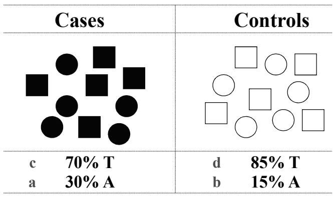
심포지엄 | 제 2 세미나실

심혈관질환의 유전체학 : 혈압 GWAS를 중심으로

오 범 석

경희의대 의공학교실

ASSOCIATION STUDY 란?
CASE-CONTROL STUDY

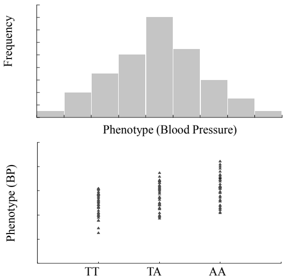


Cases		Controls	
c	70% T	d	85% T
a	30% A	b	15% A

Chi-Square test, Logistic Regression

OR = ad/bc

ASSOCIATION STUDY 란?
QUANTITATIVE TRAITS 에서



Linear Regression

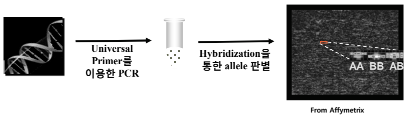
GENOME-WIDE ASSOCIATION STUDY PUBLISHED IN 2007

Vol 447|7 June 2007|**doi:10.1038/nature05911** nature

ARTICLES

Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls

The Wellcome Trust Case Control Consortium*



From Affymetrix

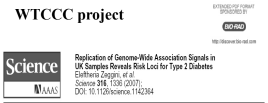
ASSOCIATION 분석의 재연성 문제를 풀다

DGI project

Genome-Wide Association Analysis Identifies Loci for Type 2 Diabetes and Triglyceride Levels
Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, *et al.*
Science **316**, 1331 (2007);
DOI: 10.1126/science.1142358

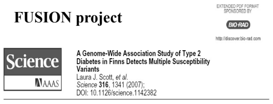
Published in Science 2007, Back-to-back !

WTCCC project



Replication of Genome-Wide Association Signals in UK Samples Reveals Risk Loci for Type 2 Diabetes
Eleftheria Zeggini, *et al.*
Science **316**, 1336 (2007);
DOI: 10.1126/science.1142364

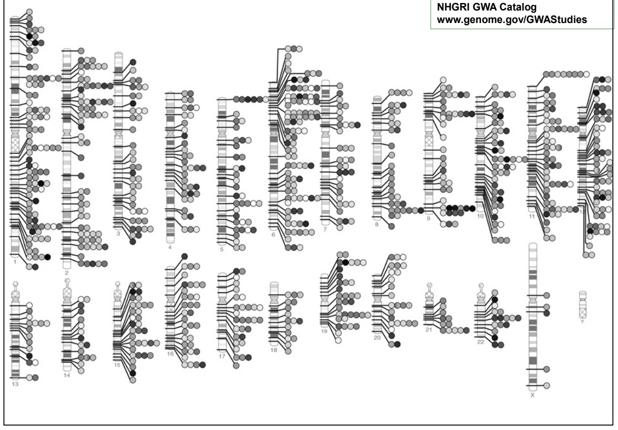
FUSION project



A Genome-Wide Association Study of Type 2 Diabetes in Finns Detects Multiple Susceptibility Variants
Laura J. Scott, *et al.*
Science **316**, 1341 (2007);
DOI: 10.1126/science.1142382

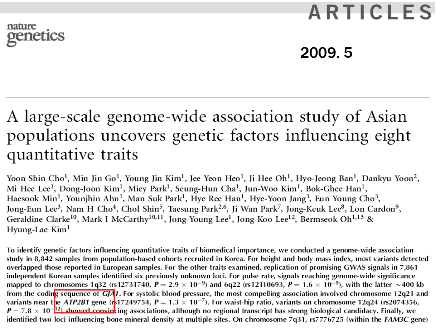
Published Genome-Wide Associations through 6/2010, 904 published GWA at $p \leq 5 \times 10^{-8}$ for 165 traits

