6) Implementation of the study

Selected men and women who are assigned to the next month's examination are invited by mail to an explanatory meeting that is held twice a month, once on Sunday and once on Monday (Fig. 2, 3). At the explanatory meeting, procedures for each examination and the importance of the continuation to follow up are fully explained. Participants are limited to those who understand all examination procedures and sign their names on a written form (informed consent).

The Department of Epidemiology of the NILS is taking the initiative for all examinations and investigations. All participants are examined from 9 am to 5 pm at a special examination center within a facility at the Chubu National Hospital located next door to the NILS (Fig. 4). To examine 2,400 men and women in two years, that is 1,200 men and women per year, six or seven participants are to be examined each day, 4 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 tested. Each examination is to be extensive and most up-to-date, aiming at the internationally highest level. The follow up period is to be up to 30 years, but we hope to get significant longitudinal results within 5 to 10 years. By the end of September 1999, 1,643 men and women had completed their first wave examinations.

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

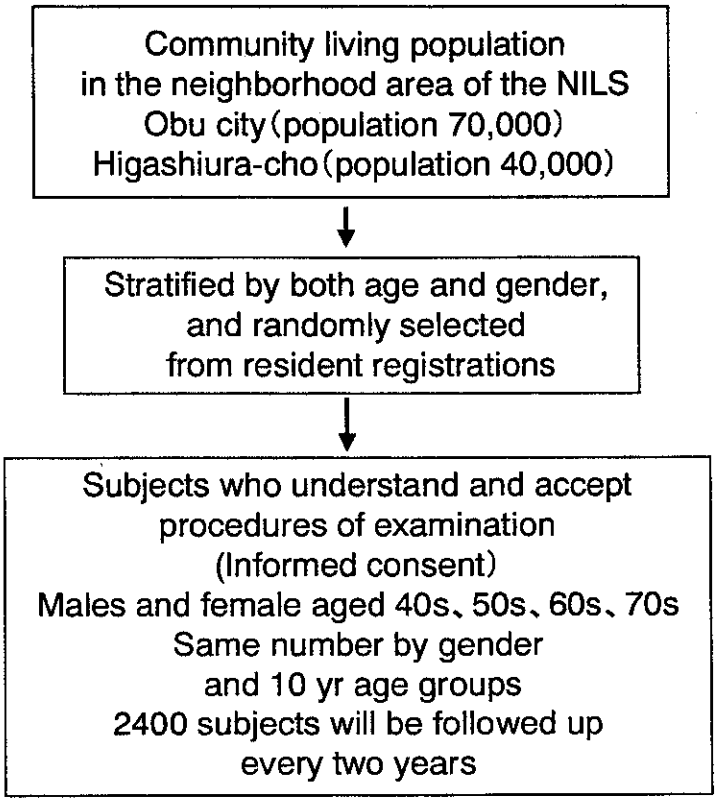

Fig. 2 Selection of the subjects in the NILS-LSA.

