Original Article

Exome Sequencing Identifying LRP2 Gene (rs2228171) Related Hyperuricemia in Thai Patients with Non-communicable Diseases

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Background: Although genome-wide association studies have been conducted to investigate the association between genomic loci associated with urate concentrations and gout in a large population. However, there is a lack of information in the Thai population.

Objective: To identify the new genetic predisposition of hyperuricemia (HUA) and gout in non-communicable disease patients (NCDs).

Materials and Methods: A whole-genome sequencing (WGS) using the Illumina HiSeq X Ten platform (Macrogen, Korea) was performed on the genomic DNA of 4 adult men (HUA, gout, early-onset gout, and normal subjects) who selected from 250 individuals of Gout among Thai Population Study. Then the candidate gene was identified the association of HUA in Thai NCDs patients (n=550).

Results: The data set comprised 118,599 single-nucleotide variants were selected in all 4 participants. The missense Ala17Thr (G>A) GLUT9 mutation was found only in early-onset gout. The synonymous Pro1146Pro, A>G RREB1 mutation was identified in HUA and gout. WGS also identified synonymous Ile223Ile (C>T) ABCC4) and non-synonymous Ala2872Thr (G>A) LRP2 mutation in patients with HUA, early-onset gout, and gout. Because a missense of LRP2 (rs2228171) was found in the HUA subject. Thus the frequencies and association of rs2228171 in patients with HUA, hypertension, diabetes mellitus, heart disease, obesity, dyslipidemia, and stroke by using polymerase chain reaction and DNA sequencing analysis were investigated. Seventy-eight of 550 NCDs patients were selected. As result, an association between LRP2 and HUA was not found. The genotypes GA (adjusted OR 0.11, p=0.040), AA (adjusted OR 0.05, p=0.017) were associated with hypertension. However, the effect of rs2228171 in hypertension was still controversial due to the small population.

Conclusion: Our study is the first cross-sectional study of the rs2228171 related HUA in Thai NCDs patients. Furthermore, the study will be done to clarify the effect of rs2228171 and metabolic diseases such as hypertension.

Keywords: LRP2 rs2228171, Serum uric acid levels, Non-communicable disease, Single nucleotide polymorphism

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Hyperuricemia (HUA) is an imbalance of the endogenous production and renal excretion of uric acid[1]. The prevalence of HUA was predominant in adult men (24 to 29%) more than in adult women (2.6 to 20%). But, there is a rare prevalence in children[2-5]. Some previous studies in the Khon Kaen population, Thailand showed that the prevalence of HUA was higher in Thai men than women (14.9% vs. 8.6%, respectively)[6]. Currently, a study in Thai health people had found the prevalence of HUA was 31.1%[7]. Males had a higher prevalence than females (42.7 versus 24.8%)[7]. Several studies have been reported the association of HUA and diseases such as diabetes mellitus type 2 (DM2), metabolic syndrome[8-13], and cardiovascular disease (CVD)[14-23]. But the evidence of renal destruction from HUA is still controversial[18,20,22,24-30]. There is evidence of the increase in uric acid levels can promote endothelial dysfunction and development of cardiovascular disease[31].

Uric acids in blood vessels are freely filtered, reabsorbed, and secreted at the glomerulus, and again reabsorbed by urate transporters protein in the proximal tubule. Serum uric acids were secreted and filtrated only 10% at the late proximal tubule[32,33], as approximately 75% of uric acid was excreted by the kidney. Therefore, the main cause of HUA is a defect in the renal excretion of urate[34].

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Currently, it has been claimed that several risk factors affect the level of serum uric acid, such as genetics factors, obesity, alcohol, smoking, and their interactions[17,35-39]. Moreover, many previous studies reported that a relationship between genetic and serum uric acid ranged from 25 to 63%[4,5,40].

Urate transporters are proteins coded in the genes. Mutations in these genes may prompt disturbances in uric acid reabsorption and could lead to the development of hypouricemia, HUA, gout, and other metabolic diseases. Today many genetic variants for the main urate transporters are known, including SLC22A12 (URAT1) and SLC2A9 (URATv1/GLUT9), which have the greatest influence on urate excretion[8-13].

Moreover, The SLC22A6 (OAT1), SLC22A8 (OAT3), SLC17A3 (NPT4), ABCC4 (MRP4), and ABCG2 (BCRP) are the most important transporters involved in renal secretion[41-45]. Therefore, these dysfunctions cause aberrant urate transport disorders leading to HUA and gout. The previous evidence showed that the heritability of serum urate concentrations is estimated at 40 to 70%. Previous genome-wide association studies (GWAS) combining data from more than 140,000 individuals of European ancestry within the Global Urate Genetics Consortium (GUGC) identified and replicated 28 genome-wide significant loci in association with serum urate concentrations[46]. Many genes reported the association with serum uric acids such as LRP2, ACVR2A, ASAH2, C17ORF82, GCKR, HLF, HNF4G, IGF1R, INHBB, INHBE, LTBP3, MAF, MLXIPL, MUSTN1, NFAT5, NRG4, PDZK1, PKLR, PRKAG2, PRPSAP1, PTPN11, RREB1, SLC16A9, SLC22A11, STC1, TMEM171, and VEGFA[47].

Because NCDs are the causes of mortality in worldwide and Thailand. Several studies were reported the association of HUA and CVD, DM2, metabolic disease as mentioned above. So, we conducted the study in a patient with NCDs to whether we would find the association of genes involving uric acid and HUA including NCDs.

Knowledge of genes associated with NCDs may lead to the early detection and prevention of these diseases.

