European Review for Medical and Pharmacological Sciences 2022; 26: 8873-8878

PSMD9 is linked to T2D age of onset, years of isolated and combined insulin therapy, irregular menses, and hot flashes

L. DEL BOSQUE-PLATA[1], M. AMIN[2,3], C. GRAGNOLI[4,5,6]

[1]National Institute of Genomic Medicine, Nutrigenetics and Nutrigenomic Laboratory, Mexico City, Mexico
[2]INSERM, US14-Orphanet, Paris, France
[3]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Al-Neelain University, Khartoum, Sudan
[4]Division of Endocrinology, Department of Medicine, Creighton University School of Medicine, Omaha, NE, USA
[5]Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA, USA
[6]Molecular Biology Laboratory, Bios Biotech Multi-Diagnostic Health Center, Rome, Italy

Abstract. – OBJECTIVE: ***PSMD9*** **is a ubiquitous protein present at high concentrations in eukaryotic cells. It contributes to the degradation of intracellular proteins in the immune system. It is part of the 26S proteasome complex, and its regulatory role on proteasomal activity as well as its effect on genetic transcription have been recognized.** ***PSMD9*** **has been related with insulin secretion, and it regulates the ligand-dependent retinoid-target genes transcription. Importantly,** ***PSMD9*** **rs74421874 (*IVS3+nt460*-G>A), rs3825172 (*IVS3+nt437*-C>T), and rs14259 SNPs have been previously linked to type 2 diabetes (T2D), maturity-onset diabetes of the young 3 (MODY3), overweight status and waist circumference, hypertension, hypercholesterolemia, cardiovascular disease, microvascular disease (retinopathy, neuropathy, and nephropathy), carpal tunnel syndrome, depression, anxiety, insomnia, and sleep hours.**

MATERIALS AND METHODS: **In this study, we analyzed the above-mentioned** ***PSMD9*** **rs74421874 (*IVS3+nt460*-G>A), rs3825172 (*IVS3+nt437*-C>T), and rs14259 SNPs for linkage to the T2D quantitative traits of T2D age of onset, duration in years of combined oral hypoglycemic agents and insulin therapy and only insulin therapy, stress, and the birth weight of the subjects' children; and with the T2D qualitative phenotypes of irregular menses, couple infertility, and menopausal hot flashes.**

RESULTS: **We found that** ***PSMD9*** **was linked to irregular menses of reproductive age, menopausal hot flashes, T2D age of onset, years of combined oral and insulin therapy and of insulin therapy; we also found that it shows only a tendency towards linkage to stress, birthweight, and couple infertility.**

CONCLUSIONS: **This is the first time that this gene is implicated with irregular menses of reproductive age (a trait of polycystic ovarian syndrome), hot flashes, T2D onset age, and duration years of combined oral and insulin therapy and only insulin therapies.**

Key Words:
PSMD9, p27, Rpn4, Bridge-1, Proteasome, Type 2 diabetes, T2D, Age of onset, Irregular menses, Couple infertility, Polycystic ovarian syndrome, Birth weight, Stress, Duration years, Combined therapy, Oral hypoglycemic agents, Insulin, Menopausal hot flashes, Cardiovascular disease, Microvascular, Macrovascular, Retinopathy, Neuropathy, Nephropathy, Carpal tunnel syndrome, Overweight, Waist circumference, Depression, Insomnia, Sleep hours, Anxiety, Linkage, Association, Variance component, Trait, Phenotype, Heritability, Italian, families, Gene 12q24 locus, Pleiotropy, Merlin.

