# Proceedings of the 15th International SHR Symposium/ the 48th Scientific Meeting of the Japanese Society for Hypertension-Related Disease Model Research

*September 27- 28, 2012, Melbourne, Australia*

**IDENTIFICATION OF "NEUTRALIZING" GENE-GENE INTERACTION IN THE REGULATION OF BLOOD PRESSURE IN THE SHR-RELATED STRAINS**

Koichi Akiyama[1], Yi-Qiang Liang[1], Norihiro Kato[1]
*[1]Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan*

Recently, a number of genetic loci associated with blood pressure (BP) or hypertension have been identified through genome-wide association studies in human. Such a susceptibility locus can, however, only indicate a chromosomal region showing significant association with the trait, and is a long way from the goal of elucidating pathogenic mechanisms and eventual identification of causal genes or variants. In the present study, as an experimental tool to investigate one of the difficulties, i.e., gene-gene interaction, we developed a series of chromosome 1 (RNO1) congenic strains from the spontaneously-hypertensive rat (SHR) and stroke-prone SHR (SHRSP). In a previous study, we found the most significant linkage signal with BP to be in the broad region on RNO1. We first introgressed a 270-Mbp RNO1 region *en masse* from the normotensive strain, WKY, on to SHRSP (hereafter SHRSPwch1). In SHRSPwch1, BP was 29.3 mm Hg lower in males and 16.0 mm Hg lower in females at 16 weeks of age ($P<1x10^{-20}$). Next, we developed an additional panel of 15 subcongenic strains, in which various sizes of target fragments were introgressed from WKY to SHRSP. Phenotypic analysis of the congenic/subcongenic rats indicated that at least 10 quantitative trait loci for BP (Bp1-1 to BP1-10) were located on RNO1. In a subcongenic strain (SHRSPwch1-4) carrying a WKY-allele of Bp1-3 locus (93.8-94.8 Mbp), BP was significantly lower ($p=2x10^{-13}$). In another subcongenic strain (SHRSPwch1-3) carrying WKY-alleles of both Bp1-2 and Bp1-3 loci (90.4-94.8 Mbp), BP was 7-12 mm Hg higher compared to SHRSPwch1-4. We found that 28 genes of the kallikrein (*Klk*) family were clustered in the BP1-3 region and that mRNA expression levels were 2-12 fold higher ($p<0.05$) for 8 (of 28) *Klk* genes in SHRSPwch1-4, compared to SHRSP, whereas in SHRSPwch1-3 they were expressed at the same level as in SHRSP; that is, *Klk* gene expression differences were "neutralized" by the co-existence of Bp1-2. Therefore, we assume that the eight *Klk* genes in combination are responsible for the reduced BP conferred by the Bp1-3 locus. Thus, we confirmed that two QTLs, Bp1-2 and Bp1-3, on RNO1 would interact with each other and neutralize their actions on BP regulation and mRNA expression in SHRSP.

**EVALUATION OF HUMAN LIPID-ASSOCIATED GENES AND METHYLATION IN SHR FED ON HIGH LIPID DIET**

Koichi Akiyama[1], Yi-Qiang Liang[1], Norihiro Kato[1]
*[1]Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan*

Recently, a number of genetic loci associated with lipid traits have been identified through genome-wide association studies (GWAS). Such a susceptibility locus, however, can only indicate a chromosomal region showing significant association with the trait, and is still far from the goal of elucidation of pathogenic mechanisms and eventual identification of causal genes or causal variants. In the present study, to explore the possibility that lipid-associated genes detected in human GWAS show some expression changes, we evaluated whole-genome mRNA expression in the spontaneously hypertensive rat (SHR) with and without being fed a high-fat/high-cholesterol (HFHC; 5 % lard, 2 % cholesterol) diet for four weeks from 12 weeks of age. After HFHC diet, serum total cholesterol concentration was significantly increased, as compared to a rat chow-diet (59.9 vs. 142.2 mg/dl, $P<0.001$) in SHR. On analyzing mRNA expression changes by microarray, 222 and 82 genes were differentially (fold change >2, $P<0.05$) expressed in the liver and retroperitoneal fat-pad, respectively, between HFHC and rat chow dietary interventions. Among the genes common in both types of tissues, we identified *LRP5*, which had been known to be involved in cholesterol metabolism. Clustering analysis further showed that HFHC dietary intervention could stimulate biological pathways involved in the MHC class II-mediated antigen presentation, chemokine activity, lymphocyte proliferation, and gluconeogenesis. We then examined rat homologues of human genes located near 95 loci, which were significantly associated with lipid traits in GWAS, and confirmed mRNA expression changes in 26 out of the 95 genes (27 %) in the liver and/or fat-pad of SHR between HFHC and rat chow dietary interventions. Our study demonstrates that almost one quarter of the candidate lipid genes originally identified in human GWAS are differentially expressed upon HFHC dietary intervention in rats, indicating the usefulness of rat models in pursuing their functional relevance to lipid metabolism.

**INTERLEUKIN-1β SIGNAL ACCELERATES THE ONSET OF STROKE IN STROKE-PRONE SHR**

Tsuyoshi Chiba[1], Tatsuki Itoh[2], Masaki Tabuchi[3], Toru Nakazawa[2], Takao Satou[2]
*[1]Information Center, National Institute of Health and Nutrition, Tokyo, Japan, [2]Department of Pathology, Department of Biochemistry, Kinki University School of Medicine, Osaka, Japan, [3]First Institute of New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan*

Hypertensive subjects or patients with stroke have high blood levels of inflammatory cytokines. In addition, it is reported that immune cells existed in stroke lesions. But it is not clear whether inflammatory events are a cause or a result of stroke. To address this issue, we examined the effect of inflammation on the onset of stroke in an animal model. Plasma levels of inflammatory cytokines were measured in male Wistar Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), and stroke-prone spontaneously hypertensive rats (SHRSP). At 10 weeks of age, the plasma level of interleukin (IL)-1β, but not IL-6 or TNF-α, was significantly higher in SHRSP than in WKY or SHR. IL-1β signal-related gene expression and NF--κB protein levels in cerebrovascular endothelial cells (CVECs) from each strain were then measured. Expression of IL-1β signal-related genes, such as IL-1β, IL-1 receptors (IL-1RI and IL-1RII), IL-1β processing enzyme (caspase-1), and downstream genes of IL-1β (monocyte chemoattractant protein-1 and intercellular adhesion molecule-1) in CVECs were significantly greater in SHRSP than in WKY or SHR. Protein levels of NF-κB subtypes, p50 and RelB in CVECs were also greater in SHRSP than in WKY or SHR. To elucidate the direct effect of IL-1β on stroke onset, subcutaneous IL-1β (2 μg/day) was administered continuously in SHR or SHRSP using an osmotic pump. Continuous administration of IL-1β significantly accelerated the onset of stroke in SHRSP, and continuous administrated of IL-1β for 4 weeks increased the incidence of stroke in SHR. In conclusion, SHRSP showed a higher inflammatory state compared to WKY and SHR prior to the onset of stroke. Exogenous IL-1β accelerated the onset of stroke in SHRSP. These results suggested that a stimulated IL-1β signal might be one of causes of stroke in SHRSP.