Materials and Methods

Experimental design

Although several studies were reported the association of genes involving serum uric acid and gout, there is lacking data on the Thai population. The objective of our study was to identify the gene associated with HUA in NCDs patients. Phase 1 of the study was conducted to identify new genes associated with HUA. The association of new genes and HUA in NCD patients was explored in phase 2 of the study.

Study participants in phase 1

The study in phase 1 was approved by the review board and ethical committee of Srinakharinwirot University, Bangkok, Thailand (MEDSWUEC-148/60E). The cross-sectional study "Gout among Thai Population Study (GUHGTHS)" was conducted during 2017 to 2018. All 250 participants including gout, HUA, and healthy were included in this study. We selected 4 male participants (1 HUA subject, 1 patient with gout, 1 patient with early-onset gout, and 1 patient with no HUA). HUA, gout, and no HUA subjects were matched in age (±5 years). But, early-onset gout did not match with anyone. In HUA subject did not receive any medication or herbal or food that effect to serum uric acid. All subjects with gout and early-onset gout were diagnosed by a rheumatologist. The overall flowchart for phase 1 is presented in Figure 1.

Definition of HUA and gout

Gout was diagnosed using clinical criteria from the American College of Rheumatology/European League Against Rheumatism collaborative initiative (ACE/EULAR) and confirmed by a rheumatologist[34,48,49]. HUA was defined as SUA levels over 7 mg/dL in men, and over 6 mg/dL in women[50].

Genetic analysis phase 1

DNA extraction and whole-genome sequencing

Four DNA samples were extracted from peripheral blood by the standard phenol/chloroform method[51]. Red blood cells were lysed using an RBC lysis buffer. Subsequently, the blood was centrifuged by 30 microliters of 20 mg/ml proteinase K (Invitrogen, Carlsbad, CA, USA). Three hundred microliters lysis buffer was added for protein denaturation. DNA precipitation was obtained by absolute alcohol. After precipitation, the DNA pellet was recovered and separated by centrifugation. 70% ethanol was used to clean the DNA samples. The DNA pellets for each sample

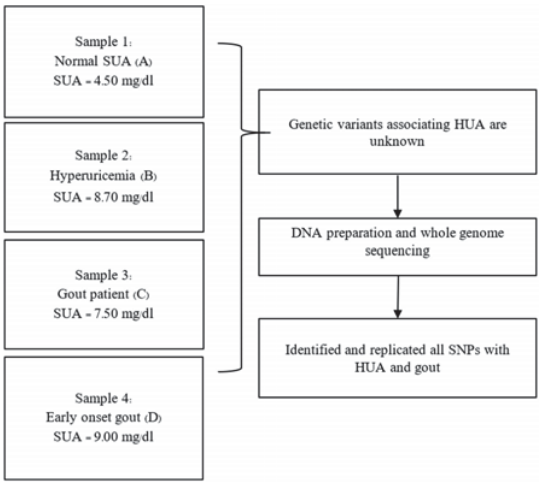


Figure 1. Overall flowchart for investigating variants associated with hyperuricemia and gout in phase 1.

were re-suspended by TE buffer when it's nearly dry. DNA (ng/µL) was measured by a Nanophotometer. The ratio of absorbance at 260 nm and 280 nm was used to assess the optimal and adequate quality of DNA.

Whole-genome sequencing was performed at Macogen Inc, Korea (Illumina Inc., San Diego, CA, USA).

SNPs variants analysis

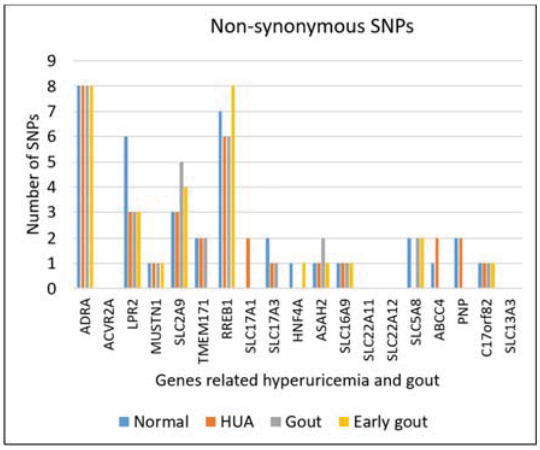
All DNAs were sequenced using the Illumina HiSeq X Ten platform (Macrogen). Sequence reads were quality checked by FastQC[52], and mapped by the Burrows-Wheeler Alignment tool (BWA version 0.7.17) (http://bio-bwa.sourceforge.net/)[53] using the human reference genome GRCh37 (hg19) downloaded from the UCSC Genome Browser. Variants were called by SAMtools version 1.10[54] and BCFtools version 1.10.2[55], followed by the variant annotation by SnpEff version 4.3t[56] using the dbSNP (https://www.ncbi.nlm.nih.gov/snp/), 1,000 Genomes (https://www.internationalgenome.org/), ESP6500 (https://evs.gs.washington.edu/EVS/), and ClinVar databases (http://www.ncbi.nlm.nih.gov/clinvar/). The variants were compared and visualized by using the BasePlayer version[57] and IGV version 2.8.2[58].

Target SNPs selected

All exons were analyzed by WGS, 112 variants of 17 genes in normal, 109 variants of 16 genes in HUA subject, 95 variants of 16 genes in gout subject, and 91 variants of 15 genes in early onset-gout were identified. The non-synonymous variant of genes was shown in Figure 2. The LRP2 variant (rs2228171), Ala2872Thr (G>A)was found in HUA, and gout samples, respectively. Moreover, the GLUT9 Ala17Thr was identified only in gout samples. Because the LRP2 variant was not present in the normal HUA sample so it may be the candidate variant that promotes HUA and gout. Change of nucleotide from G to A at position 2,872 resulted in the conversion of alanine to threonine. The missense mutation may lead to disorders of uric acid homeostasis. Of all the above, we selected LRP2 rs2228171 to identify the association of HUA in NCD patients.

