Predictors of dementia and mortality in Down's syndrome

Tonnie Coppus

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We are not actually made for time.
A fish lives in the water and isn't troubled by the water,
But we live in time and are constantly troubled by it.
Therefore we were not made for time.
We were made for eternity.
We are timeless creatures.

CS Lewis

Ter nagedachtenis aan mijn ouders
en Els

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Chapter 1

Introduction

Chapter 1.1

General Introduction

CHARACTERISTICS

Down's syndrome (DS) is a common genetic disorder affecting around 1 in 800 live births in the human population. The syndrome was first described by Down in 1866, in which he marked a collection of symptoms observed in 10% of the children with intellectual disability[1]. In 1959 Lejeune, Gautier and Turpin discovered the relation between DS and the triplication of chromosome 21[2]. The diagnosis of DS is made by chromosome analysis, which can be initiated prenatally or postnatally based on the characteristic appearance of the newborn child.

Down syndrome is in 95% of the cases caused by the presence of an entire extra copy of chromosome 21. A small number, 4% of the cases, have a partial trisomy, resulting from a translocation, and the attachment of an extra long arm of chromosome 21 to chromosome 14, 21 or 22. These infrequent cases have allowed the definition of the Down Syndrome Critical Region (DSCR) of chromosome 21 within the q22 region (about 5.4Mb in length)[3] that includes a subset of genes that, when present in three copies, results in the major phenotypic features of DS, including the characteristic facial appearance, congenital heart disease, and intellectual disability[4]. Less than 1% of the cases are caused by mosaicisme, where some, but not all cells are trisomic.

The presence of an extra chromosome 21 makes DS a biologically complex disorder. Sequencing of human chromosome 21 has been completed and almost 300 genes have been identified and are publically available[5]. Overexpression of any one of these genes can influence the development of DS in itself but these gene products can also interact with each other and with genes on other chromosomes. Recent evidence has shown that gene overexpression in persons with DS can be specific for tissue type[6]. Different subsets of chromosome 21 genes are over-expressed in different cell types.

The more than 80 clinical features, characterising the phenotype of DS, can vary in number and in severity, and none of the features diagnosed in DS is unique to persons with trisomy 21[7-9]. Common DS phenotypes include intellectual disability and dysmorphic features involving the craniofacial area, neck and limbs. Manifestations include brachycephaly, epicanthal folds, flat nasal bridge, folded or dysplastic ears, open mouth, narrow palate, protruding tongue, short and broad hands, incurved fifth finger, transverse palmar lines, gap between the first and second toe, and hyperflexibility. Other clinical manifestations include cardiac malformations, thyroid dysfunctions, digestive system abnormalities, and seizures. There is also high susceptibility to leukaemia's and infections[10]. Most males with DS are infertile. Although not sterile, women with DS show often

reproductive system dysfunction and an earlier menopause compared with women in the general population[11].
None of the dysmorphic features is present in all cases, and no two persons with DS are the same, although the overall pattern is consistent and enables clinical recognition. There is an age-dependant presentation of the phenotypic features. Some aspects are congenital, as there is hypotonia, others appear in childhood; such as growth retardation and development delay, and still others occur in adulthood or in the elderly e.g. premature aging and dementia.

Three hypotheses have been singled out to explain the relationship between genotype and phenotype:

