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 the elderly population in Japanese society is rapidly increasing. In 2020, the percentage of the elderly population in Japan will be the largest in the world. Along with these changes, various medical and care-giving problems for the elderly have arisen. Longevity science, with the goal that all of elderly people 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 the elderly population.

In 1995, a new national research institute of aging in Japan, the National Institute for Longevity Sciences (NILS) was established 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 randomly selected males and females aged 40 to 79 years from the NILS area. They will be examined every two years and now the third wave examination is carrying out. Six to seven participants are now 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 the elderly 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 the elderly.

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 (Fig.1). 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.

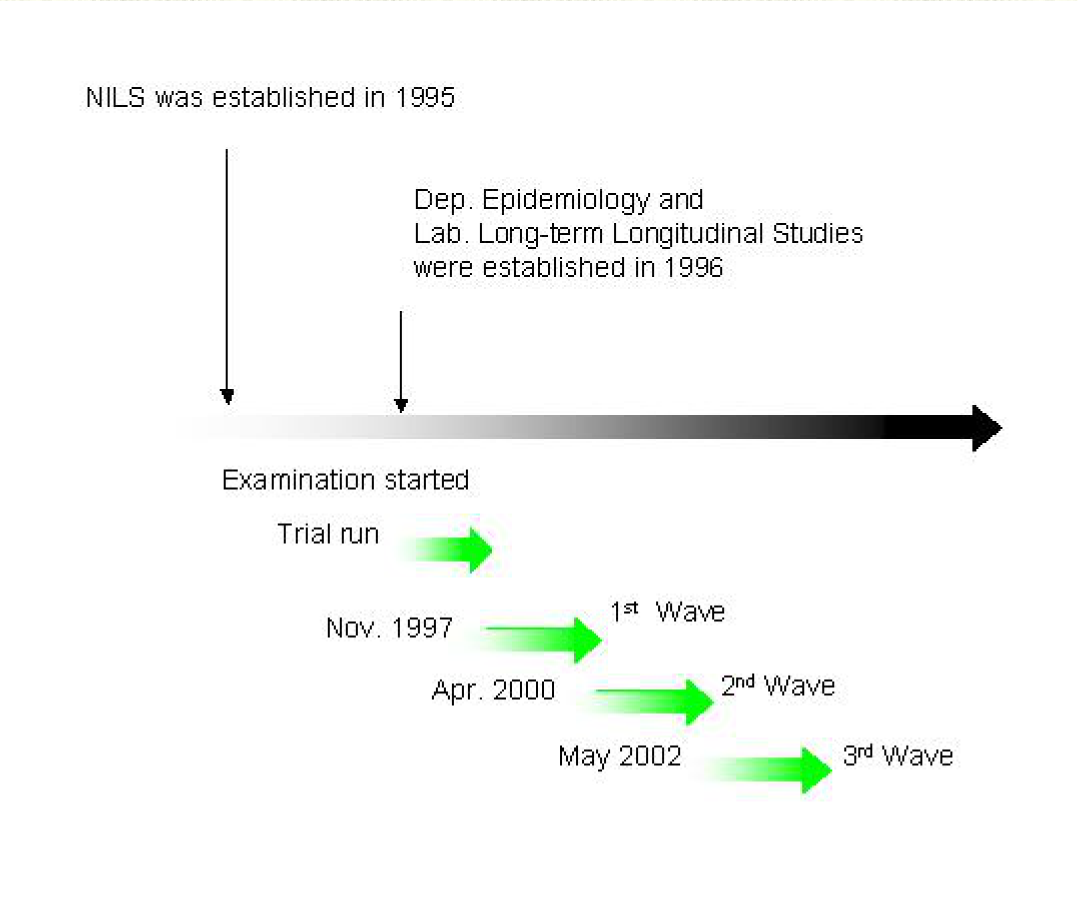

Fig. 1 Progress of the NILS-LSA

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 the elderly.
(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 Chubu National Hospital adjoins the NILS. Thus, the research area was determined to be in the neighborhood of the NILS, that is Obu city (population 70,000) and Higashiura town (population 40,000) (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.