NHGRI GWA Catalog
www.genome.gov/GWAStudies



BLOOD PRESSURE GWAS

혈압 GWAS는 한국에서 처음으로 발표



ARTICLES

nature genetics

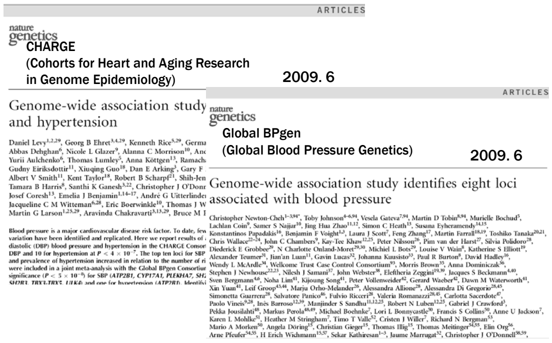
2009. 5

A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits

Yoon Shin Cho[1], Min Jin Go[1], Young Jin Kim[1], Jee Yeon Heo[1], Ji Hee Oh[1], Hyo-Jeong Ban[1], Dankyu Yoon[2], Mi Hee Lee[1], Dong-Joon Kim[1], Miey Park[1], Seung-Hun Cha[1], Jun-Woo Kim[1], Bok-Ghee Han[1], Haesook Min[1], Younjhin Ahn[1], Man Suk Park[1], Hye Ree Han[1], Hye-Yoon Jang[3], Eun Young Cho[3], Jong-Eun Lee[3], Nam H Cho[4], Chol Shin[5], Taesung Park[2,6], Ji Wan Park[7], Jong-Keuk Lee[8], Lon Cardon[9], Geraldine Clarke[10], Mark I McCarthy[10,11], Jong-Young Lee[1], Jong-Koo Lee[12], Bermseok Oh[1,13] & Hyung-Lae Kim[1]

To identify genetic factors influencing quantitative traits of biomedical importance, we conducted a genome-wide association study in 8,842 samples from population-based cohorts recruited in Korea. For height and body mass index, most variants detected overlapped those reported in European samples. For the other traits examined, replication of promising GWAS signals in 7,861 independent Korean samples identified six previously unknown loci. For pulse rate, signals reaching genome-wide significance mapped to chromosomes 1q32 (rs12731740, $P = 2.9 \times 10^{-9}$) and 6q22 (rs12110693, $P = 1.6 \times 10^{-9}$), with the latter ~400 kb from the coding sequence of *GJA1*. For systolic blood pressure, the most compelling association involved chromosome 12q21 and variants near the *ATP2B1* gene (rs17249754, $P = 1.3 \times 10^{-7}$). For waist-hip ratio, variants on chromosome 12q24 (rs2074356, $P = 7.8 \times 10^{-12}$) showed convincing associations, although no regional transcript has strong biological candidacy. Finally, we identified two loci influencing bone mineral density at multiple sites. On chromosome 7q31, rs7776725 (within the *FAM3C* gene)

본격적인 혈압 GWAS 결과 발표



ARTICLES

nature genetics

CHARGE
(Cohorts for Heart and Aging Research
in Genome Epidemiology)

2009. 6

Genome-wide association study
and hypertension

Daniel Levy[1,2,29], Georg B Ehret[3,4,29], Kenneth Rice[5,29], Germa
Abbas Dehghan[6], Nicole L Glazer[9], Alanna C Morrison[10], Anc
Yurii Aulchenko[6], Thomas Lumley[5], Anna Köttgen[13], Ramacha
Gudny Eiriksdottir[11], Xiuqing Guo[18], Dan E Arking[3], Gary F
Albert V Smith[11], Kent Taylor[18], Robert B Scharpf[21], Shih-Jen
Tamara B Harris[8], Santhi K Ganesh[3,22], Christopher J O'Donn
Josef Coresh[13], Emelia J Benjamin[1,14–17], André G Uitterlinder
Jacqueline C M Witteman[6,28], Eric Boerwinkle[10], Thomas J W
Martin G Larson[1,25,29], Aravinda Chakravarti[3,13,29], Bruce M I

