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 highest in the world. Both the absolute number and relative percentage of the elderly population in Japan are growing rapidly. Currently, the percentage of the elderly population is the largest in the world, with the number rising over 30 million. Along with these changes, various medical and care-giving problems for the elderly have come to light. Longevity science, with the goal that all elderly people can live a long life in 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 a variety of specialists. 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 to both start and continue a large-scale and comprehensive longitudinal study of aging in Japan, 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 as a research facility in Chubu National Hospital. In 1996, the Department of Epidemiology was established with the Section of Long-term Longitudinal Study, and subsequently the Section of Epidemiology for the aged was added to the Department of Epidemiology. In 1997, the NILS-LSA (NILS-Longitudinal Study of Aging) started at the Department of Epidemiology. 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 were examined every two years and the 7th wave examination was ended in 2012. Seven participants were examined on Tuesday to Friday 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 survey, and psychological assessment. The data from the study was useful to investigate the causes of geriatric diseases and health problems such as depression, cognitive impairment, restriction of ADL, low nutrition and physical activity. The data will also be useful to prevent these diseases and health problems in the elderly.

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

The NILS was reorganized to cover more areas of geriatrics and gerontology. The number of departments increased from 8 to 13. A new research section, the Section of Nutritional Epidemiology, was added to the Department of Epidemiology and the Section of Epidemiology for the Aged was reorganized to the Section of Preventive Epidemiology.

In April 2010, the six National Centers for Advanced and Specialized Medical Care in Japan were reorganized to independent administrative institutions. At the same time, the Department of Epidemiology in the NCGG was reorganized to the Department for Development of Preventive Medicine (DDPM) with two sections, the Section of Preventive Nutrition and the Section of Long-term Longitudinal Studies. A new facility, the Center for Development of Advanced Medicine for Dementia (CAMD) was established in the NCGG, and the DDPM was attached to the CAMD.

2) Progress of the NILS-LSA

In 1990, the 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 longitudinal pilot 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 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 equipment 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, seven

participants were examined at the NILS-LSA Examination Center. In the first wave of the examination which ended in April 2000, 2,267 males and females had completed the examinations. All participants were examined every two years, but some participants dropped out. Age and gender-matched random samples of the same number of dropout participants were recruited, except for participants over 79 years old. Male and female participants aged 40 years were also newly recruited every year.

The second wave of the examination started in April 2000 and ended in May 2002; a total of 2,259 participants were examined. The third wave of the examination started in May 2002 and ended in May 2004; a total of 2,378 participants were examined. The fourth wave examination started in June 2004 and ended in July 2006; a total of 2,383 participants were examined. The fifth wave examination started in July 2006, and ended in July 2008; a total of 2,419 participants were examined. The same month the sixth wave examination started and continued until July 2010, examining 2,302 participants. Lastly, the seventh wave examination started and ended in July 2012; a total of 2,330 participants were examined (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.

