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I. Objectives and Overview of the NILS-LSA

1) Background and outline of the NILS-LSA

The life expectancy of the Japanese population is the longest in the world. Both the absolute number and relative percentage of older population in Japanese society is rapidly increasing. In 2020, the percentage of older population in Japan will be the largest in the world. Along with these changes, various medical and care-giving problems for older adults have arisen. Longevity science, with the goal that all of older adults can live a long life with good physical and mental health should be promoted in Japan.

Human aging is associated with many factors, including not only physical and physiological factors but also social and psychological factors. Thus, research into human aging requires many kinds of examinations and specialists in various areas. In addition, human aging research requires long-term study in which the same subjects are measured repeatedly to observe age-related changes. However, the number of researchers and budget for studies on gerontological and geriatric epidemiology are limited. It has been very difficult in Japan to start and to continue a large-scale and comprehensive longitudinal study of aging, despite a rapid increase in older population.

In 1995, a new national research institute of aging in Japan, the National Institute for Longevity Sciences (NILS) was established as a research facility in Chubu National Hospital and in 1997 the NILS-LSA (NILS-Longitudinal Study of Aging) started. The participants in the NILS-LSA of the first wave were 2,267 males and females aged 40 to 79 years randomly selected from the NILS area. They will be examined every two years and now the third wave examination is carrying out. Six to seven participants were examined every day at the NILS-LSA examination center. The aging process is

assessed by detailed questionnaires and examinations including clinical evaluation, body composition and anthropometry, physical functions, nutritional analysis, and psychological assessments. The data from the study will be useful to investigate the causes of geriatric diseases and health problems in older adults such as depression, mental disturbance, restriction of ADL, low nutrition and physical activity. The data will also be useful to prevent these diseases and health problems in older adults.

In March 2004, Chubu National Hospital and NILS were reorganized to establish the National Center for Geriatrics and Gerontology as a new national facility for research and medical care. There are six National Centers for Advanced and Specialized Medical Care in Japan. Other National Centers are located in five areas; Cancer Center in Chuo-ku, Tokyo and in Kashiwa-shi, Chiba, Cardiovascular Center in Suita-shi, Osaka, Center of Neurology and Psychiatry in Kodaira-shi, Tokyo and Ichikawa-shi, Chiba, International Medical Center in Shinjuku-ku, Tokyo, and Center for Child Health and Development in Setagaya-ku, Tokyo. They provide advanced medical care and conduct researches in each special medical area.

Chubu National Hospital was reorganized as National Hospital for Geriatric Medicine. The NILS was also reorganized to cover more area of geriatrics and gerontology. The number of department increased from 8 to 13. A new research section, the Section of Nutritional Epidemiology was added to the Department of Epidemiology and the Laboratory of Epidemiology for the Aged was reorganized to the Section of Preventive Epidemiology.

2) Progress of the NILS-LSA

In 1990, projects of “Comprehensive Research on Aging and Health” were started by the Ministry of Health and Welfare to promote longevity sciences in commemoration of the 60th year in the reign of Emperor Showa. A research group for a longitudinal study of aging was organized as one of these projects. Indices of aging were evaluated, the methodology for the longitudinal study was assessed, and many problems in actual longitudinal follow-ups using existing cohorts were analyzed by this research group in order to start a new comprehensive longitudinal study of aging in Japan. A pilot longitudinal study on aging started in 1992. A manual of the many procedures used in the study was published in 1996.

In July 1995, the National Institute for Longevity Sciences (NILS) was established as the leading national research center for aging and geriatrics in Obu city in the suburbs of Nagoya. In 1996, the Laboratory of Long-term Longitudinal Studies was established in the Department of Epidemiology to start a new longitudinal study of aging in Japan.

Various equipments necessary for geriatric research, such as magnetic resonance imaging (MRI) and peripheral quantitative computed tomography (pQCT) were set up in the NILS, and a special examination center for longitudinal study was established in the Chubu National Hospital. Physicians, psychologists, nutritionists, epidemiologists, and exercise physiologists were assigned to the Laboratory of Long-term Longitudinal Studies and the Department of Epidemiology.