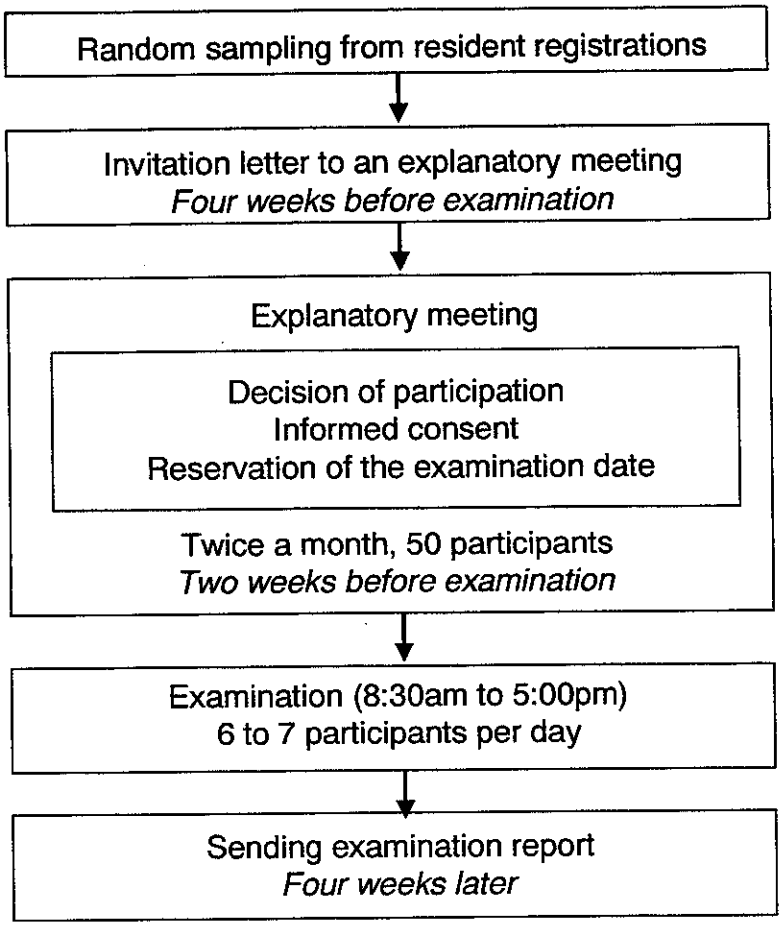

Fig. 3 Examination schedule in the NILS-LSA.

7) Informed consent

All participants are fully informed of the following items. Only subjects who understand and accept examination procedures, and sign their names to a written form to participate in the study (informed consent) are included. This informed consent includes; (1) purpose o f the study; (2) detailed procedures for each examination; (3) predictable danger; (4) participation in the examinations totally depends on free will, without any enforcement, and refusal to participate has no disadvantage; (5) to keep secret personal data from the examination.

We are paying particular attention to genetic analysis and preservation of blood and urine samples for future examinations. The Ethical Committee of the Chubu National Hospital has already approved all procedures of the NILS-LSA.

8) Examinations and tests

The normal aging process is assessed by detailed examinations including clinical evaluation, sensory aging, body composition and anthropometry, physical functions, nutritional analysis, and psychological tests (Table 1).

Table 1. Examinations and tests in the NILS-LSA

Health related questionnaire

Self-rated Health (SRH), Medical history, Clinical symptoms, Medical care, 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

GOT, GPT, γGTP, 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

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

Urine analysis: Protein, Sugar, Urobilinogen, Ketone, pH, Occult blood, Nitrite

Sensory aging

Visual system

Visual acuity: near vision (33 cm), distant vision (5 m), Kinetic visual Acuity, Refraction, Visual field, Retinal camera, Intraocular pressure, Color perception, Stereoscopic vision, Contrast sensitivity, Quantitative test of lens opacity

Auditory system

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

Skin sensory system

Quantitative sensory test (Neurometer), Skin discrimination test

Medical examinations

Automatic EKG analyzer

Cardiac ultrasonic tomography

Pulse wave (digital plethysmography)

Pulmonary functions (spirometer)

Blood oxygen saturation (Pulse oxymeter)

DXA (Dual Energy X-ray Absorptiometry)

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

High Quality Peripheral Quantitative CT (pQCT)

Intima thickness of carotid artery)

Head MRI (Magnetic resonance imaging system)

DNA phenotype and disease markers

Alzheimer's disease

Apolipoprotein E phenotype, Protease phenotype, Peptidase activity and inhibitors, beta-amyloid peptide concentration accumulative beta-amyloid autoantibody, DLST phenotype, Mitochondria CCO

Stroke and arteriosclerosis

Angiotensin converting enzyme (ACE) phenotype, Platelet-activating factor acetylhydrase activity (PAF-AH) and phenotype

Osteoporosis

Transforming growth factor beta 1 (TGF-b1) phenotype, Osteocalcin, Bone alkaline phosphatase, Aminoterminal cross-links of type I collagen (urine)

Parkinson's disease

N-methyl transferase activity and phenotype

Obesity and diabetes

CCK-A receptor phenotype, beta 3-adrenaline receptor phenotype, Leptin, Sex hormones (testosterone, free testosterone, estradiol, sex hormone binding globulin)

Prostate hypertrophy

Alpha-1 adrenaline receptor phenotype

Aging

Mitochondria 5178 phenotype

Body Composition

Body fat measurement

Air displacement (Bodpod), Impedance body fat measurement, DXA

Body fluid measurement (Bioimpedance spectroscopy)

Ultrasonic tomography

Intrabdominal fat, Muscle thickness, Subcutaneous fat thickness

Anthropometric measurements

Physical function

Exercise test system

Grip power, Sit-ups, Anteflexion, Static balance, Leg extension power, Static leg strength, Reaction time.

Walking test (pitch, stride, speed),

3D motion analyzer (four cameras and two force plates)

Balance test (stabilometer)

Physical activity (questionnaire)

Electric pedometer (7 days average)

Psychological tests

Interview

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

Questionnaire

Depression (CES-D), Personality (Self-esteem, EPSI, Locus of control), Social environment (Social support, Social network), Family Relations, QOL (LSI-K, SWLS), Stress coping, Instrumental ADL, Death Anxiety

Nutrition analysis

Food and nutrition Intake

Nutrition Diary (3 days) using scales and disposable camera

Food frequency questionnaire

Dietary habit questionnaire

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1. Routine clinical evaluations

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

Life-style, medical history and prescribed drugs are examined by questionnaires. These questionnaires are checked by a physician at the medical examination. All drugs used during the previous two years are to be documented by participants; the physician confirms them at interview and codes drugs used during the last two weeks.

