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An Overview of Multidisciplinary Research Resources at the Osaka University Center for Twin Research

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Osaka University Center for Twin Research is currently organizing a government-funded, multidisciplinary research project using a large registry of aged twins living in Japan. The purpose of the project is to collect various information as well as biological resources from registered twins, and to establish a biobank and databases for preserving and managing these data and resources. The Center is collecting data from twin pairs, both of whom have agreed to participate in a one-day comprehensive medical examination. The following data are being collected: physical data (e.g., height, body mass, blood pressure, theoretical visceral fat, pulse wave velocity, and bone density), data regarding epidemiology (e.g., medical history, lifestyle, quality of life, mood status, cognitive function, and nutrition), electrocardiogram, ultrasonography (carotid artery and thyroid), dentistry, plastic surgery, positron emission tomography, magnetoencephalogram, and magnetic resonance imaging of brain. These data are then aggregated and systematically stored in specific databases. In addition, peripheral blood is obtained from the participants, and then genomic DNA is purified and sera are stored. A wide variety of studies are ongoing, and more are in the planning stage.

■ **Keywords:** Twin Research Center, twin biobank, epigenetics

The Osaka University Center for Twin Research (OUCTR) was founded in 2009 as a branch of the Osaka University Graduate School of Medicine. Its original twin registry was launched before 1980 and has been expanded to become one of the largest twin registries in Japan (Hayakawa et al., 2002, 2006a, 2006b). Twin pairs have been recruited by newspaper advertisements, posters in hospitals, referrals from nurse midwives, and follow-ups of previous studies conducted by retired twin researchers, some of whom collected data from elementary schools before World War II (Hayakawa & Shimizu, 1982). The total number of registrants enrolled exceeds 12,000. A remarkable feature of our registry is that most of the registrants are elderly people aged 60 years or older. An additional strength is that our center is a multidisciplinary organization comprising experts in a wide variety of specialties in clinical practices, as well as health and social sciences. Because of its uniqueness, the OUCTR has been financially supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) since 2011 as a 4-year project. The purpose of the project is to collect various information, as well as biological resources from registered twins, and to establish a biobank and databases for preserving and managing these data and resources.

Organization of OUCTR

The OUCTR is a research center that belongs to the Graduate School of Medicine, Osaka University (Dean: Prof. Yoshihiro Yoneda). The main office of the OUCTR is a setup in the Division of Health Sciences of Graduate School of Medicine, Osaka University (Director: Prof. Toshisaburo Nagai).

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TABLE 1

List of Data Being Collected from Blood Samples

Blood tests	Leucocyte, erythrocyte, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophil, lymphocyte, monocyte, eosinophil, basophil
Biochemical tests	Hemoglobin A1c, total protein, albumin (improved BCP methods), albumin (BCG methods), uric acid, creatinine, urea nitrogen, calcium, inorganic phosphorus, iron, unsaturated iron binding capacity, total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, amylase, gamma glutamil-transpeptidase, lactate dehydrogenase, creatine kinase, rheumatoid factor, antistreptolysin-O, Cystatin C, apolipoprotein A1, apolipoprotein B, apolipoprotein E, lipoprotein (a), C-reactive protein (CRP), immunoglobulin G, immunoglobulin A, immunoglobulin M, C3, C4, transferin, retinol-binding protein, transthyretin, magnesium, small dense LDL, Na, K, Cl, total IgE
Tumor makers	Alpha-fetoprotein (AFP), CA15-3, carsinoembryonic antigen (CEA), CA125, CA19-9, prostate-specific antigen (PSA), thyroglobulin
Endocrinological test	Cortisol, dehydroepiandrosterone sulfate (DHEA-S), Estradiol, erythropoietin, ferritin, folate, free thyroxine (FT4), free triiodothyronine (FT3), thyroid stimulating hormone (TSH), human follicle-stimulating hormone (hFSH), growth hormone, human luteinizing hormone (hLH), insulin, prolactin, progesteron, parathyroid hormone (PTH), beta-human chorionic gonadotropin (β-hCG), vitamin B12
Autoantibodies	Anti-thrombopoietin

Note: BCP = bromcresol purple, BCG = bromcresol green.