In October 1997, a trial run of the examinations was conducted, and in November 1997, the NILS-LSA began as a large-scale and comprehensive longitudinal study of aging in Japan. Every day, six or seven participants were examined at the NILS-LSA Examination Center. In the first wave of the examination finished in April 2000, 2,267 males and females had completed the examinations. All participants will be examined every two years. The second wave of the examination started in April 2000 and finished in May 2002. Total number of participants of the second wave examination was 2,259. From May 2002, the third wave examination started. The third examination finished in May 2004 and 2,378 participants were examined. The fourth wave examination started in June 2004 (Fig. 1), and finished in July 2005, total 2,383 participants were examined. . The number of examined variables was over 1,000, including

various areas of gerontology and geriatrics such as medical examinations, anthropometry, body composition, physical functions, physical activities, psychological assessments, nutritional analysis and molecular epidemiology.

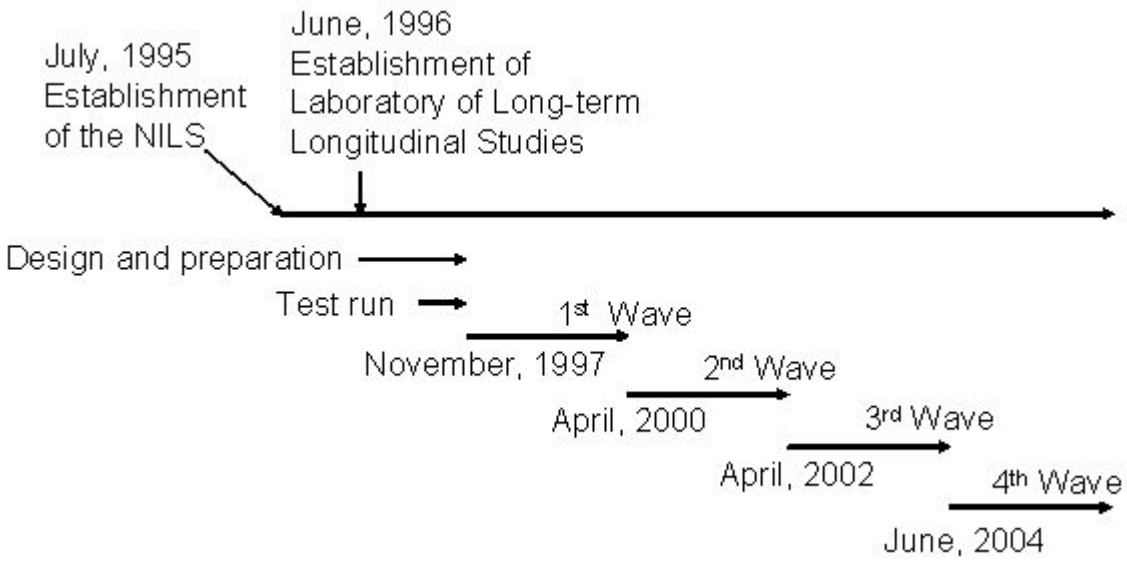

Fig. 1 Progress of the NILS-LSA

3) Objectives of the NILS-LSA

1. Main purpose

Systematic observation and description of the process of normal aging in humans.

(1) To quantify normal and successful aging.
(2) To determine the reference values in normal aging process by longitudinal observation.

2. Additional purpose

(1) To find out early markers of age-related diseases
(2) To clarify molecular genetic factors of aging and geriatric diseases
(3) To find out factors associated with longevity
(4) To examine the effects of life-style, stress, life events and disease on aging process
(5) To separate normal aging and age-related disease
(6) To assess the influence of age on progressive changes of various diseases
(7) To determine predictors of age at death and risk factors for diseases as well as institutionalization and loss of independence
(8) To examine race difference by international comparative study
(9) To assess social and economical changes with age in older adults
(10) To develop indices of biological age
(11) To prepare basic population for the research of clinical and social medicine

4) Research area

The NILS-LSA is a facility-based study using various equipments including MRI, DXA and pQCT for

the detailed and comprehensive assessments of aging and geriatric disease. The facility of examinations is located at the NILS. Thus, the research area was determined to be in the neighborhood of the NILS, that is Obu city (population 79,000) and Higashiura town (population 48,000) (Fig. 1). This area is located in the south of Nagoya, and is a bedroom town and also industrial area of the Toyota group, but still has many orchards and farms, having both urban and rural characteristics.

This research area is geographically located at the center of Japan, and the climate is almost Japanese average. We examined the representativeness of the area via national postal questionnaire of prefecture-stratified random samples of 3,000 households from all prefectures in Japan, and found that the life-style of this area was the most typical of all areas in Japan. It is expected that the results of examinations in this area will represent Japan.