Study participants in phase 2

The cross-sectional study was conducted from November 2018 to January 2019. Subjects were selected from the NCDs who participated in Gout among Thai Population study Nakhon Nayok hospital. After screening 550 individuals, 78 males with no prior history of gout. were selected (flow chart was shown in figure 1). All participants had no cancer, acute heart disease, receiving uric lowering agents, and on dialysis. Patients who had incomplete data were excluded from our study. All participants were given informed consent, reviewed a history, and collected blood sampling including genotyping. Clinical profiles of our subjects including age, gender, comorbidities, smoking, alcohol use, blood pressure (BP), waist circumference (WC), hip circumference (HC), and body mass index (BMI) were collected. Blood chemistry such as fasting plasma glucose



(A) Genetic linkage related diseases: SLC2A9, SLC17A1, SLC17A3, and SLC16A9.
(B) Genetic linkage to be investigated for its: SLC22A11, SLC22A12, SLC5A8, ABCC4, and SLC13A3 (Adenosine adenase RNA specific (ADAR), Low-density lipoprotein receptor-related protein 2 (LRP2), Musculoskeletal embryonic nuclear protein 1 (MUSTN1), Soluble carrier family 2 member 9 (SLC2A9) or glucose transporter 9 (GLUT9), Transmembrane protein 171(TMEM171), Ras responsive element-binding protein 1(RREB1), Soluble carrier family 17 member 1 (SLC17A1), Soluble carrier family 17 member 3 (SLC17A3), N-acylspingosine amidohydrolase 2 (ASAH2), Soluble carrier family 16 member 9 (SLC16A9), Soluble carrier family 22 member 12 (SLC22A12), Soluble carrier family 5 member 8 (SLC5A8), ATP-binding cassette subfamily C member 4 (ABCC4), Purine nucleoside phosphorylase (PNP), Chromosome 17 open reading frame 82 (C17orf82) and Soluble carrier family 13 member 3 (SLC13A3)

Figure 2. Show non-synonymous SNPs in hyperuricemia and gout.

(FPG), serum uric acid (SUA), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglyceride (TG), HbA1C, blood urea nitrogen (BUN), creatinine (Cr), and glomerular filtration rate (GFR) were collected from all participants. Genomic DNA was extracted from peripheral blood to find the association of LRP2 rs2228171 in patients with HUA, DM2, hyperlipidemia, hypertension (HT), heart disease, and overweight. The ethics committee of Nakhon Nayok hospital, Thailand approved the study protocol (EC-011/2018).

Definition of overweight, HT, DM, dyslipidemia, stroke, and heart disease

Overweight was defined as BMI 25 to 29.99 kg/m^2 followed by WHO criteria. Diagnosis of DM was described as fasting blood sugar (FBS) equal to or over 126 mg/dL or 2-hours after an oral glucose tolerance test plasma glucose equal or over 200 mg/dL or HbA1C equal or over 6.5%[59].

HT was diagnosed follow by systolic BP equal to or over 140 mmHg or diastolic BP equal to or over 90 mmHg or antihypertensive medication. Dyslipidemia was included cholesterol (over 200 mg/dl), hypertriglyceridemia (over 150 mg/dl), and low-density lipoprotein cholesterol (LDL-Cover 160 mg/dl) or received lipid-lowering agents. Heart disease consists of coronary heart disease, ischemic heart disease, and hypertensive heart disease, cardiomyopathy excluding congenital heart disease, heart valve diseases, and arrhythmias. The stroke was diagnosed by a physician.

Genetic analysis phase II

DNA extraction phase II

Genomic DNA was isolated from peripheral blood by QIAamp DNA Blood Mini kit (QIAGEN, Germany) and stored at -20 C. The DNA concentration and quality were determined using a Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA), a 260/280 ratio of ~1.8, and a 260/230 ratio between 2.0-2.2 were generally accepted.

Polymerase chain reaction (PCR)

Forward and reverses primers for rs2228171 were designed. Primer sequences of forwarding 5' - 3' and reverse 5' - 3' were TCCATTTTCCAGGCTCAGTC and AGCATCAATCAGCAGCTTCC, respectively. Forward and reverse primers were checked with the Primer Blast program. PCR product size was 477 based pair. PCR products were done by PCR Thermal Cycler (Bio-Rad). Then PCR products were run by agarose gel electrophoresis, eluted and purified, respectively.

DNA sequencing analysis

DNA sequencing from PCR products was performed using Sanger Sequencing (Macrogen). A chromatogram was analyzed to identify the genotypes of all participants.

Statistical analysis

All baseline characteristic analyses were performed using STATA version 14 (Stata, College Station, TX). The Hardy-Weinberg equilibrium (HWE) was used to describe genotype and allele distribution[60]. A Chi-square test was used to determine the association of genes and factors ($p<0.05$).