**DELAY OF STROKE ONSET BY MILK PROTEINS IN STROKE-PRONE SHR**

Tsuyoshi Chiba[1], Tatsuki Itoh[2], Masaki Tabuchi[3], Takao Satou[2], Osamu Ezaki[4]
*[1]Information Center, National Institute of Health and Nutrition, Tokyo, Japan, [2]Department of Pathology, Kinki University School of Medicine, Osaka, Japan, [3]Department of Biochemistry, Kinki University School of Medicine, Osaka, Japan, [4]Department of Nutritional Science, National Institute of Health and Nutrition, Tokyo, Japan*

There is an inverse association between dairy food consumption and incidence of stroke in observational studies. It is not, however, known whether the relationship is causal or, if so, what components in milk are responsible for reducing the incidence of stroke. To address this issue, stroke-prone spontaneously hypertensive rats (SHRSP) were fed diets comprising amino acids, proteins from different sources (casein, whey, soybean, or egg white), fats from different sources (butter, beef tallow, or cocoa butter), or peptides (Ile-Pro-Pro, IPP; Val-Pro-Pro, VPP), and the onset of stroke and lifespan were examined. Increasing the amounts of dietary casein (5 to 55 % of caloric intake) markedly delayed the onset of stroke. However, when SHRSP were fed diets containing 55 % of caloric intake as protein, rats fed casein or whey protein, a major component of milk, displayed a delayed onset of stroke compared to rats fed soybean or egg white protein. Rats fed a diet of amino acids containing the same amino acid composition as casein did not have a delay in the onset of stroke. In addition, anti-hypertensive peptides (IPP or VPP) derived from casein failed to delay the onset of stroke.

Increasing dietary fats, including butter, as well as beef tallow and cocoa butter, did not affect the onset of stroke. All diets did not affect blood pressure in the early stage. In conclusion, these data suggest that the inverse association between dairy food consumption and incidence of stroke in epidemiological studies is causal, and that peptides (but not IPP and VPP) in milk protein might be responsible for this effect.

## BERBERINE INHIBITS DIFFERENTIATION TO ADIPOCYTES AND REACTIVE OXYGEN SPECIES PRODUCTION IN 3T3-L1CELLS

Shi-Fen Dong[1], Naomi Yasui[2], Yukari Sazawa[2], Mai Nishimura[2], Yukio Yamori[2], Jian-Ning Sun[2], Katsumi Ikeda[2]
*[1]Department of Pharmacology, Beijing University of Chinese Medicine, Beijing, China, [2]School of Pharmacy and Pharmaceutical Sciences, Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan*

The aim of the present study was to investigate the inhibitory effect of berberine on the differentiation of 3T3-L1 mouse fibroblasts to adipocytes. Two-day postconfluent 3T3-L1 cells were incubated with 10 % FBS/high glucose Dulbecco's modified Eagle's medium (HG-DMEM), 500 μM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone and 5 μg/ml insulin for 3 days (Day 0-2). Berberine diluted in 10 % FBS/HG-DMEM and insulin at final concentrations of 5, 10 and 20 μM, were given to cells at Day 3 and 4, then the medium was changed to 10 % FBS/HG-DMEM with berberine for the last five days (Day 5-9). Triglyceride concentration was measured by ultraviolet spectrophtometric method, and Oil-Red-O staining was used to detect droplets in 3T3-L1. Reactive oxygen species (ROS) production was detected by the NBT assay. RNA was extracted from cultured cells, and analyzed using RT-PCR. Berberine at concentrations of 5, 10 and 20 μM dose-dependently reduced triglyceride content, and suppressed lipid accumulation in adipocytes. Transcription factors during the differentiation process, including CCAAT/enhancer-binding protein β (C/EBP β), C/EBPα and peroxisome proliferator-activated receptor γ (PPAR γ), were significantly decreased with berberine treatment. The addition of berberine to differentiatied cells reduced ROS content ($P$<0.05). The genes involved in lipid metabolism, including fatty acid synthase (FAS) and adipocyte-specific acid binding protein (aP2), were also significantly decreased with berberine treatment. Berberine 5, 10 and 20 μM dose-dependently reduced adipokines such as leptin and resistin. Berberine 20 μM was able to inhibit angiotensinogen (AGT), plasminogen activator inhibitor-1 (PAI-1) and monocyte chemoattractant protein-1 (MCP-1). Berberine could inhibit differentiation of 3T3-L1 fibroblasts to adipocytes through suppressing C/EBP β, C/EBP α, PPARγ, FAS and aP2 expression. At the same time it suppressed adipokines including leptin, resistin, AGT, PAI-1 and MCP-1 expression. Berberine also suppressed ROS production. These results may indicate the protective effect of berberine on oxidative stress related disease.

## THE RECENT PROGRESS IN SHR RESEARCH

Noboru Fukuda[1]
*[1]Division of Nephrology, Hypertension and Endocrinology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan*

SHR and SHRSP are genetic animal models to investigate the precise pathogenesis and to develop fundamental treatment of human essential hypertension related cardiovascular diseases. Blood pressure begins to increase in SHR from 3 weeks of age accompanied by increases in sympathetic nerve activity, local renin-angiotensin systems, oxidative stress and the insulin resistance. SHR show cardiovascular remodelling, such as the cerebral ischemia, cardiac hypertrophy, and glomerulosclerosis and tubulointestial fibrosis. Interestingly, enhanced DNA synthesis and organ hypertrophy are observed prior to the elevation of blood pressure in SHR. In addition, SHR-derived vascular smooth muscle cells (VSMC) show exaggerated growth in culture. These findings indicate that the SHR is a genetical model having intrinsic and genetic abnormalities independent of pressure overload. We have demonstrated that de-differentiation of mesenchymal tissues such as VSMC and renal mesengial cells in SHR, induces angiotensin II generation and cardiovascular remodelling. We found that complement 3 (C3) is expressed only in VSMCs from SHR, not in cells from WKY rats, by microarray analysis. We also demonstrated that C3 changes VSMCs to the synthetic phenotype by activation of a transcription factor KLF-5. Thus, C3 may be a primary factor that is genetically involved in the cardiovascular and renal remodelling in hypertension. To investigate the contribution of C3 in the pathogenesis of SHR, we will generate C3 knockout SHR by the zinc-finger nuclease method. The SHR Disease Model Cooperative Research Association performed whole genome sequencing in the WKY/Izm rat, SHR/Izm and SHRSP/Izm in colloboration with EuraTrans in MDC. We have been analysing the gene structural and functional abnormalities in SHR in comparison to those in WKY rats. Thus much progress has been made in SHR research and we are making inroads on the identification of the critical abnormalities involved in the pathogenesis of hypertension and other perturbations in the SHR. Current limitations of SHR research are translating the findings to an understanding of the pathogenesis of the important cardiovascular diseases of human essential hypertension and how to apply the gene abnormalities identified in SHR to human essential hypertension.

## ALTERED RENAL CORTICAL AND MEDULLARY BLOOD FLOW DISTRIBUTION BY SYMPATHETIC NERVOUS ACTIVATION IN SHR

Takuzo Hano[1], Naomi Iwane[1], Noriko Miyoshi[1], Masato Mizukoshi[1]
*[1]Wakayama Medical University Graduate School of Medicine, Medical Education and Population-based Medicine, Wakayama, Japan*