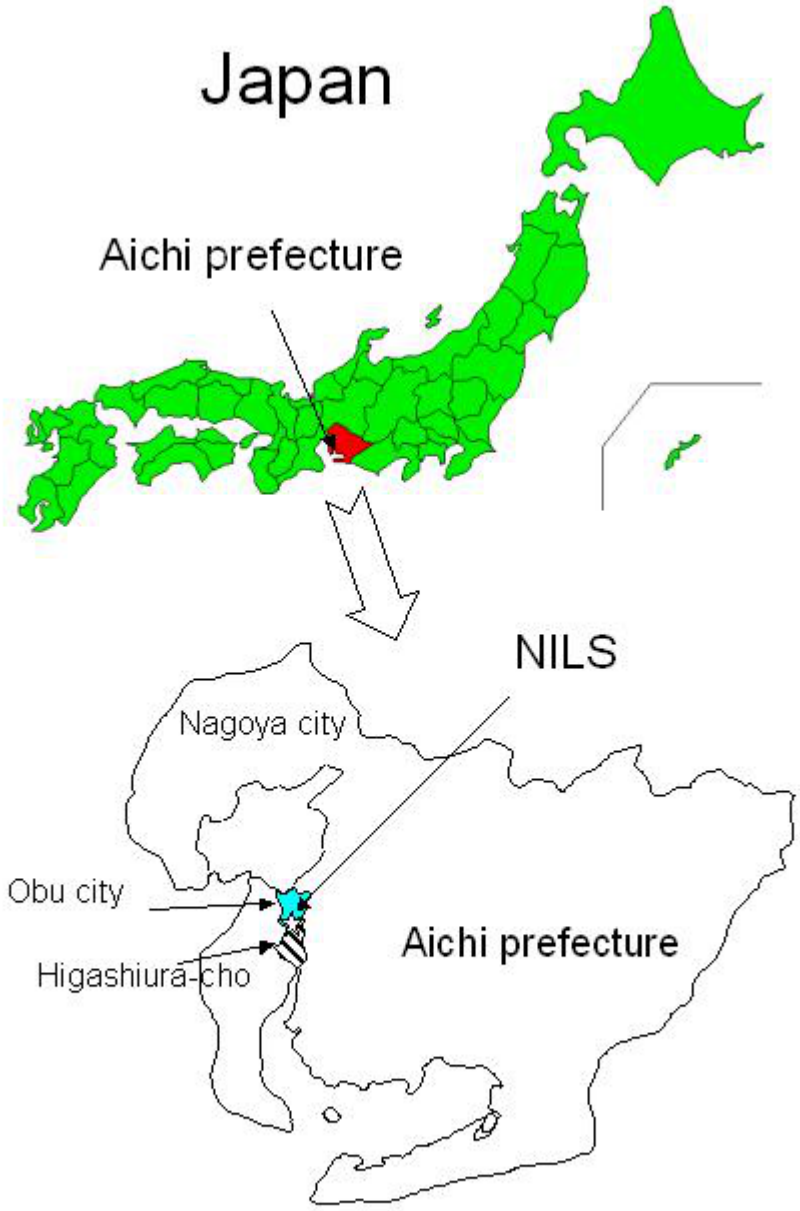

Fig. 2 Research area of the NILS-LSA

5) Subjects

The subjects of baseline examination of the NILS-LSA were males and females aged 40 to 79 years old. The population of Obu city and Higashiura town was stratified by both age and gender, and randomly selected from resident registrations in cooperation with the local governments (Fig.3). The number of males and females was to be equal to test gender difference, and the number of participants in each decade (40s, 50s, 60s, 70s) was also to be equal. The total number of participants was to be 2,400, that is 300 males and 300 females for each decade. They will be followed up every two years. Age and gender-matched random samples of the same number of dropout participants will be recruited except the participants over 79 years old. The male and female participants aged 40 years will be also newly recruited every year (Fig.4). Table 1 shows age and gender distribution of the participants in the first wave examination. Table 2, Table 3, and Table 4 also show age and gender distribution of the second, third, and fourth wave participants, respectively. About two third (65.8%) of the first wave subjects were participated in the fourth wave examination (Table 5), and 84.7% of the third wave participants came back for the fourth wave examination (Table 6).

Recruitment and follow up of volunteers would be much easier than with random samples. However, these samples generally tend to be interested in health, and observation of these samples would produce biased results. Examinations in random samples are necessary to observe the aging process of ordinary Japanese who live ordinary lives.