Introduction

Within the 12q24 locus/NIDDM2 locus lies the 26S Proteasome Non-ATPase Regulatory Subunit 9 [*PSMD9* (mouse homologous Bridge-1), also known as p27 or Rpn4], an insulin gene transcription coactivator that is highly expressed in pancreatic islets[1]. PSMD9 is part of the 26S proteasome complex and its regulatory role on proteasomal activity is well-known as well as its effect on genetic transcription[2]. The 26S proteasome also regulates ligand-dependent retinoid-target genes transcription; and the PSMD9 PDZ [post

Corresponding Author: Claudia Gragnoli, MD, Ph.D; e-mail: claudia.gragnoli@gmail.com

synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1)] interaction domain plays a part in mRNA processing and mRNA editing, transcriptional regulation, hormone and receptor activity, and protein translation[3]. Pancreatic overexpression in mice of the homologous *Bridge-1* causes insulin deficiency, diabetes, and hypertriglyceridemia[4]; its transcription inhibition *in vitro* reduces insulin secretion[1]. Thus, *PSMD9* variants might impair insulin transcription and cause beta-cell dysfunction[1]. Recently, transomic analyses using lipid regulatory networks in 107 genetically distinct mice strains allowed the identification and validation of PSMD9 as a previously unknown lipid regulatory protein[5].

The *PSMD9* rs74421874 (*IVS3+nt460*-G>A*)*, rs3825172 (*IVS3+nt437*-C>T), and rs14259 single nucleotide polymorphisms (SNPs) have been significantly linked to T2D and digenic type of inheritance maturity-onset diabetes of the young 3 (MODY3) at least in Italian families[6,7], and they have also been linked to several T2D-related long-term complications, including T2D-related nephropathy[8], T2D-related neuropathy[9], T2D-related retinopathy[10] and T2D-related micro- and macro-vascular pathology[11,12]. *PSMD9* has also been linked to several conditions associated with T2D, such as hypertension[13], coronary artery disease (CAD), stroke and/or transitory ischemic attacks[12], hypercholesterolemia[14], overweight and waist circumference[15], carpal tunnel syndrome[16], anxiety[17], insomnia and sleep hours[18], depression[19,20] and anti-depressant response[21], and schizophrenia (SCZ)[22]. *PSMD9* clearly shows strong pleiotropic effects. In the phenotypes associated with T2D and atherosclerosis, one potential role of *PSMD9* is related to the pathogenesis of inflammation[23]. The mouse homologous gene of *PSMD9*, *Bridge-1*, has been implicated in ovarian follicular maturation[24], and might thus, if impaired, underlie the pathogenesis of anovulation, a trait of polycystic ovarian syndrome (PCOS). PCOS usually manifests, among other phenotypes, with irregular menses or anovulation, insulin resistance, obesity[25], and increased risk for infertility[26]. PCOS is genetically determined[27] and beyond being associated with infertility[26], it is commonly associated with T2D and cardiovascular disease[28,29].

All of these traits can co-occur with T2D and may be due to a commonly shared pathogenesis[28]. We hypothesized that the *PSMD9* gene could be linked to other T2D- or PCOS-related endophenotypes, such as T2D age of onset or menopausal hot flashes[30] – both genetically determined traits [31,32] – and possibly also to other unknowingly heritable traits, such as irregular menses and duration of T2D treatment. Of note, T2D age of onset has already been linked to the 12q24 locus[33], which harbors the *PSMD9* gene. The 12q24 locus shows disease pleiotropy, especially for cardiovascular, metabolic, and mental phenotypes, which can be genetically comorbid and contributed by *PSMD9*[34]. If *PSMD9* is linked to T2D[6], it may be linked to the associated traits of stress[35] and birth weight[36], both of which confer risk for cardiovascular disease[37,38]; menopausal hot flashes, a phenotype associated with a vulnerable vascular phenotype among midlife women[39]; and couple infertility and irregular menses of reproductive age[26].

We, therefore, aimed to test three SNPs in the *PSMD9* gene for linkage to both the T2D quantitative traits of T2D age of onset, duration years of combined oral hypoglycemic agents and insulin therapy and only insulin therapy, stress, the birth weight of the subjects' children; and the T2D qualitative phenotypes of irregular menses, couple infertility, and menopausal hot flashes.