The OUCTR (Head: Prof. Kazuo Hayakawa) consists of four main divisions: Division of Ethics and Data Management (Chief: Prof. Hiroshi Mikami; Drs. Kenji Kato, and Mikiko Ito), Division of Research Infrastructure (Chief: Prof. Yoshinori Iwatani and Dr. Mikio Watanabe), Division of Data Collection (Chief: Prof. Shiro Yorifuji; Drs. Chika Honda, Chisato Hayashi, and Akiko Akiyama), and Division of Research (Chief: Prof. Jun Hatazawa; Dr. Ko Hosokawa, and Kazunori Ikebe).

The steering committee, mentioned at the end of this paper, supervises these divisions and is responsible for all functions of the center, including research collaboration.

Participants

We send correspondence to pairs of registered twins (aged 20 years or older) living all over Japan. When both twins of a pair agree to participate, we invite them to Osaka University (located in central Japan). Participants then undergo a 1-day comprehensive medical examination. As of July 1, 2012, more than 60 pairs of twins have visited our center to participate in the comprehensive medical examination. The mean age of the participants is 70 years (range: 37–87 years), and 53% of the participants are male twins.

As the majority of registrants of our original twin registry are aged over 60 years, we have started to recruit adult twins of all ages living in Japan for new enrollment. Our efforts include advertising in newspapers, distributing posters and leaflets, and organizing public lectures and symposia.

Data Collection

We collect physical data (e.g., height, body mass, waist circumference, grip strength, hearing level, blood pressure, theoretical visceral fat, pulse wave velocity, and bone density), those regarding epidemiology (e.g., medical history, lifestyle, quality of life, mood status, cognitive functions, and nutrition), electrocardiogram (ECG), ultrasonography (carotid artery and thyroid), dentistry, plastic surgery, positron emission tomography (PET), magnetoencephalogram (MEG), and magnetic resonance imaging (MRI) of brain. These data are then aggregated and systematically stored in specific databases.

In addition, we obtain peripheral blood from the participants. Genomic DNAs is purified and sera are separated from their blood, and these samples are preserved in deep freezers to be provided for future research. We perform laboratory tests shown in Table 1 to assess the clinical condition of each participant.

Illumina BeadArray™ Technology

Using Illumina BeadArray™ technology (http://www.illumina.com/technology/beadarray˙technology.ilmn), we have genotyped more than 4.3 million single nucleotide polymorphisms in DNA, and evaluated methylation levels in more than 450 thousand CpG sites in DNA to analyze epigenetic state. Furthermore, we are planning to conduct transciptomic analysis using RNA in collaboration with a Japanese research institute.

All data collection has been approved by the Research Ethics Committee, Osaka University. Requests for joint research are subject to inspection in advance by the steering committee of OUCTR.

Zygosity Determination

Zygosity of same-sex pairs was confirmed using the 15 loci of short tandem repeat (STR) markers (Krenke et al., 2002). A completely concordant pair with these STRs is diagnosed as a monozygotic twin pair. All other pairs are designated as dizygotic.

Overview of Ongoing Research

Genomic Medicine

It is well known that a pair of monozygotic twins can be considered an ideal case-control pair if the twins are discordant in terms of a certain phenotype of interest. Through the comprehensive medical examinations, including blood

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sampling, we are currently gathering plenty of phenotypic, genotypic, and epigenetic information from middle-aged and older twins. Analyzing discordant monozygotic twin pairs enables us to identify or clarify the contributions of environmental factors, such as disease susceptibility, pathophysiology, abnormal clinical findings, and physical functions, including brain, to such phenotypes.

The objectives of our research in this area can be summarized as follows:

1. To identify environmental factors influencing disease susceptibility and pathophysiology with adjustment for individual genetic factors.
2. To evaluate environmental influences on genetic expression from an epigenetic perspective.
3. To predict the onset of diseases and their prognosis.
4. To improve personalized health management and disease prevention.

Neurology

The human brain has both innate and acquired functions, but it is unclear how they interact with each other and then lead to individual differences. For example, the function of mirror neurons is highly heritable, whereas language ability is acquired after birth and is substantially influenced by environmental factors. If differences in brain functions are detected within twin pairs and then compared with differences among unrelated subjects, we will be able to disentangle the relationship between innate and acquired functions. Intrapair comparison of MRI and MEG imaging examinations will also shed light on the influences of these functions on the deficit and impairment of higher brain functions associated with senescence. We expect that our study in this area can improve general understanding of preventive lifestyle against dementia and cognitive impairment.