In addition to the usual b lood and urine analysis, renal and l iver f unctions, serum protein and lipids, and complete blood count, lipid peroxide, sex hormones and geriatric disease markers are also examined. Serum, DNA and urine samples are stored in deep freezers for future examinations. As for DNA analysis, genotypes which are related geriatric diseases such as Alzheimer's disease, arteriosclerosis, osteoporosis, benign prostate hypertrophy and diabetes mellitus are examined with the agreement of the participants.

2. Physiological examinations

For physiological examinations, a head MRI is taken for all participants and stored in an image database. Intracranial tumors and vascular lesions are checked and brain volume is estimated via a computerized trace of the MRI. Pulmonary functions are e xamined w ith a s pirometer. Blood oxygen s aturation is a lso ch ecked w ith a n oxymeter. Blood pressure is measured by a physician as well as with an automatic blood pressure manometer. Electrocardiograms are assessed by computerized automatic d iagnosis a nd M innesota c odes of t he di agnosis a re s tored i n a da tabase. Cardiac functions and intima-media thickness of the carotid artery are assessed by ultrasonic tomography. Peripheral vascular function is assessed using a digital plethysmogram.

3. Sensory aging

Sensory functions are profoundly associated with QOL in the elderly. Visual and auditory disturbance causes v arious difficulties i n the daily li ves of the el derly. Sensory aging, including visual and auditory functions will be examined in detail. As for visual acuity, both distant vision (5 m) and near vision (33 cm) are assessed.

Kinetic visual acuity, stereoscopic vision, color perception, contrast sensitivity, visual field, and intraocular pressure are also examined. An anterior eye segment analysis system is used for the assessment of cataracts. Fundus photographs are taken with a Topcon fundus camera (TRC-NW5S). Autorefraction is done with the NIDEK-ARK700A. Refractive errors, in the spherical equivalent, are assessed.

Hearing acuity is assessed by pure-tone audiometry air conduction at 500Hz to 8000Hz in all participants and bone conduction in participants with hearing disturbance by a ir c onduction. Middle e ar function is a lso a ssessed b y impedance a udiometry. Peripheral skin sensory function is assessed using current perception thresholds at three different frequencies: 5, 250 and 2000 Hz. This is a non-invasive procedure to examine the function of three different sensory nerve fibers, that is Aβ fiber, Aäfiber, and C fiber. Cognitive sensory function at the parietal lobe of the brain is assessed by a skin discrimination test.

4. Body composition and anthropometry

Osteoporosis is one of the major geriatric diseases. Osteoporosis causes chronic l umbago and b one f racture that disturbs activity i n daily li fe i n the elderly. Bone mineral density is measured by dual X-ray absorptiometry (DXA). Four scans, including whole body, lumbar spine L2 to L4, right and left femoral bone neck, are taken. Moreover, bone density is also measured by high quality peripheral quantitative computed tomography (pQCT).

For anthropometry measurements, height, weight, abdominal depth, circumferences of waist, hip, thigh and upper arm and other parameters are taken. Using ultrasonic tomography, intrabdominal and subcutaneous fat thickness and muscle thickness are evaluated. Intra- and extra-cellular fluid is measured via bioimpedance spectroscopy. Body fat is assessed by impedance measurement, air displacement and DXA.

5. Exercise examinations

Grip power, leg extension power, sit-ups and static balance, reaction time, and anteflexion are measured with a computerized automatic diagnosis system. Pitch, stride and speed of walking are assessed by the 10m walking test system using four video cameras and two force plates. Physical activities are checked by detailed interview using job-specific questionnaire sheets. Seven day averages of physical activity are also measured with an electric pedometer.

6. Nutritional survey

Nutritional intakes are assessed by three day dietary records using scales. Scales are handed out to all participants to record the weight of all foods intake over three days. If it is impossible to weigh the food, size and approximate amount of food are noted. During lunchtime on the day of the examination, dieticians explain to each participant how to weigh foods and how to determine the size and approximate amount. Moreover, for more accurate assessment of food intake, disposable cameras are also handed out to all participants. Before and after each meal, participants take pictures of all food eaten to record what kind of foods and how much food were eaten, and how much food is not eaten. Using these dietary records and photographs, dieticians estimate actual food intake.