Sympathetic activation causes blood pressure elevation *via* renin release and sodium reuptake in renal tubules. The precise hemodynamic distribution of blood in the renal cortex and medulla in response to sympathetic nerve activation in hypertension remain to be determined. Endothelial cells represent an important target "organ" in hypertension. Bone marrow-derived endothelial progenitor cells (EPCs) have been shown to be incorporated into sites of physiological and pathological neo-vascularization *in vivo*. The present study was carried out to clarify the effect of renal nerve stimulation on renal cortical and medullary blood flow and senescence of bone-marrow derived EPCs from stroke-prone spontaneously hypertensive rats (SHRSP). Renal nerves of the left renal artery were stimulated by electrical pulses of 2, 5, 10 and 15 Hz in 6-week old Wistar rats, 17-week old SHRSP rats and WKY rats under isoflurane anesthesia. Renal blood flow was measured by a laser Doppler flow meter using a noncontact probe for cortical flow and a contact glass-fiber needle type probe for medullary flow. EPCs were isolated and cultured from peripheral blood and senescent EPCs were detected by acidic β-galactosidase staining. Renal cortical blood flow increased in response to 2 to 10 Hz and decreased if the stimulus was over 15 Hz in 6-week old Wistar rats. Renal medullary blood flow showed small changes compared with cortical blood flow. The increased component of blood flow was suppressed after treatment with the NO synthase inhibitor, L-NAME. Aged SHRSP showed a decrease in cortical and medullary blood flow in response to electrical nerve stimulation of 10 to 15 Hz. In contrast, young SHRSP and WKY showed increased cortical blood flow at 2 Hz and decreased flow at 5 to 15 Hz. Medullary blood flow was increased at 2 to 15 Hz and this increase was abolished after treatment with L-NAME. The number of senescent EPCs in SHRSP was high and telomerase activity was blunted compared with that in WKY. The reduction in medullary blood flow after sympathetic activation was protected in young and normotensive rats through nitric oxide production. Cortical flood flow was reduced in an age-related manner and in hypertension. Medullary protection was impaired in SHR. This may be caused by endothelial dysfunction *via* senescence of EPC.

## INCREASED EXPRESSION OF GLUCOCORTICOID SYNTHESIS-RELATED FACTORS IN THE ADRENALS OF SHRSP.*Z-LEPR*$^{FA}$/IZMDMCR RATS

Naoki Harikai[1]
*[1]School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan*

We previously reported increased expression of glucocorticoid (GC) synthesis-related factors in the adrenals of obese Zucker rats and

spontaneously hypertensive rats (SHR). In the present study we investigated the expression of GC synthesis-related factors in obese hypertensive SHRSP.Z-*Lepr*$^{fa}$/IzmDmcr rats (ZSP(fa)), which are congenic with obese Zucker rats in the stroke-prone SHR background, and exhibit a metabolic syndrome-like phenotype. Real-time PCR showed that gene expression levels of steroidogenic acute regulatory protein (StAR), cytochrome P450 side chain cleavage enzyme and 11β-hydroxylase were higher in ZSP(fa) and their lean and hypertensive littermates (ZSP(+)) than in normotensive control Wistar Kyoto rats (WKY), but were not different between ZSP(fa) and ZSP(+). Melanocortin 2 receptor (Mc2r) was only increased in ZSP(+). There were no differences in expression levels of the transcription factor steroidogenic factor 1 (SF-1), which stimulates expression of the above genes, while levels of DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome gene 1), which negatively regulates the transcriptional activity of SF-1, were markedly lower in ZSP(fa) and ZSP(+) than in WKY. Furthermore, the protein levels of StAR, SF-1 and DAX-1 determined by western blot correlated well with the gene expressions. These results suggest that the increased expression of GC synthesis-related factors, targeted by SF-1, may be caused by the low levels of DAX-1 in ZSP(fa) and ZSP(+). In addition, apart from Mc2r, we found no differences in expression of GC synthesis-related factors between ZSP(fa) and ZSP(+).

## GROWTH HORMONE SECRETAGOGUE RECEPTOR (GHSR) GENE POLYMORPHISMS ARE ASSOCIATED WITH RENAL FUNCTION IN JAPANESE ELDERLY

Yoshinari Hatanaka[1], Akira Haketa[1], Takahiro Ueno[1], Noboru Fukuda[1], Tomohiro Nakayama[3], Kaoru Sugama[2], Nobutaka Doba[4], Shigeaki Hinohara[4], Masayoshi Soma[2]
*[1]Division of Nephrology, Hypertension and Endocrinology, Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan, [2]Division of General Medicine, Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan, [3]Division of Laboratory Medicine, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan, [4]The Life Planning Center, Tokyo, Japan*

The growth hormone secretagogue receptor (GHSR) gene resides at human chromosome 3q26, and GHSR has been identified in the myocardium, brain stem, blood vessels and kidney. GHSR also plays an important role in the regulation of energy metabolism, heart protective effects, and renal protective effects by binding ghrelin that is mainly secreted by gastric endocrine cells. We hypothesized that the GHSR gene is a candidate gene for susceptibility to healthy aging. We tested the relation between three single nucleotide polymorphisms (SNPs; *rs535438*, *rs495225*, *rs1403637*) by genotyping these in the GHSR gene in relation to clinical phenotypes (height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), bone mineral density (BMD), blood urea nitrogen (BUN), creatinine (Cr), estimated glomerular filtration rate (eGFR), total cholesterol(T-cho), serum cortisol (F), and walking speed) in 316 Japanese healthy elderly people (average age 77.9 years; 155 men and 161 women). To calculate eGFR, we used the "Equation for estimating glomerular filtration rate in Japanese people", issued by the Japanese Society of Nephrology. In association analyses of genotype frequencies of the SNPs and eGFR, *rs535438* and *rs495225* showed a significant association with eGFR (*rs535438*; $P$=0.009, *rs495225*; $P$=0.009). Allele frequencies of *rs1403637* showed no association with eGFR ($P$=0.38). We did not find any significant associations between the GHSR gene polymorphisms and height, weight, BMI, SBP, DBP, BMD, BUN, Cr, T-cho, F, and walking speed. The GHSR gene may affect renal function of Japanese healthy elderly subjects.

## POSSIBLE INVOLVEMENT OF MESOCORTICAL DOPAMINERGIC SYSTEM IN FRONTOCORTICAL DYSFUNCTION OF SHRSP/EZO

Sachiko Hiraide[1], Machiko Matsumoto[1], Yoshiki Yanagawa[1], Taku Yamaguchi[2,3], Mitsuhiro Yoshioka[2], Hiroko Togashi[1]
*[1]Department of Pharmacology, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Japan, [2]Department of Neuropharmacology, Hokkaido University, Sapporo, Japan, [3]Department of Pharmacology, Nagasaki International University, Nagasaki, Japan*

Adolescent SHRSP/Ezo, a substrain of SHRSP, has been used as an appropriate animal model of attention-deficit/hyperactivity disorder (ADHD), which fulfils symptomatic features of ADHD, i.e., inattention/hyperactivity and/or impulsivity. The prefrontal cortex is a critical brain region in the pathogenesis of ADHD, and is expressed as "hypofrontality". The purpose of the present study was to elucidate neurotransmission in the medial prefrontal cortex (mPFC) of an ADHD animal model, SHRSP/Ezo, focusing on the possible involvement of mesocortical dopaminergic neurons originated from the ventral tegmental area (VTA). Using *in vivo* intracerebral microdialysis and electrophysiological techniques, cortical DA levels and evoked potentials in the hippocampal CA1-mPFC pathway were determined in male SHRSP at 6-8 weeks of age. SHRSP/Ezo exerted synaptic dysfunction, expressed as the impaired long-term potentiation (LTP) in the hippocampal CA1-mPFC pathway. Intraperitoneal methylphenidate (1 mg/kg) and atomoxetine (3 mg/kg), which are ADHD drugs, reversed the LTP impairment, while a selective dopamine re-uptake inhibitor GBR12909 (3 mg/kg) did not. Extracellular DA levels in the mPFC were low in SHRSP/Ezo as compared with those in a genetic control WKY/Ezo. Methylphenidate and atomoxetine, but not GBR12909, significantly increased the cortical level of DA in SHRSP/Ezo. Electrical stimulation of VTA produced a significant increase in the cortical DA with a higher threshold than WKY, which ameliorated the cortical LTP impairment in SHRSP. Our findings suggest that mesocortical dopaminergic mechanisms are responsible for the impaired frontocortical neurotransmission, which may underlie the ADHD-like behavioral disturbance in SHRSP/Ezo. Thus, the present findings further provide insights into potential mechanisms for monoamine reuptake inhibitors to cure ADHD.

