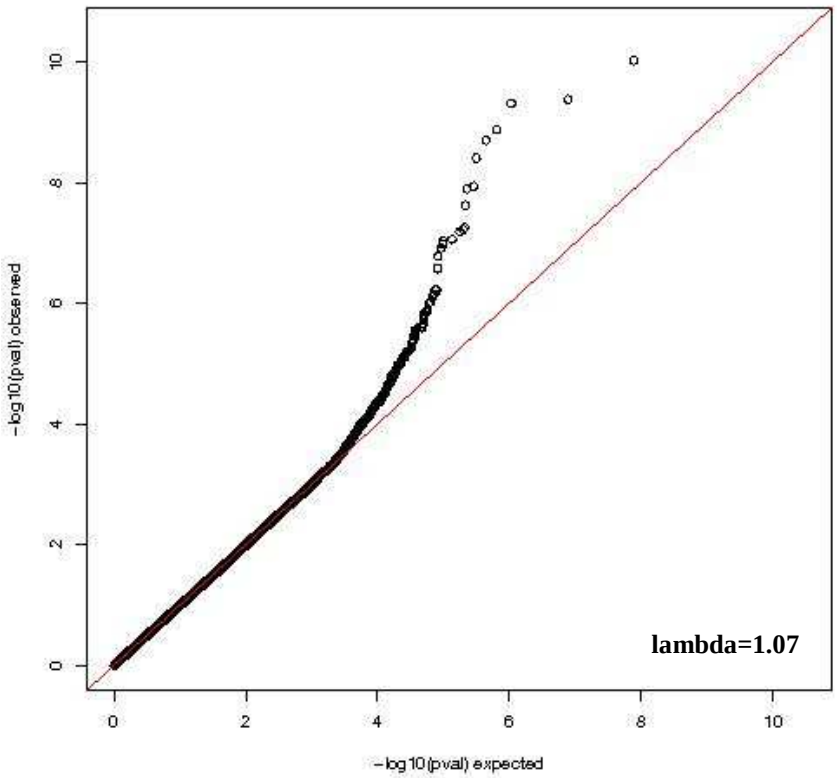


Figure 5. Q-Q plot of GWAS of LVH. The Q-Q plot depicts the observed versus the expected test statistics under the null hypothesis of no association. The black dots denote single nucleotide polymorphisms (SNPs) in regions known to be associated. The lambda denoted is the inflation factor estimated from chisq test statistic obtained from the logistic regression on LVH.

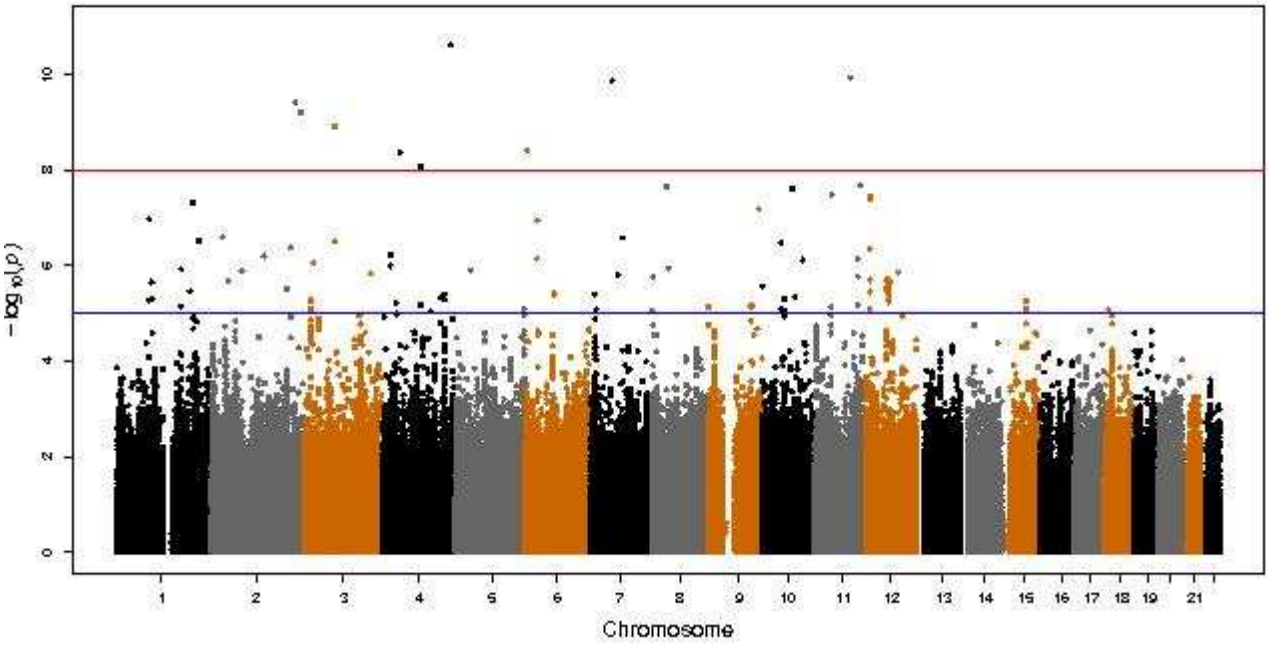


Figure 6. Manhattan plot displays results of the genome-wide association analysis on LVH. Shown are the $-\log_{10}(P)$ of all single nucleotide polymorphisms (SNPs) sorted by position. SNPs above the blue solid line (lower one) represent suggestive findings at P value < $1x10^{-5}$ whereas SNPs above the red solid line (upper one) represent findings at P value < $1x10^{-8}$.

From the genome-wide association (GWA) results on left ventricular hypertrophy (LVH), we identified 19 variants (shown in Table 2) at a significant value P < $5x10^{-7}$, a priori threshold used by the Wellcome Trust Case-Control[51]. Within the 19 GWAS hits for LVH, two variants were identified on chromosome 1 in *C1orf106* (rs6427864, P = $1.21x10^{-7}$), and in *MCOLN2* (rs1030932; P = $2.61x10^{-7}$); two nearby *IGBP5* (rs13389579, P = $1.33x10^{-9}$) and in *SP140* (rs4972945, P = $2x10^{-9}$); one on chromosome 3 nearby *ZNF717* (rs686591, P = $3.93x10^{-9}$); three on chromosome 4 in *VEGFG* (rs4557213, P = $9.52x10^{-11}$), in *GABRB1* (rs728294, P = $1.28x10^{-8}$), and in *ADH1C* (rs283410, P = $2.36x10^{-8}$); two on chromosome 6 nearby a pyruvate kinase pseudogene (rs93992718, P = $1.15x10^{-8}$) and in *HLA-DRA* (rs6911419, P = $2.69x10^{-7}$); one on chromosome 7 in *VSTM2A* (rs1403237, P = $4.90x10^{-10}$); one on chromosome 8 nearby *KCNU1* (rs7464912, P = $5.97x10^{-8}$); one on chromosome 9 in *ABL1* (rs10556171, P = $1.67x10^{-7}$); three on chromosome 11 between *HEPHL1* and *PANX1* (rs4753538, P = $4.19x10^{-10}$), in *ARHGEF12* (rs11217837, P = 5.54 $x10^{-8}$), in *SLC35C1*

(rs7130656, P = 8.71x10^{-8}); and two on chromosome 12 nearby *KLRA1* (rs11053849, P = 9.43x10^{-8} and rs10845156, P = 1.06x10^{-7}).

Table 2. Results of GWAS of left ventricular hypertrophy (LVH) at P value < 5x10^{-7}

SNP identifier	Chr	Position	Gene (nearest genes)	CA/ NCA	MAF	Beta	s.e.	*P*	P_G
rs6427864	1	199,126,036	*C1orf106* *(GPR25, C1orf106)*	C/T	0.225	0.855	0.156	4.73E-08	1.21E-07
rs1030932	1	85,179,836	*MCOLN2* *(LPAR3, MCOLN3)*	C/T	0.231	0.760	0.143	1.07E-07	2.61E-07
rs13389579	2	217,338,593	*(IGFBP5)*	A/G	0.435	-0.694	0.111	3.90E-10	1.33E-09
rs4972945	2	230,857,341	*SP140* *(SP110, SP140L)*	C/T	0.405	0.778	0.126	6.03E-10	2.00E-09
rs686591	3	76,134,314	*(ZNF717)*	A/G	0.378	-0.767	0.126	1.24E-09	3.93E-09
rs4557213	4	177,926,127	*VEGFC* *(SPCS3, NEIL3)*	A/G	0.492	-0.675	0.101	2.36E-11	9.52E-11
rs728294	4	47,052,170	*GABRB1* *(GABRA4,COMMD8)*	A/G	0.233	-0.876	0.149	4.32E-09	1.28E-08
rs283410	4	100,483,422	*ADH1C* *(ADH1B, ADH7)*	A/G	0.473	0.655	0.114	8.30E-09	2.36E-08
rs9392718	6	5,776,566		A/G	0.233	0.929	0.158	3.86E-09	1.15E-08
rs6911419	6	32,517,765	*HLA-DRA* *(BTNL2, HLA-DRB9)*	C/T	0.241	-0.803	0.151	1.10E-07	2.69E-07
rs1403237	7	54,602,061	*VSTM2A*	C/T	0.358	-0.799	0.124	1.35E-10	4.90E-10
rs7464912	8	36,981,063	*(KCNU1)*	A/C	0.345	-0.684	0.122	2.23E-08	5.97E-08
rs1056171	9	132,750,822	*ABL1* *(EXOSC2, QRFP)*	A/G	0.128	-1.278	0.237	6.67E-08	1.67E-07
rs2255649	10	79,013,818	*KCNMA1*	C/T	0.169	1.089	0.195	2.42E-08	6.45E-08
rs4753538	11	93,490,696	*(HEPHL1, PANX1)*	C/T	0.307	0.844	0.131	1.14E-10	4.19E-10
rs11217837	11	119,737,976	*ARHGEF12* *(TMEM136, GRIK4)*	A/G	0.304	-0.726	0.129	2.06E-08	5.54E-08
rs7130656	11	45,789,085	*SLC35C1* *(DKFZp779M0652, CRY2)*	A/G	0.370	-0.664	0.120	3.33E-08	8.71E-08
rs11053849	12	10,571,483	*(KLRA1)*	C/T	0.160	1.067	0.194	3.62E-08	9.43E-08
rs10845156	12	10,567,665	*(KLRA1)*	C/G	0.156	-1.080	0.197	4.09E-08	1.06E-07

SNP, single nucleotide polymorphism; Chr, chromosome; CA, coded allele; NCA, non-coded allele; MAF, minor allele frequency; Beta, genetic effect, se, standard deviation of beta; P, nominal P value; P_G, adjusted P value for genomic control;

The regional association plots of the first top GWA hits for LVH are illustrated below as well as brief discussion concerning the associated genetic finding.

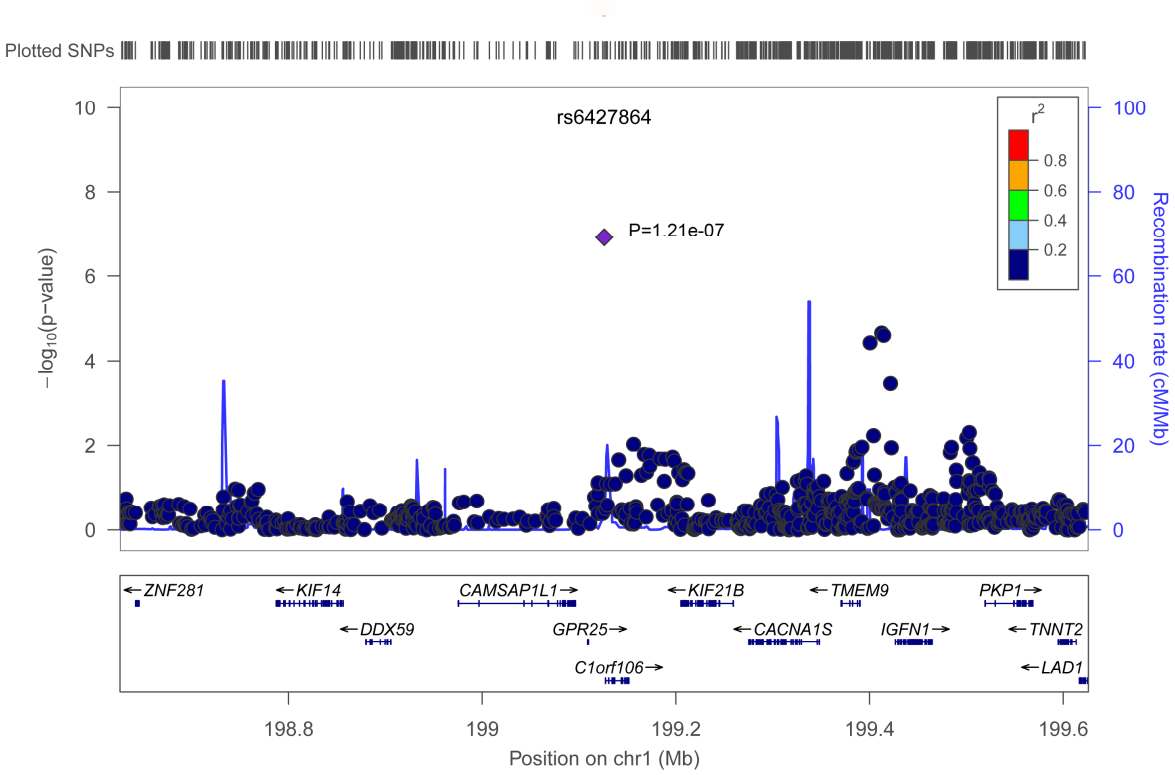


Figure 7. Regional association plot for 1q32.1. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The chromosome 1 open reading frame 206 (*C1orf106*) is a common susceptible locus for complex diseases, such as Crohn's disease, and ulcitis disease[51]. Its exact cause is unknown, but it has been shown recently that *C1orf106* interacts with TEC tyrosine kinase[52], a set of proteins involved in intracellular signaling mechanisms of cytokine receptors, lymphocyte surface antigens, heterotrimeric G-proteins coupled receptors, and integrin molecules. Furthermore, TEC tyrosine kinase proteins are key regulatory molecules in cardiac injury and protection as well as in physiological response to angiotensin II[53]. The role

of tyrosine kinases in cardiac remodeling has been well documented. A recent study has shown that an TEC isoform, *Bmx* is implicated in angiogenesis and that is a necessary component of compensatory cardiac hypertrophy[54].