Materials and Methods

We used the previously collected deidentified data of 200 Italian T2D affected siblings and families. We excluded all families with identical twins and confirmed that families were at least three generations Italian. Italians are a homogeneous population living in a peninsula, and they are suitable for genetic studies due to lack of genetic admixture. Individuals were previously recruited from central Italy following the Helsinki declaration guidelines, and subjects provided written informed consent prior to participation. The study was institutionally approved by Penn State College of Medicine. We amplified the *PSMD9* SNPs rs74421874 (*IVS3+nt460*-A/G), rs3825172 (*IVS3+nt437*-C/T), and rs14259 (*E197G*-A>G) of exon 5 in the affected and unaffected family members by using PCR; the PCR products were directly sequenced, status post-purification via EXOSAP-IT, on an automated ABI 3730 Sequencer. We analyzed the SNPs rs74421874, rs3825172, rs14259 for linkage to the quantitative traits: age at T2D onset; total years of combined therapy of oral hypoglycemic agents and insulin and the total years of only insulin therapy; self-perceived lifetime stress (defined by a scale 1-4, 1 = no stress, 2 = minimal-level of stress,

Table I. Non-parametric Linkage Analysis of *PSMD9* Single Nucleotide Polymorphisms *IVS3+nt460*, *IVS3+nt437*, *E197G* with Phenotypes of 200 Italian Families.

Phenotype	Prevalence	Families	Lod Score	*p*
Hot Flashes	50.90%	10	1.33	0.007
Irregular Menses	28.70%	5	0.66	0.040
Couple Infertility	27.60%	8	0.54	0.060

Prevalence = phenotype prevalence among the family subjects studied; Families = families number considered informative by Merlin for the tested single nucleotide polymorphisms; Lod score = derived from the non-parametric linkage analysis by Merlin.

3 = medium-level of stress, 4 = maximum-level of stress), and the birth weight of the subjects' children; and for linkage to the qualitative traits: irregular menses (defined as less than 9 cycles per year); couple infertility (defined as inability to conceive after 1 year of unprotected intercourse); and, menopausal hot flashes. Non-parametric linkage analysis was performed for the qualitative phenotypes and variance component analysis and association testing were performed for the quantitative traits by using Merlin software[40].

Statistical Analysis

The non-parametric linkage analysis for the qualitative traits and the variance component analysis and association testing for the quantitative traits were considered significant if the p was ≤ 0.05.

In Silico Analysis

We analyzed the 3 variants using several functional and regulatory *in silico* prediction tools: pathogenicity [SIFT][41] and [PolyPhen][42]; protein stability [I-Mutant][43]; splicing [SpliceAI][44]; transcription-factor binding [SNPnexus][45] and SNP2TFBS[46]; regulatory potential [RegulomeDB][47]; and, miRNA binding [mirSNP][48]. We found that the 3 risk variants intersect with actively transcribed chromatin state in the brain and pancreatic tissues. These findings corroborate the results of *Bridge-1 – PSMD9* homologous - pancreatic overexpression in mice causing insulin deficiency, diabetes, and hypertriglyceridemia, all of which can manifest with insulin resistance, T2D, and PCOS[4]. The rs14259 variant was also predicted to be damaging for the non-ATPase subunit of the 19S regulator of the PSMD9 complex. In addition, the mutant residue of the rs14259 variant decreased the stability of the protein by –1.23 Kcal/mol.

Results

The lod scores and corresponding p of the analyses are reported in Table I (qualitative phenotypes) and in Table II (quantitative traits). The results of our analysis showed that the *PSMD9* SNPs studied and/or any gene variants in linkage disequilibrium with them are linked to the age of onset of T2D with an estimated trait heritability of

Table II. Analysis of *PSMD9* Single Nucleotide Polymorphisms *IVS3+nt460*, *IVS3+nt437*, *E197G* with Quantitative Traits of 200 Italian Families.