Blood pressure is a major cardiovascular disease risk factor. To date, few
variation have been identified and replicated. Here we report results of
diastolic (DBP) blood pressure and hypertension in the CHARGE Consor
DBP and 10 for hypertension at $P < 4 \times 10^{-7}$. The top ten loci for SBP
and prevalence of hypertension increased in relation to the number of ri
were included in a joint meta-analysis with the Global BPgen Consortiu
significance ($P < 5 \times 10^{-8}$) for SBP (*ATP2B1, CYP17A1, PLEKHA7, SH2*
SH2B3, TBX3-TBX5, ULK4) and one for hypertension (*ATP2B1*). Identifyi

ARTICLES

nature genetics

Global BPgen
(Global Blood Pressure Genetics)

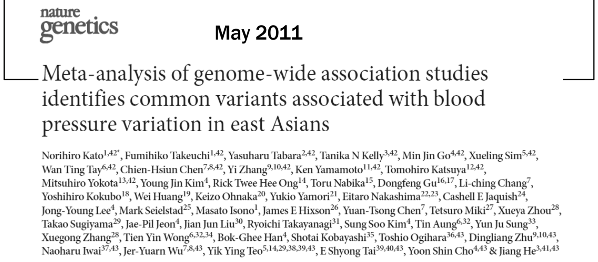
2009. 6

Genome-wide association study identifies eight loci associated with blood pressure

Christopher Newton-Cheh[1–3,94*], Toby Johnson[4–6,94], Vesela Gateva[7,94], Martin D Tobin[8,94], Murielle Bochud[5], Lachlan Coin[9], Samer S Najjar[10], Jing Hua Zhao[11,12], Simon C Heath[13], Susana Eyheramendy[14,15], Konstantinos Papadakis[16], Benjamin F Voight[1,3], Laura J Scott[7], Feng Zhang[17], Martin Farrall[18,19], Toshiko Tanaka[20,21], Chris Wallace[22–24], John C Chambers[9], Kay-Tee Khaw[12,25], Peter Nilsson[26], Pim van der Harst[27], Silvia Polidoro[28], Diederick E Grobbee[29], N Charlotte Onland-Moret[29,30], Michiel L Bots[29], Louise V Wain[8], Katherine S Elliott[19], Alexander Teumer[31], Jian'an Luan[11], Gavin Lucas[32], Johanna Kuusisto[33], Paul R Burton[8], David Hadley[16], Wendy L McArdle[34], Wellcome Trust Case Control Consortium[93], Morris Brown[35], Anna Dominiczak[36], Stephen J Newhouse[22,23], Nilesh J Samani[37], John Webster[38], Eleftheria Zeggini[19,39], Jacques S Beckmann[4,40], Sven Bergmann[4,6], Noha Lim[41], Kijoung Song[41], Peter Vollenweider[42], Gerard Waeber[42], Dawn M Waterworth[41], Xin Yuan[41], Leif Groop[43,44], Marju Orho-Melander[26], Alessandra Allione[28], Alessandra Di Gregorio[28,45], Simonetta Guarrera[28], Salvatore Panico[46], Fulvio Ricceri[28], Valeria Romanazzi[28,45], Carlotta Sacerdote[47], Paolo Vineis[9,28], Inês Barroso[12,39], Manjinder S Sandhu[11,12,25], Robert N Luben[12,25], Gabriel J Crawford[3], Pekka Jousilahti[48], Markus Perola[48,49], Michael Boehnke[7], Lori L Bonnycastle[50], Francis S Collins[50], Anne U Jackson[7], Karen L Mohlke[51], Heather M Stringham[7], Timo T Valle[52], Cristen J Willer[7], Richard N Bergman[53], Mario A Morken[50], Angela Döring[15], Christian Gieger[15], Thomas Illig[15], Thomas Meitinger[54,55], Elin Org[56], Arne Pfeufer[54,55], H Erich Wichmann[15,57], Sekar Kathiresan[1–3], Jaume Marrugat[32], Christopher J O'Donnell[58,59],