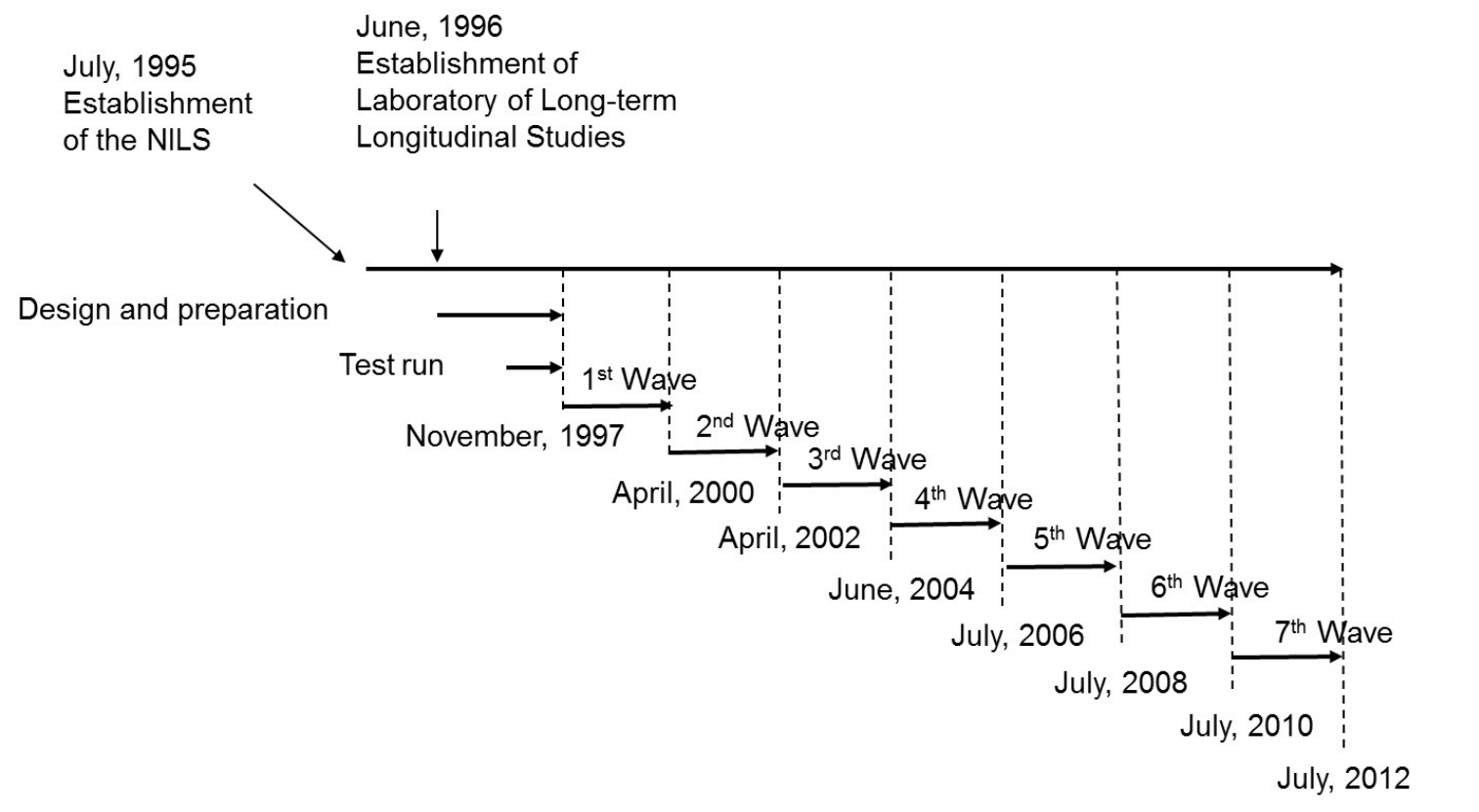

Fig. 1 Progress of the NILS-LSA

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 identify early markers of age-related diseases;
(2) To clarify molecular genetic factors of aging and geriatric diseases;
(3) To identify factors associated with longevity;
(4) To examine the effects of lifestyle, stress, life events and disease on the 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 disease 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 a basic population for research in clinical and social medicine..

4) Research area

The NILS-LSA is a facility-based study using various equipment including MRI, DXA and pQCT for the detailed and comprehensive assessment of aging and geriatric disease. The examination facility is located at the NILS. Thus, the research area was determined to be in the neighborhood of the NILS: Obu (designated a city; population 79,000) and Higashiura (designated a town; population 48,000) (Fig. 2). This area is located south of Nagoya, a bedroom suburb with an industrial area for the Toyota group including many orchards and farms, and has both urban and rural characteristics.