Quantitative trait	Prevalence	Families	Trait heritability	Gene trait heritability	Lod score	*p*
Variance component linkage analysis						
Age of onset of T2D	94.50%	189	60.02%	60.52%	1.34	0.00700
Years of pills/Insulin Therapy	90.00%	180	85.56%	100.00%	5.58	0.00000
Years of insulin therapy	89.00%	178	100.00%	100.00%	3.82	0.00001
Association testing						
Age of onset of T2D	94.50%	189	60.00%	1.18%	0.832	0.050
Birth Weight	31.50%	63	100.00%	3.31%	0.773	0.059
Stress	65.00%	130	31.97%	1.44%	0.788	0.057

Prevalence = trait prevalence among the family subjects studied; Families = families number number considered informative by Merlin for the tested single nucleotide polymorphisms (SNPs); Trait heritability = heritability of the quantitative trait calculated by Merlin; Gene trait heritability = heritability attributable to the PSMD9 SNPs. Lod score = derived from the quantitative trait analysis by Merlin.

60.52%. We also found that *PSMD9* is linked to the duration in years of combined oral hypoglycemic agents and insulin therapy and of only insulin therapy, both with an estimated gene-related heritability of 100%. We found an association between the intronic IVS3+nt460 and IVS3+437 alleles for age of onset of T2D, and we found an association trend with perceived lifetime stress level and the birth weight of the children born to the subjects. Additionally, we found that the *PSMD9* SNPs are linked to irregular menses of reproductive age, the menopausal hot flashes, and shows only a tendency towards linkage to couple infertility.

Discussion

Since the *PSMD9* gene has been previously linked to overweight condition and waist circumference[16] and given our finding that *PSMD9* is linked to irregular menses of reproductive age and shows a tendency towards linkage to couple infertility and based on the fact that some overweight subjects with irregular menses and/or couple infertility have PCOS, the gene may lead to PCOS. Of interest, *PSMD9* expressed in the human ovary induces follicle maturation and has a role in activin A signaling in the human granulosa cell line HGL-5. Expression of *PSMD9* can be stimulated by activin A, an important factor for follicle maturation[25].

This is the first time that *PSMD9* has been shown to be involved with irregular menses of reproductive age (a trait of PCOS), menopausal hot flashes, T2D age of onset, and duration years of combined oral and insulin therapy and only insulin therapies. Our findings should be replicated in other cohorts and ethnicities.

Notably, *PSMD9* has pleiotropic effects and might contribute to the 12q24 linkage to multiple somatic disorders and mental diseases. However, the variant function involved in these linkages still needs to be characterized using diverse models. Recent technological advancement in next generation sequencing (NGS), in particular whole exome sequencing (WES), can help lead to the identification of novel causative variants in *PSMD9*.

Conclusions

Molecular and genetic data have linked *PSMD9* to a diverse range of important human conditions, including our novel findings that it is linked to T2D age of onset, duration years of T2D therapy, irregular menses, and hot flashes, thereby highlighting its potential impact as a therapeutic target in these multiple phenotypes and/or traits.

Conflict of Interest

The authors declare that they have no conflict of interests.

Acknowledgements

We thank the families who participated in the study, and we thank Bios Biotech Multi-Diagnostic Health Center, Rome, Italy, for data access and for financial, medical, and laboratory staff support. This study was supported by Penn State College of Medicine.

Ethics Approval

Families were recruited following the Helsinki declaration guidelines, and individuals provided written informed consent prior to participation. The study was institutionally approved by Penn State College of Medicine.

Funding

This work was supported by Penn State College of Medicine.

Authors' Contribution

L.D.B. (https://orcid.org/0000-0003-3224-378X) contributed to manuscript drafting and data interpretation. M.A. (https://orcid.org/0000-0003-2876-0784) helped with manuscript drafting and *in silico* analysis. C.G. (https://orcid.org/0000-0002-3873-6617) conceived and performed the study and drafted the manuscript.

Data Availability Statement

The study data are available on reasonable request, and due to lacking specific patients' consent and privacy restrictions, they are not publicly available.