GENETIC LOCI FOR BP

Genes	SNP	Chr. Pos.	P-value	BP
ATP2B1	rs2681492	12	3.0E-11	SBP
CYP17A1	rs11191548	10	7E-24	SBP
CYP1A2-CSK-ULK3	rs1378942	15q24	1E-23	DBP
FGF5	rs16998073	4q21	1E-21	DBP
MTHFR	rs17367504	1p36	2E-13	SBP
C10ORF107	rs1536440	10q21	1E-09	DBP
ZNF652	rs16948048	17q21	5E-09	DBP
PLCD3	rs12946454	17q21	1E-08	SBP
PLEKHA7	rs381815	11	1.89E-09	SBP
CACNB2	rs11014166	10	1.24E-08	DBP
TBX3-TBX5	rs2384550	12	3.75E-08	DBP
ULK4	rs9815354	3	2.54E-09	DBP
CASZ1	rs880315	1	2.1E-07	SBP
SH2B3-ATXN2	rs653178	12q24	3E-18	DBP
ITGA9	rs7640747	3	4.8E-07	Hyp



nature genetics

May 2011

Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians

Norihiro Kato[1,42*], Fumihiko Takeuchi[1,42], Yasuharu Tabara[2,42], Tanika N Kelly[3,42], Min Jin Go[4,42], Xueling Sim[5,42], Wan Ting Tay[6,42], Chien-Hsiun Chen[7,8,42], Yi Zhang[9,10,42], Ken Yamamoto[11,42], Tomohiro Katsuya[12,42], Mitsuhiro Yokota[13,42], Young Jin Kim[4], Rick Twee Hee Ong[14], Toru Nabika[15], Dongfeng Gu[16,17], Li-ching Chang[7], Yoshihiro Kokubo[18], Wei Huang[19], Keizo Ohnaka[20], Yukio Yamori[21], Eitaro Nakashima[22,23], Cashell E Jaquish[24], Jong-Young Lee[4], Mark Seielstad[25], Masato Isono[1], James E Hixson[26], Yuan-Tsong Chen[7], Tetsuro Miki[27], Xueya Zhou[28], Takao Sugiyama[29], Jae-Pil Jeon[4], Jian Jun Liu[30], Ryoichi Takayanagi[31], Sung Soo Kim[4], Tin Aung[6,32], Yun Ju Sung[33], Xuegong Zhang[28], Tien Yin Wong[6,32,34], Bok-Ghee Han[4], Shotai Kobayashi[35], Toshio Ogihara[36,43], Dingliang Zhu[9,10,43], Naoharu Iwai[37,43], Jer-Yuarn Wu[7,8,43], Yik Ying Teo[5,14,29,38,39,43], E Shyong Tai[39,40,43], Yoon Shin Cho[4,43] & Jiang He[3,41,43]

- Meta-analysis of GWASs in 19,608 East Asians from AGEN-BP consortium
- Follow-up (n=10,518) and replication (n=20,247) in east Asians
- Identify six new loci for BP

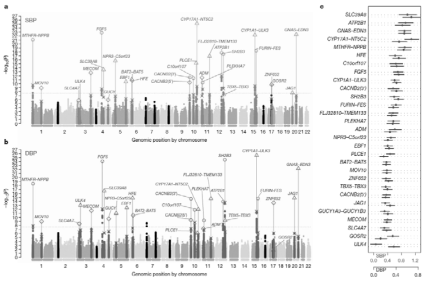
LETTER

doi:10.1038/nature10405

Oct 2011

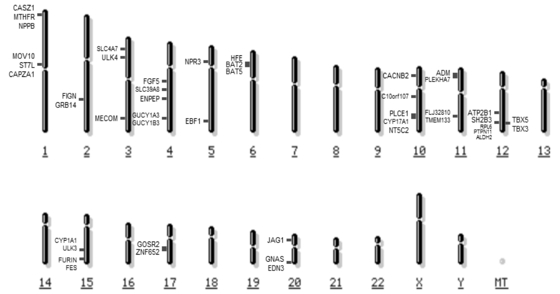
Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk