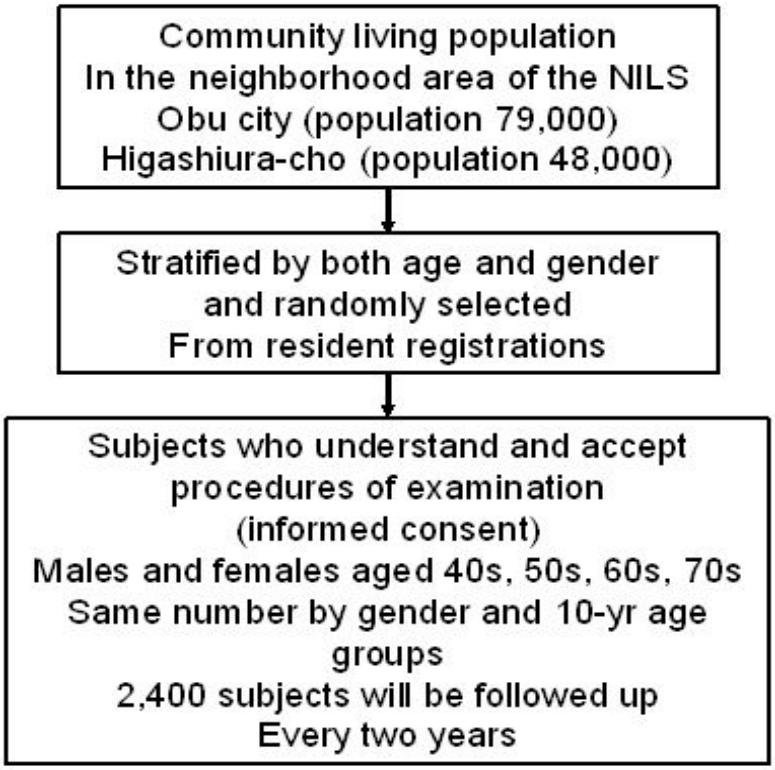

Fig. 3 Selection of the subjects in the NILS-LSA.

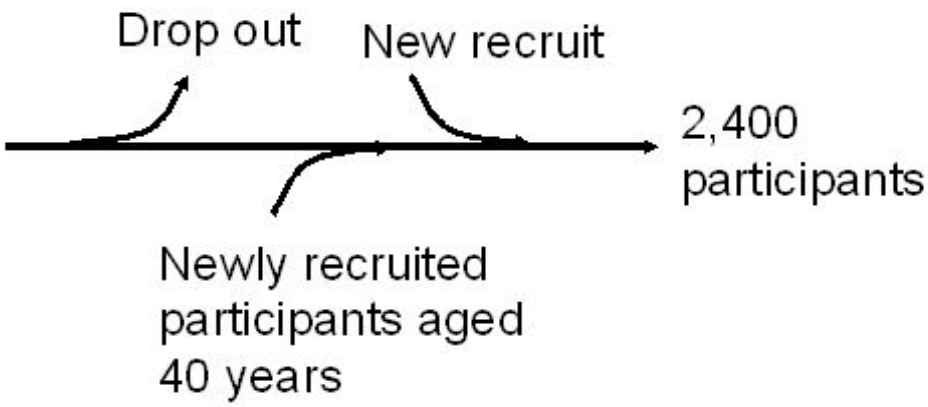

Fig. 4 NILS-LSA as a dynamic cohort

Age	Male	Female	Total
40 – 49	291	282	573
50 – 59	282	279	561
60 – 69	283	285	568
70 – 79	283	282	565
Total	1,139	1,128	2,267

Table 1. Age and gender distribution of the first wave participants

Age	Male	Female	Total
40 – 49	273	261	534
50 – 59	296	284	580
60 – 69	291	271	562
70 – 79	275	269	544
80 –	17	22	39
Total	1,152	1,107	2,259

Table 2. Age and gender distribution of the second wave participants

Age	Male	Female	Total
40 – 49	266	294	560
50 – 59	331	285	616
60 – 69	297	286	583
70 – 79	267	275	542
80 –	43	34	77
Total	1,204	1,174	2,378

Table 3. Age and gender distribution of the third wave participants

Age	Male	Female	Total
40 – 49	286	294	580
50 – 59	295	283	578
60 – 69	300	273	573
70 – 79	255	285	540
80 –	53	59	112
Total	1,189	1,194	2,383

Table 4. Age and gender distribution of the fourth wave participants

	Male	Female	Total
First wave	1,139	1,128	2,267
First and fourth wave	766	726	1,492
Percentage	67.3%	64.4%	65.8%

Table 5. Number of males and females who participated both the first and fourth wave examinations

	Male	Female	Total
Third wave	1,204	1,174	2,378
Third and fourth wave	1,018	997	2,015
Percentage	84.6%	84.9%	84.7%

Table 6. Number of males and females who participated both the second and third wave examinations

6) Implementation of the study

Randomly selected males and females who were assigned to the examination were invited by mail to an explanatory meeting that was held (Fig. 5). At the explanatory meeting, procedures for each examination and the importance of continuation to follow up were fully explained. Participants were limited to those who accept examination procedures and sign their names on a written form (informed consent).