## ROLE OF C3A-C3A RECEPTOR SYSTEM IN CARDIO-VASCULAR AND RENAL REMODELINGS IN SHR

Kazuya Ikeda[1], Noboru Fukuda[1], Han Ying[1], Takahiro Ueno[1], Masayoshi Soma[1]
*[1]Division of Nephrology, Hypertension and Endocrinology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan*

We have demonstrated that vascular smooth muscle cells (VSMCs) from SHR show a synthetic phenotype that induces angiotensin (Ang) II generation and cardiovascular remodeling. We performed microarray analysis and found that C3 is expressed only in VSMCs from SHR, not in cells from WKY rats. We also demonstrated that C3 changes VSMCs to the synthetic phenotype through KLF-5. In the present study, we investigated the contributions of C3 to phenotypic changes and the exaggerated growth of VSMCs and mesangial cells (MCs) from SHR and SHRSP *in vitro*, and cardiovascular and renal organ growth in SHRSP *in vivo*. We examined the effect of the C3a inhibitor SB290157 on proliferation, expression of phenotype marker and KLF-5 mRNAs in VSMCs or MCs from WKY/Izm rats, SHR/Izm rats and SHRSP/Izm rats. We examined the effects of SB290157 on Ang II production in VSMCs or MCs measured by radioimmunoassay in conditioned medium. We examined effects of SB290157 on PCNA staining in aorta and kidney from the three strains *in vivo*. SB290157 significantly inhibited proliferation, expression of the synthetic phenotype markers and KLF-5 mRNAs, and significantly suppressed Ang II production in VSMCs and MCs from SHR and SHRSP. The C3a inhibitor significantly suppressed PCNA staining in kidney from SHR/Izm and SHRSP/Izm *in vivo*. These findings indicate that the C3a-C3a receptor system is a primary factor genetically involved in cardiovascular and renal remodeling in hypertension.

## CAFFEIC ACID PHENETHYL ESTER INHIBITS THE INFLAMMATORY RESPONSES IN HYPERTROPHIC ADIPOCYTES THROUGH LPS STIMULATED MACROPHAGES

Sachiko Juman[1], Naomi Yasui[1], Katsumi Ikeda[1], Ai Ueda[1], Hiroto Okuda[1], Tomohiro Miki[1]
*[1]School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan*

Obesity is a condition in which excess body fat accumulates due to lipid-producing adipocytes and an increased number of differentiated mature cells. This process is regulated by genetic and environmental factors, such as nutrient intake. Recently, new findings have shown that macrophages infiltrate into adipose tissues and produce various pro-inflammatory cytokines in obese subjects. Caffeic acid phenethyl ester (CAPE) is an active component of propolis from honeybee hives. CAPE is known to have many functions, including antibacterial, anticancer and anti-inflammatory properties, but there is no evidence of its effect on the inflammatory responses in adipocytes. This study investigated the effect of CAPE on macrophages and hypertrophic adipocytes. CAPE significantly suppressed the levels of lipopolysaccharide (LPS)-induced interleukin (IL)-1-β, tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 from a macrophages cell line, RAW 264.7. Supernatants of stimulated RAW264.7 significantly increased mRNA levels of pro-inflammatory cytokines such as IL-6, MCP-1 and TNF-α in 3T3-L1 hypertrophic adipocytes. CAPE also significantly and dose-dependently reduced the gene expression of these cytokines. Our findings indicate that CAPE has inhibitory effects on the production of pro-inflammatory cytokines from LPS-stimulated RAW264.7. In addition, CAPE suppressed gene expressions of cytokines under inflammatory conditions in hypertrophic adipocytes, suggesting that it may have the potential to suppress inflammation by macrophage infiltration into adipose tissue in obese patients.

## ANTIHYPERTENSIVE EFFECTS OF GAMMA-AMINOBUTYRIC ACID GLOBAL GENETICS OF BLOOD PRESSURE

Norihiro Kato[1]
*[1]Department of Gene Diagnostics and Therapeutics, National Center for Global Health and Medicine, Tokyo, Japan*

A new understanding of the genetic basis of cardiovascular conditions including BP elevation (or hypertension) has emerged recently from genome-wide association (GWA) studies. Despite the lack of clear physiological evidence about the target traits, the GWA findings are expected to provide clues to the precise biological mechanisms. Human biology is, however, so complex that proving their validity and translating the findings from GWA studies into clinical applications requires a lot more information (e.g., fine mapping and functional analysis of causative genes) than assumed initially. Along this line, highly sophisticated bioinformatics tools have become indispensable for medical geneticists to analyze the high-throughput experiment data. In-depth examination and integration of individual molecules are also required to give an overall picture of cardiovascular conditions. This process will be assisted by formation of a multi-center, trans-ethnic collaboration. The importance of racial or ethnic differences will be discussed in this presentation. At present, approximately a quarter of BP-associated loci that have been reported in four meta-analyses of GWA studies (i.e., 8 out of 34 loci) appear to be common across 3 ethnic groups – Europeans, East Asians, and South Asians. We believe that 'trans-ethnic' BP meta-analysis will be useful not only for revealing novel susceptibility loci and pathophysiological pathways but also for facilitating the fine mapping of common causal variants.

## GENOME-WIDE PHARMACOGENOMIC RESPONSE TO ANTIHYPERTENSIVE MEDICATION USING HOME BLOOD PRESSURE MEASUREMENTS – THE HOMED-BP-GENE DRUG STUDY

Tomohiro Katsuya[1], Kei Kamide[1], Kei Asayama[2,3], Takayoshi Ohkubo[3,4], Mitsuru Ohishi[1], Yuhei Kawano[5], Toshio Ogihara[6], Hiromi Rakugi[1], Jan A. Staessen[2,7], Yutaka Imai[3]
*[1]Departments of Geriatric Medicine and Nephrology and Clinical Gene Therapy, Osaka University, Suita, Japan, [2]Department of Cardiovascular Diseases, University of Leuven, Belgium, [3]Department of Planning for Drug Development and Clinical Evaluation, Tohoku University Graduate School of Pharmaceutical Sciences, Sendai, Japan, [4]Department of Health Science, Shiga University of Medical Science, Otsu, Japan, [5]Division of Hypertension and Nephrology, National Cerebro and Cardiovascular Research Center, Tokyo, Japan, [6]Morinomiya University of Medical Sciences, Osaka, Japan, [7]Department of Epidemiology, Maastricht University, The Netherlands*