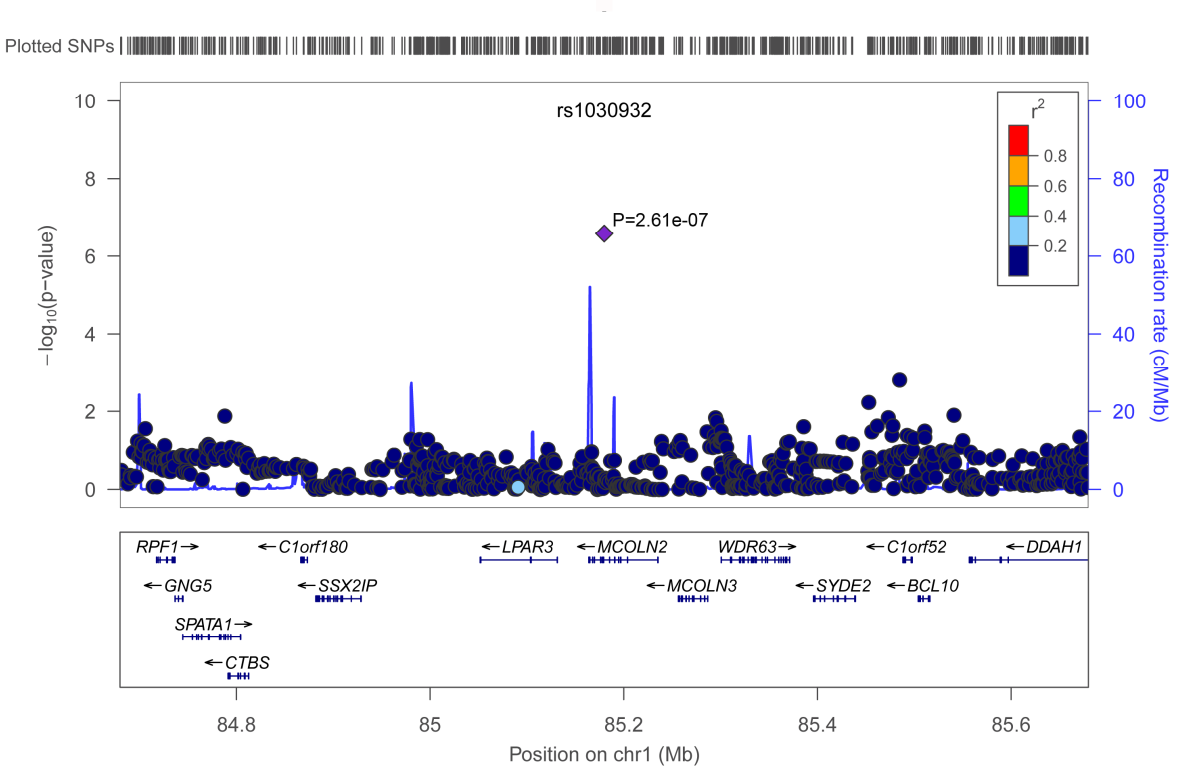


Figure 8. Regional association plot for 1p22.3. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The mucolin 2 (*MCOLN2*) is a member of the Mucolilins proteins, a family of ion channel proteins with homology to the transient receptor potential (TRP) superfamily of cation channels[55]. These proteins play various roles in the regulation of membrane and protein sorting. In the heart, *MCONL1* is widely expressed. Although the function of *MCONL2* is not well known and it has not been associated with any human pathology, evidence shows that the *MCONL2* channel is functional at the plasma membrane and is characterized by a

significant inward rectification similar to other *MCONL* mutant isoforms[56]. Moreover, biochemical interactions have been observed between in homo- and hetero-multimeric combinations[57].

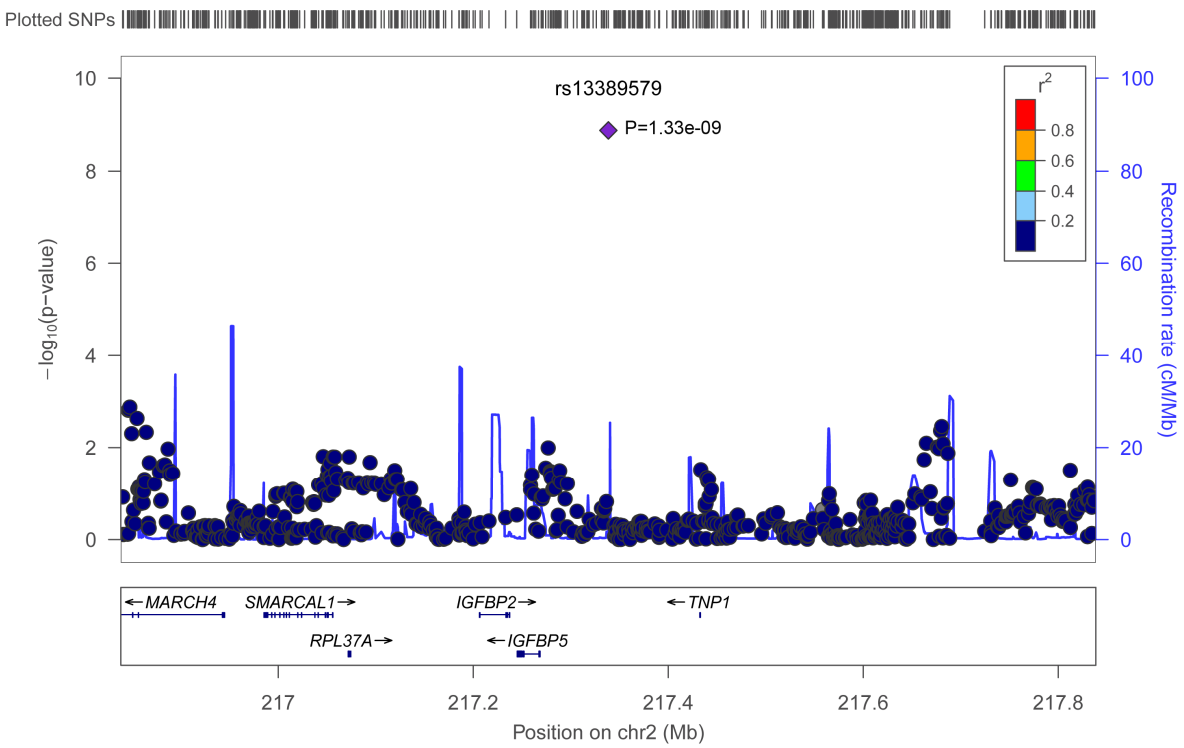


Figure 9. Regional association plot for 2q35. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The insulin-like growth factor binding protein 5 *(IGFBP5)* is a member of the IGFBP family of six secreted proteins that play an important role in the regulation of insulin growth factors (*IGF1* and *IGF2)*. IGFBPs exhibit distinct structural and biochemical properties, function as carrier proteins in the circulation, and regulate insulin growth factor (*IGF*) turnover, transport, and half-life of circulating IGFs[58]. The functional role of *IGFBP5* in the heart is currently unclear, but IGFBPs that are similar to *IGFBP5*, such as *IGFBP2,* have been shown to be involved in cardiovascular defects in targeted knockdown zebra fish

embryos[59]. Likewise, *IGFBP1* has been associated with mediating hypoxia-induced growth and developmental retardation in embryo and adult zebra fish[60-62].

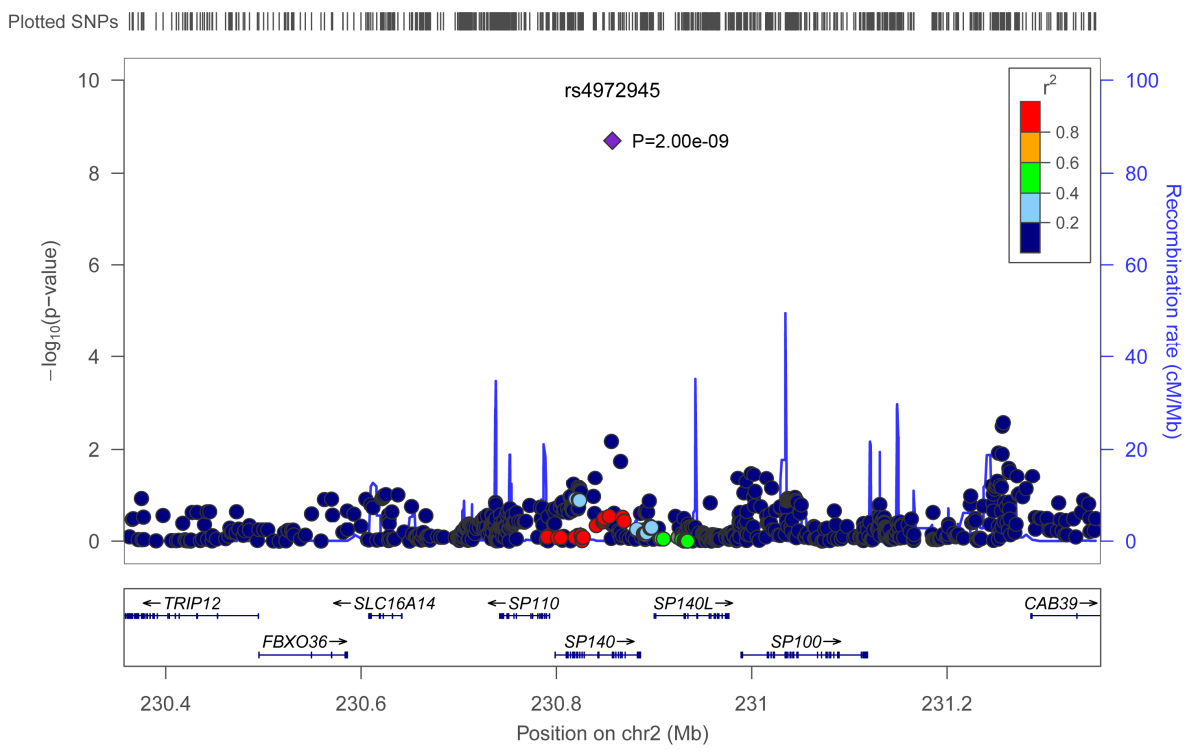


Figure 10. Regional association plot for 2q37.1. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The *SP140*, a nuclear body protein, is implicated in the control of cellular differentiation, cell growth, and gene transcription.[63, 64] The biological processes of this gene makes it a potential candidate for the development of LVH. *SP140* is upregulated in response to stimuli by cytokines, such as interferons (INFs), a group of proteins involved in pro-inflammatory activities.

In pathological LVH, an increase of interferons has been shown to activate macrophages to produce nitric oxide, which increases vascular permeability across hypertrophied cardiomyocytes[65]. Moreover, activated macrophages produced abundant

ACE, resulting in local increased production of angiotensin II (Ang II), the effector peptide of the rennin-angiotensin system (RAS) that regulates the volume and electrolyte homeostasis and is involved in vascular and cardiomyocyte growth[66].

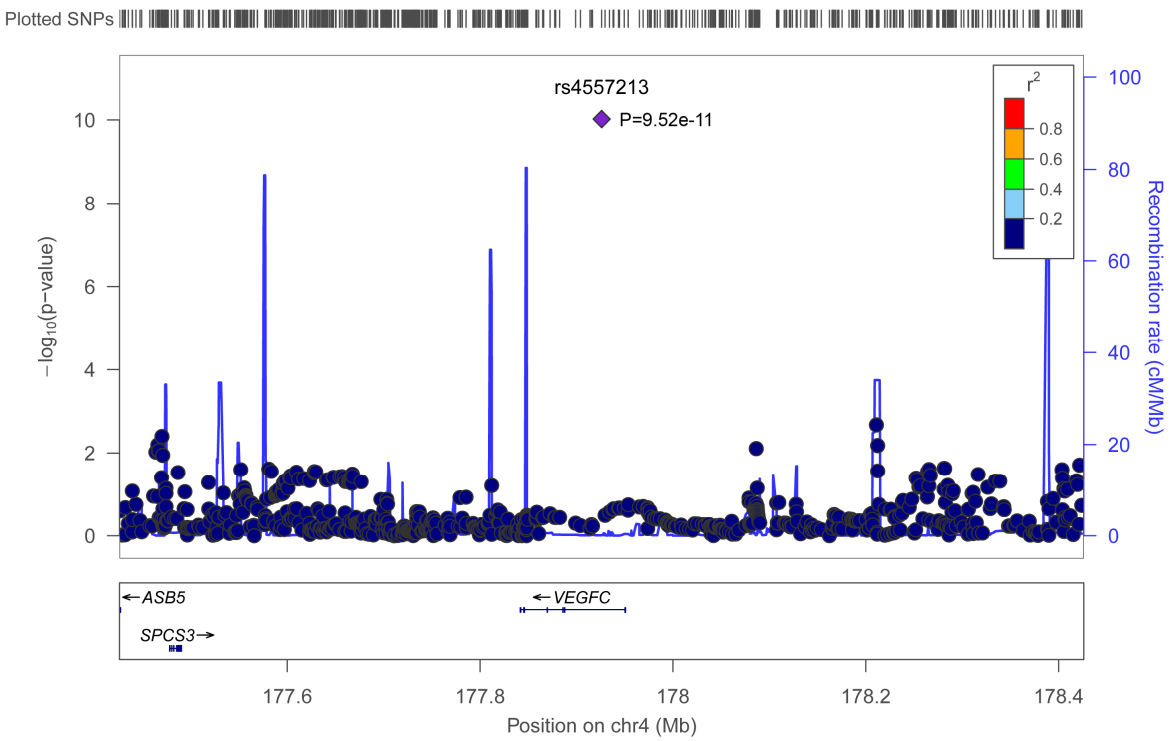


Figure 11. Regional association plot for 4q34.3. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The vascular endothelial growth-factor C (*VEGF-C*) is homologous with other members of the *VEGF*/platelet derived growth factor (*PDGF*) family and is a novel regulator of endothelia. *VEGF*-C is produced as a precursor protein, which is proteolitically processed, and binds to ligands, *VEGFR-2* and *VEGFR-3,* inducing tyrosine autophosphorylation[67], thus inducing myocardial vascularization during embryogenesis and in adult mouse heart[68, 69]. *VEGF-C* is localized in epicardial vessels[70] and expression of ligand *VEGFR-3* is highly present throughout the ventricles[68]. Furthermore, *VEGF-C* is highly expressed in

cardiomyocytes following at myocardial infarction, particularly around the lesion and in all stages of remodeling[71].