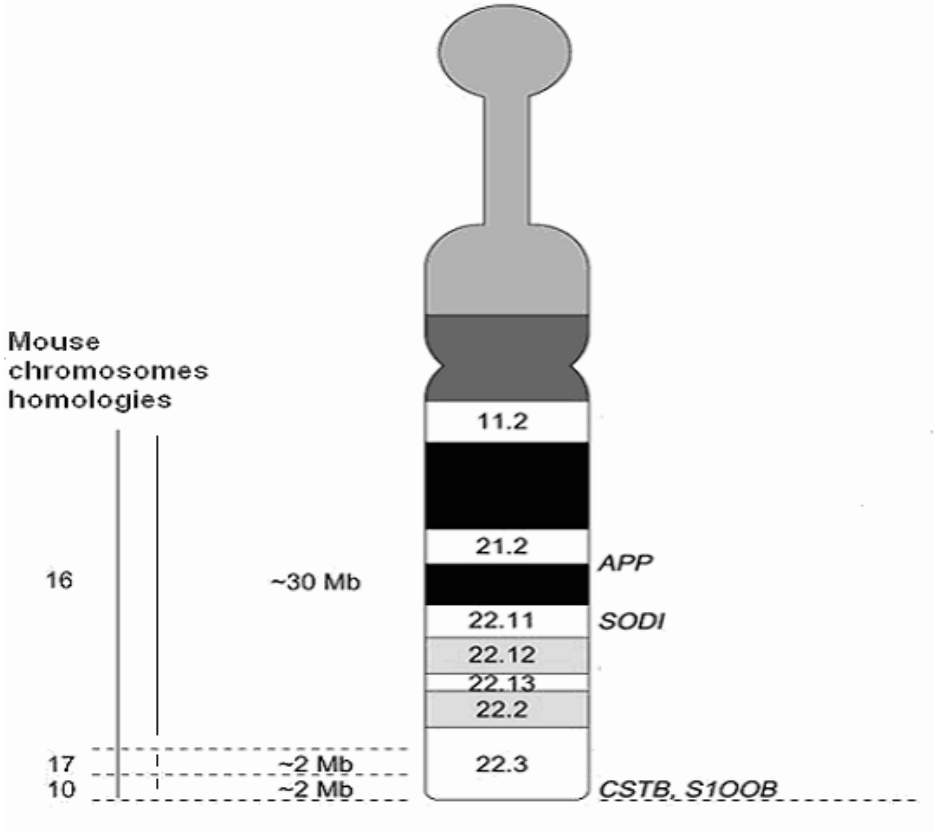
1. "The gene dosage effect" hypothesis proposes that dosage imbalance of specific genes on chromosome 21 causes specific DS phenotypes[12,13]. A restricted number of genes, located on the DSCR that are over-expressed due to the trisomy contribute to the phenotype.
2. Specific phenotypes arise from a small set of triplicated genes, but only when other genes (on chromosome 21 or on other chromosomes) are also present and active[14].
3. The "developmental instability" predicts that non-specific phenotypes result from genetic imbalance and over-expression in combination with environmental interactions[7,15].

The use of models of DS in mice is one of the promising approaches to understanding the genotype-phenotype relationship. The genes found on chromosome 21 are found on three mouse chromosomes; 16,17 and mouse chromosome 10 (see figure 1)[10].

ETIOLOGY

The majority of trisomy 21, or Down's syndrome, is caused by the failure of chromosomes to separate properly during meiosis. This process is also known as chromosome nondisjunction.
At least 90% of the cases of chromosome 21 nondisjunction are due to maternal meiotic errors during oogenesis.
Meiosis I is initiated in oocyte during fetal life, at about 11-12 weeks of gestation and, also after pairing, synapsis and recombination, arrests in prophase I until just prior to ovulation. At that time, the oocyte completes MI and progresses to metaphase II. Meiosis II extends over 3-4 days during ovulation period and is completed after fertilisation. Thus, homologous chromosomes are arrested in prophase I for 10 to 50 years.
It has been established that increased maternal age, the most significant risk factor for nondisjunction, is associated with errors occurring during oogenesis. For chromosome 21 nondisjunction,

Figure 1 Chromosome 21 in humans and in mice



APP: amyloid precursorprotein
SOD-1: super oxid dismutase
CSTB: Cystathionine β-synthase
S100B: S100 β

advanced maternal age is associated with both maternal meiosis I and meiosis II errors[16-18]. The risk of having a child with DS increases with maternal age. While a 20 year old woman has a risk of 1/1400, the risk increases to 1/100 at the age of 40 years [18].

Paternal nondisjunction of chromosome 21 accounts for 5-10% of DS cases and is not associated with advanced paternal age. Spermatogenesis starts at puberty and cells entering meiosis move from one stage to the next without delay.