The research area is located at the center of Japan geographically, and experiences a very average Japanese climate. We examined the representativeness of the area via a national postal questionnaire of prefecture-stratified random samples of 3,000 households from all prefectures in Japan, and found that the lifestyle of this area was the most typical of all Japan. It is expected that the examination results in this area will be representative of Japan as a whole.

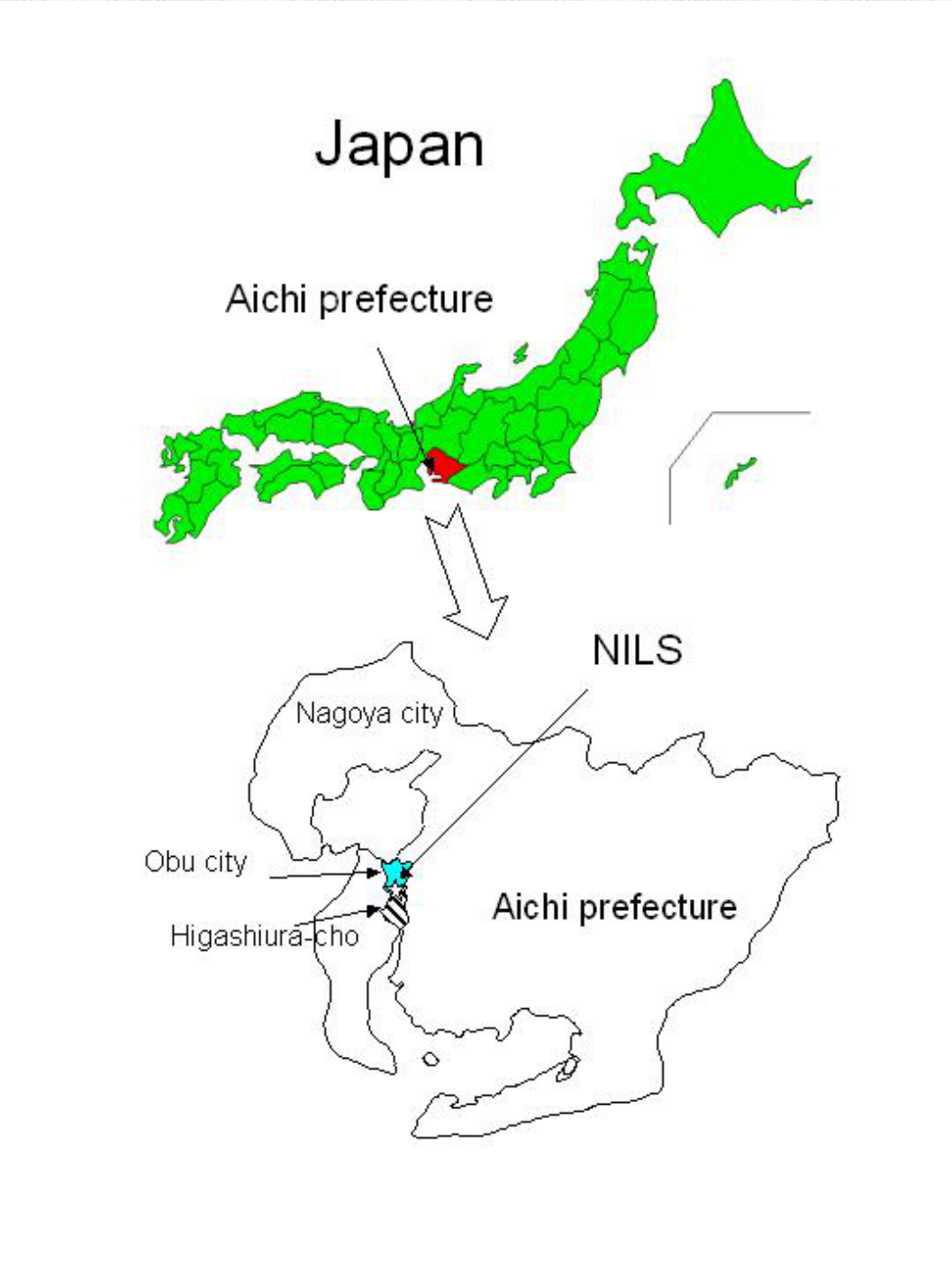

Fig. 2 Research area of the NILS-LSA

5) Subjects

The subjects in baseline examination of the NILS-LSA consisted of both men and women, aged 40 to 79 years. They were randomly selected from resident registrations in Obu and Higashiura stratified by gender and age decade (Fig. 3). The number of males and females was equal to test gender differences, and the number of participants in each decade (40s, 50s, 60s and 70s) was also equal. The total number of participants was 2,400, meaning there were 300 males and 300 females in each