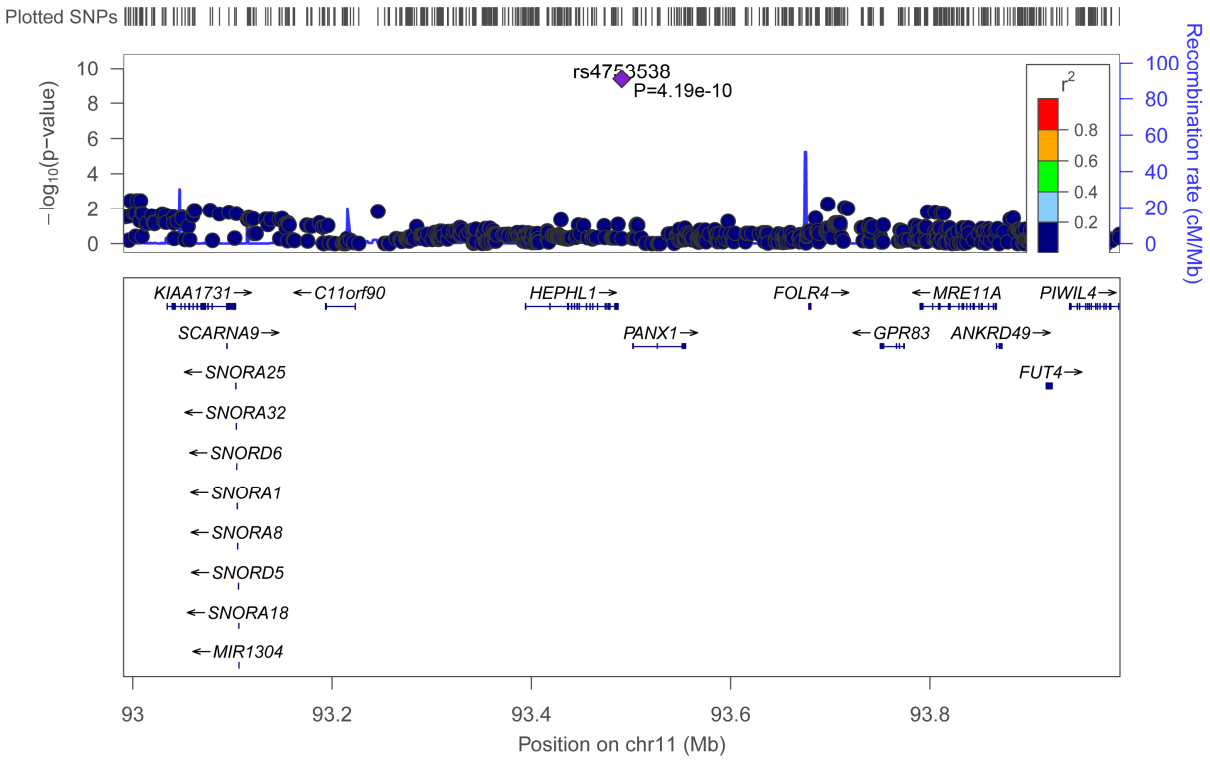


Figure 12. Regional association plot for 11q21. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The hephaestin-like 1 (*HEPHL1*) is similar to the hephaestin (*HEPH*), a homologue of the ceruloplasmin (*CP*) gene. Little is known about the biological functionality of the *HEPHL1* or its relationship with cardiomyocyte hypertrophy. However, the protein encoded by the *HEPHL1* gene shares similar properties with proteins encoded by the *CP* and *HEPHL* genes, which are ferroxidase enzymes involved in the heart iron and copper metabolism[72, 73]. Copper reverses hypertrophic cardiomyopathy induced by pressure overload in mice model through *VEGF/VEGF-R1* signaling pathway[74, 75]; whereas, iron overload in the heart leads to iron deposition in the myocardium leading to cardiomyopathy[76].

The pannexin 1 (*PANX1*) is homologous to the invertebrate innexins, the structural components of gap junctions[77]. Pannexins and innnexins share considerable structural similarities, but their sequence homology is different from connexins (Cx), the typical gap junction proteins. *PANX1*, one of three in the pannexin gene family, is ubiquitously expressed in the body, including in the cardiomyocytes. *PANX1* biological functionality relates to mediation of ATP release in intracellular calcium wave initiation and propagation upon mechanical stress (or other stimuli, such as depolarization) and release ATP to the extracellular medium[78]. Hypertrophied cardiomyocytes are characterized by multiple genetic changes that modify the integration of pathways utilized for energy synthesis and intracellular calcium homeostasis[79]. Moreover, in the hypertrophied heart where ischemia-conditions are constantly threatening host cells, contraction and relaxation patterns are modified by molecular mechanisms in order to meet oxygen and metabolic demands in the heart. A recent study has shown that *PANX1* is active during ischemic-conditions in cardiac tissue[80].

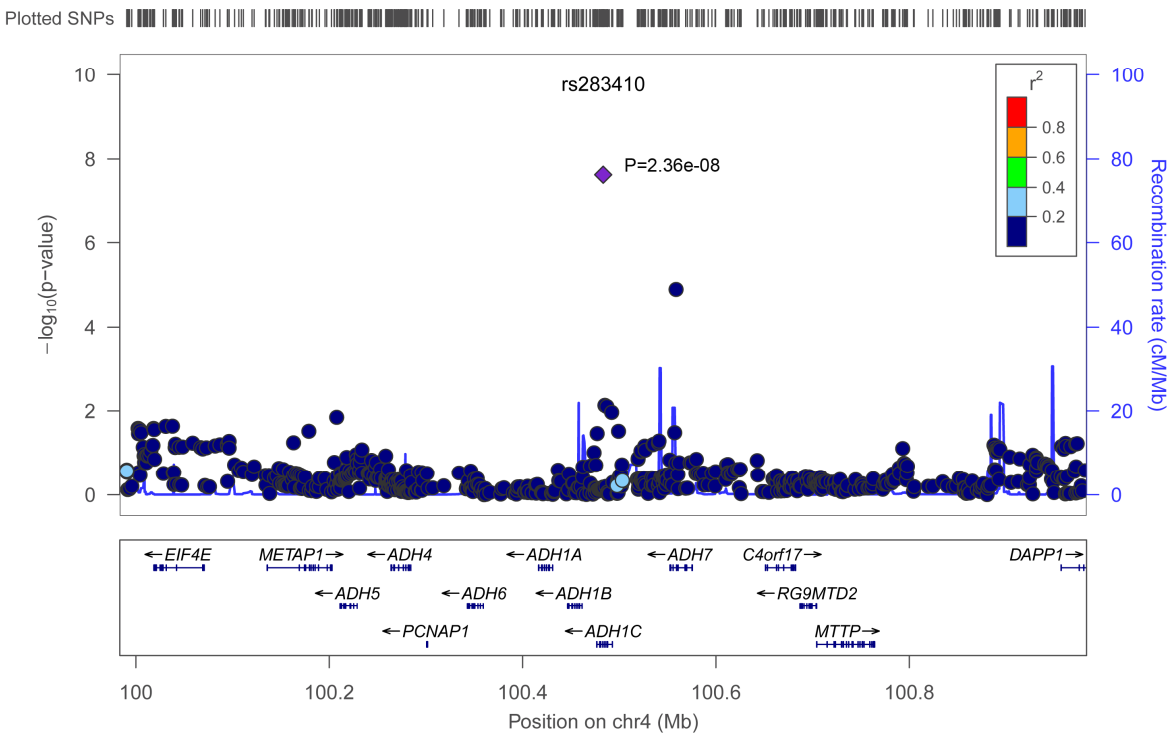


Figure 13. Regional association plot for 4q23. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The alcohol dehydrogenase 1C (class 1), gamma polypeptide (*ADH1C*), a cytoplasmic protein that encodes a class I alcohol dehydrogenase, gamma subunit. *ADH1C* exhibits high activity for ethanol oxidation and metabolizes a wide variety of substrates, including other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products (www.genecards.org). Functional relevant polymorphisms are found in the genes encoding class I alcohol dehydrogenase (ADH) isoenzymes, *ADH1C* and *ADH1B*, affecting ethanol degradation rates and alcohol intake in white populations[81]. Expression of *ADH1C* is found ubiquitously in the whole body, including the liver, kidney, skeletal muscle, lung, heart, and digestive tract.

Additional variants identified at suggestive *P* value < $1x10^{-5}$ are given in Supplementary Table 1 in the Appendix.

GWAS results of left ventricular mass index

The genome-wide association results for left ventricular mass index (LVMI) illustrated by the Q-Q plot and Manhattan plot, given in Figure 14 and Figure 15, respectively.

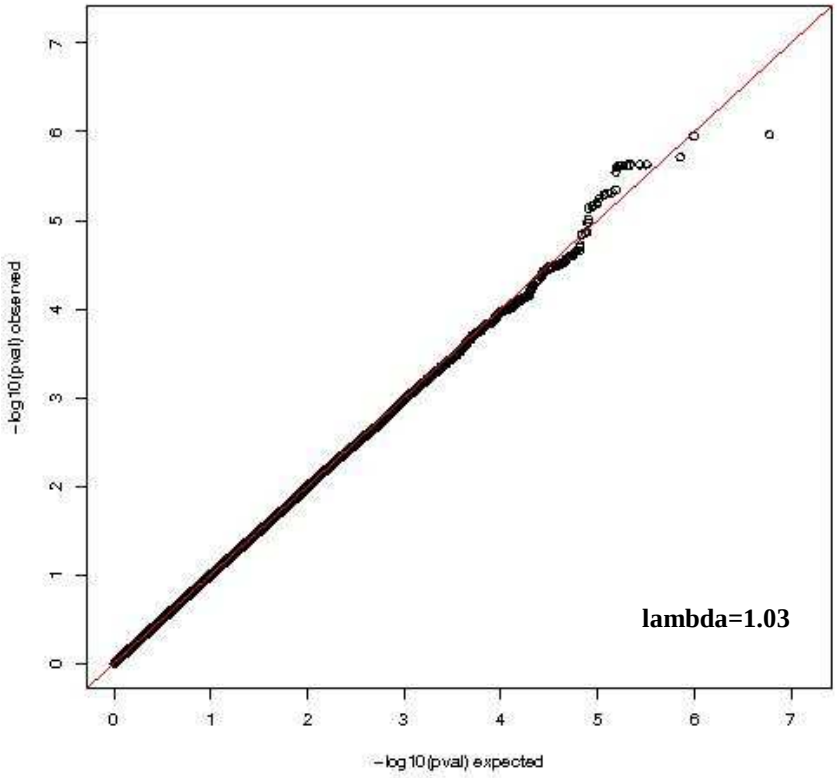


Figure 14. Q-Q plot of GWAS of LV mass index. The Q-Q plot depicts the observed versus the expected test statistics under the null hypothesis of no association. The black dots denote single nucleotide polymorphisms (SNPs) in regions known to be associated. The lambda denoted is the inflation factor estimated from chisq test statistic obtained from the linear regression on LV mass index.

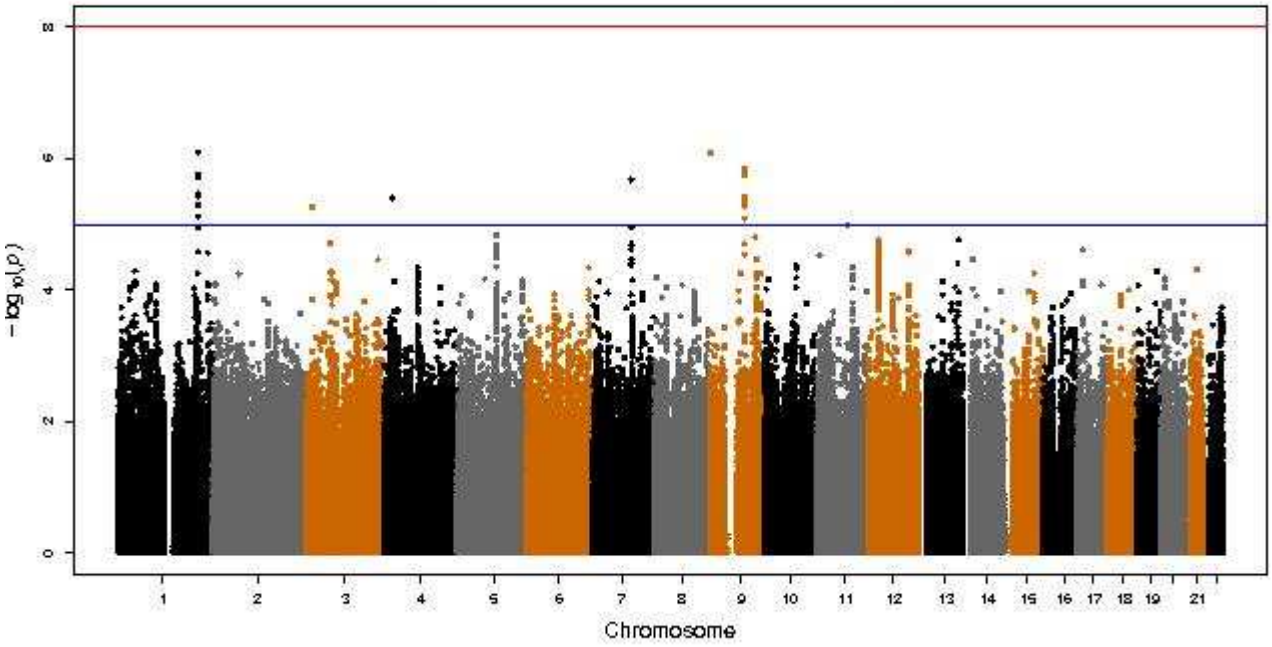


Figure 15. Manhattan plot displays results of the genome-wide association analysis on LV mass index. Shown are the $-\log_{10}(P)$ of all single nucleotide polymorphisms (SNPs) sorted by position. SNPs above the blue solid line (lower one) represent suggestive findings at *P* value < $1x10^{-5}$ whereas SNPs above the red solid line (upper one) represent findings at *P* value < $1x10^{-8}$.

The GWAS results for LVMI yielded no associated variant at *P* value < $5x10^{-7}$. However, at suggestive *P* value < $1x10^{-5}$, we identified 23 variants that were associated with LVMI as shown in Table 3.

Interestingly for this trait, the majority of suggestive findings fell within a 97.6 Kb region (from 208,466,275 to 208,368,666) on chromosome 1 in *SYT14* and within a 3.4 Kb region (from 88,225,212 to 88,221,807) on chromosome 9 in the vicinity of *GAS1*.