The HOMED-BP-GENE Drug study was embedded in the Hypertensive Objective Treatment Based on Measurement by Electrical Devices of Blood Pressure Trial, in which 3518 patients were randomly allocated to first-line treatment with an angiotensin II receptor blockade (ARB), an angiotensin-converting enzyme inhibitor (ACEI), or a calcium channel blocker (CCB). The HOMED-BP-GENE ancillary project involved 300 patients with mild to moderate essential hypertension, of whom 267 (101 for ARB, 72 for ACEI, and 94 for CCB) completed the study. Home blood pressure (HBP) was measured for 5 days off-treatment before randomization and for 5 days after 4 weeks on randomized drug treatment. The responses were analyzed as a quantitative trait. Genotyping was performed by 500K DNA micro-array chips (Affymetrix Genome Wide Human Array 5.0). Systolic/diastolic HBP decreased by 7.8/3.7, 3.8/2.4, and 9.4/4.2 mm Hg on ARBs, ACEIs, and CCBs, respectively. In multivariable-adjusted analyses (sex, age, body mass index, diabetes mellitus, HBP level before the treatment, duration of taking drugs, and the defined daily doses of each drug), of genome wide association data significant associations ($p<10^{-5}$) with drug responses for ARBs (rs10021898, 17278162, 1283807, 704209, 728329), ACEIs (rs9262636, 16943088, 9262635, 10937304, 548987), and CCBs (rs16867780). For replication of SNPs with $p<10^{-4}$, we used the Gene Evaluation for Antihypertensive Effects of drugs study (GEANE) study, in which patients were randomized to valsartan or amlodipine. The following SNPs were replicated: rs1283807 in an intron of the ATP-binding cassette subfamily C member 9 gene (*ABCC9*) for the ARB response, and rs1377446 in the ankyrin repeat and rs1530955 in the SOCS box-containing 18 gene (*ASB18*) for the CCB response. These two SNPs had only a marginal association of $P<10^{-4}$ in the HOMED-BP-GENE study. In conclusion, our findings, for the first time based on high-fidelity phenotyping by HBP measurement, potentially identified susceptible SNPs for the blood pressure response to ARBs and CCBs. If confirmed, our current observations might help in moving pharmacogenomics towards personalized treatment of hypertension.

## (GABA)-ENRICHED BROWN RICE IN SHR

Kohei Kawakami[1], Kazuo Yamada[2], Satoru Tsuchikura[3], Rei-ichiro Ohno[1], Miki Tongu[1], Akiko Fujita[4], Koji Kawakami[4], Takaya Yamada[1], Masato Nomura[5], Yuta Kobayashi[1], Toru Nabika[6]
*[1]Department of Experimental Animals, Shimane University, Izumo, Japan, [2]Department of Biochemistry, Shimane University, Izumo, Japan, [3]Disease Model Cooperative Research Association, Hamamatsu, Japan, [4]Satake Co. Ltd., Higashi-Hiroshima, Japan, [5]Department of Biotechnology and Chemistry, Kinki University, Higashi-Hiroshima, Japan, [6]Department of Functional Pathology, Shimane University, Izumo, Japan*

Gamma-aminobutyric acid (GABA), a ubiquitous non-protein amino acids known to be an inhibitory neurotransmitter, has attracted much interest due to its antihypertensive effect. If dietary intake of GABA can be increased through dietary means, it may provide substantial benefit to public health by lowering the incidence of hypertension. In the present context, we investigated the effect of GABA-enriched brown rice (developed by Satake Co Ltd, Hiroshima, Japan) on blood pressure in the spontaneously hypertensive rat (SHR). Twenty-four 4 week-old female SHRs were used in the experiments. As the average intake of rice by a standard adult Japanese was estimated 450 g/day, rats were fed 1.1 (low-GABA, comparable with 450 g/day in humans) and 2.1 g/day (high-GABA) of the GABA-enriched rice through a gastric tube (resulting in a GABA intake of 0.52 and 0.99 mg/day, respectively). Corn starch was given to control rats. Systolic blood pressures (SBP), body weight (BW) and food consumption were checked once a week. SBP was significantly lower in the rats fed with the GABA-enriched rice than in the control rats after 3 weeks of treatment (167.0±0.9, 168.5±2.2 and 181.4±1.6 mm Hg for the high-GABA, the low-GABA and the control, respectively, $P<0.01$). BW and food consumption were not different among the three groups. These data indicated that a diet of GABA-enriched rice at a normal level of consumption could ameliorate high blood pressure.

## BETTER HEART RATE RESPONSE DURING INCREASED LOCOMOTOR ACTIVITY IN CONGENIC RAT THAN STROKE-PRONE SHR

Hiroshi Kawamura[1], Hiromi Mitsubayashi[2], Noboru Saito[3], Katsumi Ikeda[4], Kohhei Kawakami [5], Toru Nabika [6]
*[1]MGJ Cardiovascular Institute, Saitama, Japan, [2]Department of Medicine, Nippon Dental University, Tokyo, Japan, [3]Kusatsu General Hospital, Shiga, Japan, [4]Department of Pharmacology, Mukogawa Women's University, Nishinomiya, Japan, [5]Department of Experimental Animals Shimane University, Izumo, Japan, [6]Department of Functional Pathology, Shimane University Faculty of Medicine, Izumo, Japan*

Stroke-prone spontaneously hypertensive/Izumo (SHRSP/Izm) rats display hypertension, stroke, and a non-dipping blood pressure status. There have been no studies, however, about the circadian variations in blood pressure, heart rate (HR), and locomotor activity (ACT) in congenic rats (SHRSPwch1.0). SHRSPwch1.0 were derived from SHRSP/Izm and Wistar-Kyoto/Izumo (WKY/Izm) rats. In particular the interrelationships amongst systolic arterial pressure (SAP), HR and ACT have not to date been studied in SHRSPwch1.0. We evaluated the responses of SAP and HR during increased ACT in SHRSPwch1.0 and compared these to those in SHRSP/Izm. We used ten male mature SHRSPwch1.0 and 10 age-sex matched SHRSP/Izm, and monitored SAP, HR and ACT using radio-telemetry. The responses of SAP and HR during increase ACT were calculated by (mean night-time SAP – mean daytime SAP)/(mean night-time ACT – mean daytime ACT) and that of HR by (mean night-time HR – mean daytime HR)/(mean night-time ACT – mean daytime ACT). SAP in SHRSPwch1.0 was lower than in SHRSP/Izm (mean±SD, 194±9 vs. 229±15 mm Hg, $P$=0.0012). HR in SHRSPwch1.0 was slower than in SHRSP/Izm (310±9 vs. 381±45 beats/min, $P$=0.0110). ACT in SHRSPwch1.0 was higher than that in SHRSP/Izm (62±47vs. 26±27, median, 58 vs.18, IQR, 33 vs.18 counts/10 s, all $P$<0.0010). SAP response during increased ACT in SHRSPwch1.0 did not differ from that in SHRSP/Izm. HR response during increased ACT was, however, greater in SHRSPwch1.0 than that in SHRSP/Izm (4.75±4.01 vs. 0.16±0.24 beats/counts/10 s, $P$=0.0055). Therefore, the relationship between HR and ACT may be better maintained in the congenic rat than that in SHRSP. Thus, these improved HR responses during increased ACT might increase the lifespan of the congenic rat.

## FEMORAL HEAD OSTEONECROSIS RESEARCH IN SHRSP

Kenji Kumagai [1], Masami Niwa [2]
*[1]Department of Orthopedic Surgery, Nagasaki Genbaku Hospital, Nagasaki, Japan, [2]Department of Pharmacology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan*

Over the past 15 years we have advanced research on avascular osteonecrosis of the femoral head (ONFH) by using SHR. Use of SHR as a model of idiopathic osteonecrosis of the femoral head has produced consistent experimental results showing a high frequency of necrosis incidence. It was necessary to determine optimum conditions in response to medications. We found that ONFH in SHRSP was more common than in SHR, had typical histological features, with a predilection from 15 weeks to 17 weeks of age, and was facilitated by steroid hormone (SH) treatment. A small number of ONFH was detected in 40 week-old long-lived SHRSP. To determine the cause of ONFH, a high fat cholesterol (HFC) diet was fed to SHRSP. This resulted in hyperlipidemia and adipocyte hyperplasia in the femoral head, but did not result in ONFH. Marked oxidative stress and apoptosis was observed in the area of necrosis and in the non-necrotic area of the femoral head after SH loading. In recent research, it was recognized that SH administration causes adipocytes to undergo not only hyperplasia but results in production of various cytokines, especially PAI-1, suggesting that these participate in the osteonecrosis. Our research on prevention showed that administration of Warfarin led to a decrease the incidence of ONFH in SHR. Furthermore, pravastatin, an inhibitor of HMG-CoA reductase enzyme activity, was found to prevent ONFH in SHRSP. Thus research findings on ONFH using the SHRSP should guide research and treatment of OHFN in humans.