Authors' Information

C.G. is Professor of Medicine and Chief of Endocrinology, Creighton University School of Medicine, Omaha, NE, and Adjunct Professor of Public Health Sciences, Penn State University College of Medicine, Hershey, PA; M.A. has an MD and a PhD in Genetics (University of Paris) and is a geneticist at Orphanet | INSERM-US14, Paris, France, and member of the executive committee, European Reference Network for the Intellectual Disabilities (ERN-ITHACA) and the International Consortium of Gene Curation Coalition (GenCC); L.B.P. is a PhD research scientist at the National Institute of Genomic Medicine, Nutrigenetics, and Nutrigenomic Laboratory, Mexico City, Mexico.

References

1) Thomas MK YK, Tenser MS, Wong GG, Habener JF. Bridge-1, a novel PDZ-domain coactivator of E2A-mediated regulation of insulin gene transcription. Mol Cell Biol 1999; 19: 8492-8504.
2) Dalton WS. The proteasome. Semin Oncol 2004; 31: 3-9; discussion 33.
3) Sangith N, Srinivasaraghavan K, Sahu I, Desai A, Medipally S, Somavarappu AK, Verma C, Venkatraman P. Discovery of novel interacting partners of PSMD9, a proteasomal chaperone: role of an atypical and versatile PDZ-domain motif interaction and identification of putative functional modules. FEBS Open Bio 2014; 4: 571-583.
4) Volinic JL, Lee JH, Eto K, Kaur V, Thomas MK. Overexpression of the coactivator bridge-1 results in insulin deficiency and diabetes. Mol Endocrinol 2006; 20: 167-182.
5) Parker BL, Calkin AC, Seldin MM, Keating MF, Tarling EJ, Yang P, Moody SC, Liu Y, Zerenturk EJ, Needham EJ, Miller ML, Clifford BL, Morand P, Watt MJ, Meex RCR, Peng KY, Lee R, Jayawardana K, Pan C, Mellett NA, Weir JM, Lazarus R, Lusis AJ, Meikle PJ, James DE, de Aguiar Vallim TQ, Drew BG. An integrative systems genetic analysis of mammalian lipid metabolism. Nature 2019; 567: 187-193.
6) Gragnoli C. PSMD9 gene in the NIDDM2 locus is linked to type 2 diabetes in Italians. J Cell Physiol 2010; 222: 265-267.
7) Gragnoli C. PSMD9 is linked to MODY3. J Cell Physiol 2010; 223: 1-5.
8) Gragnoli C. T2D-nephropathy linkage within 12q24 locus. Diabetes Res Clin Pract 2011; 92: e73-e75.
9) Gragnoli C. PSMD9 is linked to type 2 diabetes neuropathy. J Diabetes Compl 2011; 25: 329-331.
10) Gragnoli C. Proteasome modulator 9 gene is linked to diabetic and non-diabetic retinopathy in T2D. Ophthalmic Genet 2011; 32: 228-230.
11) Gragnoli C. Proteasome modulator 9 is linked to microvascular pathology of T2D. J Cell Physiol 2012; 227: 3116-3118.
12) Gragnoli C. Proteasome modulator 9 and macrovascular pathology of T2D. Cardiovasc Diabetol 2011; 10: 32.
13) Gragnoli C. Proteasome modulator 9 SNPs are linked to hypertension in type 2 diabetes families. Cardiovasc Diabetol 2011; 10: 77.