Results

Candidate SNPs should be related to HUA and gout

The 4 participants were identified by numbers A, B, C, and D. Their anthropomorphic and clinical parameters are summarized in Table 1. WGS was performed on genomic DNA from 4 adult men participants. The data set comprised 118, 599 single-nucleotide variants (SNVs) were selected. Figure 2 showed non-synonymous or missense mutation comparing to 4 male participants. In our study, we would

Table 1. Summary of clinical observations and genetic variants discovered by whole-genome sequencing in 4 participants of phase 1 study

Factors	Subject A	Subject B	Subject C	Subject D
Groups	Normal	HUA	Gout	Early gout
Age (y)	43	43	37	21
BMI	22.75	24.83	26.89	29.40
SBP	129	128	121	119
DBP	85	60	84	61
HDL	34	55	41	39
LDL	137.0	160.0	137.0	109.8
TG290	66	152	166	
TC 197	207	209	182	
FBS	344	83	87	82
Genetic Variants				
GLU9	Not found	Not found	Not found	Ala17Thr
ABCC4	Not found	Ile223Ile	Ile223Ile	Ile223Ile
RREB1	Not found	Pro1146Pro	Pro1146Pro	Not found
LRP2	Not found	Ala2872Thr	Ala2872Thr	Ala2872Thr

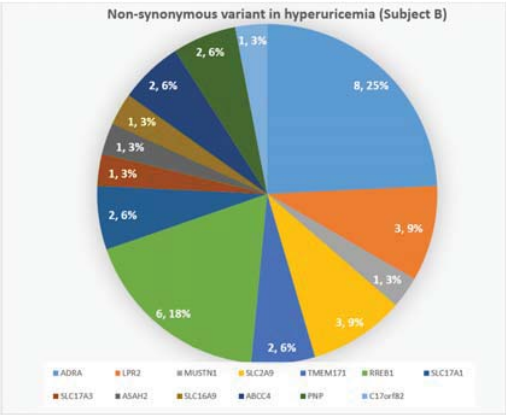
BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; TG = Triglyceride; TC = Total cholesterol; FBG = Fasting blood sugar; GLU9 = glucose transporter 9; ABCC4 = ATP Binding Cassette Subfamily C Member 4; RREB1 = Ras responsive element binding protein 1; LRP2 = LDL receptor-related protein 2; Ala = Alanine; Thr = Threonine; Ile = Isoleucine; Pro = Proline

like to find a novel variant of genes that may be associated with HUA. So the genes in the HUA sample (Subject B) were selected. The variants in HUA were classified into 4 groups by 1) urate transporter gene that evidence to related diseases 2) urate transporter gene which needs to investigated for its relation to diseases 3) genes involving in the metabolic pathway of urate and 4) unclear of the pathway (Figure 3).

Genetic variants identified compare to 4 participants were described in Table 1.

The early onset gout (subject D) was found a missense Ala17Thr, C>T variants (heterozygosity) in the founder mutation of GLU9, which is a gene located on 4p16.1. The Pro1146Pro synonymous mutation, a known variant of RREB1, was found as heterozygous in HUA (subject B), gout patient (subject C), and early-onset gout (subject D). The Ile223Ile (C>T) synonymous mutation of ATP binding protein cassette subfamily C member 4 (ABCC4) was identified as heterozygous in gout patients (subject C). However, the Ile223Ile (C>T) mutation was homozygous in both HUA (subject B) and early-onset gout subjects (subject D). A missense Ala2872Thr (G>A) variant in LDL receptor-related protein 2 (LRP2) gene was identified as heterozygous in HUA (subject B), gout (subject C), and early-onset gout (subject D).

In phase 2 we were interested in LRP2 (rs2228171) because this variant did not find in the normal subject. So we identified the association of rs2228171 and HUA, DM2, dyslipidemia, heart disease, stroke, HT, and overweight. Baseline characteristics of 78 participants were shown in Table 2.



(A) pathway of uric: salvage pathway; ADRA, and PNP
(B) Urate transporter (strong evidence); SLC2A9, SLC17A1, SLC17A3, and SLC16A9
(C) Urate transporter (evidence to be investigated); ABCC4
(D) Unclear pathway; LRP2, MUSTN1, TMEM171, RREB1, ASAH2, and C17orf82

Figure 3. Classification of SNPs in hyperuricemia (Subject B).

Correlation of serum uric acid and factors

There was no correlation between serum uric acid and genotype GG, GA, and AA. But the negative correlation was identified between the level of serum uric acid and DM2 (r=-0.284, 95% CI -0.476 to -0.065, p=0.012) (data as shown in Table 3).

Association between LRP2 (rs2228171) and HUA

Focusing on the genetic variation of LRP2, the allelic frequencies and heterozygosity between HUA and non- HUA were clarified. There were 34, 36, and 8 who had homozygous major G, heterozygous, and homozygous minor A-allele carriers, respectively. There was no deviation of genotype frequencies between groups from HWE SNP (Table 4).

Analysis of baseline characteristics between HUA and non- HUA groups found that no difference between age, smoking status, alcohol status. But BP, TC, TG, LDL, HDL, FBS, BUN, Cr was found more than in the HUA group (Table 2). The correlation between uric acid and genotype was not identified (data shown in Table 3). Unfortunately, there was no association between HUA and LRP2 genotypes (Table 5).

Association between LRP2 (rs2228171) and DM2

The allelic frequencies and heterozygosity were identified in diabetes mellitus. There were 34, 36, and 8 patients who carried homozygous G, heterozygous, and homozygous A, respectively (Table 4). There was no significant difference in genotypic frequencies from HWE SNP between DM and no DM.

In data, the diabetic patients have more than alcohol use, WC, HC, SBP, FBS, HbA1C, BUN, and GFR (Table 2). However, the association between DM2 and LRP2 genotype was not identified (Table 5).

Association between LRP2 (rs2228171) and HT

Thirty-three, 28, and 5 of patients with HT had homozygous G, heterozygous and homozygous A, respectively (Table 4). There was a deviation in genotypic frequencies from HWE SNP between HT and non-HT (p=0.010). The patients with HT had older age, smoking, alcohol use, a greater level of HC, WC, TC, TG, LDL, SUA, BUN, and Cr than the non-HT group (Table 2). Moreover, the genotypes GA and AA were associated with HT (Table 5).