## EXPLORATION OF GENETIC LOCI CONTROLLING SERUM CHOLESTEROL CONCENTRATION IN THE SHR-RELATED STRAINS: IDENTIFICATION OF TWO NOVEL CHOLESTEROL LOCI

Yi-Qiang Liang[1], Koichi Akiyama[1], Norihiro Kato[1]
*[1]Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan*

Serum cholesterol concentration is a hereditary trait, and hypercholesterolemia is one of the major risk factors of cardiovascular disorder. It is important to elucidate the genetic mechanisms in view of possible cooperative interactions with other atherosclerosis risk factors such as hypertension. Although lipid metabolism in the rodent is partly distinct from that in the primate, the rat model seems to be suitable for genetic analysis to investigate the pathophysiology. In a previous whole-genome linkage scan, we found that quantitative trait loci for serum cholesterol concentration were located on chromosome 15 (RNO15). Therefore, to identify genetic loci controlling serum cholesterol concentration, we developed congenic strains from the spontaneously hypertensive rat (SHR) and the stroke-prone SHR (SHRSP) using a marker-assisted selection method involving ~210 polymorphic markers, and examined the genetic influences on total cholesterol (TC), triglyceride, free fatty acids, blood pressure (BP), body weight (Bw) and Bw-adjusted heart weight (Hw/Bw). First, we introgressed a 110-Mbp RNO15 fragment *en masse* from the normotensive strain, WKY, on to SHR and SHRSP (hereafter SHRwch15 and SHRSPwch15, respectively). In both congenic strains, TC concentration was 6.9-23.8 % lower in males ($P$<0.006) and 24.6-29.3 % lower in females ($P$=$10^{-14}$) than in the recipient progenitor strains. BP was also significantly lower at 16 weeks of age. Next, we developed subcongenic strains by roughly dividing the RNO15 fragment into two parts, and analyzed a total of 6 congenic/subcongenic strains. We found that there were at least two QTLs for TC (TC15-1 at 0-61 Mbp, TC15-2 at 61-110 Mbp) on RNO15. The genetic effects at TC15-2 appeared to act in the opposite directions between SHR- and SHRSP-derived strains. *Acox2* and *Ephx2*, both located at TC15-1, were considered as candidate genes for lipid metabolism. The expression levels of *Acox2* and *Ephx2* mRNA in the liver of SHR and SHRSP were 1.6-fold higher (P=2x$10^{-7}$) and >5-fold lower (P=4x$10^{-6}$) as compared to those of WKY. Thus, our results suggest that there are at least two loci controlling TC levels on RNO15, and that *Acox2* and *Ephx2* at TC15-1 are candidate genes for the trait.

## EXPLORATION OF CARDIAC HYPERTROPHY LOCI IN THE SHR-RELATED STRAINS: CONFIRMATION OF THE ENDONUCLEASE G LOCUS AND IDENTIFICATION OF NOVEL LOCI

Yi-Qiang Liang[1], Koichi Akiyama[1], Norihiro Kato[1]
*[1]Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan*

Cardiac hypertrophy (CH) is associated with increased morbidity and mortality in various cardiovascular disorders. While elevated blood pressure or hypertension has been considered as a principal cause of CH, it has been pointed out recently that some genetic mechanisms underly the development of CH independent of hypertension. In the present study, we developed congenic strains from the spontaneously hypertensive rat (SHR) and the stroke-prone SHR (SHRSP) to identify genetic loci responsible for CH. We have previously found by whole-genome linkage scans that quantitative trait loci for a CH parameter, heart weight adjusted for body weight (Hw/Bw), are located on chromosome 3 (RNO3). We therefore developed congenic strains targeted to RNO3 by a marker-assisted selection method with ~210 polymorphic markers, and assessed the genetic influences on Bw, Hw/Bw, blood pressure (BP) and retroperitoneal fat-pad weight (fat/Bw). We first introgressed a 171-Mbp fragment of RNO3 *en masse* from the normotensive donor strain, WKY, on to the recipient strains, SHR and SHRSP (hereafter SHRwch3 and SHRSPwch3, respectively). The Hw/Bws of congenic strains were 4.4-8.4 % lower ($P$<0.002) in males and 1.6-6.5 % lower ($P$=0.12-5x$10^{-5}$) in females than the recipient progenitor strains. BP levels were also significantly lower at 16 weeks of age. Next, we divided the WKY-derived congenic fragments roughly into three parts, and developed three subcongenic

strains involving each of them. We then further developed two subcongenic strains such that each had two adjacent parts. The phenotypic analyses of the congenic/subcongenic strains indicated that three independent loci were associated with CH, partly independent of hypertension, on RNO3. The likely positions of the three genetic loci were narrowed down to the intervals from 1-25 Mbp (Hw-1), 50-100 Mbp (Hw-2) and 115-150 Mbp (Hw-3). Endonuclease G (*Endog*), located in the Hw-1 region, has been reported recently as a gene responsible for CH. Our data showed that the mRNA expression level of *Endog* was ~1/5 fold ($P<2x10^{-7}$) lower in the hearts of SHR and SHRSP than in that of WKY, in accordance with our previous study. In conclusion, we have identified three genetic loci responsible for CH, and confirmed that *Endog* is one of the candidate CH genes on RNO3.

## ANTIOXIDANT ACTION OF SOLID PREPARATION OF XINGNAOJING IN SHRSP

Yang Liu[1], Naomi Yasui[2], Ming Gao[1], Yukio Yamori[2], Jian-ning Sun[1], Katsumi Ikeda[2]
*[1]Department of Pharmacology, School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, China, [2]School of Pharmacy and Pharmaceutical Sciences, Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan*

We investigated the antioxidant action of a solid preparation of Xingnaojing (XNJ) and ascorbic acid (AA). Male stroke-prone spontaneously hypertensive rats (SHRSP) were divided into five groups: Group A rats served as a control, group B rats were treated with ascorbic acid (AA, 1000 mg/d), group C rats were treated with XNJ (2.5 mg/100 g per day), group D rats were treated with XNJ (5.0 mg/100 g per day), and E group rats were treated with AA (1000 mg/d) + XNJ (5.0 mg/100 g per day). All rats were given salt (4 g/100 g of diet) during 6-week treatment. Total antioxidant status (TAS) in plasma, systolic blood pressure (SBP), and heart rate (HR) were measured every 2 weeks, and lipid peroxidation (LP), expressed as thiobarbituric acid-reactive substances (TBARS) in plasma, was measured in week. The results showed that AA and XNJ significantly increased TAS in plasma ($P<0.01$) and reduced malondialdehyde (MDA) in plasma ($P<0.01$). The treatment had no significant effect, however, on SBP and HR. These data suggest that during 6-weeks of administration to SHRSP, XNJ has antioxidant action which may relate to its generalized inhibition of lipid peroxidation and promotion of TAS.