14) Gragnoli C. Hypercholesterolemia and a candidate gene within the 12q24 locus. Cardiovasc Diabetol 2011; 10: 38.
15) Gragnoli C. Overweight condition and waist circumference and a candidate gene within the 12q24 locus. Cardiovasc Diabetol 2013; 12: 2.
16) Gragnoli C. Proteasome modulator 9 and carpal tunnel syndrome. Diabetes Res Clin Prac 2011; 94: e47-e49.
17) Gragnoli C. Proteasome modulator 9 gene SNPs, responsible for anti-depressant response, are in linkage with generalized anxiety disorder. J Cell Physiol 2014; 229: 1157-1159.
18) Hao H, Haas MJ, Wu R, Gragnoli C. T2D and depression risk gene proteasome modulator 9 is inked to insomnia. Sci Rep 2015; 5: 12032.
19) Gragnoli C. Proteasome modulator 9 and depression in type 2 diabetes. Curr Med Chem 2012; 19: 5178-5180.
20) Wong ML, Dong C, Andreev V, Arcos-Burgos M, Licinio J. Prediction of susceptibility to major depression by a model of interactions of multiple functional genetic variants and environmental factors. Mol Psychiatry 2012; 17: 624-633.
21) Wong ML, Dong C, Maestre-Mesa J, Licinio J. Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response. Mol Psychiatry 2008; 13: 800-812.
22) Lee YH, Kim JH, Song GG. Pathway analysis of a genome-wide association study in schizophrenia. Gene 2013; 525: 107-115.
23) Wick G, Millonig G, Xu Q. The Autoimmune Pathogenesis of Atherosclerosis: An Evolutionary-Darwinian Concept. In: Shoenfeld Y, Harats D, Wick G, eds. Atherosclerosis and Autoimmunity. Elsevier 2001; pp. 5-16.
24) Banz C, Munchow B, Diedrich K. Bridge-1 is expressed in human granulosa cells and is involved in the activin A signaling cascade. Fertil Steril 93: 1349-1352.
25) Al-Jefout M, Alnawaiseh N, Al-Qtaitat A. Insulin resistance and obesity among infertile women with different polycystic ovary syndrome phenotypes. Sci Rep 2017; 7: 5339.
26) Hart R. PCOS and infertility. Panminerva Med 2008; 50: 305-314.
27) Mykhalchenko K, Lizneva D, Trofimova T, Walker W, Suturina L, Diamond MP, Azziz R. Genetics of polycystic ovary syndrome. Expert Rev Mol Diagn 2017; 17: 723-733.
28) Zhu T, Cui J, Goodarzi MO. Polycystic ovary syndrome and risk of type 2 diabetes, coronary heart disease, and stroke. Diabetes 2021; 70: 627-637.
29) Osibogun O, Ogunmoroti O, Michos ED. Polycystic ovary syndrome and cardiometabolic risk: opportunities for cardiovascular disease prevention. Trends Cardiovasc Med 2020; 30: 399-404.
30) Ogbera A, Fasanmade O, Kalra S. Menopausal symptoms and the metabolic syndrome in Nigerian women with type 2 diabetes mellitus. Climacteric 2011; 14: 75-82.
31) Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP. Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. Am J Hum Genet 1999; 64: 1127-1140.