decade. Participants were followed up every two years. Age and gender-matched random samples of the same number of dropout participants were recruited except for participants over 79 years old. The male and female participants aged 40 years were also newly recruited every year (Fig. 4). Table 1 shows age and gender distribution of the participants in the first wave examination. Tables 2, 3, 4, 5, 6 and 7 also show age and gender distribution of the second, third, fourth, fifth, sixth, and seventh wave participants, respectively. More than half of the first wave subjects participated in the seventh wave examination (Table 8). The number of cumulative examinations from the first to the seventh waves was 16,338, and total number of participants

through all waves was 3,983. Of these participants, 955 men and women attended all examinations from the first to the seventh wave (Table 9).

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.

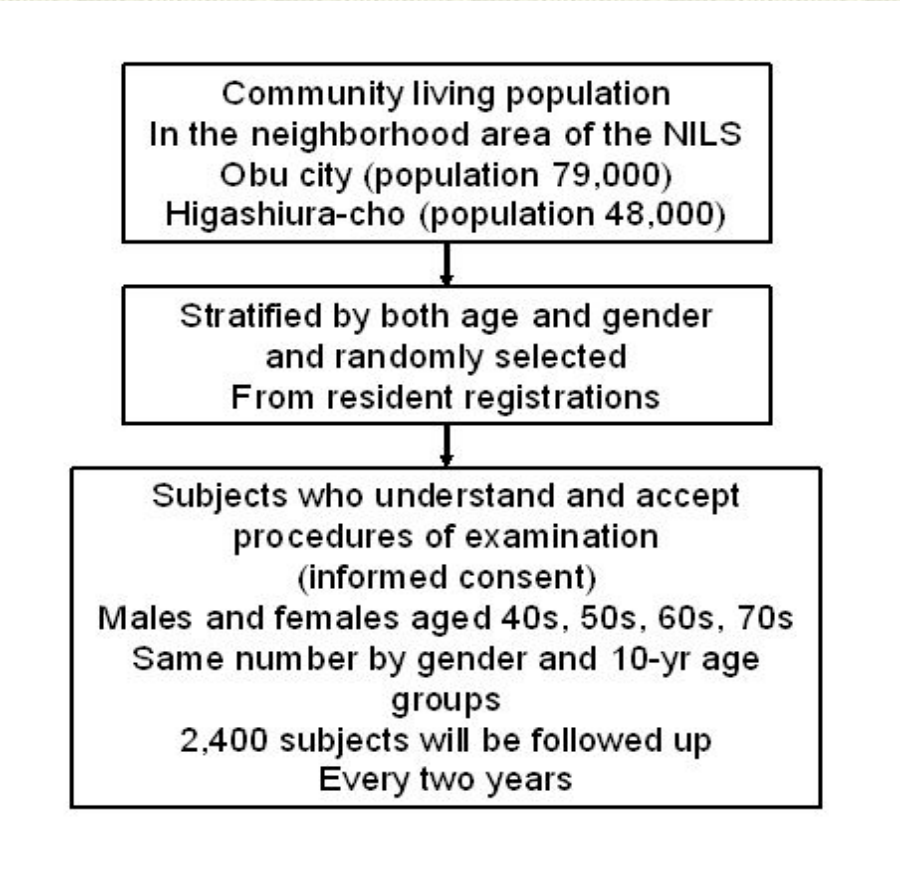

Fig. 3 Selection of the subjects in the NILS-LSA.