The Department of Epidemiology of the NILS was taking the initiative for all examinations and investigations. The participants were examined from 8:50 am to 4:00 pm at a special examination center within a facility at the the NILS. To examine 2,400 males and females in two years, that is, 1,200 males and females per year, six or seven participants were to be examined each day, four days a week from Tuesday to Friday, 200 days (50 weeks) a year. Taking advantage of the fact that all participants can be examined at the center, detailed examinations including not only medical evaluations, but also examinations of exercise physiology, body composition, nutrition, and psychology can be done. Each examination was to be extensive and most up-to-date, aiming at keeping the internationally highest level. The follow up period is to be up to 30 years, but we hope to get initial significant longitudinal results within 5 to 10 years.

Information from the examinations that would be helpful to manage the health was returned to individual participants as a report from the NILS-LSA.

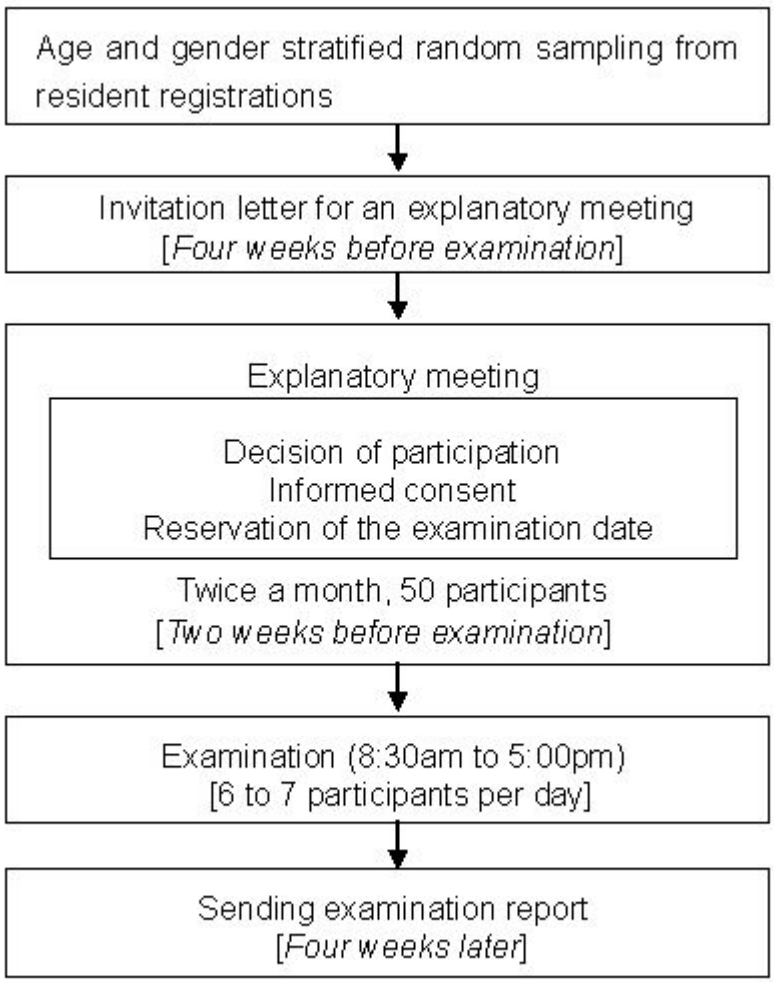

Fig. 5 Examination schedule in the NILS-LSA.

7) Informed consent

Participation in the examinations totally depended on free will, without any enforcement. All participants were fully informed of the following items. Only subjects who understood and accepted examination procedures, and signed their names on a written form to participate in the study (informed consent) were included. This informed consent included; (1) purpose of the study; (2) detailed procedures for each examination; (3) gene analysis; (4) preservation of blood, urine and DNA samples for future examinations; (5) to send examination report to the participants; (6) to keep personal data secret. The Ethical Committee of the National Center for Geriatrics and Gerontology had already approved all procedures of the NILS-LSA.