32) Crandall CJ, Diamant AL, Maglione M, Thurston RC,Sinsheimer J. Genetic variation and hot flashes: a systematic review. J Clin Endocrinol Metab 2020; 105: e4907-e4957.

33) Wiltshire S, Frayling TM, Groves CJ, Levy JC, Hitman GA, Sampson M, Walker M, Menzel S, Hattersley AT, Cardon LR, McCarthy MI. Evidence from a large U.K. family collection that genes influencing age of onset of type 2 diabetes map to chromosome 12p and to the MODY3/NIDDM2 locus on 12q24. Diabetes 2004; 53: 855-860.

34) Postolache TT, Del Bosque-Plata L, Jabbour S, Vergare M, Wu R, Gragnoli C. Co-shared genetics and possible risk gene pathway partially explain the comorbidity of schizophrenia, major depressive disorder, type 2 diabetes, and metabolic syndrome. Am J Med Genet B Neuropsychiatr Genet 2019; 180: 186-203.

35) Hackett RA, Steptoe A. Type 2 diabetes mellitus and psychological stress - a modifiable risk factor. Nat Rev Endocrinol 2017; 13: 547-560.

36) Huang T, Wang T, Zheng Y, Ellervik C, Li X, Gao M, Fang Z, Chai JF, Ahluwalia TVS, Wang Y, Voortman T, Noordam R, Frazier-Wood A, Scholz M, Sonestedt E, Akiyama M, Dorajoo R, Zhou A, Kilpeläinen TO, Kleber ME, Crozier SR, Godfrey KM, Lemaitre R, Felix JF, Shi Y, Gupta P, Khor CC, Lehtimäki T, Wang CA, Tiesler CMT, Thiering E, Standl M, Rzehak P, Marouli E, He M, Lecoeur C, Corella D, Lai CQ, Moreno LA, Pitkänen N, Boreham CA, Zhang T, Saw SM, Ridker PM, Graff M, van Rooij FJA, Uitterlinden AG, Hofman A, van Heemst D, Rosendaal FR, de Mutsert R, Burkhardt R, Schulz CA, Ericson U, Kamatani Y, Yuan JM, Power C, Hansen T, Sørensen TIA, Tjønneland A, Overvad K, Delgado G, Cooper C, Djousse L, Rivadeneira F, Jameson K, Zhao W, Liu J, Lee NR, Raitakari O, Kähönen M, Viikari J, Grote V, Langhendries JP, Koletzko B, Escribano J, Verduci E, Dedoussis G, Yu C, Tham YC, Lim B, Lim SH, Froguel P, Balkau B, Fink NR, Vinding RK, Sevelsted A, Bisgaard H, Coltell O, Dallongeville J, Gottrand F, Pahkala K, Niinikoski H, Hyppönen E, Pedersen O, März W, Inskip H, Jaddoe VWV, Dennison E, Wong TY, Sabanayagam C, Tai ES, Mohlke KL, Mackey DA, Gruszfeld D, Deloukas P, Tucker KL, Fumeron F, Bønnelykke K, Rossing P, Estruch R, Ordovas JM, Arnett DK, Meirhaeghe A, Amouyel P, Cheng CY, Sim X, Teo YY, van Dam RM, Koh WP, Orho-Melander M, Loeffler M, Kubo M, Thiery J, Mook-Kanamori DO, Mozaffarian D, Psaty BM, Franco OH, Wu T, North KE, Davey Smith G, Chavarro JE, Chasman DI, Qi L. Association of birth weight with type 2 diabetes and glycemic traits: a Mendelian randomization study. JAMA Netw Open 2019; 2: e1910915.

37) Kivimaki M, Steptoe A. Effects of stress on the development and progression of cardiovascular disease. Nat Rev Cardiol 2018; 15: 215-229.

38) Mohseni R, Mohammed SH, Safabakhsh M, Mohseni F, Monfared ZS, Seyyedi J, Mejareh ZN, Alizadeh S. Birth weight and risk of cardiovascular disease incidence in adulthood: a dose-response meta-analysis. Curr Atheroscler Rep 2020; 22: 12.

39) Thurston RC, Chang Y, Barinas-Mitchell E, Jennings JR, Landsittel DP, Santoro N, von Kanel R, Matthews KA. Menopausal hot flashes and carotid intima media thickness among midlife women. Stroke 2016; 47: 2910-2915.

40) Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002; 30: 97-101.

41) Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003; 31: 3812-3814.

42) Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet 2013; 7: 7.20.

43) Capriotti E, Fariselli P, Casadio R. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res 2005; 33: W306-W310.

44) Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, Darbandi SF, Knowles D, Li YI, Kosmicki JA, Arbelaez J, Cui W, Schwartz GB, Chow ED, Kanterakis E, Gao H, Kia A, Batzoglou S, Sanders SJ, Farh KKH. Predicting splicing from primary sequence with deep learning. Cell 2019; 176: 535-548.e24.

45) Dayem Ullah AZ, Oscanoa J, Wang J, Nagano A, Lemoine NR, Chelala C. SNPnexus: assessing the functional relevance of genetic variation to facilitate the promise of precision medicine. Nucleic Acids Res 2018; 46: W109-W113.

46) Kumar S, Ambrosini G, Bucher P. SNP2TFBS - a database of regulatory SNPs affecting predicted transcription factor binding site affinity. Nucleic Acids Res 2017; 45: D139-D144.

47) Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM, Snyder M. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 2012; 22: 1790-1797.

48) Liu C, Zhang F, Li T, Lu M, Wang L, Yue W, Zhang D. MirSNP, a database of polymorphisms altering miRNA target sites, identifies miRNA-related SNPs in GWAS SNPs and eQTLs. BMC Genomics 2012; 13: 661.