About 5% of cases of trisomy 21 are probably due to mitotic (postzygotic) nondisjunction of a chromosome 21 in the early embryo[19]. The mititotic errors are not associated with advanced maternal age.

Mosaicisme with a normal cell line occurs in about 2-4% of the cases. DNA polymorphism analysis in 17 families with mosaic trisomy 21 probands showed that the majority resulted from a trisomic zygote with mitotic loss of one chromosome[20].

EPIDEMIOLOGY

In the Netherlands, data concerning incidence and life expectancy of persons with DS are not available. In Europe as a whole, DS accounts for 8% of all registered cases of congenital anomalies, with an estimated prevalence in the Netherlands of 10 to 14 per 10.000 live births[21]. The prevalence of DS among live births in the Netherlands, registered by the Dutch Paediatric Surveillance Unit in 2003, 16 per 10.000 live births, was much higher than might have been expected based on previous registrations, and higher than suggested in literature[21]. The data from 2003 concerning terminated pregnancies reveals a total prevalence of DS (both live births and still births) of 26.8 per 10.000 pregnancies[22,23].

Worldwide the overall prevalence of DS is 10 per 10.000 live births, which seem to have increased in recent years. In countries in which abortion is illegal, such as Ireland and the United Arab Emirates, prevalence is higher, varying from 17 to 31 per 10.000 live births. Conversely, the prevalence in France is low (7.5 DS per 10.000), due to high percentage (77%) of DS pregnancy terminations[24]. In Europe, the proportion of mothers aged 36 and above has increased from 8% to 25% over the past 20 years[24,25]. Despite the availability of advanced prenatal screening tests, the influence of increasing maternal age outweighed the effect of antenatal diagnosis and termination of pregnancies.

Survival rates of children with DS continue to improve[22,25,26]. Infant survival after live birth is now close to 100%. This is partly due to better surgical management of gastrointestinal and cardiovascular malformations. Exceeding expectations formed by previous literature[27], the large majority of persons with DS are now expected to live into adult life. In 2000 the median life expectancy, in Australia, was 61.1 years for men and 57.8 years for women[26].

In this respect, future studies should focus on probable changes in long term DS morbidity, as there is a growing cohort of survivors with DS, with their own specific health problems. Special attention is required for mobility, the presence of epilepsy, visual and hearing impairments and the development of Alzheimer's disease.

DEMENTIA

The first English description of the link between DS and Alzheimer's disease (AD) is found in a publication by Jervis in 1948[28], who described three persons with DS in their fourth decade whom were found to have intellectual deterioration, neuronal cell loss, senile plaques and neurofibrillary tangles. Present evidence suggests that persons with DS suffer exactly the same pathological process in later life as patients with AD in the general population[29].
In DS, AD is assumed to be caused by the triplication and over-expression of the gene for amyloid precursor protein (APP), located on chromosome 21 and leading to the accumulation of cerebral β-amyloid[30].The neuropathology of AD and some degree of the neuroanatomical changes associated with AD have been reported to occur in the brains of almost all persons with DS aged 40 years and over[30]. However, clinical and epidemiological studies have shown that age-specific rates of AD among person with DS are lower than would be expected given the extensive amyloid and neurofibrillary pathology that is found at autopsy[31,32]. Even at ages associated with extensive neuropathology, a proportion of elderly persons with DS remain functionally unimpaired.
In persons with DS, the average age of onset of dementia ranges from 38 to 70 years, with most cases occurring between 50 and 55 years of age. The prevalence of clinical AD has been examined for several decades[31-52]. Prevalence rates have varied from 4% in a community sample[53] to 88% in an institution- based sample[42]. All studies showed an exponential increase in prevalence with age. Although triplication of the gene for APP may act to increase deposition of amyloid β in diffuse plaques in persons with DS, explanations other than increased APP alone are needed to account for the wide range in age at onset of the clinical signs of AD.