Table 3. Results of GWAS of left ventricular mass index (LVMI) at suggestive *P* value < 1x10-5

SNP identifier	Chr	Position	Gene (nearest gene)	CA/ NCA	MAF	Beta	s.e.	*P*	P_G
rs12739243	1	208,368,666	*SYT14 (C1orf107, C1orf133)*	C/T	0.170	5.783	1.292	7.55E-06	9.80E-06
rs11119421	1	208,387,666	*SYT14 (C1orf107, C1orf133)*	A/G	0.219	-5.334	1.119	1.89E-06	2.54E-06
rs11119422	1	208,389,448	*SYT14 (C1orf107, C1orf133)*	C/T	0.219	-5.350	1.121	1.83E-06	2.46E-06
rs7516843	1	208,389,552	*SYT14 (C1orf107, C1orf133)*	A/G	0.218	-5.355	1.122	1.81E-06	2.43E-06
rs12029138	1	208,391,580	*SYT14 (C1orf107, C1orf133)*	A/G	0.218	-5.360	1.122	1.78E-06	2.40E-06
rs12130989	1	208,394,563	*SYT14 (C1orf107, C1orf133)*	A/G	0.107	7.711	1.692	5.17E-06	6.77E-06
rs11119423	1	208,395,015	*SYT14 (C1orf107, C1orf133)*	C/T	0.217	-5.361	1.122	1.75E-06	2.36E-06
rs4537554	1	208,398,208	*SYT14 (C1orf107, C1orf133)*	C/T	0.217	-5.355	1.121	1.77E-06	2.38E-06
rs4609425	1	208,401,689	*SYT14 (C1orf107, C1orf133)*	A/G	0.218	5.632	1.141	7.92E-07	1.09E-06
rs1338298	1	208,462,685	*(SYT14, C1orf133)*	A/G	0.317	-4.646	1.004	3.69E-06	4.87E-06
rs1473696	1	208,464,175	*(SYT14, C1orf133)*	C/T	0.316	4.617	0.994	3.43E-06	4.54E-06
rs677520	1	208,466,275	*SYT14, C1orf133*	C/T	0.320	-4.620	0.999	3.74E-06	4.93E-06
rs656312	1	208,486,479	*(SERTAD4)*	C/G	0.402	-4.732	0.990	1.74E-06	2.33E-06
rs3887276	3	14,861,366	*FGD5 (C3orf20, NR2C2)*	C/T	0.279	5.142	1.132	5.56E-06	7.26E-06
rs12509827	4	22,959,397	*(GBA3, PPARGC1A)*	A/G	0.131	-8.544	1.852	3.98E-06	5.25E-06
rs2074685	7	100,648,984	*ZNHIT1 (PLOD3,*	C/T	0.324	-4.846	1.022	2.11E-06	2.82E-06

			CLDN15)						
rs7860714	9	1,856,537	*(DMRT2, SMARCA2)*	A/G	0.407	-7.950	1.612	8.17E-07	1.12E-06
rs11792039	9	88,221,807	*(GAS1)*	A/C	0.182	6.138	1.349	5.36E-06	7.01E-06
rs11141385	9	88,222,194	*(GAS1)*	A/G	0.191	-6.439	1.336	1.43E-06	1.94E-06
rs11141386	9	88,223,866	*(GAS1)*	A/T	0.187	6.386	1.336	1.75E-06	2.35E-06
rs12377012	9	88,224,793	*(GAS1)*	A/G	0.171	-6.291	1.362	3.88E-06	5.12E-06
rs12375764	9	88,225,190	*(GAS1)*	A/T	0.170	6.242	1.358	4.30E-06	5.65E-06
rs11141387	9	88,225,212	*(GAS1)*	A/T	0.169	-6.181	1.352	4.87E-06	6.38E-06

SNP, single nucleotide polymorphism; Chr, chromosome; CA, coded allele; NCA, non-coded allele; MAF, minor allele frequency; Beta, genetic effect, se, standard deviation of beta; *P*, nominal *P* value; P_G, adjusted *P* value for genomic control;

Regional association plots for LVMI at suggestive *P* value for interesting loci are illustrated below.

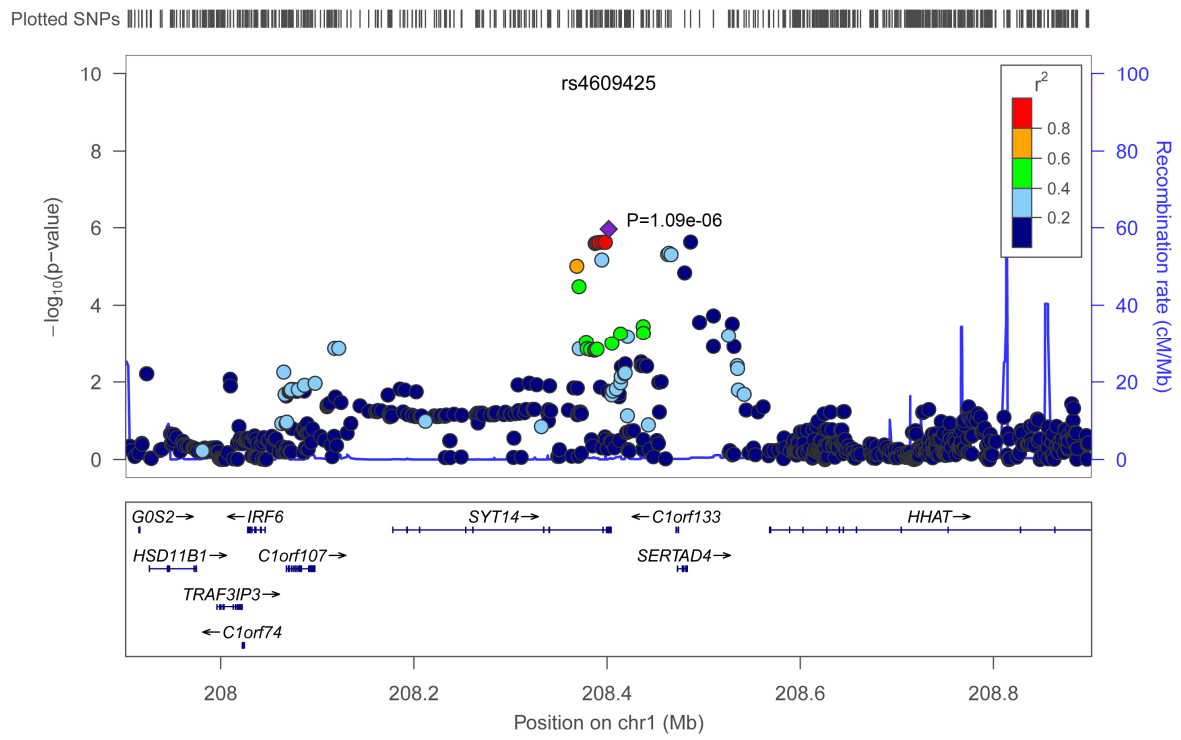


Figure 16. Regional association plot for 1q32.2. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The synaptotagmin 14 (*SYT14*) is a member of Sypnaptotagmins, a large family of putative membrane trafficking proteins that are involved in various biological functions, including regulation of Ca^{2+}-triggered cellular events through Ca^{2+}-regulated membrane trafficking[82]. Microarray data indicate that *SYT14* is expressed ubiquitously in the body in various ways, including upregulation in the atrioventricular node[82]. There is not direct relationship between *SYT14* and hypertrophic cardiomyocytes; however, a homologue member of the Syt family, *SYT1* interacts with the SNARE protein in rises of cytosolic Ca^{2+}. The SNARE protein has been shown to regulate cardiac potassium channels and natriuretic factor secretion in cardiomyocytes, particularly during conditions of severe stress, including myocardial ischemia, hypoxia, and metabolic inhibition[83].

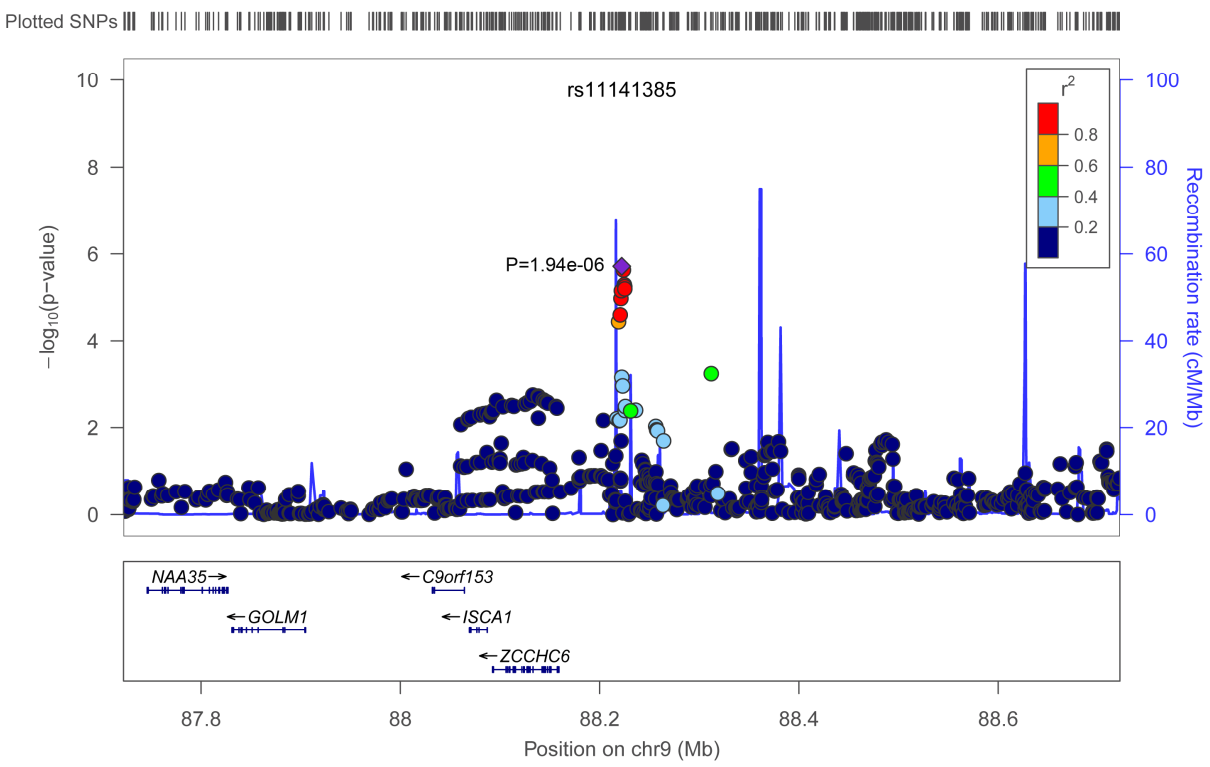


Figure 17. Regional association plot for 9q.21.33. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

The protein encoded by the growth arrest-specific 1 (*GAS1*) gene is involved in growth suppression, preventing normal and transformed cells at G_0 from traversing into the S phase[84, 85]. During cardiac morphogenesis, *GAS1* is highly expressed in the endocardium and myocardium of the developing heart[86]. Recent data show that in the adult mice heart, *GAS1* is expressed higher in the left ventricle than in any other anatomical cardiac structure under normal conditions, leading the authors to hypothesize that *GAS1* is one of the genes that would be a potential regulator, playing a causal role under hypertrophic cardiomyocyte stimulation[87]

DISCUSSION

Left ventricular hypertrophy is a heterogeneous disorder in which patients can be stratified by pathophysiological characteristics, i.e., expressing distinct cardiac hypertrophic phenotypes. Some causes of cardiac hypertrophic can be identified (as in monogenic forms), and some can be suspected (as in the adaptive hypertrophy for the athletic heart). Although aspects of the pathophysiology of cardiac hypertrophic can be identified in many patients, the molecular mechanisms involved in pathological LVH are not yet known. Furthermore, left ventricular hypertrophy encloses a vast etiologic variability, reflected by genetic and phenotypic heterogeneity.

Many studies have demonstrated that 60% of LV mass variations is heritable and that most likely genetic factor play a major role. However, the genetics of left ventricular hypertrophy is poorly understood. Moreover, the fact that the prevalence of LVH varies within and across populations, demonstrate that the etiology of LVH is a combination of genetic predispositions and environmental influencing the features manifested in the phenotype of LVH. As for the Mendelian forms of LVH, this only account for small percentage of all LVH cases in a population, leaving the remaining unaccounted for causes to be identified.

Additionally, apart from the combination or the pooling of genetic and environmental factors, most plausibility there is a set of multiple gene interactions working together or independently at many different cellular levels (i.e., gene-gene interactions), as well as a set of gene influenced by environmental factors that are likely to induce epigenetic changes affecting DNA transcriptional activity (gene-environment interactions). A graphical interpretation of these interactions can be captured by the following illustration.

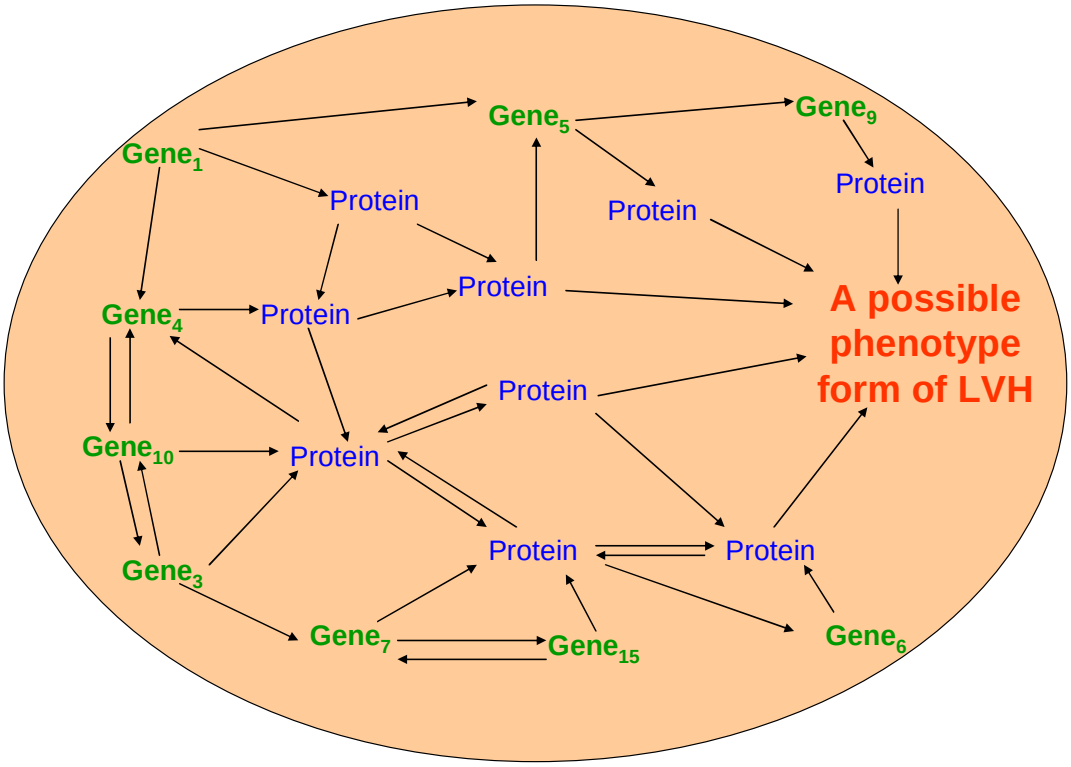


Figure 18. Illustration of linked mechanisms involving gene-gene interactions for developing for possible phenotype form of LVH

Thus, the genetic variations of LVH are plausibly caused by polymorphic genetic differences, implicating complex interactions among various genes, and interactions among genetic and environmental factors. Several strategies have begun to address the genetic basis of left ventricular hypertrophy.

One approach has been to seek variant candidate genes and associate them with the risk of LVH as it has been explained in previously. A much more advanced approach is by means of genome-wide association studies (GWAS), a high throughput genotyping, utilizing single nucleotide polymorphism (SNP) arrays having hundreds of thousands of SNPs as DNA markers distributed across the human genome. These markers can identify the location of genes that influence the susceptibility to LVH by exploiting various phenotypic forms of LVH or related-quantitative traits that influence the LVH phenotype, such as LV mass.

In this work, performing GWA analysis allowed to identify loci associated with left ventricular hypertension and LV mass index anywhere across the genome with more

certainty and without many of the hurdles and limitations encountered as in other types of genetic studies. Although the ability to detect a locus depends on many factors (i.e., the model design and assumptions, the population genetic homogeneity, the sampling bias, the genetic structures, the strength of the effect of each locus, the linkage disequilibrium between the markers tested at the locus, and the environmental factors accounted in the study), this study aimed to examine a small part of the vast complexity that left ventricular hypertrophy encompasses. From the GWA analysis on left ventricular hypertrophy (LVH) and left ventricular mass index (LVMI) conducted in a population study of 1,212 individuals from white European ancestry, 19 variants associated with LVH were identified at a *P* value < $5x10^{-7}$, the pre-specified genome-wide significance threshold in the Welcome Trust Case-Control study[51]. These 19 variants correspond to 12 intronic and 7 intergenic markers located in different genetic loci, including *C1orf106, MCOLN2, IGFBP5, SP140, ZNF717, VEGFG, GABRB1, ADH1C, HLA-DRA, VSTM2A, KCNU1, ABL1, KCNMA1, HEPHL1, PANX1, ARHGEF12, SLC35C1, and KLRA1* genes. Additionally, two more other potential loci of interest (*SYT14* and *GAS1*) associated with LV mass index merit to be further studied.