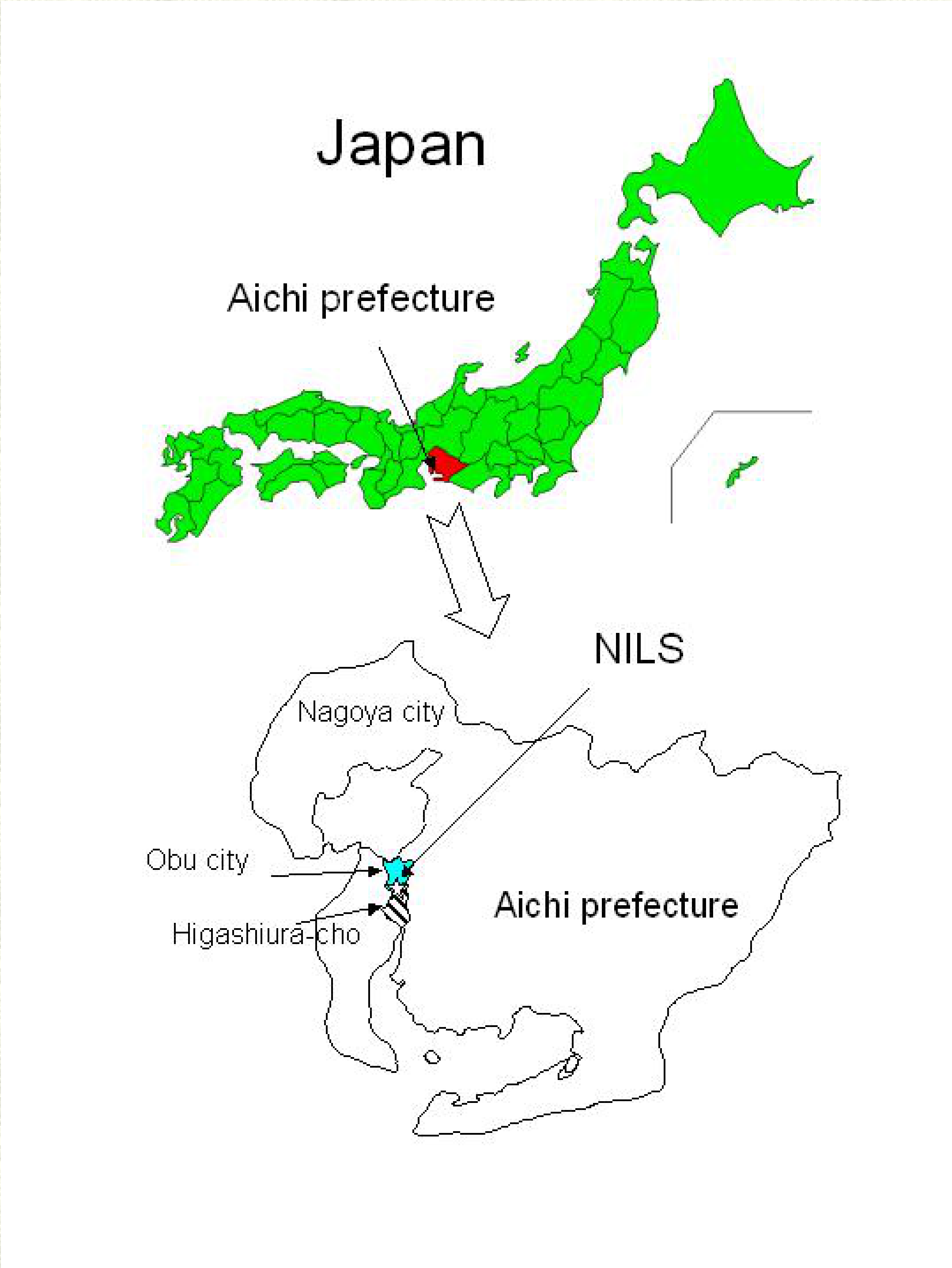

Fig. 2 Research area of the NILS-LSA

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 shows age and gender distribution of the second wave participants. Eighty percent subjects of the first wave examination participated the second wave examination again (Table 3).

Recruitment and follow up of convenient samples 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.

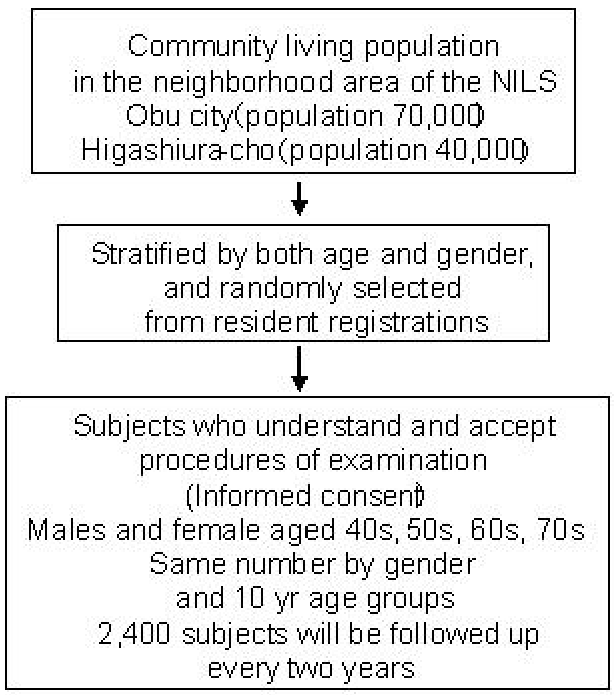

Fig. 3 Selection of the subjects in the NILS-LSA.