Age

The factor that has been conclusively linked to an increase in risk for AD in DS is increasing age[54]. All studies showed an exponential increase in prevalence with age[40,43,47-49,51,52,55-59]. There is an ongoing debate on the percentage of DS persons that will eventually develop dementia if they reach a very old age. Several studies, mostly neuropathological, suggested that all patients develop AD[31,60-63]. Further research is required to investigate the age associated prevalence and incidence of dementia in persons with DS.

There are at least 10 known chromosome 21 genes that are involved in brain structure and function, but only 3 have been implicated in the neuropathology of DS[64]. These are the genes for amyloid β precursor protein (APP), for superoxide dismutase (SOD1), and for the astrocyte-derived neurotrophic factor S100β[4,65,66] (see figure 1).

Amyloid precursor protein (APP)

From a genetic perspective, Alzheimer disease (AD) is almost unavoidable in DS. In 1987, the gene that encodes the precursor protein of amyloid β was found to be located on chromosome 21[67,68]. Triplication of the gene for APP is not necessary for the development of phenotypic characteristics of DS, but does seem necessary for the development of AD in persons with DS[12,64]. Prasher[69] described in a case report, a 78 year old woman with an appearance suggestive of DS, with partial trisomy 21 that included the genes for SOD-1 and S100β but did not include the APP gene. This woman showed neither dementia nor significant AD pathology. She had only two copies of the gene for APP, suggesting that triplication of this gene is necessary for the development of AD in DS.

Superoxide dismutase (SOD-1)

Oxidative stress is caused by an imbalance between the production of reactive oxygen and the biological system's ability to detoxify the reactive intermediates or repair the resulting damage. Oxidative damage is considered to be a causal factor in the brain changes seen in AD within the general population. As a factor in the pathogenesis of AD in DS, the role of superoxide dismutase (SOD-1)[65] may be relevant. The free radical, superoxide O_2^-, is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. Hydrogen peroxide is then detoxified by the enzymes catalase and glutathione peroxidase. Overexpression of SOD-1 has been found in all tissues including the foetal brain in DS[70]. Although SOD-1 may be an important component in the mechanism of protection against free radicals, it can also have a negative effect. It is possible that in DS, increase SOD-1 activity results in the overproduction of H_2O_2 and hydroxyl radicals which compromise cellular functioning in the brain, contributing to neurodegeneration[54,71,72].

S100 β

S100 β, is a small, soluble, astrocyte derived protein, which is encoded by a gene mapped in the Down's Syndrome Critical Region. It has trophic effects on neurons including proliferation and differentiation. In addition to these trophic effects, S100β has potentially neurotoxic effects at higher concentrations[64]. S100 β induces astrocytic expression of nitric oxide synthase with release of nitric oxide (NO). S100 β is overexpressed by activated astrocytes, associated with the amyloid plaques of AD and tissue levels of biologically active S100 β are markedly increased in the brain of AD patients.

In persons with DS, S100 β expression, is elevated in the brain even at fetal stage, making S100 β-overexpression one of the earliest Alzheimer-associated brain alterations. This S100 β-overexpression increases progressively throughout life in persons with DS[73,74] and the degree of this S100 β-overexpression correlates with the degree of amyloid deposition in brain.

Apolipoprotein E (APOE)

It is well established that one of the major risk factors for the development of AD in the general population, is the presence of the allele ε4 of the Apolipoprotein E (APOE) on chromosome 19[75,76]. APOE is involved in cholesterol transport and lipid metabolism in plasma and is associated with greater accumulation of beta-amyloid in the brains of the elderly in the general population[12,77,78]. Numerous studies have shown that the presence of the ε4 allele is associated with early onset of AD, whereas the presence of the ε2 allele is associated with a delay in disease onset and is possibly protective[79,80].
In persons with DS, the ε4 allele is associated with a 1.6 to 2-fold increased risk of AD and is associated with an early onset of the disease. The relation between APOE ε2 and the risk and onset of AD has been a controversial issue[50,81-84].