A limitation of the study is the 62% of subjects were receiving antihypertensive therapy at the time of the study, which may resulted in underestimation of the role of genetic factors of LVH and possibly reserve the hypertrophic changes on the LV mass. Nevertheless, we found potential findings that need to be further validated in other cohorts as well as proven their functionality in animal model settings.

APPENDIX

Supplementary Table 1 Results of GWAS of left ventricular hypertrophy (LVH) at suggestive *P* value < $1x10^{-5}$

SNP identifier	Chr	Position	Gene (nearest gene)	CA/ NCA	MAF	Beta	s.e.	*P*	P_G
rs6427864	1	199,126,036	*C1orf106 (GPR25, C1orf106)*	C/T	0.225	0.855	0.156	4.73E-08	1.21E-07
rs1030932	1	85,179,836	*MCOLN2 (LPAR3, MCOLN3)*	C/T	0.231	0.760	0.143	1.07E-07	2.61E-07
rs12734277	1	213,029,394	*(CENPF)*	C/T	0.326	0.664	0.130	3.06E-07	7.01E-07
rs12409341	1	169,030,050	*(PRRX1, C1orf129)*	A/G	0.274	-0.734	0.151	1.21E-06	2.56E-06
rs281959	1	91,514,643	*HFM1*	C/T	0.380	0.660	0.139	2.19E-06	4.48E-06
rs4329494	1	190,342,868	*(RGS18)*	C/T	0.223	0.664	0.143	3.52E-06	6.99E-06
rs1059267	1	92,747,850	*EVI5 (GFI1, RPL5)*	A/C	0.208	0.662	0.145	5.05E-06	9.83E-06
rs13389579	2	217,338,593	*(IGFBP5)*	A/G	0.435	-0.694	0.111	3.90E-10	1.33E-09
rs4972945	2	230,857,341	*SP140 (SP110, SP140L)*	C/T	0.405	0.778	0.126	6.03E-10	2.00E-09
rs3820821	2	27,009,352	*DPYSL5 (MAPRE3)*	C/T	0.245	-0.754	0.146	2.58E-07	5.98E-07
rs12694040	2	206,690,791	*(NDUFS1)*	C/T	0.328	0.661	0.131	4.20E-07	9.45E-07
rs3769027	2	135,393,505	*CCNT2 (YSK4)*	C/T	0.363	-0.613	0.123	6.24E-07	1.37E-06
rs4853363	2	78,120,485	*(CYCSP6)*	G/T	0.433	0.476	0.098	1.32E-06	2.77E-06
rs10180152	2	41,821,726	*(LDHAL3)*	A/G	0.286	-0.619	0.130	2.11E-06	4.31E-06
rs2718443	2	193,983,293		C/T	0.199	-0.742	0.159	3.12E-06	6.24E-06
rs1371639	2	193,979,588		A/C	0.198	0.747	0.160	3.12E-06	6.24E-06
rs686591	3	76,134,314	*(ZNF717)*	A/G	0.378	-0.767	0.126	1.24E-09	3.93E-09
rs676433	3	76,116,213	*(ZNF717)*	A/T	0.288	0.748	0.146	3.15E-07	7.21E-07
rs9864800	3	21,783,852	*(ZNF385D)*	C/T	0.321	0.687	0.139	8.57E-07	1.85E-06
rs7611653	3	169,406,166	*(GOLIM4)*	G/T	0.114	-1.227	0.255	1.45E-06	3.04E-06
rs4685016	3	13,783,011	*(FBLN2, WNT7A)*	C/T	0.290	0.539	0.118	5.11E-06	9.94E-06
rs4557213	4	177,926,127	*VEGFC (SPCS3, NEIL3)*	A/G	0.492	-0.675	0.101	2.36E-11	9.52E-11
rs728294	4	47,052,170	*GABRB1 (GABRA4,*	A/G	0.233	-0.876	0.149	4.32E-09	1.28E-08

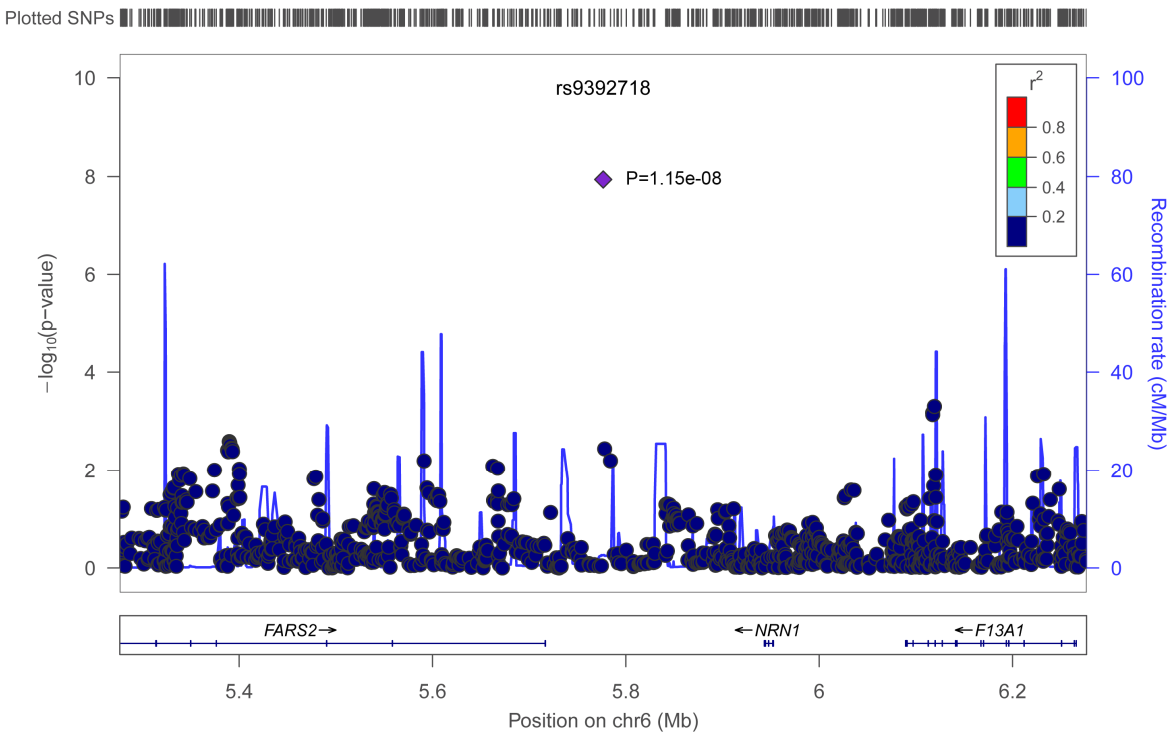
			COMMD8)						
rs283410	4	100,483,422	*ADH1C (ADH1B, ADH7)*	A/G	0.473	0.655	0.114	8.30E-09	2.36E-08
rs12509827	4	22,959,397	*(GBA3, PPARGC1A)*	A/G	0.131	-0.946	0.189	5.93E-07	1.31E-06
rs1364841	4	20,801,088	*KCNIP4 (PACRGL, NCRNA00099)*	A/G	0.427	-0.591	0.121	1.01E-06	2.15E-06
rs6858366	4	162,053,730	*(RAPGEF2, FSTL5)*	C/G	0.327	-0.594	0.129	3.91E-06	7.72E-06
rs12641441	4	152,827,073	*PET112L (FAM160A1, FBXW7)*	A/G	0.222	0.649	0.142	4.67E-06	9.13E-06
rs7669761	4	152,828,754	*PET112L (FAM160A1, FBXW7)*	A/G	0.222	-0.649	0.142	4.68E-06	9.14E-06
rs7724224	5	38,310,069	*EGFLAM (GDNF, LIFR)*	A/G	0.268	-0.682	0.141	1.23E-06	2.60E-06
rs9392718	6	5,776,566		A/G	0.233	0.929	0.158	3.86E-09	1.15E-08
rs6911419	6	32,517,765	*HLA-DRA (BTNL2, HLA-DRB9)*	C/T	0.241	-0.803	0.151	1.10E-07	2.69E-07
rs3115672	6	31,835,876	*MSH5 (CLIC1, C6orf26)*	C/T	0.345	-0.627	0.126	6.90E-07	1.51E-06
rs2917891	6	74,644,390	*(CD109)*	G/T	0.206	-0.725	0.157	3.71E-06	7.35E-06
rs3012568	6	74,644,421	*(CD109)*	G/T	0.205	0.725	0.158	4.16E-06	8.19E-06
rs1403237	7	54,602,061	*VSTM2A*	C/T	0.358	-0.799	0.124	1.35E-10	4.90E-10
rs1228867	7	83,869,480	*(SEMA3A, HMG17P1)*	C/T	0.144	1.074	0.209	2.61E-07	6.05E-07
rs6965211	7	69,735,201	*AUTS2 (STAG3L4, WBSCR17)*	C/T	0.302	0.647	0.135	1.58E-06	3.29E-06
rs2189349	7	11,309,599	*(PHF14, THSD7A)*	C/T	0.400	0.535	0.116	3.95E-06	7.80E-06
rs7464912	8	36,981,063	*(KCNU1)*	A/C	0.345	-0.684	0.122	2.23E-08	5.97E-08
rs12164144	8	43,820,269	*(ASNSL1)*	C/T	0.165	-0.844	0.173	1.13E-06	2.39E-06
rs4875308	8	4,048,012	*CSMD1 (MYOM2)*	A/G	0.272	-0.651	0.136	1.70E-06	3.53E-06
rs1056171	9	132,750,822	*ABL1 (EXOSC2, QRFP)*	A/G	0.128	-1.278	0.237	6.67E-08	1.67E-07

rs2255649	10	79,013,818	*KCNMA1*	C/T	0.169	1.089	0.195	2.42E-08	6.45E-08
rs7075976	10	49,284,283	*MAPK8 (ARHGAP22)*	A/G	0.328	-0.602	0.118	3.34E-07	7.63E-07
rs4918120	10	106,372,348	*(CCDC147, SORCS3)*	C/T	0.405	0.560	0.113	7.58E-07	1.65E-06
rs2813452	10	1,557,300	*ADARB2 (C10orf109)*	C/T	0.422	-0.528	0.113	2.70E-06	5.44E-06
rs1324245	10	85,284,722		C/T	0.190	0.813	0.177	4.46E-06	8.74E-06
rs10160121	10	56,836,579	*(PCDH15)*	A/G	0.226	0.687	0.151	5.05E-06	9.83E-06
rs4753538	11	93,490,696	*(HEPHL1, PANX1)*	C/T	0.307	0.844	0.131	1.14E-10	4.19E-10
rs11217837	11	119,737,976	*ARHGEF12 (TMEM136, GRIK4)*	A/G	0.304	-0.726	0.129	2.06E-08	5.54E-08
rs7130656	11	45,789,085	*SLC35C1 (DKFZp779M0652, CRY2)*	A/G	0.370	-0.664	0.120	3.33E-08	8.71E-08
rs1369817	11	112,433,374	*NCAM1*	G/T	0.325	0.622	0.125	7.01E-07	1.53E-06
rs2574829	11	112,433,167	*NCAM1*	C/G	0.214	-0.943	0.197	1.66E-06	3.45E-06
rs11053849	12	10,571,483	*(KLRA1)*	C/T	0.160	1.067	0.194	3.62E-08	9.43E-08
rs10845156	12	10,567,665	*(KLRA1)*	C/G	0.156	-1.080	0.197	4.09E-08	1.06E-07
rs10844135	12	9,636,873	*(OVOS, KLRB1)*	A/C	0.262	-0.723	0.143	4.47E-07	1.00E-06
rs11613874	12	83,236,046		A/G	0.335	-0.594	0.123	1.36E-06	2.86E-06
rs2255074	12	55,316,293	*BAZ2A (RBMS2, ATP5B)*	C/T	0.127	-0.977	0.205	1.93E-06	3.98E-06
rs2536929	12	9,572,295	*(DDX12, KLRB1)*	A/G	0.382	-0.723	0.152	1.98E-06	4.07E-06
rs1795819	12	53,288,281	*(PPP1R1A, LACRT)*	C/T	0.225	-0.677	0.142	2.00E-06	4.10E-06
rs10784385	12	62,853,955	*(SRGAP1, C12orf66)*	G/T	0.309	-0.597	0.126	2.15E-06	4.40E-06
rs4466908	12	60,074,978	*(PGBD3P1, FAM19A2)*	A/T	0.144	0.835	0.178	2.80E-06	5.64E-06
rs4429133	12	60,074,999	*(PGBD3P1, FAM19A2)*	G/T	0.146	0.829	0.177	2.83E-06	5.69E-06
rs10877635	12	60,074,852	*(PGBD3P1, FAM19A2)*	C/T	0.144	0.838	0.179	2.89E-06	5.81E-06
rs1716354	12	53,287,894	*(PPP1R1A, GLYCAM1)*	G/T	0.216	0.677	0.145	2.90E-06	5.83E-06
rs1716353	12	53,287,858	*(PPP1R1A, GLYCAM1)*	A/G	0.216	-0.676	0.145	2.99E-06	6.00E-06