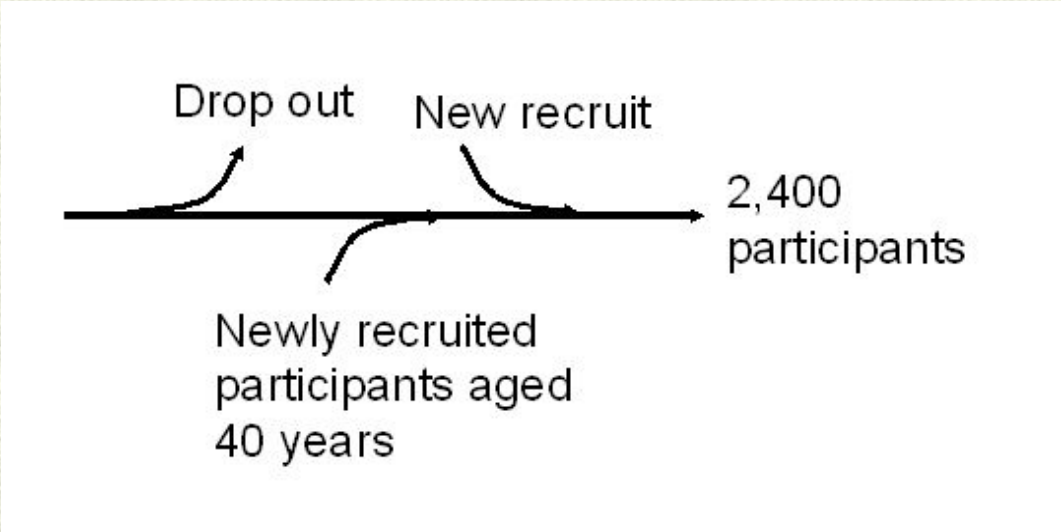

Fig. 4 NILS-LSA as a dynamic cohort

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

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 2. Age and gender distribution of the second 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 3. Age and gender distribution of the third 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 4. Age and gender distribution of the fourth 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 5. Age and gender distribution of the fifth wave participants

Age	Male	Female	Total
40 − 49	279	295	574
50 − 59	289	277	566
60 − 69	274	281	555
70 − 79	283	278	561
80 −	75	88	163
Total	1,200	1,219	2,419

Table 6. Age and gender distribution of the sixth wave participants

Age	Male	Female	Total
40 - 49	257	262	519
50 - 59	274	249	523
60 - 69	276	271	547
70 - 79	270	249	519
80 -	96	98	194
Total	1,173	1,129	2,302

Table 7. Age and gender distribution of the seventh wave participants

Age	Male	Female	Total
40 - 49	268	276	544
50 - 59	265	260	525
60 - 69	284	265	549
70 - 79	262	246	508
80 -	99	105	204
Total	1,178	1,152	2,330

Table 8. Number of males and females who participated both the first and the seventh wave examinations

	Male	Female	Total
First wave	1,139	1,128	2,267
First and sieventh wave	598	570	1,168
Percentage	52.5%	50.5%	51.5%

Table 9. Number of males and females who participated from the first to the seventh wave examinations

	Male	Female	Total
Total examinations	8,235	8,103	16,338
Total participants	1971	2012	3,983
Participants attending all examinations	496	459	955

6) Implementation of the study

The men and women, selected randomly from gender- and age decade-stratified resident registration, were invited by mail to an explanatory meeting (Fig. 5). At the meeting, procedures for each examination and the importance of continuation through to follow-up were fully explained. The participants were limited to those who accepted the examination procedures and signed their names on a written form

(informed consent).