Estrogen

In women who are pre-menopausal, estrogen promotes the growth, prolongs survival of cholinergic neurons and has antioxidant properties. Women with DS appear to have an earlier age at menopause than women in the general population[11,85-87]. It is hypothesised that the earlier menopause, including the reduction in estrogens following menopause, contributes to the earlier onset and increased risk of Alzheimer's Dementia (AD) in women with DS[88].

Pre-existing levels of cognitive ability

An explanation for the variability in age of onset and prevalence rates of AD in persons with DS may be found in the level of intellectual disability prior to the onset of neuropathological changes[54]. The reserve capacity model implicates that persons with DS with severe and profound intellectual disability should have a higher risk of AD than those with mild and moderate intellectual disability. In order to establish a diagnosis of dementia, a key criterion is that there is a decline in cognitive functioning from a person's baseline level. The measurements have to be sensitive and the changes measured should be greater than found in normal aging[44,89,90]. The presence of severe and profound pre-morbid intellectual disability in persons may make additional cognitive deficits

associated with early stages of dementia difficult to detect. The existing literature on AD in DS in relation to pre-morbid intellectual disability, so far, is inconclusive[42,51,59,91,92].

PERIPHERAL BIOCHEMICAL MARKERS IN PERSONS WITH DOWN'S SYNDROME

It has become clear that amino acids are not only essential for various metabolic pathways such as the synthesis of the major neurotransmitters, but also for maintenance of cell structure and functionality of many organs. In addition, amino acids are involved in immune responses[93,94].
Over the past decade, several studies have shown that serum amino acid concentrations in persons with DS differ from concentrations in healthy controls[95-97]. At this moment, little is known about DS-specific biochemical pathways that are thought to be related to accelerated aging, to increased susceptibility to infectious diseases, to autoimmune disorders and to the development of AD.

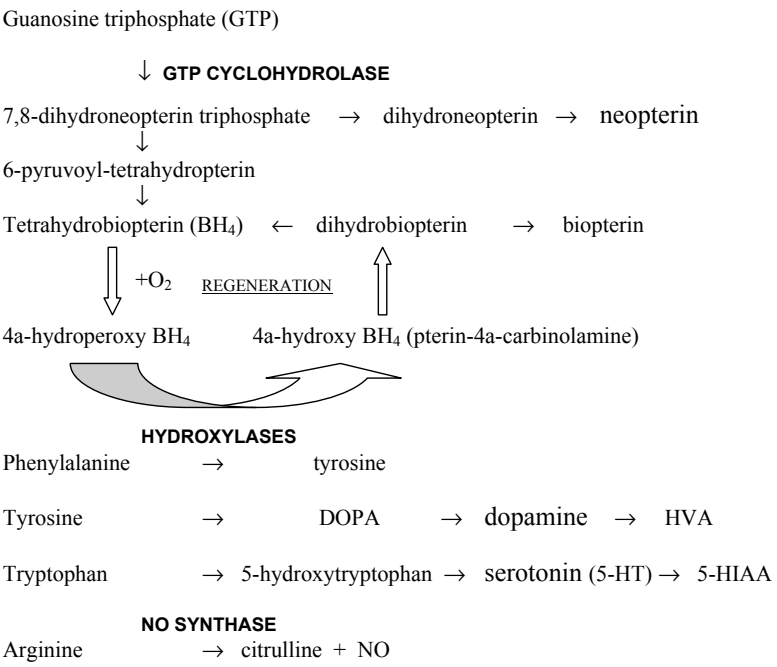
Cystathionine β-synthase

The gene encoded for the enzyme Cystathionine β-synthase (CBS) is located on chromosome 21 (21q22.3)(see figure 1) and is overexpressed in persons with DS. CBS catalyses the conversion of homocysteine and serine into the dipeptide cystathionine. It shunts homocysteine away from the methionine cycle to the transsulfuration pathway resulting in the synthesis of cysteine and glutathione. An increase in the transsulfuration, due to CBS overexpression, reduces the methionine synthase activity and results in lower conversion of 5-methyltetrahydrofolate to tetrahydrofolate (THF). This is the metabolically active form of folate, required for the novo synthesis of nucleotides for RNA and DNA synthesis. With respect to the CBS-related parameters, plasma concentrations of amino acids measured in persons with DS are in accordance with an overexpression of the CBS gene[95,97,98].