Association between LRP2 (rs2228171) and heart disease

Of 10 patients with heart disease, there were 2, 7, and 1 patient who carried homozygous G, heterozygous, and homozygous A, respectively (Table 4). There was no genotypic frequencies difference from HWE SNP between the heart and no heart disease. The greater level of age, WC, HC, TG, LDL, FBS, HbA1C, BUN, and Cr was found in the heart disease group (Table 2). The association of LRP2 genotype and heart disease was not

Table 2. Baseline characteristics of all patients in phase II study (n=78)

Factors	HUA/ no HUA	DM/ no DM	HT/ no HT	Heart dz/ no Heart dz	CVA/ no CVA	DLP/ no DLP	Overweight/ no overweight
Number	40/38	44/34	66/12	10/68	7/71	53/25	37/41
Age (years)	55.6±9.7/ 55.6±9.9	55.5±9.8/ 55.8±9.8	56.7±9.5/ 49.5±8.9	56.8±7.1/ 55.41±10.1	53±12.5/ 55.9±9.5	55.2±10.2/ 56.4±8.7	53.3±10.5/ 57.6±8.6
Smoking	20/20	21/19	36/4	5/35	5/35	25/15	18/22
History smoking	10/9	12/7	17/2	3/16	2/17	13/6	11/8
Current smoking	10/11	9/12	19/2	2/19	3/18	12/9	7/14
Alcohol	24/24	27/21	41/7	6/42	4/44	31/17	23/25
History alcohol	8/4	8/4	12/0	3/9	2/10	8/4	8/4
Current alcohol	16/20	19/17	29/7	3/33	2/34	23/13	15/21
Exercise	25/28	30/23	42/11	7/46	5/48	39/14	22/31
WC (cm)	90.8±10.7/ 90.0±12.3	92.4±12.5/ 87.9±9.4	90.6±11.0/ 89.6±14.2	94.1±12.6/ 89.9±11.2	93.1±15.6/ 90.2±11.1	91.6±10.8/ 88.0±12.5	98.4±9.6/ 83.2±7.6
HC (cm)	97.4±11.4/ 98.3±34.2	99.7±10.2/ 95.5±10.0	98.2±10.4/ 96.3±9.8	102.6±10.2/ 97.2±10.2	100±11.0/ 97.7±10.3	99.2±9.2/ 95.0±12.0	104.5±8.4/ 91.9±7.9
SBP (mmHg)	135.5±13.5/ 133.0±14.2	133.7±15.2/ 135±11.7	135.6±13.0/ 127.1±16.1	131.2±9.9/ 134.7±14.2	128.9±7.5/ 134.8±14.1	132.8±13.0/ 137.4±15.0	135.8±12.6/ 132.9±14.7
DBP (mmHg)	82.8±10.6/ 80.0±10.6	81.4±10.6/ 82.1±11.9	81.9±10.1/ 78.8±13.8	83.1±11.6/ 81.2±10.5	78.0±12.7/ 81.8±10.4	79.0±10.0/ 86.6±10.3	81.8±8.3/ 81.1±12.5
TC (mg/dL)	179.4±40.8/ 172.5±36.9	167.8±40.5/ 186.7±34.4	177.8±40.3/ 166.1±29.0	163.1±50.1/ 177.9±37.0	179.1±35.4/ 175.7±39.4	179.4±40.3/ 169.9±35.1	178.4±35.2/ 178.3±42.2
TG (mg/dL)	158.1±75.5/ 145.8±102.0	143.9±83.6/ 162.7±95.7	155.7±91.1/ 132.4±77.1	180.3±109.9/ 148.0±85.7	137.6±34.9/ 153.5±92.7	160.3±92.5/ 134.7±80.1	162.1±96.7/ 143.1±81.6
HDL (mg/dL)	49.9±21.6/ 48.0±10.3	45.7±10.8/ 52.1±22.3	48.2±17.9/ 50.1±10.8	38.8±12.5/ 49.9±17.2	51.4±19.4/ 48.2±16.9	47.3±13.3/ 51.1±23.0	44.4±9.3/ 52.1±21.2

Data are presented as mean ± standard deviation (SD).
heart dz = heart disease; WC = waist circumference; HC = Hip circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein cholesterol; LDL = Low-density lipoprotein cholesterol; FBS = fasting blood sugar; SUA = serum uric acid; BUN = blood urea nitrogen; GFR = Glomerular filtration rate

Table 2. Cont.