However, there are significant seasonal differences in daily food intake in Japan. Food intakes are also assessed by a food frequency and dietary habit questionnaire excluding seasonal differences. The average of amounts and frequencies of 166 representative foods eaten during the previous year are written. A dietician interviews the subjects to confirm the amounts and frequencies.

7. Psychological test

All participants are interviewed by psychology specialists. Cognition and intelligence are 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 are also assessed by interview. Basic ADL is checked via the Katz index.

Depressive state using CES-D (the Center for Epidemiologic Studies Depression Scale), personality, self-esteem, social environment including social support, social network and family relations, life satisfaction scale (SWLS; Satisfaction with Life Scale) and QOL, stress coping, instrumental ADL and death anxiety are assessed using a questionnaire.

The examined variables number over 1,000, including various areas of gerontology and geriatrics and these variables will be checked repeatedly every two years in the 2,400 participants. The staff of the NILS-LSA are full time researchers, researchers from hospitals and universities, research assistants such as administrators, clinical technicians, dieticians, psychologists, programmers and radiologists. The total number of staff is now 73.

9) Major outcomes

The recent major outcomes of the projects by cross-sectional analysis are as follows.

1. Clinical Evaluations and Medical Examinations

About 88% of the subjects felt themselves to be healthy. The prevalence of smoking was 41.8% in men and 8.9% in women. The prevalence of the past or present illness, such as cerebrovascular disease, hypertension, cardiac disease, hyperlipidemia, diabetes mellitus and osteoporosis, were 4.0%, 23.1%, 10.0%, 11.4%, 9.6% and 4.1%, respectively. The prevalence of postmenoposal women was 73.1%.

There was a significant gender difference ($p<0.01$) in the combined thickness of the intima and media of common carotid artery (IMT). IMT positively correlated with age after adjustment for gender ($r=0.33$, $p<0.0001$). In a logistic analysis, higher IMT (1.1mm and over) significantly related with stroke volume of left ventricle and the history of diabetes or hyperlipidemia among men, and fasted glucose level among women.

Magnetic r esonance images (MRI) of t he b rains w ere pe rformed in a ll t he subjects except contraindication of the examination and the analysis is now in progress.

Bone mineral density (BMD) measured by dual X-ray Absorptiometry (DXA) was significantly higher in men than in women and it negatively associated with age in both gender. BMD measured by high quality peripheral quantitative computed tomography (pQCT) was highly correlated with that measured by DXA. BMD of D50 or D100 measured by pQCT, which was mainly influenced by trabecular bone, showed higher association than that of P100, which was mainly influenced by compact bone, with total or lumbar spine BMD measured by DXA, especially in the group of 40-59 years.

As for visual function, age groups of 40s and 50s had higher mean distant corrected visual acuity and contrast sensitivity than 60s and 70s. Kinetic visual acuity and the mean sensitivity of visual field decreased with age. Red-green color discrimination was the lowest in 70s. Blue-yellow color discrimination was significantly lower in 60s and 70s than 40s and 50s. Stereo acuity was significantly lower in 60s and 70s than in 40s and 50s.

The hearing threshold over each frequency of 500, 1,000, 2,000, 4,000 and 8,000 Hz, was positively associated with age (right ear; $r=0.36$-0.67, $p<0.0001$) . The thresholds over frequencies 1,000Hz and over were significantly higher in men than in

women.

Blood and urine are sampled for ongoing chemical analysis, and serum , extracted DNA and urine are stored for future studies.

2. Anthropometry and Body Composition

As anthropometry measurements, height, weight, abdominal depth, circumference o f w aist, h ip, t high a nd u pper a rm and o ther pa rameters w ere taken. Using ultrasonic tomography, Intrabdominal and subcutaneous fat thickness and muscle thickness were evaluated. Intra-and extra-cellular fluid measured by bioimpedance spectroscopy showed negative relationship with age.

Percent of body fat measured by DXA positively correlated with age and it also highly correlated with percent body fat measured by air displacement method (BOD POD) (men; r=.922, p<.0001, women; r=.865, p<.0001). But the difference of body f at m easured by these two methods related with age and waist circumference. Some consideration to age and figure might be needed using BOD POD instead of DXA for estimating percent body fat.

3. Physical Functions and Physical Activities

Grip power, leg extension power, sit-ups and static balance inversely correlated with age, and reaction time was prolonged with aging. Ante-flexion showed no relationship with age. Pitch, stride and speed in 10 m walking test showed negative correlation with age. There were significant positive relationship between leg extension power with pitch, stride and speed.