Nitric Oxide

A growing body of evidence indicates that there is an increased oxidative stress in AD brains and it is hypothesised that nitric oxide (NO) can be related to many of the pathophysiological mechanisms of AD[99,100]. The presence of any stimulant that leads to an overproduction of NO will probably cause neuronal damage[101]. The biologically active molecule NO is formed during the conversion of L-arginine to L-citrulline, a reaction catalysed by the enzyme nitric oxide synthase. In this process tetrahydrobiopterin (BH4) is the necessary cofactor. The ratio of the plasma concentrations of citrulline (Cit) and arginine (Arg), the so-called Cit-Arg ratio, is regarded as an index of NO synthesis[102].

Figure 2 Bio synthesis of pterins and biogenic amines.



Brain inflammation is mainly caused by activation of micro-glia cells that produce a variety of pro-inflammatory and neurotoxic factors, including free radicals such as NO[103,104]. Inflammation and immune system activation are considered to play an important role in the development and progression of dementia[105].

Neopterin

A marker of cell-mediated immune activation is the pteridine neopterin[106]. Neopterin is formed as a bypass product in the synthesis of BH4, which is a cofactor required for the biosynthesis of catecholamines, serotonin, and nitric oxide (see figure 2).

Monocytes and macrophages produce neopterin upon stimulation by interferon (IFN) γ[106]. Neopterin, besides being a marker of IFN activity, induces and enhances cytotoxicity and is a proinflammatory mediator. Higher levels of neopterin have been found in patients with inflammatory diseases of the central nervous system, infectious diseases, and tumours[107].

Peripheral levels of neopterin increase with age. Recent studies showed higher levels of neopterin in persons with DS with and without AD[95,108] .

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Chapter 1.2

Scope of the Thesis

SCOPE OF THIS THESIS

The aim of the present longitudinal population based study on persons with Down's syndrome (DS), aged 45 years and older is to study determinants of dementia and mortality, and to obtain new insights into biological pathways involved in the development of dementia in DS.

An overview of the study design, population characteristics and the instruments used is presented in chapter 2.1. The large number of subjects included in the study enables us to give more precise age-specific estimates for the prevalence and incidence of dementia and mortality.

Apolipoprotein E (APOE), especially the APOEε4 allele, is consistently associated with dementia in the general population. Findings on the role of this gene in persons with DS are inconclusive. In chapter 2.2 we describe the effects of APOE on mortality and dementia in our study population of persons with DS (n=425), demented and non-demented. In a meta-analysis, we also compare our findings with those of the existing literature, focusing on the effect in elderly DS persons. Although life expectancy increases enormously, our knowledge of factors that influence survival in older persons with DS is still very limited. In Chapter 2.3 the impact of cognitive and functional decline, and physical comorbidity, on mortality risk in the course of time is examined. DS is considered as a model of precocious aging and of Alzheimer's disease (AD). In Chapter 2.4, as an indication of this precocious aging in women with DS, the early menopause is described and related to survival and age at diagnosis of dementia.

Chapter 3 focuses on biochemical pathways possibly involved in the development of dementia in persons with DS. In chapter 3.1, first we describe peripheral biochemical parameters that reflect changes in homocysteine metabolism, immune function and monoaminergic neurotransmission in a small group of relatively healthy persons with DS aged 45 years and older. The study group comprised 48 persons with DS, without signs of dementia, psychiatric or somatic comorbidity and free of medication and was compared with 48 age and sex matched healthy controls.

Among the various pathophysiological mechanisms involved in dementia in the general population, inflammation and immune system activation are considered to play an important role in the development and progression of dementia. In Chapter 3.2, we present whether the plasma neopterin level, a marker for cell mediated immune activation and inflammation, is associated with an increased risk of dementia in persons with Down syndrome.