## THE PROSPECT FOR ANIMAL RESEARCH IN JAPAN ~DISEASE MODELS BEYOND SHR~

Toru Nabika[1]
*[1]Department of Functional Pathology, Shimane University School of Medicine, Izumo, Japan*

The spontaneously hypertensive rat (SHR) and its stroke-prone substrain (SHRSP) have made huge contribution in the cardiovascular research. In spite of that, we do not know the genetic mechanisms of hypertension and stroke in these models yet. On the other hand, GWA studies in humans have made substantial progression in the last decade to identify many candidate genes responsible for hypertension and other cardiovascular diseases. In the past years, QTL analysis using genetic model animals was the mainstream to identify candidate genes (or genomic fragments) for complex diseases. Don't we need such genetic models anymore because of the success of GWAS in humans? What are new roles, if any, of genetic model animals in the study of complex diseases? Two directions of animal study in the research of complex diseases may be proposed; 1) In the research of hypertension, we have accumulated a vast amount of physiological and biochemical data on genetic model rats such as SHR and Dahl rats. To take further advantage of them, congenic strains mimicking the phenotype of SHR (or SHRSP/Dahl) now need to be constructed with a limited number of congenic fragments. Such congenic strains may become new genetic models that can reduce noises of 'strain difference' substantially. 2) For the diseases not enough studied physiologically, new animal models need to be constructed either by 'classical' selection breeding or through application of gene targeting technologies established recently. I would like to introduce a new genetic model for non-alcoholic steatohepatitis (NASH) derived from SHRSP as an example.

## OVEREXPRESSION OF UNCOUPLING PROTEIN-2 STIMULATED BY FREE FATTY ACID WITH REACTIVE OXYGEN SPECIES IN HEART OF A NEW RAT MODEL OF METABOLIC SYNDROME

Hiroko Negishi[1], Naomi Yasui[2], Norie Ide[1], Yasuo Nara[3], Yukio Yamori[2], Katsumi Ikeda[2]
*[1]Nara Women's University, Nara, Japan, [2]Mukogawa Women's University, Nishinomiya, Japan, [3]Fukuyama University, Hiroshima, Japan*

Experimental and genetic evidence relate dysfunctions in uncoupling proteins (UCPs) with metabolic syndrome. In the present study, we have investigated the links between serum free fatty acid (FFA), peroxisome proliferator-activated receptor (PPARs) and UCPs in SHRSP.Z-*Lepr*$^{fa}$/Izm Dmcr rats (SHRSP·ZF) known as a model of metabolic syndrome. Twelve-week-old, female Wistar-Kyoto (WKY) rats, stroke-prone spontaneously hypertensive rats (SHRSP) and SHRSP·ZF were used in the study. We measured metabolic parameters. Furthermore, the expression of UCP2 mRNA was measured by real-time PCR, and the PPARα, PPARγ and Sirt1 protein levels were measured by western blotting in liver, heart, kidney, skeletal muscle and mesenteric adipose tissue (Mes). The mean level of serum lipid peroxidation (thiobarbituric acid [TBARS] assay) in SHRSP·ZF was significantly higher than in SHRSP. In the Mes and kidney of SHRSP·ZF, PPARα and Sirt1 expression was downregulated. Furthermore, in the heart of SHRSP·ZF, UCP2 mRNA and Sirt1 protein levels were increased, and PPARα expression was downregulated. No differences were found in skeletal muscle. These findings suggest that the overexpression of UCP2 leading to an increased export of fatty acid anions, allowing continued fatty acid oxidation, and a down-regulation of PPARα activity by generation of reactive oxygen species, may affect and contribute to the metabolic syndrome.

## SEARCH FOR THE GENES RESPONSIBLE FOR EXAGGERATED SYMPATHETIC RESPONSE TO STRESS IN SHRSP: GENETIC AND MOLECULAR BIOLOGICAL APPROACH USING CONGENIC STRAINS

Hiroki Ohara[1], Mohammed Zubaerul Ferdaus[1], Minoru Isomura[1], Toru Nabika[1]
*[1]Department of Functional Pathology, Shimane University School of Medicine, Izumo, Japan*

The stroke-prone spontaneously hypertensive rat (SHRSP) is well known to have an exaggerated sympathetic response to stress, which may be causally related to its severe hypertension. Based on the results of quantitative trait locus (QTL) analysis using F2 hybrid rats generated between SHRSP and normotensive Wistar-Kyoto (WKY) rats, a chromosomal segment responsible for exaggerated sympathetic response to stress was mapped on chromosome 1 (Chr1) of SHRSP. We constructed a series of congenic strains targeting the Chr1 segment and their sympathetic response to cold and restraint stress were analyzed with three different methods (urinary norepinephrine excretion, blood pressure monitored with a telemetry system and the powerspectral analysis of heart rate variability). Based on the difference in sympathetic response to stress between congenic strains, we narrowed down the range of the candidate region on Chr1 to a 1.2-Mbp fragment (maximally 2.4-Mbp). After excluding genes of the olfactory receptors as well as genes without definite annotations, we focused on 9 genes located in the target region. Gene expression analysis by qRT-PCR revealed that expression levels of genes in the brain stem were not significantly different between WKY/Izm and SHRSP/Izm. Expression analysis of the genes in response to cold stress and their sequence analysis are now in progress.

## EFFECTS OF ELASTIN PEPTIDE FROM BONITO BULBUS ARTERIOSUS ON VASCULAR STRUCTURE AND FUNCTION IN SHR

Kumiko Takemori[1], Ei Yamamoto[2], Hiroyuki Ito[2]
*[1]Department and Food and Nutrition, Faculty of Agriculture, Kinki University, Nara, Japan, [2]Department and Biomedical Engineering,*

*Faculty of Biology-Oriented Science and Technology, Kinki University, Kinokawa, Japan*

It is well known that the degeneration or degradation of elastic fibers in the arterial wall is responsible for the development of hypertension and vascular injuries. In the present study, we examined the effects of elastin peptide from bonito bulbus arteriosus for vascular morphology and function using the spontaneously hypertensive rat strain SHR/Izm. Experiment 1 – Effects of elastin peptide: male SHR aged 20 weeks were divided into two groups. Elastin peptide (600 mg/kg) from bonito bulbus was administered orally for 5 weeks. Although no significant difference was found in blood pressure, body weight and connective tissue related gene expression in aorta between the control and the elastin treated group, the contractile response to PHE was suppressed in the treated group. Experiment 2 – Effects of Proryl glysin (a degradation product of elastin peptide): male SHR/Izm and WKY/Izm aged 15 weeks were used. PG was infused intravenously by osmotic mini pump for 4 weeks at 104 nmol/h. Vasodilation in the PG group was higher than in the control SHR, but lower than in the WKY. In the PG group, the surface of the endothelium was smooth and the endothelial changes such as bleb or crater-like structure were much less intense as compared to those in the control groups. These results indicate that the elastin peptide has beneficial effects on vascular function due to its protective effects against endothelial injuries. Further research will be conducted to elucidate the mechanisms of such beneficial effects of elastin peptide. These will include studies of the elastin receptor, signal transduction, etc, using cultured endothelial cells and smooth muscle cells.

## COMPLEMENT 3 INDUCES RENAL RENIN GENERATION THROUGH THE EPITHELIAL-MESENCHYMAL TRANSITION

Zhou Xueli[1], Noboru Fukuda[1], Takahiro Ueno[1], Koichi Matsumoto[1], Masayoshi Soma[1]
*[1]Division of Nephrology, Hypertension and Endocrinology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan*

Complement 3 (C3) excerts pleiotrophic effects to maintain the stemness and the synthetic phenotype of mesenchymal cells. We have demonstrated that vascular smooth muscle mesenchymal cells and mesangial cells from SHR produce C3. Mature tubular epithelial cells in adult kidney can undergo epithelial-to-mesenchymal transition (EMT), a phenotypic[c] conversion that is fundamentally linked to the pathogenesis of renal interstitial fibrosis. We investigated the role of C3 in EMT phenomena and the renal renin-angiotensin system (RAS). Mouse TCMK-1 epithelial cells were incubated with C3a *in vitro*. We induced EMT by unilateral ureteral obstruction in wild type B6 or C3 knockout (KO) mice. EMT was evaluated by decreases in E-cadherin and increases in α-smooth muscle actin of *h*-caldesmon. The expression for C3 and renin was examined by immunocytochemistry, Western Blot and real-time PCR. Expression of C3, renin, LXRα and prorenin receptor were evaluated by immunohistochemistry and real-time PCR. C3a induced EMT in TCMK-1 cells in which renin was expressed, with nuclear localization of LXRα. In wild-type mice, C3 and renin stained strongly in the degenerated nephrotubulus at this location, with increases in expression of LXRα and prorenin receptor. In C3 KO mice, the EMT phenomenon was suppressed with no expression of renin and C3. C3 induced the EMT to dedifferentiate epithelial cells that produce renin through the induction of LXRα in kidney. C3 may be a primary factor in activation of the renal RAS and so induce hypertension.