Factors	HUA/ no HUA	DM/ no DM	HT/ no HT	Heart dz/ no Heart dz	CVA/ no CVA	DLP/ no DLP	Overweight/ no overweight
LDL (mg/dL)	103.2±46.6/ 96.2±36.8	98.8±47.0/ 101.1±35.2	101.7±44.2/ 89.4±25.4	111.1±65.9/ 98.1±37.7	100.3±45.1/ 99.7±42.0	100.9±40.6/ 97.5±45.7	97.2±35.3/ 102.2±47.6
FBS (mg/dL)	152.7±107.0/ 139.8±45.2	174.6±86.8/ 110±60.7	139.0±63.4/ 187.4±147.7	159.3±89.1/ 144.5±82.1	106.6±30.2/ 150.4±85.1	145.2±85.9/ 149.0±76.7	156.2±93.3/ 137.6±71.6
SUA (mg/dL)	8.3±0.9/ 5.7±0.9	6.69±1.60/ 7.51±1.40	7.08±1.48/ 6.77±2.01	7.05±1.92/ 7.03±1.52	7.41±1.23/ 7.00±1.60	7.00±1.58/ 7.09±1.56	6.96±1.80/ 7.10±1.33
HbA1C	6.9±2.2/ 7.3±1.7	7.4±2.0/ 5.5±0.5	7.1±2.0/ 7.5±1.5	7.9±3.0/ 7.0±1.7	8.7±3.6/ 7.1±1.9	7.5±2.0/ 6.7±1.8	7.2±2.1/ 7.2±1.8
BUN (mg/dL)	15.5±6.4/ 14.0±6.1	15.2±6.3/ 14.2±6.3	14.9±6.6/ 13.8±4.5	19.8±8.0/ 14.0±5.7	15.0±4.4/ 14.7±6.5	14.5±5.3/ 15.3±8.0	14.7±4.4/ 14.8±7.7
Creatinine (mg/dL)	1.17±0.36/ 1.01±0.26	1.10±0.35/ 1.09±0.28	1.12±0.32/ 0.96±0.21	1.31±0.39/ 1.07±0.30	1.17±0.25/ 1.09±0.33	1.09±0.3/ 1.11±0.36	1.11±0.35/ 1.08±0.29
GFR (mL/min/1.73 m^2)	75.6±22.6/ 85.4±18.0	80.6±22.2/ 80.2±19.6	78.1±20.8/ 92.9±18.0	67.6±21.3/ 82.3±20.4	74.0±17.4/ 81.0±21.3	80.8±20.5/ 79.4±22.3	81.2±21.6/ 79.7±20.6

Data are presented as mean ± standard deviation (SD).
heart dz = heart disease; WC = waist circumference; HC = Hip circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein cholesterol; LDL = Low-density lipoprotein cholesterol; FBS = fasting blood sugar; SUA = serum uric acid; BUN = blood urea nitrogen; GFR = Glomerular filtration rate

Table 3. Correlation of serum uric acid with factors (n=78)

Factors	r	95% CI	p-value
GG	-0.061	-0.280 to 0.164	0.596
GA	0.058	-0.167 to 0.277	0.058
AA	0.005	-0.218 to 0.227	0.968
Hypertension	0.109	-0.116 to 0.324	0.342
Dyslipidemia	-0.042	-0.262 to 0.182	0.714
Diabetes mellitus	-0.284	-0.476 to -0.065	0.012
Heart disease	0.037	-0.187 to 0.257	0.750
Stroke	0.088	-0.138 to 0.304	0.445
Alcohol drinking	-0.006	-0.228 to 0.217	0.959
Smoking	0.085	-0.140 to 0.302	0.457
Exercise	-0.099	-0.315 to 0.126	0.386
Overweight	-0.030	-0.251 to 0.194	0.797
Systolic blood pressure	0.079	-0.146 to 0.297	0.490
Diastolic blood pressure	0.025	-0.199 to 0.246	0.828

r = correlation, Hyperuricemia was defined as serum uric acid over 7 mg/dL; Diabetic mellitus was defined as fasting blood sugar more than or equal to 126 mg/dL or receiving the antidiabetic drug, Hypertension was defined SBP over 140 mmHg or DBP over 90 mmHg; Heart diseases were diagnosed by a physician; Stroke was diagnosed by a physician; Dyslipidemia was defined as total cholesterol over 200 mg/dL, triglyceride over 150 mg/dL, low-density lipoprotein cholesterol over 160 mg/dL or received lipid-lowering agents; Overweight was defined as BMI 25.00 to 29.99 kg/m^2

Table 4. Frequency of rs2228171 in HUA, DM, HT, Heart disease, Stroke, Dysplipidemia, and Overweight (n=78)

Group	n	Genotype, n (%)			Allele, n (%)		HWE p-value
		G/G	G/A	A/A	G	A	
HUA	40	17 (42.50)	19 (47.50)	4 (10.00)	53 (66.25)	27 (33.75)	0.970
No HUA	38	17 (44.74)	17 (44.74)	4 (10.52)	51 (67.11)	25 (32.89)	
DM	44	20 (45.45)	19 (43.18)	5 (11.36)	59 (67.05)	29 (32.95)	0.820
No DM	34	14 (41.18)	17 (50.00)	3 (8.82)	45 (66.18)	23 (33.82)	
HT	66	33 (50.00)	28 (42.42)	5 (7.58)	94 (71.21)	38 (28.79)	0.010
No HT	12	1 (8.33)	8 (66.67)	3 (25)	10 (41.67)	14 (58.33)	
Heart disease	10	2 (20.00)	7 (70.00)	1 (10.00)	11 (55.00)	9 (45.00)	0.220
No heart disease	68	32 (47.06)	29 (42.65)	7 (10.29)	93 (68.38)	43 (31.62)	
Stroke	7	0 (0.00)	6 (85.71)	1 (14.29)	6 (42.86)	8 (57.14)	0.013
No Stroke	71	34 (47.89)	30 (42.25)	7 (9.86)	98 (69.01)	44 (30.99)	
Dyslipidemia	53	23 (43.40)	24 (45.28)	6 (11.32)	70 (66.04)	36 (33.96)	0.900
No dyslipidemia	25	11 (44.00)	12 (48.00)	2 (8.00)	34 (68.00)	16 (32.00)	
Overweight	39	16 (41.03)	19 (48.72)	4 (10.26)	51 (65.38)	27 (34.62)	0.890
No overweight	39	18 (45.15)	17 (43.59)	4 (10.26)	53 (67.95)	25 (32.05)	