In Chapter 3.3 we focus on the spectrum of amino acids and the role of Nitric Oxide (NO) and Branched Chain Amino Acids (BCAA) in our study cohort of persons with DS with and without dementia. Special attention was given to those amino acids, involved in the oxidative processes, and their relation to the development of Alzheimer dementia.

The findings of this study are summarized and discussed in light of the literature in chapter 4.

Chapter 2

Dementia and Mortality in persons with Down's syndrome

Chapter 2.1

Dementia and Mortality in Persons with Down's syndrome: a population based study

ABSTRACT

Numerous studies have documented that persons with Down's syndrome (DS) are at an increased risk of Alzheimer's disease (AD). However, at present it is still not clear whether or not all persons with DS will develop dementia as they reach old age.

We studied 506 people with DS, aged 45 years and above. A standardized assessment of cognitive, functional, and physical status was repeated annually. If deterioration occurred, the patients were examined and the differential diagnosis of dementia was made according to the revised Dutch consensus protocol and according to the ICD-10 Symptom Checklist for Mental Disorders. We compared our findings with those reported in the literature.

The overall prevalence of dementia was 16.8%. Up to the age of 60, the prevalence of dementia doubled with each 5-year interval. Up to the age of 49, the prevalence is 8.9%, from 50 to 54 it is 17.7%, and from 55 to 59 it is 32.1%. In the age category of 60 and above, there is a small decrease in prevalence of dementia to 25.6%. The lack of increase after the age of 60 may be explained by the increased mortality among elderly demented DS patients (44.4%) in comparison with non-demented patients (10.7%) who we observed during a 3.3-year follow-up. There was no decrease in incidence of dementia in the age group of 60 and above. Our findings are very similar to those published in the literature. Patients with dementia were more frequently treated with anti-epileptic, anti-psychotic and anti-depressant drugs. The history of depression was strongly associated with dementia.

Our study is one of the largest population-based studies to date. We found that despite the exponential increase in prevalence with age, the prevalence of dementia in the oldest persons with DS was not higher than 25.6%.

INTRODUCTION

Down's syndrome (DS) is the most common cause of intellectual disability (ID). The syndrome is clinically characterised by congenital malformations, especially of the heart and the gastrointestinal tract, which result in high mortality rates early in life. As a result of many improvements in the medical treatment of people with DS, the survival rate has increased in comparison with the past. Estimates of life expectancy suggest that about 44% of persons with DS will reach the age of 60 and 14% will reach the age of 68[1]. At present, Alzheimer's disease (AD) appears to be the most important cause of morbidity and mortality among elderly DS persons. The neuropathology of AD includes the deposition of extra cellular beta-amyloid (Aß) in neuritic plaques and intracellular accumulation of neurofibrillary tangles. The neuropathological manifestations of AD in DS have been attributed to triplication and over-expression of the gene encoding for the ß-amyloid precursor protein (APP), located on chromosome 21.

The prevalence, of clinical AD, has been examined extensively[2-23]. The average age at the onset of dementia was found to be between 50 and 55 years, with a range from 38 to 70 years. Prevalence rates have varied from 4% in a community sample[24] to 88% in an institution-based sample[12]. Previous studies varied considerably in the populations studied, in the design of the study, and in the size of the population studied. Studies conducted with institutionalized patients may have been biased in that they included a selection of more severely intellectually disabled or demented patients. The advantage of a population-based study, including persons living in a community setting as well as institutionalized persons, is that there is no selection. Another major limitation of the previously listed studies is their small size, varying from 50 to 307 subjects. In particular, there were only small numbers of elderly DS persons included, which will have limited the interpretation and analysis of the prevalence of AD. This may also explain the diversity in the findings. At present it is still not clear whether or not all persons with DS will eventually develop dementia as they reach old age.