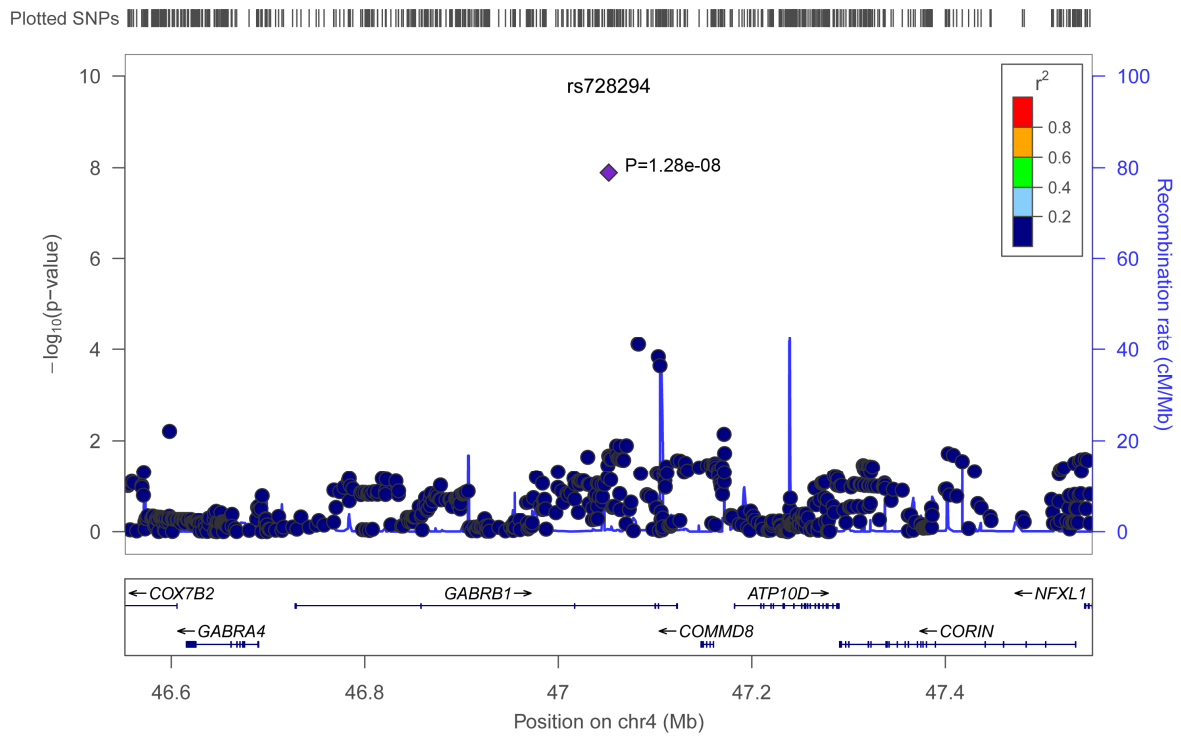
rs1691625	12	53,287,784	*(PPP1R1A, GLYCAM1)*	A/C	0.216	-0.675	0.145	3.07E-06	6.14E-06
rs11173956	12	60,075,591	*(PGBD3P1, FAM19A2)*	C/T	0.140	0.855	0.183	3.07E-06	6.16E-06
rs1691627	12	53,287,982	*(PPP1R1A, GLYCAM1)*	A/G	0.203	-0.677	0.145	3.09E-06	6.19E-06
rs7312486	12	60,076,227	*(PGBD3P1, FAM19A2)*	A/G	0.139	0.859	0.184	3.13E-06	6.26E-06
rs1623481	12	53,287,240	*(PPP1R1A, GLYCAM1)*	G/T	0.216	-0.674	0.145	3.14E-06	6.28E-06
rs1691626	12	53,287,969	*(PPP1R1A, GLYCAM1)*	A/G	0.203	-0.676	0.145	3.18E-06	6.35E-06
rs1400054	12	60,077,052	*(PGBD3P1, FAM19A2)*	A/G	0.138	0.863	0.185	3.23E-06	6.44E-06
rs1795821	12	53,287,173	*(PPP1R1A, GLYCAM1)*	C/T	0.216	-0.673	0.145	3.23E-06	6.45E-06
rs7967816	12	60,077,314	*(PGBD3P1, FAM19A2)*	C/T	0.135	-0.873	0.188	3.36E-06	6.69E-06
rs10492163	12	9,555,916	*(DDX12, KLRB1)*	A/G	0.348	-0.933	0.201	3.53E-06	7.02E-06
rs12578125	12	60,077,474	*(PGBD3P1, FAM19A2)*	A/G	0.133	0.881	0.190	3.58E-06	7.10E-06
rs4628751	12	60,078,623	*(PGBD3P1, FAM19A2)*	C/T	0.132	-0.885	0.191	3.68E-06	7.29E-06
rs11173961	12	60,078,910	*(PGBD3P1, FAM19A2)*	A/C	0.131	-0.889	0.192	3.77E-06	7.47E-06
rs1913927	12	60,080,008	*(PGBD3P1, FAM19A2)*	A/G	0.128	-0.902	0.196	4.12E-06	8.10E-06
rs10877640	12	60,079,394	*PGBD3P1, FAM19A2*	A/C	0.134	-0.886	0.194	4.92E-06	9.58E-06

SNP, single nucleotide polymorphism; Chr, chromosome; CA, coded allele; NCA, non-coded allele; MAF, minor allele frequency; Beta, genetic effect, se, standard deviation of beta; *P*, nominal *P* value; P_G, adjusted *P* value for genomic control;

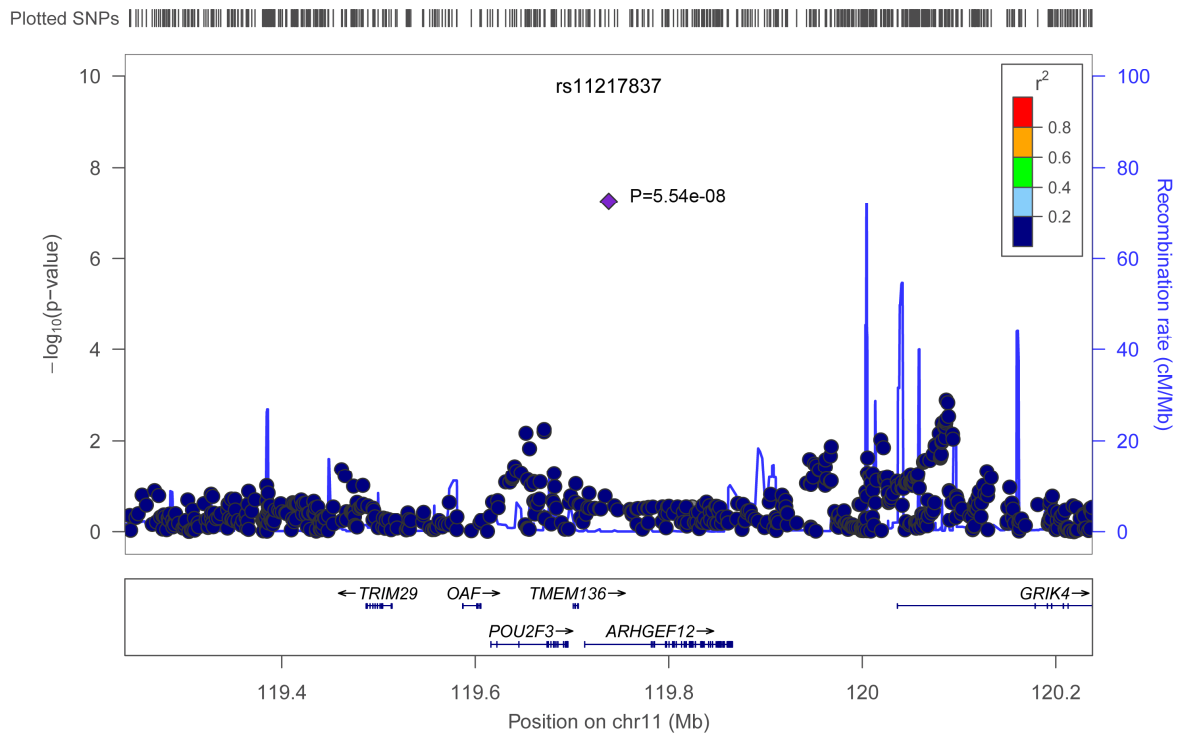
Supplementary Figures of Regional association plots for LVH at $P < 5x10^{-7}$ (as suggested by WTCC study [51])



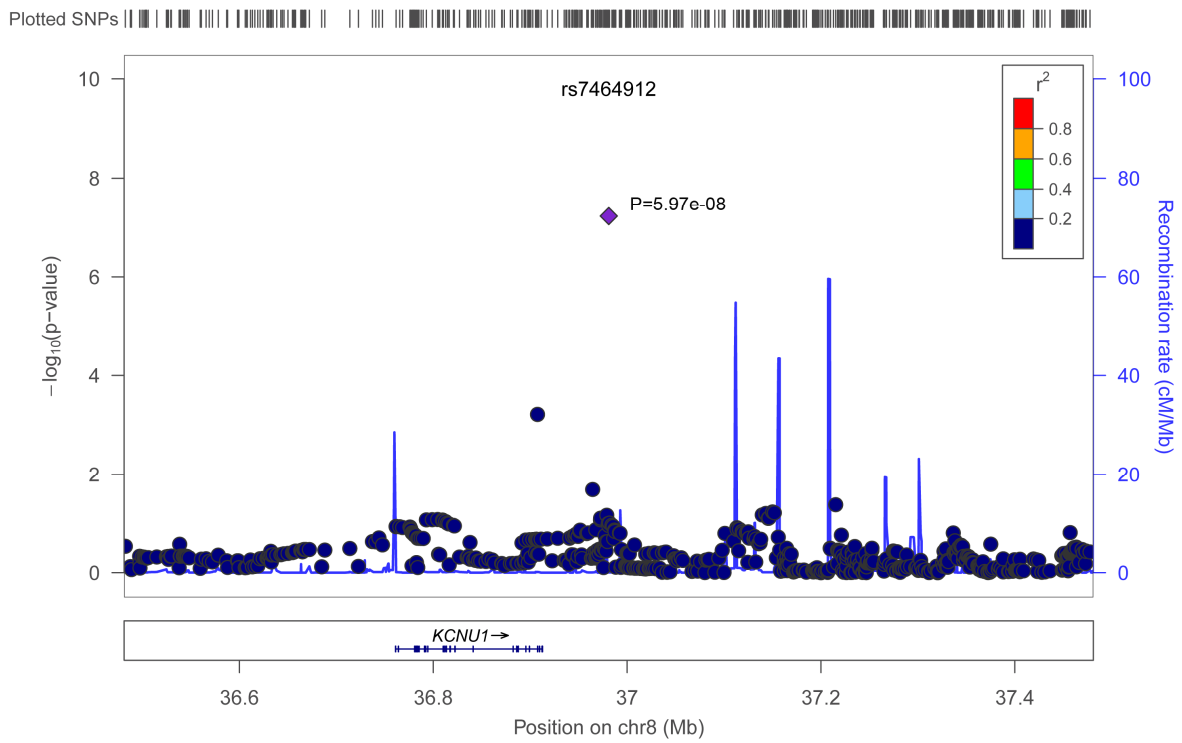
Supplementary Figure 1. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



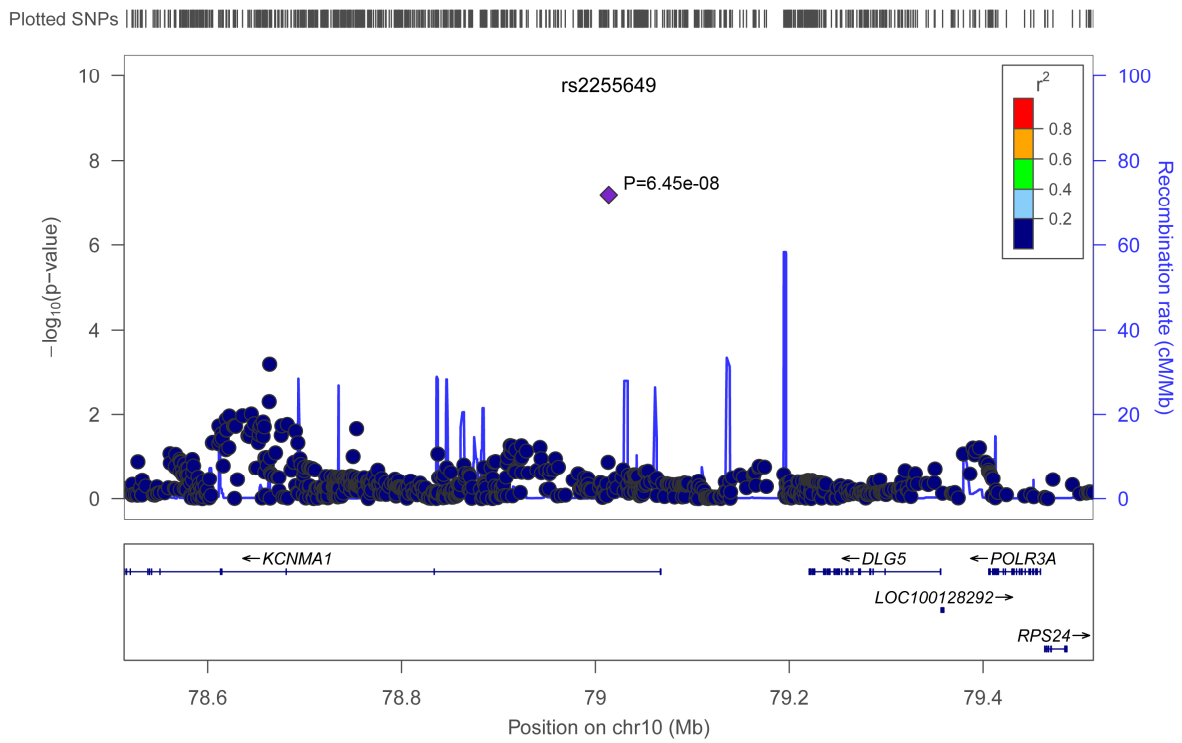
Supplementary Figure 2. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



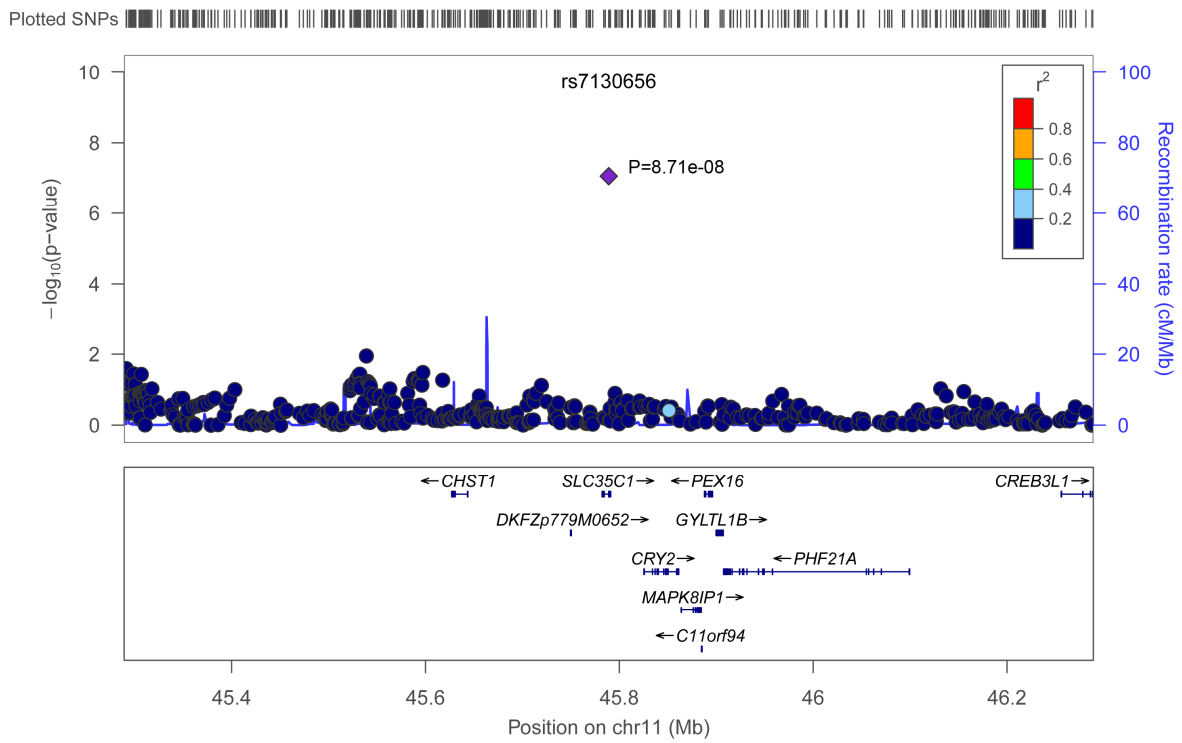
Supplementary Figure 3. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