8) Examinations and tests

The normal aging process was assessed by detailed examinations including clinical evaluation, sensory functions, body composition and anthropometry, physical functions, nutritional analysis, and psychological tests (Table 7).

The NILS-LSA is a longitudinal study to observe age related changes of various examination and tests. Thus examinations and tests of the fourth wave were basically same with the third examination.

Table 7. The fourth wave examinations and tests in the NILS-LSA

- ***Health related questionnaire***

  Self-rated Health (SRH), Medical history, Clinical symptoms, Family history, Environment, Smoking.

- ***Routine clinical evaluations***

  Physical examination
  Blood pressure
  Blood chemistry (fasting)

  GOT, GPT, gamma-GTP, Total protein, Albumin, LDH, Alkaline phosphates, Chorine esterase, Uric acid, Creatinine, Calcium, Phosphate, Total cholesterol, Trigrlyceride, HDL-cholesterol, Lipid peroxide, Fasting glucose, HbA1c, Insulin, Serum sialic acid, Fe.

  CBC: Red cell count, White cell count, Hb, Hematocrit, Platelet count

- ***Sensory examinations***

  Visual system

  Visual acuity: Presenting Visual Acuity, Best-corrected Visual Acuity (5 m), Refraction, Retinal fundus camera, Intraocular pressure, Contrast sensitivity, Corneal thickness, Contrast sensitivity

Auditory system

Audiometry (air and bone), Middle ear functions (Single frequency and Multifrequency tympanometry), Distortion product otoacoustic emission (DPOAE), Video recording of tympanic membrane

- ***Medical examinations***

ECG (Automatic ECG analyzer)
Cardiac ultrasonic tomography
Intima-media thickness of carotid artery
Head MRI (Magnetic resonance imaging system)
Thoracic and lumbar radiography

Dual energy X-ray Absorptiometry (DXA)

Lumbar spine, Right and left femur neck, Total bone density, Body fat (total and segmental fat)

- ***Oral examinations***

Dental caries
Periodontal index
Denture use
Tongue coat
Tongue wetness
Bite force

- ***Anthropometry and body composition***

Anthropometric measurements
Body fat measurement

Dual energy x-ray absorptiometry (DXA)

Thickness of fat and muscle tissue (Ultrasonic tomography)

Intra-abdominal fat, Muscle thickness, Subcutaneous fat thickness

Abdominal fat distribution (Computed tomography)

Intra-abdominal and Subcutaneous fat area

- ***Physical function***

Exercise test system

Grip strength, Sit-ups, Trunk flexion, Static balance, Leg extension power, Isometric leg strength, Reaction time, Maximum step length.

10m Walking test (pitch, step length, velocity),
Gait assessed by 3-D motion analysis system (six cameras and two force plates)
Stabilometer (with or without eye closed conditions)

Daily physical activity questionnaire

Electric pedometer (7 days average)

- ***Psychological tests***

  Interview

    Cognition (MMSE, WAIS-R), Life events, Stress, Social relations, Basic ADL (Katz Index)

  Questionnaire

    Depression (CES-D, GDS), Personality (Rosenberg Self-Esteem Scale, Locus of control, Scale of Attitude toward Death), Social relations, Subjective well-being (LSI-K), Stress checklist, Stress coping scale, Instrumental ADL, Life-style, Personal history (job, marriage, education, etc.), Social and economical back ground

- ***Nutrition analysis***

  Food and nutrition intake

    Three-day dietary record using scale and disposable camera

  Dietary supplement frequency questionnaire
  Beverage frequency questionnaire

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1. Routine clinical evaluations

First of all, physical examinations including history taking, auscultation and blood pressure were taken by a physician, and during the medical examination the physician reconfirms every participant willingness to participate in examinations. Venous blood and urine samples were collected early in the morning after at least 12 hours' fasting.

Life-style, personal history (job, marriage, education, etc.), family history, environment, smoking, social and economical back ground, health status, clinical symptoms, medical history and medication were examined by questionnaires. These questionnaires are checked by a physician at the medical examination. All drugs were to be documented by participants; the physician confirms them by interview and codes drugs used during the last two weeks.

Blood and urine analysis including renal and liver functions, serum protein and lipids, minerals, glucose, HbA1c, Insulin, Vitamin A, sialic acid, lipid peroxide, fatty acid fractions, thyroid hormones, DHEA-S, and complete blood count were also examined. DNA of the first visit participants was stored in deep freezers for future examinations. As for DNA analysis, genotypes related geriatric diseases such as Alzheimer's disease, arteriosclerosis, osteoporosis, benign prostate hypertrophy and diabetes mellitus were examined with the agreement of the participants.