We measure brain functions by using MEG and MRI. When MEG is performed, participants are asked to go through a set of 15-min questions. With regard to MRI, T1-weighted and diffusion tensor imaging are performed for the current data collection.

Epidemiology and Laboratory Sciences

We are collecting a wide range of epidemiological information to reveal lifestyle or environmental factors giving rise to phenotypic differences among monozygotic twins. As part of epidemiological data collection, we use a series of questionnaires, Japanese versions of which have been validated; for example, the Cornell Medical Index (CMI; Kanehisa & Fukamachi, 1983) for general medical conditions, Brief-Type Self-Administered Diet History Questionnaire (BDHQ; Kobayashi et al., 2011; Sasaki et al., 1998) for dietary conditions, Mini-Mental State Examination (MMSE-J; Sugishita et al., 2010) and Wechsler Memory Scale Revised (WMS-R; Matsuda et al., 1998) for cognitive ability, Profiles of Mood States (POMS; Yokoyama et al., 1990) for emotional states, WHO-QOL (Tazaki & Nakane, 1997) or EuroQOL 5D (EuroQol Group, 2010) for quality of life, and WHO-SUBI (Ohno & Yoshimura, 2001) for subjective well being. With a few exceptions, the questionnaires are performed as a one-to-one interview by trained personnel with adequate medical background.

Some anthropometric measurements (height, body mass, and waist circumstance) and basic physical functions (blood pressure, grip strength, and hearing level) are also collected. Clinical laboratory tests with medical devices provide further information, such as urine, theoretical visceral fat, bone density, pulse wave velocity, cardiac functions (ECG), and ultrasonographic images (carotid artery and thyroid).

Nuclear Medicine

The Positron Emission Tomography (PET) technique visualizes physical functions in vivo by detecting positron-emitting radioisotopes. We use ^{18}F-fluorodeoxyglucose (^{18}F-FDG) so that the distribution of glucose metabolism can be visualized. Our target organs are from brain to femoral region, and the levels of FDG aggregation for respective organs are evaluated. To our knowledge, only a few researchers have reported twin studies using FDG-PET.

We are currently examining similarities and dissimilarities of FDG aggregation between twin pairs. In particular, brain images are transformed into the standard brain and then analyzed using statistical techniques.

Dentistry

Little is known about genetic and environmental influences on oral diseases and functions in elderly people in Japan. Using elderly monozygotic twins, we aim to examine the associations of tooth loss, periodontal disease, and other oral or stomatognathic conditions with lifestyle-related diseases, cognitive function, and aging.

Data collection is conducted by dentists affiliated to OUCTR as part of the comprehensive medical examination. After dental and periodontal examinations, including panoramic X-ray imaging and dental modeling, we measure salivary flow, maximum occlusal force, and masticatory performance. Furthermore, we use the Japanese versions of Oral Health Impact Profile (OHIP-14; Ikebe et al., 2004) and OHIP-EDENT (Sato et al., 2012) to measure oral health-related quality of life.

Plastic Surgery

With the aim of contribution to anti-aging and improved quality of life, we are doing a set of facial skin measurements by using specific imaging techniques. After asking about lifestyle relevant to skin aging, such as sun exposure and skin care, we scan each participant's face by imaging devices so that we can analyze spots and redness on their facial skin. These techniques enable us to measure skin temperature, moisture content, fat quantity, viscoelasticity, and

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the stratum corneum. For a subsample, we take videos to record the movement of facial muscles linked to changes in facial expressions.

Future Plans

Other than the ongoing studies mentioned in the previous section, we plan to conduct further data collection and investigation in a variety of study areas such as epigenetics, obstetrics, pediatrics, pharmaceutics, socio-economics, and human sciences. Data analyses are in progress and the results will be published in due course.

Members of the Steering Committee (Osaka Twin Research Group)

Akiko Akiyama, Jun Hatazawa, Kazuo Hayakawa, Chisato Hayashi, Chika Honda, Ko Hosokawa, Kazunori Ikebe, Fujio Inui, Mikiko Ito, Yoshinori Iwatani, Kenji Kato, Hiroshi Mikami, Toshisaburo Nagai, Toru Nakano, Kazutomo Ohashi, Fumio Ohtake, Keiichi Ohzono, Mikio Watanabe, Yoshihiro Yoneda, and Shiro Yorifuji.

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