The Department for Development of Preventive Medicine took 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 NCGG. To examine 2,400 males and females in two years, (1,200 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 the participants were able to be examined at the center, detailed examinations including not only medical evaluations, but also examinations of exercise physiology, body composition, nutrition, and psychology were done. Each examination was to be extensive and up-to-date, aiming at keeping to the internationally highest levels.

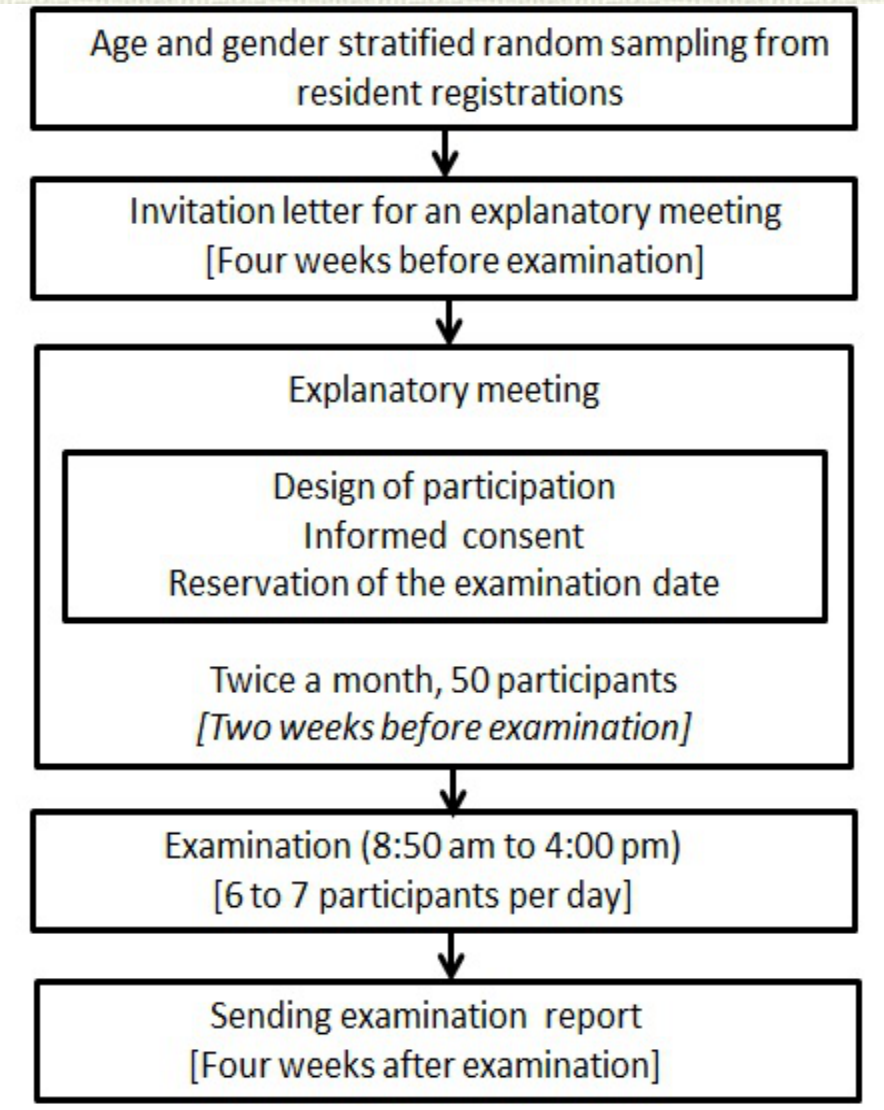

Fig. 5 Examination schedule in the NILS-LSA.

(The first examination)

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 the 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 and significance of the study;
(2) procedures and methods of each examination;
(3) place of the examination;
(4) gene analysis;
(5) preservation of blood and DNA samples for future examinations;
(5) examination report to the participants;
(6) to keep personal data secret.