The International Consortium for Blood Pressure Genome-Wide Association Studies



Meta-analysis of 29 GWASs in 69,395 Europeans
Follow-up with 133,661 Europeans

GENETIC MAP OF 33 BP LOCI

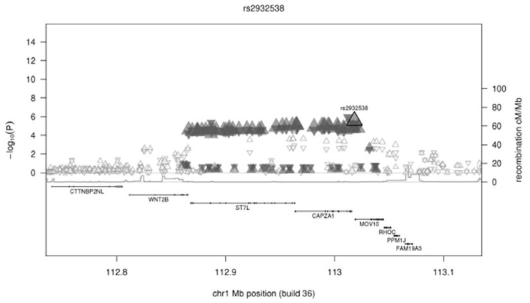


Credit by 임지은 박사

CANDIDATE GENES IN GWAS LOCI

- ENPEP in 4q25
  - Hypertensive in knockout mice
  - Converting enzyme of angiotensin II to angiotensin III
- GUCY1A3/GUCY1B3 in 4q32.1
  - Hypertensive in knockout mice
  - Guanylate cyclase catalyzing GTP to cyclic GMP
  - Activated by nitric oxide
- NPR3 in 5p13.3
  - Lowering BP in knockout mice
  - Natriuretic peptide receptor clearing circulating natriuretic peptides
- ADM in 11p15.4
  - Hypertensive in knockout mice
  - Adrenomedullin, a vasodilating peptide necessary for vascular morphogenesis
- SLC4A7 in 11q22.1
  - Hypertensive in knockout mice
  - Sodium bicarbonate cotransporter

WHY DIFFICULT TO FIND CAUSATIVE GENE?



WE NEED TO FIND CAUSATIVE GENES/VARIANTS!

- To understand how genetic variations influence blood pressure
- To predict the exact susceptibility of individual to hypertension
- To develop therapeutic drugs for hypertension

IDENTIFICATION OF CAUSATIVE GENES

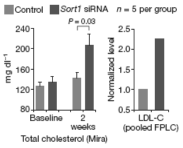
어떤 방법으로 원인유전자를 찾을 것인가?

nature Vol 466|5 August 2010|doi:10.1038/nature09266

ARTICLES

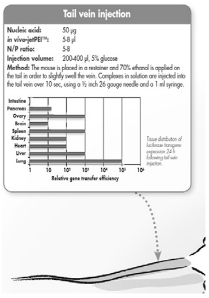
From noncoding variant to phenotype via *SORT1* at the 1p13 cholesterol locus

Kiran Musunuru[1,2,3]*, Alanna Strong[4]*, Maria Frank-Kamenetsky[5], Noemi E. Lee[1], Tim Ahfeldt[1,6], Katherine V. Sachs[4], Xiaoyu Li[4], Hui Li[4], Nicolas Kuperwasser[1], Vera M. Ruda[1], James P. Pirruccello[1,2], Brian Muchmore[7], Ludmila Prokunina-Olsson[7], Jennifer L. Hall[2,8], Eric E. Schadt[9], Carlos R. Morales[10], Sissel Lund-Katz[11], Michael C. Phillips[11], Jamie Wong[5], William Cantley[5], Timothy Racie[5], Kenechi G. Ejebe[1,2], Marju Orho-Melander[12], Olle Melander[12], Victor Koteliansky[5], Kevin Fitzgerald[5], Ronald M. Krauss[13], Chad A. Cowan[1,2], Sekar Kathiresan[1,2]* & Daniel J. Rader[4]*



Knockdown of SORT1 with siRNA
: increases LDL

siRNA IN VIVO DELIVERY

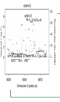


Tail vein injection

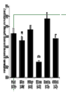
Nucleic acid: 50 µg
***in vivo-jetPEI™*:** 5-8 µl
N/P ratio: 5-8
Injection volume: 200-400 µl, 5% glucose
Method: The mouse is placed in a restainer and 70% ethanol is applied on the tail in order to slightly swell the vein. Complexes in solution are injected into the tail vein over 10 sec, using a ½ inch 26 gauge needle and a 1 ml syringe.

Tissue distribution of luciferase transgene expression 24 h following tail vein injection.

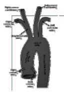
IDENTIFICATION OF CAUSATIVE GENE IN GWAS LOCUS USING siRNA INVIVO DELIVERY



Selection of candidate genes



In vitro & in vivo test of siRNA



Measurement of blood pressure