2. Physiological examinations

For physiological examinations, a head MRI was taken for the each participant and stored in an image database. Intracranial tumors and vascular lesions are checked and brain atrophy was assessed via a computerized trace of the MRI. Electrocardiograms are assessed by computerized automatic diagnosis

and Minnesota codes of the diagnosis were stored in a database. Cardiac functions and intima-media thickness of the carotid artery were assessed by ultrasonic tomography. Blood pressure was measured by a physician as well as with an automatic blood pressure manometer.

Osteoporosis is one of the major geriatric diseases. Osteoporosis causes chronic lumbago and bone fracture that disturbs activity in daily life in older adults. Thoracic and lumbar radiography was assessed for the osteoporotic changes and fracture. Bone mineral density was also measured by dual x-ray absorptiometry (DXA, Hologic QDR-4500). Four scans, including whole body, lumbar spine L2 to L4, right and left femoral bone neck, were taken.

3. Sensory examinations

Sensory functions are profoundly associated with QOL in older adults. Visual and auditory disturbance causes various difficulties in the daily lives of older adults. Sensory functions, including visual and auditory functions were examined in detail. Distant visual acuity was measured for each eye with a Landolt C letter at 5m. Contrast sensitivity and intraocular pressure were also examined. Fundus photographs were taken with a Topcon fundus camera (TRC-NW5S). Autorefraction was done with the NIDEK-ARK700A. Refractive errors, in the spherical equivalent, were assessed. Corneal thickness was obtained with the Topcon SP-2000 specular microscope.

Auditory function assessed by pure-tone audiometry (Audiometer RION AA-73A), and impedance audiometry (Middle Ear Analyzer, Grason-Stadler model 33, version 2). Air conduction thresholds at 125Hz to 8000Hz were examined in all participants. Bone conduction thresholds at 250Hz to 4000Hz were examined in participants with elevation of air conduction thresholds. Middle ear function was evaluated by impedance audiometry.

4. Oral examinations

Caries, periodontal index, and toungue coat were evaluated by awell trined dentist with a dental mirror and WHO CPI probe. All teeth were classified into intact, decayed, filled, missing, or caries observation. Periodontal index (CPI score) on 10 index teeth (17/16, 11, 26/27, 36/37, 31, 46/47) were derived according to World Health Organization standard. Score of toungue coat was given by its area on the toungue dorsum. Denture use was asked by a dentist on verbal. Score of tongue wetness was determined by putting Elsalibo R on the tongue dorsum for 10 seconds. For the assessment of bite force, each subject clenched a pressure sensitive sheet (Dental PrescaleR 50H, GC Co. Ltd.) as hard as they could at the intercuspal position for 3 seconds. Average bite force were determined by an image analyzer (Occuluzer R FPD703,GC Co. Ltd.).

5. Anthropometry and body composition

For anthropometry measurements, height, weight, trochanterion height, waist circumference at midway, waist circumference at umbilicus, hip circumference, thigh circumference at midway, claf circumference, and upper-arm circumference were taken. Using ultrasonic tomography, intrabdominal and subcutaneous fat thickness and muscle thickness were evaluated. Body fat volume and distribution were assessed by DXA. Abdominal fat distribution was evaluated as intra-abdominal and subcutaneous fat areas at the level of umbilicas using a computed tomography

6. Exercise examinations

Grip strength, leg extension power, sit-up and static balance, reaction time, and trunk flexion were measured. Step length, pitch, and velocity of walking were assessed by the 10m walking test using six video cameras and two force plates. Physical activities were checked by detailed interview using job-specific questionnaire sheets. Seven-day averages of physical activity were also measured with an electric pedmeter.

7. Nutritional survey

Nutritional intakes were assessed by three-day dietary record using a scale. The scale was handed out to each participant to record the weight of each food taken over the recording period. If it was impossible to weigh each food, approximate size and amounts of food were noted. Dieticians explained to each participant how to weigh foods and how to determine the size and approximate amount. For more accurate assessment, disposable cameras were also handed out to all participants. Before and after each meal, participants were asked to take pictures of all dishes to record what kind of foods and how much food were eaten, and how much food was not eaten. Using these dietary records and photographs, dieticians estimate actual food intake. Dietary supplement usage was also assessed by interview by dietitians in addition to three-day dietary record.