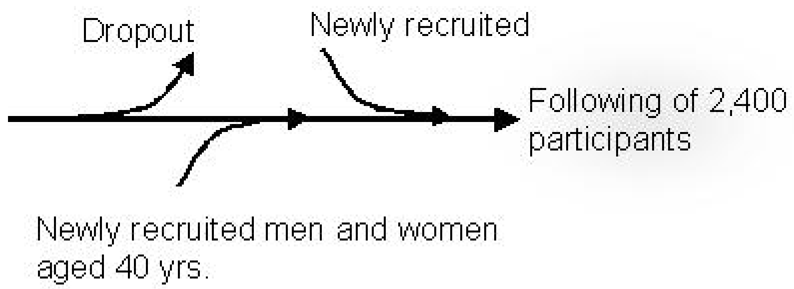

Fig. 4 NILS-LSA as a dynamic cohort

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

	Male	Female	Total
First wave	1,139	1,128	2,267
First and second wave	944	869	1,813
Percentage	82.9%	77.0%	80.0%

Table 3. Number of males and females who participated both the first and second wave examinations

6) Implementation of the study

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 8:30 am to 5 pm at a special examination center within a facility at the Chubu National Hospital located next door to 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 a 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. The first wave examination was finished by April 2000, and 2,267 male and female participants had completed their examinations. The second wave examination started soon after the first wave and finished in May 2002. At the present time the third examination is carried on.

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

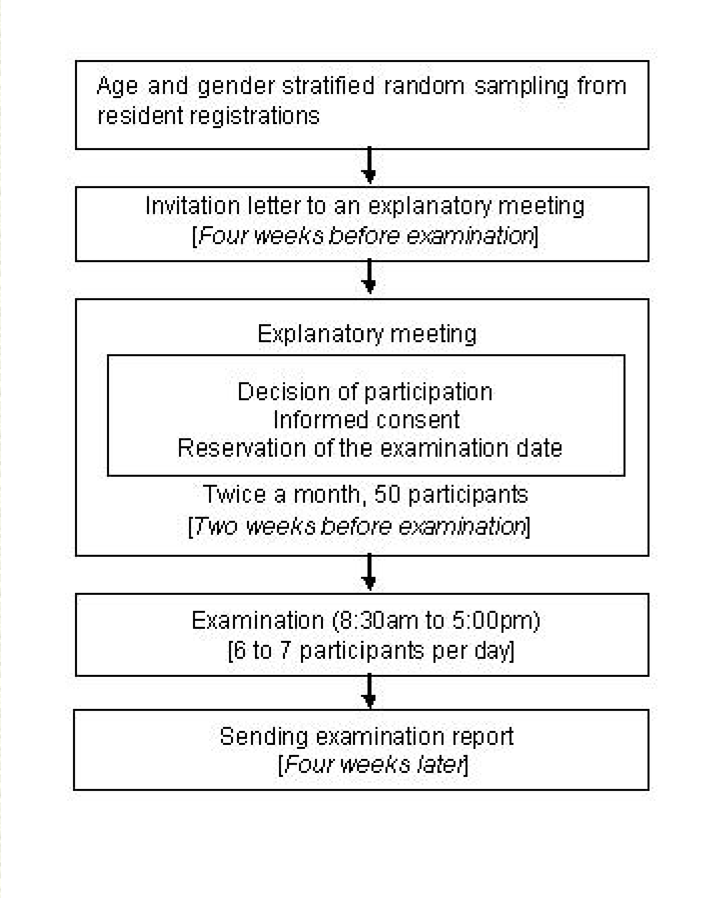

Fig. 5 Examination schedule in the NILS-LSA.

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 to 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 Chubu National Hospital 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 4).

The NILS-LSA is a longitudinal study to observe age related changes of various examination and tests. Thus examinations and tests of the second wave were basically same with the first wave except several new examinations such as evaluation of resting metabolic rate, inner ear function and supplement intakes.