4. Psychological Assessments

The prevalence of depressive symptoms measured by CES-D (Center for Epidemiologic Studies Depression Scale) was 16.2%. There was no significant differences in the prevalence among age groups in men, but in women, the group of 70s showed higher prevalence (35.1%) than other age groups. The score of somatic and retarded activity, one of the sub-scales of CES-D, showed positive relationship with age in both gender. The score of positive affect tended to decrease with age in women. The score of WAIS-R (Wechsler Adult Intelligence Scale-Revised) and MMSE (Mini-Mental State Examination) were negatively correlated with age. Life satisfaction scale (SWLS; Satisfaction with Life Scale) positively correlated with age in men but not in women.

Social support from family negatively correlated with age in men but it was

supposed to come from the decrement of negative support with age. There were no significant relationship between social support and age in women. After adjusting for gender, positive social support showed positive relationship with life satisfaction and negative relationship with depressive symptoms, while negative support showed negative relationship with life satisfaction and positive relationship with depressive symptoms.

5. Nutritional Analysis

Nutritional assessments by food frequency questionnaire, dietary habit questionnaire and dietary record using scales and disposable cameras underwent almost all subjects of the NILS-LSA. The precise results were shown in the monograph.

6. Molecular Epidemiology

The gene-related investigations of Alzheimer's disease, osteoporosis, stroke and arteriosclerosis, Parkinson's disease, benign prostate hypertrophy, and obesity are now in progress with many collaborating researchers in Japan. Several phenotypes of the genes (transforming growth factor-β 1, α 1-Adrenoreceptor, Dihydrolipoamide succinyltransferase, Mitochondrial 5178, Cholecystokinin type-A receptor, Apo E, Angiotensin converting enzyme, β3-Adrenoreceptor) and enzyme activity (N-methyl transferase) are investigated to clarify the contribution of genetic factors and other basic background factors in various age-related diseases.

10) 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 first wave examination will be completed by March 2000. The participants will be examined every 2 years. The cohort of the NILS-LSA will be a dynamic cohort, that is, new subjects will participate in the study instead of those who will 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.

The NILS-LSA includes collaborating studies 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 almost no comprehensive longitudinal studies on aging which 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. A high number of subjects and cases during follow-up need to be obtained to get significant analysis results. We are going to start multi-center collaboration with common baseline examinations that relate to the end point of the follow-up.

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.

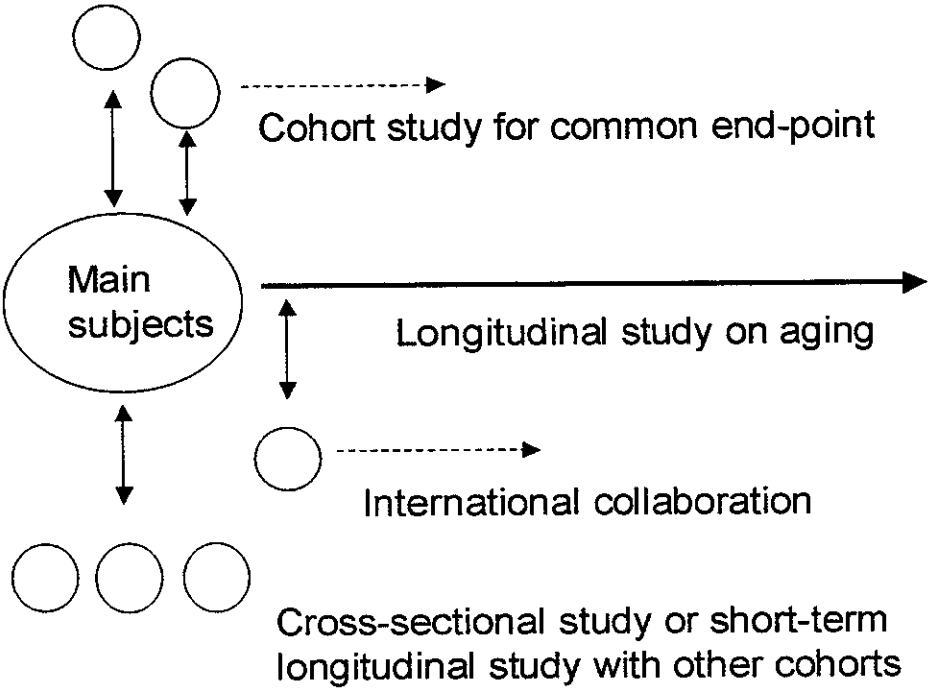

Fig. 5 Design of the longitudinal study by multi-center collaboration

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II. Background Examinations