8. Psychological test

All participants were interviewed by psychology specialists. Cognition and intelligence were assessed using the Wechsler Adult Intelligence Scale-Revised Short Form (WAIS-R-SF), as well as short-term memory function and verval memory function in all participants and the Mini-Mental State Examination (MMSE) in participants aged 60 years and over. Life events, social and domestic activities, and ssocial network were also assessed by interview. Basic ADL was checked via the Katz index.

Depressive symptoms, subjective wel-being, personality, social relations, instrumental ADL, and life-style were assessed using a questionnaire.

Over 1,000 variables, including various areas of gerontology and geriatrics will be checked repeatedly every two years in almost 2,400 participants. The staff of the NILS-LSA were consisted of full time researchers, researchers from hospitals and universities, research assistants such as administrators, clinical technicians, dieticians, psychologists, and radiologists. The total number of staff was about 90.

9) Future of the NILS-LSA

We will continue the NILS-LSA to investigate the natural course of aging and the changes that lead to disease. The participants will be examined every 2 years. The cohort of the NILS-LSA is a dynamic cohort, that is, new subjects participate in the study instead of those who do not attend their next

examination. Participants who move out of the area are to be followed up by telephone interview or postal questionnaire. Medical records of the participants who die during follow-up will be checked to find out the cause of death.

Extensive tests and examinations should be repeated in longitudinal studies on aging. However, it is actually impossible to repeat many tests and examinations in multiple research facilities with the same protocols and methods. Thus, there are almost no comprehensive longitudinal studies on aging that have been followed up for a long period by multi-center collaboration in the U.S. or other countries.

However, cohort studies with common end points such as dementia and disturbance of ADL are also important for aging studies. For these studies, a relatively large number of subjects and cases during follow-up will be required to get significant analysis results.

Comparative studies of the aging process accounting for regional and cultural differences between northern and southern areas, or between urban and rural areas, are also important. In these comparative studies, the number of common examinations and tests should be limited. The study design should be a cross-sectional or short-term longitudinal study, considering the difficulties involved continuing and repeating the examinations in all facilities with same protocols.

We are going to make the data of this study public through the Internet. We hope that the results from this large longitudinal study of aging can serve the development of health science on aging.

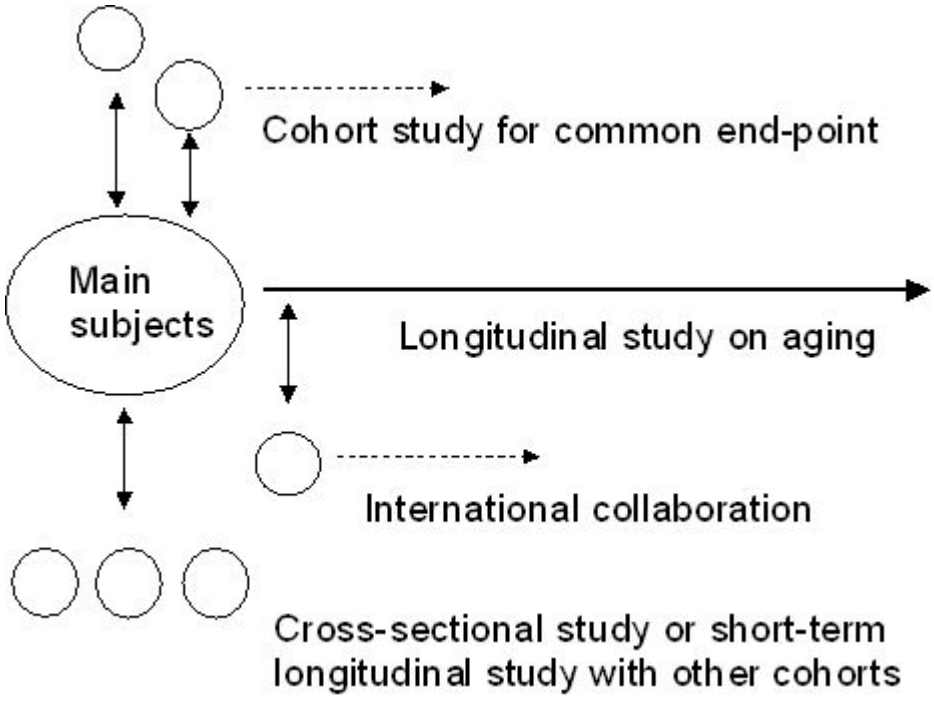

Fig. 6 Design of the longitudinal study by multi-center collaboration

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