The Ethical Committee of the National Center for Geriatrics and Gerontology had previously approved all procedures of the NILS-LSA.

8) Details of the examination

The normal aging process was assessed by detailed examinations including clinical evaluation, sensory functions, body composition and anthropometry, physical functions, nutritional survey, and psychological test batteries (Table 10).

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

Table 10. Details of the seventh wave examination in the NILS-LSA

- ***Health related questionnaire***

  Self-rated Health (SRH), Medical history, Clinical symptoms, Family history, Environment, Smoking, Menopause indices (Kupperman), SF36 physical functioning, Visual functioning questionnaire (VFQ-25),International Prostate Symptom Score (IPSS)

- ***Routine clinical evaluations***

  Physical examination
  Blood pressure, pulse rate
  Blood chemistry (fasting)

  AST, ALT, gamma-GTP, Total protein, Albumin, Chorine esterase, Uric acid, Creatinine, Calcium, Phosphate, Total cholesterol, Triglyceride, HDL-cholesterol, Fasting glucose, HbA1c, Iron

CBC: White cell count, Red cell count, Hb, Hematocrit, MCV, MCH, MCHC, Platelet count

- ***Sensory examinations***

  Visual system

    Visual acuity: Presenting Visual Acuity, Best-corrected Visual Acuity (33cm, 5 m), Refraction, Retinal fundus camera, Intraocular pressure, Contrast sensitivity

  Auditory system

    Audiometry (air and bone), Middle ear functions (Single frequency and Multifrequency tympanometry), Video recording of tympanic membrane

- ***Medical examinations***

  ECG (Automatic ECG analyzer)
  Intima-media thickness of carotid artery
  Ankle-brachial index (ABI) and pulse wave velocity were (PWV)
  Head MRI (Magnetic resonance imaging system)
  Thoracic and lumbar spine radiography

  Dual energy X-ray Absorptiometry (DXA)

    Lumbar spine, Right and left femur neck, Total bone density

- ***Anthropometry and body composition***

  Anthropometric measurements

  Body fat measurement (total and segmental fat) and lean body mass

    Dual energy x-ray absorptiometry (DXA)

  Thickness of fat and muscle tissue (Ultrasonic tomography)

    Muscle thickness and subcutaneous fat thickness

  Abdominal fat distribution (Computed tomography)

    Intra-abdominal and Subcutaneous fat area at umbilicus height

  Thigh muscle area (Computed tomography)

Physical fitness tests

Physical fitness test system

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

10m Walking test (pitch, step length, velocity),
3-D motion analysis system (six cameras and two force plates)
Stabilometer (with or without eye closed conditions)
Physical activity questionnaire
Electric uni-axial accelometer (7 days)

- ***Psychological examinations***

Depression

Center for Epidemiologic Studies Depression Scale (CES-D)

Subjective Well-being

Life Satisfaction Index-K (LSI-K)

Personality

Self-Esteem Scale
Psychological Well-Being Scale (PWB)
Attitude toward Death Scale for the middle-aged and elderly Adults (ATDS-A)

Social Relations

Social Support

Activities of Daily Living (ADL)

Katz ndex

Tokyo Metropolitan Institute of Gerontology (TMIG) Index of Competence

Intelligence and Cognitive Function

Wechsler Adult Intelligence Scale-Revised Short Forms (WAIS-R-SF)
Short-term Memory Function
Mini-Mental State Examination (MMSE)

Other Measures

Life-events Checklist
Daily Hassles Checklist
Social and Domestic Activities
Work Commitment Scale

Work-Family Conflict/ Work-Family Facilitation Scale（WFC/WFF）

Background Examinations

- ***Nutrition survey***

Food and nutrition intake

Three-day weighing dietary record using scale and disposable camera

Dietary supplement frequency questionnaire
Food frequency questionnaire

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1. Routine clinical evaluations

Medical history, family history, environment, smoking, health status, clinical symptoms, medical history and medication were examined by questionnaires. These questionnaires were checked by a physician at the physical examination. All medications were to be documented by participants; the physician confirmed them by interview and coded medications used for the last two weeks.