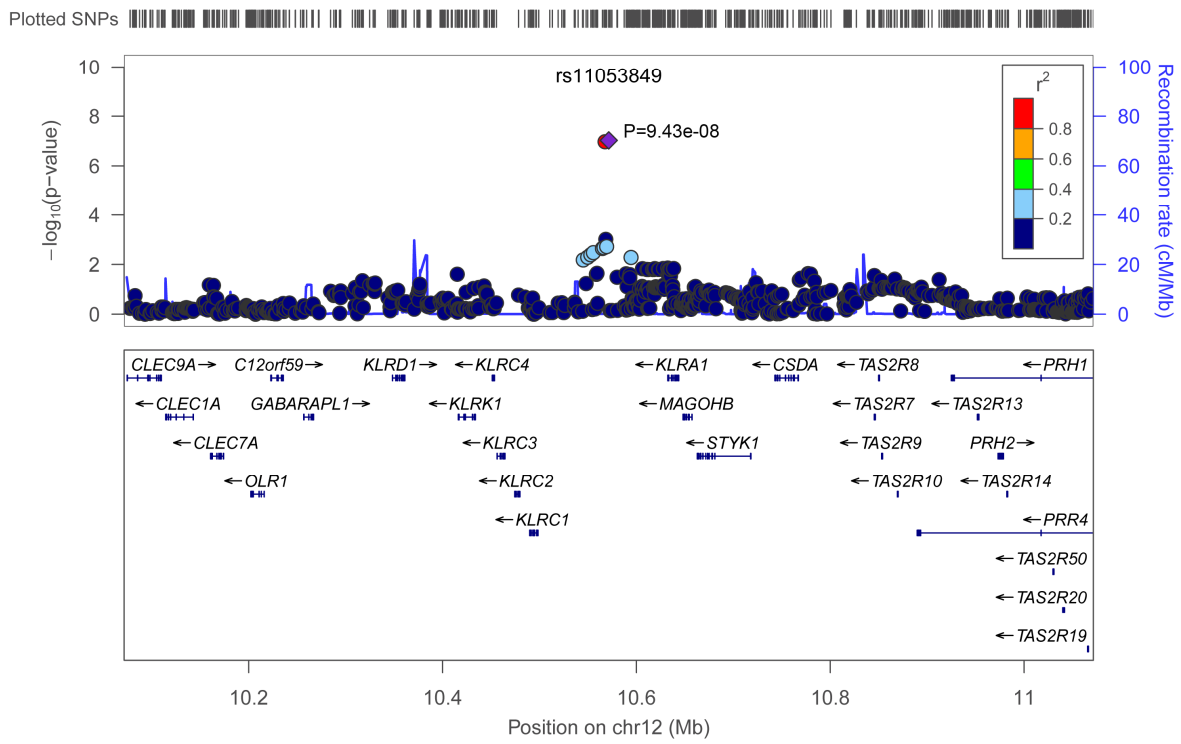
Supplementary Figure 4. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



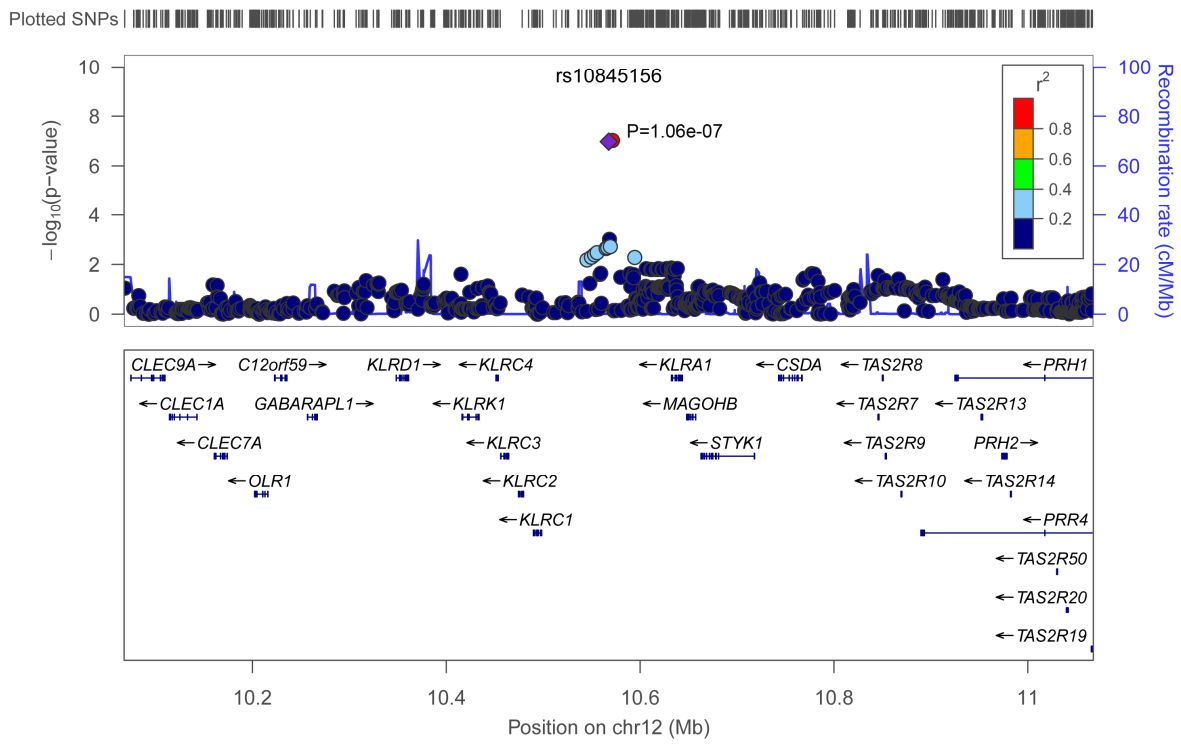
Supplementary Figure 5. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