## STRESS-STRAIN RELATIONSHIP OF CORTICAL BONE OBTAINED FROM STROKE-PRONE SHR

Ei Yamamoto[1], Yasuaki Handa[1], Yuji Miyazaki[1], Kumiko Takemori[2], Hiroyuki Ito[1]
*[1]Department of Biomedical Engineering, Faculty of Biology-Oriented Science and Technology, Kinki University, Kinokawa, Japan, [2]Department of Food Science and Nutrition, Faculty of Agriculture, Kinki University, Nara, Japan*

Epidemiological data reveal bone abnormalities in patients with hypertension. The mechanisms responsible remain, however, unclear. Bone fractures are ultimately a kind of mechanical event. Therefore, a quantitative assessment for bone biomechanical performance is essential both biologically and clinically. We hypothesized that hypertension is related to impaired material and structural properties of cortical bone. To examine this hypothesis, stress-strain relationships of the bone were determined by means of mechanical loading tests. Stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar Kyoto rats (WKY) aged 20 weeks were used for the experiments. Cortical bone specimens were obtained from the femora of these rats, and compressive forces were applied to the specimens until failure. Figure 1 shows the stress-strain relations in the WKY and SHRSP. Maximum stress in the SHRSP (104±6 MPa) was significantly lower than that in the WKY (128±11 MPa), indicative of direct evidence suggesting a possible link between hypertension and osteoporotic fracture. These findings obtained from a mechanical viewpoint support the concept that bone metabolism may be impaired in hypertensive subjects.

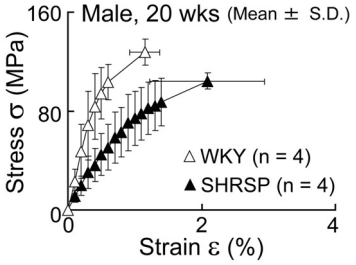


**Fig. 1.** Stress-strain relations.

## EXCESS SALT INCREASES INFARCT SIZE PRODUCED BY PHOTOTHROMBOTIC FOCAL BRAIN ISCHEMIC INJURY IN SHR

Hiroshi Yao[1], Toru Nabika[2]
*[1]Laboratory for Neurochemistry, National Hospital Organization Hizen Psychiatric Center, Saga, Japan, [2]Department of Functional Pathology, Shimane University School of Medicine, Izumo, Japan*

Excess salt plays a critical role in the pathogenesis of cardiovascular disease. In particular, the cerebral circulation is known to be vulnerable to high salt loading. To the best of our knowledge, no study has, however, investigated the relationship between excess salt and the size of brain infarction produced by middle cerebral artery (MCA) occlusion (MCAO). A total of 22 two-month-old male spontaneously hypertensive rats (SHR/Izm) were purchased from Japan SLC (Shizuoka, Japan), and used at the age of 3 months. Rats were randomly assigned to either the salt loading group (0.9 % saline as drinking water) or the control group (tap water). After 14 days of salt loading, rats were subjected to photothrombotic distal MCAO as described previously (*Cell Mol Neurobiol* **31**: 57-63, 2011), in which a krypton laser operating at 568 nm was used to irradiate the distal MCA at a power of 20 mW for 4 min with an infusion of photosensitizing dye rose bengal (20 mg/kg, i.v.). Mean arterial blood pressure (MABP) was monitored continuously. Regional cerebral blood flow (CBF) was measured at 2 mm posterior and 4 mm lateral to the bregma with laser-Doppler flowmetry. Forty-eight hours after distal MCAO, rats were decapitated under deep anesthesia, and infarct volume was determined by the method of 2,3,5-triphenyltetrazolium chloride (TTC) staining. Physiological variables (head and rectal temperatures, blood gases, and glucose) were maintained within the normal range, and were not different between the groups. Resting MABP was 137±16 (SD) mm Hg and 138±7 mm Hg in the salt-loaded and control groups, respectively. After distal MCAO, CBF was decreased more steeply to 20±4 % in the salt-loaded group than in the control group (38±14 %). The area under curve values for CBF determined at 10 min, 30 min and 60 min after MCAO were significantly different between the salt loaded and control groups (unpaired 2-tailed *t*-test, $P$=0.017). Infarct volume in the salt-loaded group was 112±27 $mm^3$, which was significantly larger than the value of 77±12 $mm^3$ in the control group ($P$=0.002). This is the first study to demonstrate the detrimental effects of salt loading on intra-ischemic CBF and subsequent brain infarction produced by photothrombotic MCAO. A future study will address the mechanisms underlying the salt sensitivity to focal brain ischemia without blood pressure changes.

**DIFFERENTIAL EXPRESSION OF MICRORNAS IN THE KIDNEYS OF SHRSP.*Z-LEP$^{FA}$*/ IZMDMCR RATS, A METABOLIC SYNDROME MODEL**

Naomi Yasui[1], Maho Itoko[1], Haruka Ueda[1], Manami Ojima[1], Fuki Otani[1], Midori Kondo[1], Tomoyo Matsumuro[1], Hiroko Negishi[2], Yukio Yamori[3], Katsumi Ikeda[1]

*[1]School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan, [2]Department of Environmental Health, Nara Women's University, Nara, Japan, [3]Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan*

Metabolic syndrome is a risk factor for the development of diabetes and cardiovascular disease, and was recently linked to chronic kidney disease, but the resposible molecular mechanisms have not been fully elucidated. Several metabolic disorders similar to metabolic syndrome patients occur in SHRSP.Z-*Lep$^{fa}$*/ IzmDmcr rats (SHRSP.ZF), which have a miss-sense mutation of leptin receptor gene from the genetic background of spontaneously hypertensive rats (SHRSP), these phenotypes accelerate renal injury such as diabetic nephropathy with aging. As various biological processes are known to be regulated by micro RNAs (miRNAs), or small non-coding RNAs, we decided to investigate whether disorders of miRNAs system were involved in the pathophysiological mechanisms of renal disease in metabolic syndrome. We examined the expressions of miRNA in the kidney of SHRSP.ZF in comparison with their lean littermates (Lean). We measured metabolic parameters in SHRSP.ZF at 12 weeks of age, and examined miRNA expressions using microarray miRNA profiling analysis of the kidney. Both SHRSP.ZF and Lean had hypertension because of the genetic background of SHRSP. SHRSP.ZF showed obesity, hyperglycemia, dislipidemia, and hyperinsulinemia as well as increased urinary excretion of albumin. We identified 18 and 26 miRNAs were up- and downregulated in SHRSP.ZF compared with Lean. Moreover, the expression levels of miR-29a, miR-378, miR-758*, miR-872*, and miR-3596c in the kidney were detected by real-time RT-PCR. Mir-29a and miR378 were significantly decreased in SHRSP.ZF, and it may be related to renal disease in SHRSP.ZF.