HWE = Hardy-Weinberg equilibrium test; SNPs = single nucleotide polymorphisms; HUA = Hyperuricemia, HUA was defined as serum uric acid over 7 mg/dL; DM = Diabetic mellitus, DM was defined as fasting blood sugar more than or equal to 126 mg/dL or receiving the antidiabetic drug; HT = Hypertension, HT was defined SBP over 140 mmHg or DBP over 90 mmHg, Heart diseases were diagnosed by a physician, Stroke was diagnosed by a physician; DLP = dyslipidemia, Dyslipidemia was defined as total cholesterol over 200 mg/dL, triglyceride over 150 mg/dL, low-density lipoprotein cholesterol over 160 mg/dL or received lipid-lowering agents, Overweight was defined as BMI 25.00 to 29.99 kg/m^2

Table 5. Odds ratio for association of genotypes and factors

Genotypes	OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Hyperuricemia				
G/G	0.91 (0.37 to 2.24)	0.840	1.00	
G/A	1.12 (0.46 to 2.72)	0.810	1.12 (0.44 to 2.86)	0.816
A/A	0.94 (0.22 to 4.08)	0.940	1.00 (0.21 to 4.67)	1.00
Diabetes mellitus				
G/G	1.19 (0.48 to 2.94)	0.706	1.00	
G/A	0.76 (0.31 to 1.87)	0.550	0.96 (0.35 to 2.60)	0.930
A/A	1.32 (0.29 to 5.98)	0.715	1.43 (0.25 to 8.29)	0.687
Hypertension				
G/G	11.0 (1.34 to 90.12)	0.025	1.00	
G/A	0.37 (0.10 to 1.35)	0.131	0.11 (0.012 to 0.90)	0.040
A/A	0.25 (0.05 to 1.21)	0.084	0.05 (0.004 to 0.59)	0.017
Heart disease				
G/G	0.28 (0.06 to 1.42)	0.125	1.00	
G/A	3.14 (0.75 to 13.18)	0.118	3.86 (0.74 to 20.11)	0.108
A/A	0.97 (0.11 to 8.82)	0.977	2.29 (0.18 to 28.86)	0.523
Dyslipidemia				
G/G	0.98 (0.37 to 2.54)	0.960	1.00	
G/A	0.90 (0.35 to 2.33)	0.822	0.96 (0.35 to 2.60)	0.930
A/A	1.47 (0.27 to 7.85)	0.653	1.43 (0.25 to 8.29)	0.687
Overweight				
G/G	0.79 (0.32 to 1.94)	0.606	1.00	
G/A	1.21 (0.50 to 2.95)	0.675	1.27 (0.49 to 3.25)	0.622
A/A	1.12 (0.26 to 4.84)	0.878	1.27 (0.27 to 5.92)	0.764

Hyperuricemia was defined as serum uric acid over 7 mg/dL; Diabetic mellitus was defined as fasting blood sugar more than or equal to 126 mg/dL or receiving the antidiabetic drug, Hypertension was defined SBP over 140 mmHg or DBP over 90 mmHg; Heart diseases were diagnosed by a physician; Stroke was diagnosed by a physician; Dyslipidemia was defined as total cholesterol over 200 mg/dL, triglyceride over 150 mg/dL, low-density lipoprotein cholesterol over 160 mg/dL or received lipid-lowering agents; Overweight was defined as BMI 25.00 to 29.99 kg/m^2

identified (Table 5).

Association between LRP2 (rs2228171) and stroke

There were 6, and 1 patient in the stroke group with heterozygous, and homozygous A. The homozygous G genotype was not identified in stroke patients (Table 4). There was a deviation of genotypic between-group from HWE SNP (p=0.013). The factors such as older age, a greater level of WC, HC, TC, HDL, LDL, SUA, HbA1C, BUN, and Cr were found in the stroke group when compare with non-stroke patients (Table 2). Due to the lack of genotype GG in a patient with stroke, so the association of genotypes and stroke could not identify.

Association between LRP2 (rs2228171) and dyslipidemia

The variation of LRP2 among the dyslipidemia group was analyzed. Homozygous major G-allele carriers, heterozygous carriers, and homozygous major AA were found 34, 36, and 8, respectively. The genotypic frequencies between-group were not differenced from HWE SNP (Table 4). Patients with smoking, and alcohol found a higher level of WC, HC, TC, TG, LDL, HbA1C, GFR in DLP group compare with non-DLP group (Table 2). Nevertheless, the association of LRP2 genotypes and dyslipidemia was not identified (Table 5).

Association between LRP2 (rs2228171) and overweight

In our participants, half of the patients coexisted with overweight. Sixteen, 19, and 4 patients had carried homozygous G, heterozygous, and homozygous A, respectively. The genotypic frequencies did not deviate between groups (Table 4). The frequencies of smoking were

predominant in the overweight group. Moreover, the level of WC, HC, SBP, DBP, TG, FBS, and GFR were increased in the overweight group (Table 2). However, there was no association between genotypes and overweight (Table 5).

Discussion

From phase 1 of the study, the identification of the one novel locus mapped to LRP2 (rs2228171) (Ala2872Thr, G>A) has been found in HUA, and gout in Thai men, but a small number of a participant. The LRP2 gene (rs2228171) was evaluated in a large population as a phase 2 study. Our study was designed to identify the frequency of the LRP2 gene (rs2228171) in HUA, heart disease, stroke, dyslipidemia, HT, and overweight among Thai NCD patients.