Table 4. The second wave examinations and tests in the NILS-LSA

- ***Health related questionnaire***

  Self-rated Health (SRH), Medical history, Clinical symptoms, Life style, Personal history (job, marriage, education, etc.), Family history, Environment, Alcohol, Smoking, Social and economical back ground

- ***Routine clinical evaluations***

  Physical examination
  Blood pressure
  Blood chemistry (fasting)

    GOT, GPT, gamma-GTP, Total protein, Albumin, LDH, Alkaline phosphates, Chorine esterase, Uric acid, Urea nitrogen, Creatinine, Calcium, Total cholesterol, Trigrlyceride, HDL-cholesterol, Lipid peroxide, Fasting glucose, HbA1c, Insulin, Vitamin A, Serum sialic acid, Fe, Cu, Mg, Zn, free T3, free T4, TSH, DHEA-S, DHA, EPA, arachidonic acid, Dihome-gamma-linolenic acid

  CBC: Red cell count, White cell count, Hb, Hematocrit, Platelet count
  Urine analysis: Protein, Sugar, Urobilinogen, Ketone, pH, Occult blood, Hemoglobin, Nitrite

- ***Sensory examinations***

  Visual system

    Visual acuity: distant vision (5 m), Refraction, Retinal camera, Intraocular pressure, Stereoscopic vision, Contrast sensitivity, Quantitative test of lens opacity, Corneal thickness and cell number

  Auditory system

    Audiometry (air and bone), Middle ear functions (Impedance audiometry)

Inner ear function (Distortion product otoacoustic emission)

- ***Medical examinations***

ECG (Automatic ECG analyzer)
Cardiac ultrasonic tomography
Intima-media thickness of carotid artery
Head MRI (Magnetic resonance imaging system)
Pulse wave (digital plethysmography)
Pulmonary functions (spirometer)
Blood oxygen saturation (Pulse oxymeter)
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)

High Quality Peripheral Quantitative CT (pQCT)

Radial bone mineral density

Resting metabolic profiles (Computerized indirect calorimetry system)

Resting energy expenditure and Respiratory exchange ratio

Aging and geriatric disease related genotypes

Alzheimer disease and dementia related genotypes
Osteoporosis related genotypes
Obesity and diabetes related genotypes
Parkinson disease related genotypes
Cardiovascular disease related genotypes

- ***Anthropometry and body composition***

Anthropometric measurements
Body fat measurement
Air displacement plethysmography (BOD POD), Bioelectrical impedance body fat measurement, Dual energy x-ray absorptiometry (DXA)
Body fluid measurement (Bioimpedance spectroscopy)
Intra- and Extra-cellular fluid
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 (four cameras and two force plates)
Stabilometer (with eye-open and 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 (NEO-FFI, Self-esteem, Locus of control, Type A behavior patterns), Social relations, Subjective well-being (LSI-K), Hassles, Stress coping, Instrumental ADL

- ***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, 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 at interview and codes drugs used during the last two weeks.

Blood and urine analysis including renal and liver functions, serum protein and lipids, and complete blood count, lipid peroxide, fatty acid fractions, sex hormones and geriatric disease markers were also examined. Serum and DNA samples were 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 volume was estimated 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. Peripheral vascular function was assessed using a digital plethysmogram. Pulmonary functions were examined with a spirometer. Blood oxygen saturation was also checked with an oxymeter. 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 the elderly. Bone mineral density was 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. Moreover, bone density was also measured by high quality peripheral quantitative computed tomography (pQCT, Dinsiscan 1000).

Resting metabolic profiles were presented as resting energy expenditure, oxygen intake and carbon dioxide excretion, and respiratory exchange ratio on resting using a computerized, open-circuit, indirect calorimetry system.

3. Sensory examinations

Sensory functions are profoundly associated with QOL in the elderly. Visual and auditory disturbance causes various difficulties in the daily lives of the elderly. 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. An anterior eye segment analysis system is used for the assessment of cataracts. 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 and endothelial cell density were obtained with the Topcon SP-2000 specular microscope.

Auditory function assessed by pure-tone audiometry (Audiometer RION AA-73A), impedance audiometry (Middle Ear Analyzer, Grason-Stadler model 33, version 2) and distortion product otoacoustic emission (Otoacoustic Distortion Product Analyser, ILO92(F) Otodynamics). 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. Inner ear function was also assessed by distortion product otoacoustic emission (DPOAE).