Renal and liver functions, serum protein and lipids, minerals, glucose, HbA1c, and complete blood count were also examined by blood analyses. Blood samples for DNA of the first visit participants was stored in deep freezers for future examination.

2. Physiological examinations

For physiological examinations, electrocardiograms were assessed by computerized automatic diagnosis and Minnesota codes of the diagnosis were stored in a database (NEC KARTIER5571). Intima-media thickness of the carotid artery was assessed by ultrasonic tomography (Hitachi EUB-5500). Blood pressure was measured by a physician as well as with an automatic blood pressure manometer. Ankle-brachial index (ABI) and pulse wave velocity (PWV) were also assessed (Colin).

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. Osteoporotic fractures of the spinal bones were assessed by x-ray examination.

3. Head MRI examinations

A head MRI was taken for each participant and stored in an image database. Intracranial tumors and vascular lesions were checked and brain atrophy, ventricular dilatation, and white matter lesion were assessed.

4. 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 5 m, and near visual acuity was measured at 33 cm. Contrast sensitivity and intraocular pressure were also examined. Fundus photographs were taken with a Topcon fundus camera (TRC-NW6S). Autorefraction was done with the NIDEK-ARK700A. Refractive errors, in the spherical equivalent, were assessed.

Auditory function assessed by pure-tone audiometry (Audiometer RION AA-78), and impedance audiometry (Middle Ear Analyzer, GSI TympStar, version 2). Air conduction thresholds at 125 Hz to 8000 Hz were examined in all participants. Bone conduction thresholds at 250 Hz to 4000 Hz were also examined. Middle ear function was evaluated by impedance audiometry. Video image of tympanic membrane was recorded by CCD camera (DPM-6, RF SYSTEM lab) and perforation, calcification and adhesion of tympanic membrane were assessed.

5. Anthropometry and body composition

For anthropometry measurements, height, weight, circumferences of waist, hip, thigh and upper arm and other parameters were taken. Using ultrasonic tomography, subcutaneous fat thickness and muscle thickness were evaluated. Total and segmental body fat and lean body mass were assessed by DXA. Abdominal fat distribution was evaluated as intra-abdominal and subcutaneous fat areas at the level of umbilicus using a computed tomography. Thigh muscle area was also evaluated by computed tomography. Thigh muscle area was also evaluated by a computed tomography.

6. Physical fitness tests

Grip strength, leg extension power, sit-up, one leg standing balance, reaction time, and trunk flexion were measured with a computerized automatic diagnosis system. Gait performance was assessed by the 10 m walking test (pitch, step length, velocity) along with six cameras and two force plates (motion analysis). Physical activities were checked by detailed interview using job-specific questionnaire sheets. Seven-day averages of physical activity were also measured with an electric pedometer.

7. Nutritional survey

Nutritional intakes were assessed by three-day dietary records using a scale. Scales were given to each participant to record the weight of each food item consumed over the recording period. If it was impossible to weigh each food item, approximate size and amount of food were noted. Dieticians explained to each participant how to weigh food 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 food how much food was consumed, and how much food was not. Using these dietary records and photographs, nutritionists estimated actual food intake. Dietary supplement usage was also assessed by interview. Beverage, alcohol, and citrus intakes were assessed over the previous year by food frequency questionnaire.

8. Psychological examinations

All participants were interviewed by psychology specialists. Cognitive function 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 old and over. Life events and daily hassles were also assessed by interview.

Depression, personality, subjective well-being, social and domestic activities, work commitments, work-family conflict and facilitation, and ADL were assessed using a questionnaire.

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

9) Staff of the seventh wave examinations

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