LRP2 gene encodes low-density lipoprotein receptor-related protein 2 (megalin), it is a member of the low-density lipoprotein receptor (LDLR) family. LRP2 is expressed on the apical surface of absorptive epithelial tissues such as mainly in the kidney, especially in glomeruli and proximal tubular cells[61]. LRP2 is a glycoprotein, its molecular weight is about 600 KD. LRP2 has a large amino-terminal extracellular domain that consists of a single transmembrane domain and a short carboxy-terminal cytoplasmic tail. The extracellular domain has four clusters (I-IV) of complement type (LDLR class A) that replicates on ligand-binding regions. Its ligands include apolipoproteins B, E, and lipoprotein lipase[62,63]. It is not clear the lipid-soluble signaling molecules to target cells.

Interestingly, the previous study demonstrated that the LRP2 protein is critical for the reuptake of numerous ligands, including lipoproteins, nutrients, morphogens, protease-protease inhibitor complexes, and vitamin-vitamin binding protein complexes[61,64]. This protein also has a role in cell signaling. Mutations in this gene cause Donnai-Barrow syndrome (DBS) and facio-oculoacoustico-renal syndrome (FOAR).

A recent study showed an intron variant of LRP2 rs2544390 on chromosome 2q24-31 that is associated with HUA[65]. LRP2 gene rs2544390 was demonstrated an association between body mass index and serum uric acid levels in a Japanese population[66]. LRP2 polymorphism was associated with plasma lipid levels[67].

Association between LRP2 (rs2228171) and HUA

Otherwise, LRP2 could be associated with serum uric acid variation through the endocytosis of urate-binding proteins. But there was no association of LRP2 in patients with HUA in our study. Some previous GWAS in a Japanese population identified that one common novel variant in LRP2 (rs2544390) has been reported in association with serum uric acid[65]; in contrast, another GWAS in a Chinese population revealed no significant association between rs2544390 and uric acid levels[68]. Moreover, another report demonstrated the LRP2 gene rs2544390 relation between body mass index and serum uric acid in a Japanese population[66]. Another study in a Chinese population[69] and a combined Maori and Pacific Island cohort[70] also showed that rs2543390 had a significant influence on gout susceptibility, whereas a protective effect was found in European[70] and had not associated with gout susceptibility in a Japanese population[71].

Because positive and negative results of LRP2 were reported. Therefore, the patients who carried the LRP2 variant were unconcluded to increase the risk of HUA. Moreover, the position of LPR2 in our study is different from the Japanese and Chinese populations.

Association between LRP2 (rs2228171), DM, and dyslipidemia

Because LRP2 is a member of the LDL receptor family that involve with mediate endocytosis of LDL. LRP2 is a lipoprotein receptor that play role in cholesterol transport[72], functioning along with its coreceptor cubilin[73] Moreover, megalin is a receptor for apoJ/clusterin that involve HDL particles[74,75] and Lp (1), Lp (1) is an atherogenic particle[76,77]. Apolipoprotein M is lipocalin and antiatherogenic properties found in pre-β-HDL particles, chylomicrons, VLDLs, and LDLs. Apolipoprotein M is secreted by the liver and kidney, it needs megalin receptor[78-80]. Megalin with its coreceptor cubilin help ApoAa-I and ApoA-II that they are structural components of HDL[81,82]. Thus megalin involve with the regulation of HDL metabolism. A report from the study in the Japanese population found a level of cholesterol related to genetic variation in the megalin gene[67].

DLP is known as a risk to increase insulin resistance and serum uric acid levels[83]. A high level of LDL increases the risk of insulin resistance. A report from the Japanese population showed one variant of LRP2 (rs2229268) to be an association with serum LDL levels in humans[67]. Therefore, rs2229268 seems to have an association with serum uric acid variation. But in our study showed no association of LRP2 in patients with DLP and DM2. The fact that the difference between genotypes may be due to the difference in positions of the LRP2, ethnic diversity, and the number of population studies is relatively small. Therefore, further investigation is required.

Association between LRP2 (rs2228171) and hypertension

Megalin expression, trafficking, and/or its ligands are likely involved in some disease conditions that compromise the functioning of organs such as the kidney, brain[61]. Megalin ligands such as albumin, insulin, leptin, PTH, and angiotensin II have been implicated in pathological conditions, including diabetes, hypertension, and obesity[84-90], many of which affect renal function. Similarly, megalin-interacting proteins may be involved in disease pathology. In our study, we found patients who had HT carried homozygous G and heterozygous. So variants of the LPR2 gene might be lead to an increased risk of hypertension. However, since the majority of the population studied coexisted with HT. There were few patients without HT. Therefore, we cannot be concluded whether genotypes

GA and AA decrease the risk of HT. The authors suggest further study is required.

Association between LRP2 (rs2228171) stroke, and heart disease

Several risk factors increase the risk of stroke and heart disease such as hyperlipidemia, hypertension, and diabetes mellitus. Abnormality of LRP2 receptor leading to DM, obesity, HT, DLP[84-90] that consequence to stroke. Due to the small sample in stroke and heart disease, so we cannot be analyzed of the association.

Conclusion

Our study is the first cross-sectional study of the frequency of the rs variants in the LRP2 rs2228171 gene in Thai NCD patients. The genetic frequencies of LRP2 rs2228171 with HUA, DM, DLP, heart, stroke, HT, and overweight were identified. Although our study showed no association of genes and HUA. But our results provide information on LRP2 in HT patients. Furthermore, the study will be done to clarify the effect of LRP2 variation and metabolic diseases such as HT. The next research may lead to a better understanding of the association of LRP2.

What is already known on this topic?

Several genetic variants are associated with serum uric acid, and gout.

What this study adds?

LRP2 rs2228171 was not associated with HUA. Genotype GA and AA may be a protective effect of HT.

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Potential conflicts of interest

The authors declare no conflict of interest.

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