Supplementary Figure 6. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

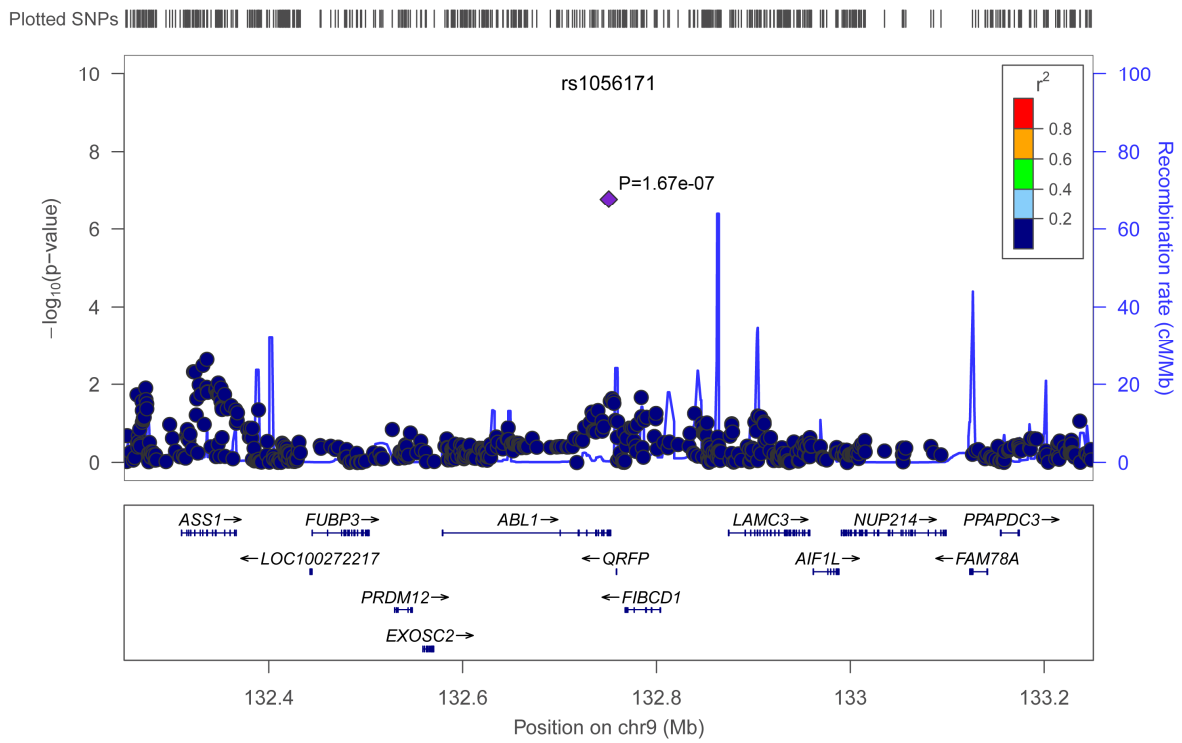


Supplementary Figure 7. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

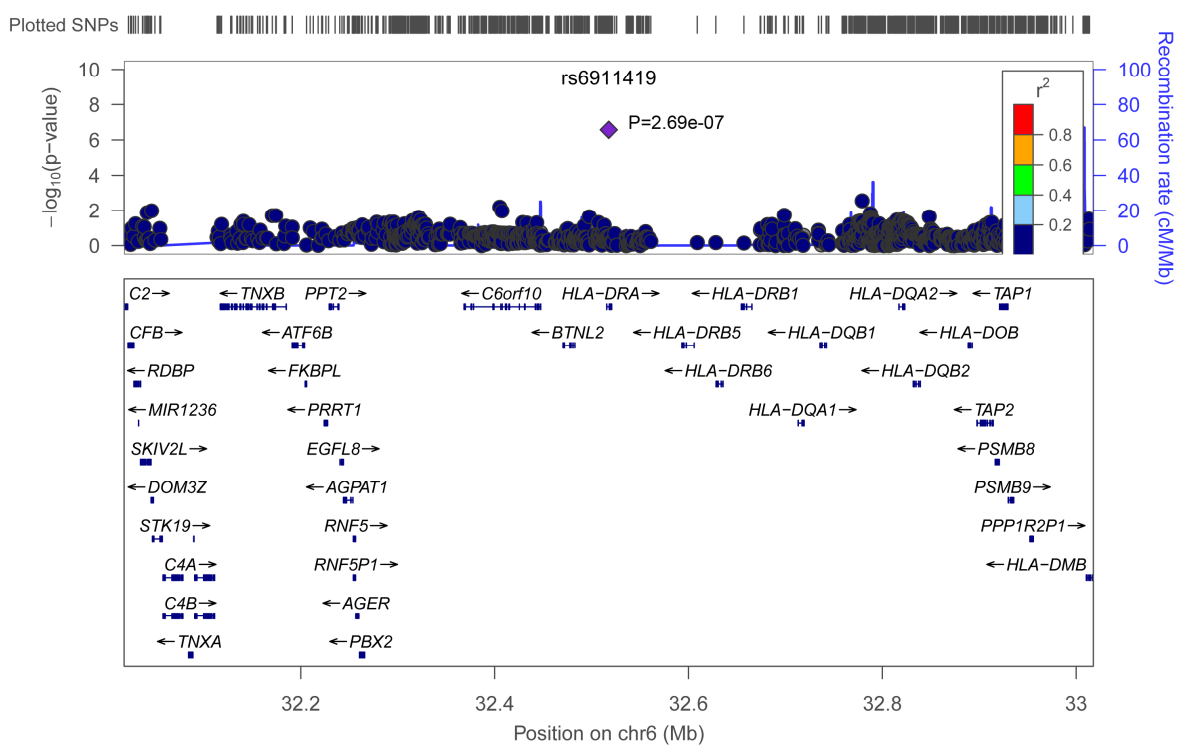


Supplementary Figure 8. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

I

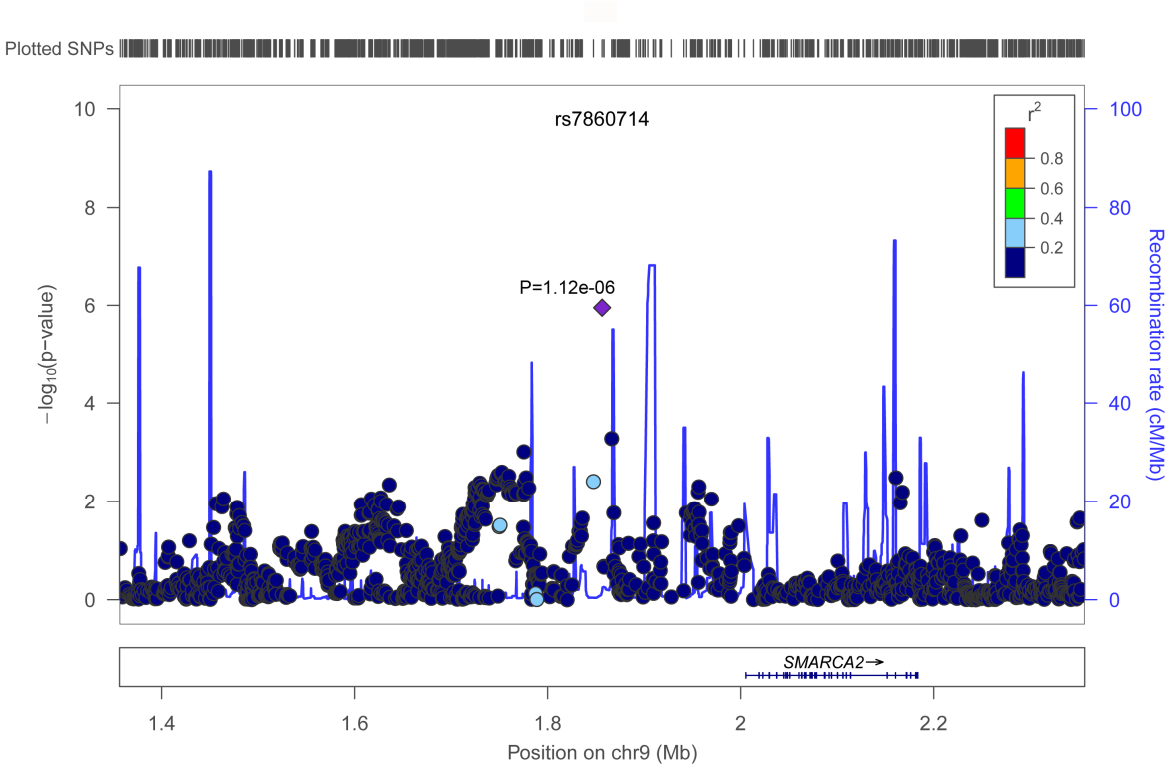


Supplementary Figure 9. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

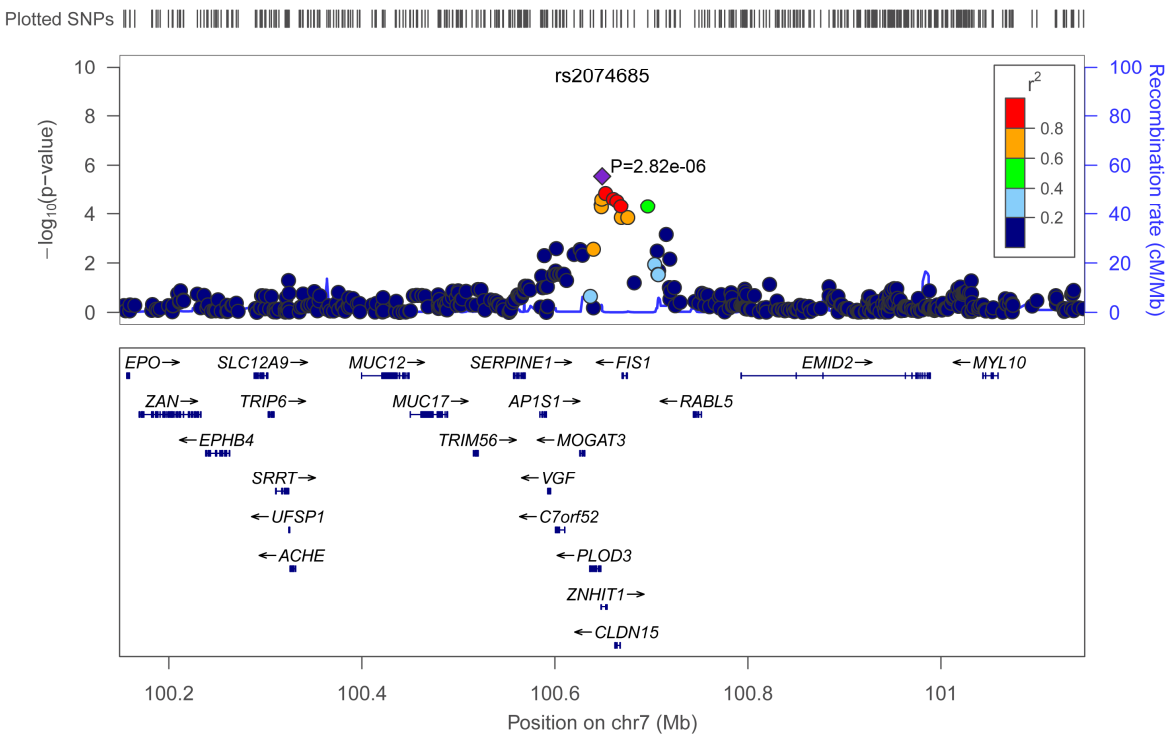


Supplementary Figure 10. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

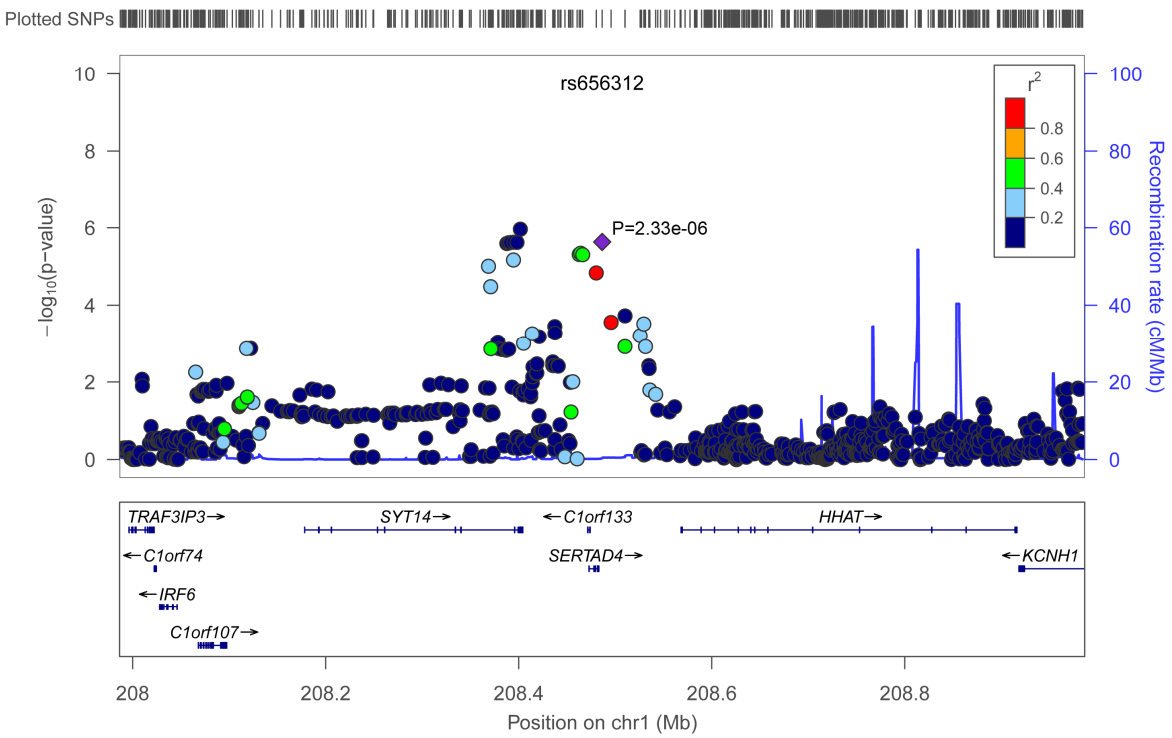
Supplementary Figures for Regional association plots for LVMI at $P < 1x10^{-5}$



Supplementary Figure 11. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



Supplementary Figure 12. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.



Supplementary Figure 13. Regional association plot. The vertical axis (on the left) shows the negative log(*P* value) of the associated single nucleotide polymorphisms (SNPs) within the illustrated region and the second vertical axis (on the right side) represents the recombination rate (centimorgams per mega bases) for measuring genetic linkage among all SNPs within this region. The horizontal axis shows the position in mega bases. Each dot represents a SNP. Genes in the region are shown below the horizontal axis.

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ABOUT THE AUTHOR

Natalia V Rivera was born on January 2, 1978, in Lima, Peru. She was raised in Lima, then moved to New York City with her family when she was thirteen. She attended Washington Irving High School in New York City, graduating with honors in 1995.

Natalia was awarded an academic scholarship to study at Syracuse University in Syracuse, New York, where she majored in Electrical Engineering, graduating in 1999. While pursuing her undergraduate studies, she focused her studies on medical instrumentation and communications engineering while working as an intern in various research centers, such as Lockheed Martin Federal Systems (Manassas, Virginia), Lucent Technologies, Bell Labs (Holmdel, New Jersey), Philips Broadband Network (Manlius, New York) and Thomson Multimedia Research Labs (Princeton, New Jersey). In 1997, she spent her third year at the university studying in the College of Engineering at City University of London, United Kingdom, as an exchange student. Upon completion of her undergraduate studies, she started work as a research engineer at Lucent Technologies, Bell Labs, in New Jersey for two years.

From 2002-2004, Natalia attended graduate school at Rutgers University in New Brunswick, New Jersey, and in 2004 obtained her Master of Science degree in Electrical Engineering, specializing in signal processing, algorithm design, and communications. During her graduate studies, she held teaching and research assistant positions in the Network Information Laboratory (WINLAB) at Rutgers University, as well as a summer internship at the Johns Hopkins University Advanced Physics Laboratory (Laurel, Maryland).

In 2004, Natalia was awarded a National Science Foundation Fellowship under the Integrative Graduate Education and Research Traineeship Program (NSF - IGERT) to pursue doctoral studies in an interdisciplinary program offered jointly by the School of Biomedical Engineering and Sciences at Virginia Polytechnic Institute and State University

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In 2007, Natalia transferred to University of Milan, Italy, to pursue doctoral studies in Molecular Medicine under the supervision of Prof. Cristina Battaglia and Prof. Gianluigi Condorelli. During her doctoral studies 2007 - 2011, she worked as a researcher in Prof. Condorelli's Laboratory of Molecular Cardiology of at the National Research Council (CNR) in Rome, as well as at Multimedica Research Hospital (IRCCS Multimedica) in Milan. In 2009, Natalia started research in the department of Genetic Epidemiology of the Erasmus MC, Rotterdam, the Netherlands, under the supervision of Prof. Cornelia van Duijn. Simultaneously, she pursued a Master of Science in Health Sciences, specializing in Genetic Epidemiology, at the Netherlands Institute of Health Sciences (NIHES), graduating in 2011. She received her Doctor of Philosophy degree in Molecular Medicine in 2012.

At present, Natalia is a guest researcher in the department of Genetic Epidemiology under the supervision of Prof. van Duijn and with Prof. Condorelli in various hypertension-related research projects collaboration with Prof. Trimarco and his team at the University of Naples Federico II, Naples, Italy. She is planning to continue working as a Post-doc research fellow while completing her medical degree.

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Introduction to Clinical and Public Health Genomics, NIHES, Rotterdam, the Netherlands	2011
Topics in Meta-analysis, NIHES, Rotterdam, the Netherlands	2011
Clinical Decision Analysis, NIHES, Rotterdam, the Netherlands	2011
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Advances in Genomics Research, NIHES, Rotterdam, the Netherlands	2011
Primary and Secondary Prevention Research, NIHES, Rotterdam, the Netherlands	2011
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Diagnostic Research, NIHES, Rotterdam, the Netherlands	2010
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Introduction of Medical Writing, NIHES, Rotterdam, the Netherlands	2010
Working with SPSS for Windows, NIHES, Rotterdam, the Netherlands	2010
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Genomics in Molecular Medicine , NIHES, Rotterdam, the Netherlands	2009
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ADDITIONAL TRAINING	
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Genome Analysis: Genetic Analysis of Multifactorial Diseases, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK, Jul 23-29	2008
4th Course in Statistical Genetic Analysis of Complex Phenotypes, European Genetics Foundation (EGF), Bertinoro, Italy, Jun 21-24	2008
Microarray Bioinformatics Course, University of Oxford, Oxford, UK, Feb 4-8	2008
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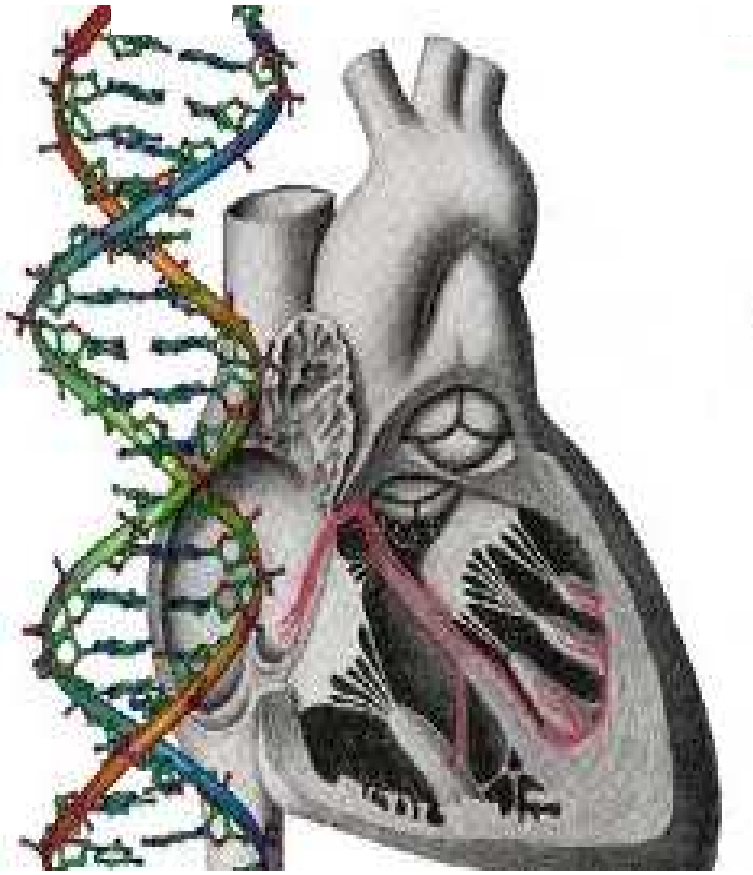
Fifth Symposium on Medical Engineering, Univ. of Patras, Greece, Jul.	2006
Third IEEE-EMBS International Summer School and Symposium on Medical Devices and Biosensors, MIT Media Laboratory, MIT, Cambridge, MA, Sept.	2006
RELEVANT PUBLICATIONS, PRESENTATIONS and POSTERS	
Rivera NV, Roncarati R, Viviani-Anselmi C, De Micco F, Mezzelani A, Condorelli G, Puca A, Airoldi F, Condorelli G, and Briguori C., "Chromosomal locus 9p21.3, An angiographic marker for CAD Patients with Severe Coronary Artery Disease", Poster presentation at American Society of Human Genetics (ASHG) Conference, Washington, DC	2010
Briguori C., Visconti G., D'andrea D., Tavano D., Focaccio A., Golia B., Giannone R., Castaldo D., **Rivera NV**, Ricciardelli B., Colombo A., "Cystatin C and Contrast-Induced Acute Kidney Injury", Circulation Journal. Accepted and waiting for publication	2010
Briguori C, Testa U, Riccioni R, Colombo A, Petricci E, Condorelli G, Mariani G, D'Andrea D, **Rivera NV**, Puca A, Peschle C, Condorelli G., "Correlations Between Progressors of Coronary Artery Disease and Circulating Endothelial Progenitor Cells", The Journal of the Federal of American Societies for Experimental Biology (FASEB). Published January.	2007
Romano G, Briguori C, Quintavalle C, Zanca C, **Rivera NV**, Colombo A, Condorelli G, "Contrast agents and renal cell apoptosis", European Heart Journal. May	2008
HONORS and AWARDS	
Award Recipient of the AHA Fellowship, 7th Hypertension Summer School 2010, Portland State University, Oregon, USA. July 31 – August 4, 2010.	2010
Award Recipient of the ERA-EDTA Fellowship, European Genetic Foundation (EGF), Bologna, Italy. March 2009	2009
Award Recipient of the Wellcome Trust Fellowship, Genetic Analysis of Multifactorial Diseases, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK. July 2008	2008
Student Award Recipient at the 3rd IEEE-EMBS International Summer School and Symposium on Medical Devices and Biosensors (ISSS-MDBS) at MIT Media Laboratory, Massachusetts Institute of Technology, Cambridge, Boston, MA.	2006
Published by the Society of Hispanic Professional Engineering Magazine, "Women on Their Way. Strategic Thinking for Engineers", SHPE Magazine, Vol. 8, No. 2, March/April 2006	2006
IREAN National Foundation Fellowship (NSF) Interdisciplinary Fellowship Recipient	2004-2006

Published by the Society of Hispanic Professional Engineering Magazine, “10 Latina Ph.D. candidates and their Cutting-Edge Research”, SHPE Magazine, Vol. 5, No. 3, August/September 2003	2003
Society of Hispanic Professional Engineers (SHPE) Scholarship Recipient	1995-2002
New York State Regents Professional Engineers Scholarship Recipient	1998-1999
Syracuse University - School of Engineering Academic Scholarship Recipient	1995-1999
Syracuse University Dean’s List	1997-1998
Amoco New York City Scholarship Recipient	1997
Aspira & Lipton Scholarship Recipient	1995-1997



This is only a small piece of the puzzle.