4. Anthropometry and body composition

For anthropometry measurements, height, weight, abdominal sagittal diameter, circumferences of waist, hip, thigh and upper arm and other parameters were taken. Using ultrasonic tomography, intrabdominal and subcutaneous fat thickness and muscle thickness were evaluated. Intra- and extra-cellular fluid was measured via bioimpedance spectroscopy. Body fat was assessed by air displacement plethysmography and DXA. Abdominal fat distribution was evaluated as intra-abdominal and subcutaneous fat areas at the level of umbilicas using a computed tomography

5. Exercise examinations

Grip strength, leg extension power, knee extension strength, sit-up, static balance, reaction time, and trunk flexion were measured with a computerized automatic diagnosis system. Step length, pitch, and velocity of walking were assessed by the 10m walking test. Linear velocity of center of mass, range of joint motion and torque were assessed using four video cameras and two force plates. Daily physical activities were checked by detailed interview using job-specific questionnaire sheets. Seven-day average of physical activity was also measured with an electric pedometer.

6. 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. At lunchtime on the day of the examination, 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.

7. 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) in all participants and the Mini-Mental State Examination (MMSE) in participants aged 60 years and over. Life events and stress coping were also assessed by interview. Basic ADL was checked via the Katz index.

Depressive symptoms, personality, subjective well-being, social relations, hassles, stress coping and ADL 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 80.

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 second wave examination was completed in May 2002. 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.

We are collaborating with other research facilities in Japan and other countries as shown in Fig. 5. 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. There are 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.

Relatively large number of subjects and cases during follow-up need 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 and measuring errors of each test and examination should be small. 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. An international comparative study collaborating with the Baltimore Longitudinal Study of Aging (BLSA) at the National Institute on Aging (NIA) in the U.S. is also planned.

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.

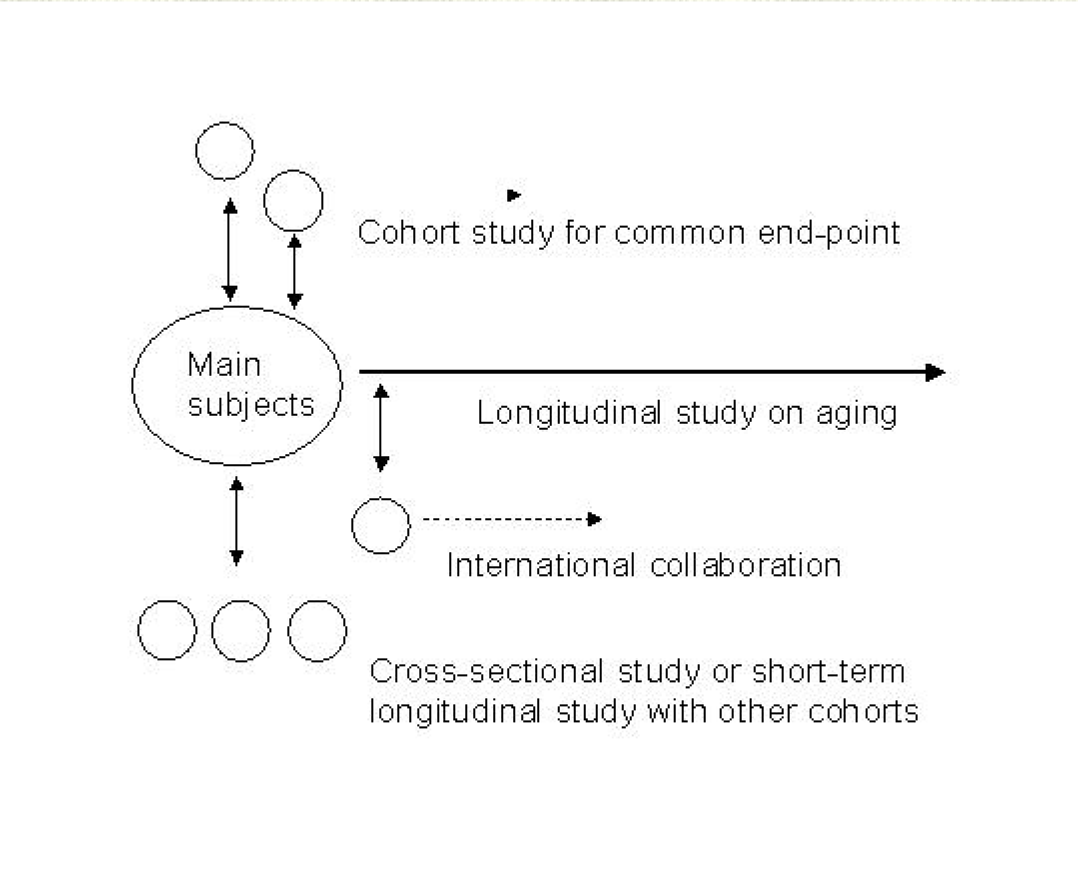

Fig. 6 Design of the longitudinal study by multi-center collaboration

10) Staff of the second wave examinations

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