In this paper, we report on a study of a population-based series of 506 patients with DS. This is the largest study conducted that includes those with DS who are institutionalized as well as those living in the community. The large size of the study enables us to give more precise age-specific estimates for the prevalence and incidence of dementia. All patients were physically examined and screened for dementia and followed over the years up to their deaths. We studied the prevalence and incidence of dementia as well as mortality in the demented and the non-demented. We compared the prevalence of dementia with that reported in the existing literature.

METHODS

Study population

In the south and southwest of the Netherlands, including the regions Rotterdam, Zeeland, Noord Brabant and Gelderland, all organizations involved in the care of intellectually disabled persons were invited to participate in this longitudinal study. Their administration departments and general practitioners were asked to recruit all those who had been diagnosed with DS and were aged 45 years and above. Only subjects in whom the diagnosis was not established were not covered by the study. Thus, the sample from these regions represents almost all the DS persons living in the community, as well as those living in institutions. The recruitment period was from 1 December 1999 to 1 December 2003.

Ethical approval for this study was obtained from the medical ethical committee of the Erasmus Medical Center. The medical ethical committees and committees consisting of relatives of DS persons residing in local institutions and organizations also approved the protocol.

Of the invited organizations, 75% (21/28) participated in the study. Reasons for not participating were: ethical problems with the study of intellectually disabled people (one organization) and increased workload for the nursing staff (six organizations). Permission for individuals to participate in the study was obtained with the help of the general practitioner or the medical officer responsible for the individual's medical care. Written informed consent to participate, and to provide blood samples, was also obtained from legal representatives (relatives and/or carers), after written information was provided. Written consent was also obtained from persons with DS who had the mental capacity to consent, after being given an explanation. The response rate in those organizations that participated is estimated to be 75%, based on a retrospective report. Due to the recruitment procedure and issues of consent, information was not available on non-responders.

All participants included in the study received a complete assessment at the baseline, including a home interview with a relative, an interview with carers and a clinical examination. Blood was taken from all participants. A standardized assessment of cognitive and functional status was repeated annually. In the event of death, permission to perform an autopsy on the brain was requested.

Medical records

For this study, the diagnosis of DS was re-evaluated based on clinical characteristics according to the criteria described by Roizen et al[25]. In 283 persons (56%) the clinical diagnosis was confirmed by available cytogenetic characterization. Of these persons, 271 (53.6%) had complete trisomy 21,

5 (1%) had a translocation of chromosome 21 and 7 (1.4%) had a mosaic form. Chromosomal analysis was not available for the remaining 223 people (44%).
Results of (pre-morbid) intelligence tests and previous levels of functioning were studied by a review of medical records. Severity of ID was classified using the International Classification of Diseases (ICD-10) criteria[26]. The general practitioner was asked to provide information about current medical and psychiatric disorders and current drug use.
The participant's medical records were reviewed to confirm the possible existence of past or present disorders. Special attention was given to psychiatric disorders, cardiovascular and cerebrovascular diseases, hypertension, thyroid disease, diabetes, sensory impairments, neoplasm and epilepsy. The use of non-steroidal anti-inflammatory drugs, estrogens and psychiatric drugs was also reviewed.

Physical examination

A comprehensive physical examination was performed at the baseline and among those who fulfilled the dementia criteria during the follow-up screening. The physical examination included measurement of height and weight and an assessment of sensory impairment (vision and hearing). A picture-graded test, the Teller acuity card, was used to assess visual acuity and distraction[27], and simple speech and whisper tests were conducted to screen hearing ability. Cardiovascular screening involved an electrocardiogram and measurements of systolic and diastolic blood pressure on both arms and legs in a lying position. The neurological examination was aimed at establishing gross and fine motor impairments, mental status, and the presence of pathological or asymmetric reflexes, and neurological signs such as myoclonus, ataxia, and Parkinsonism.

Interview

Each participant was evaluated using a semi-structured interview with his or her primary carer, sometimes a family member, but generally a professional carer. The interviewee had to have known the participant for a minimum of 6 months. Smoking habits, alcohol use, diet, mobility and general mental functioning were recorded. This interview was supplemented with the Vineland Adaptive Behavior Scales[28], the Dementia Questionnaire for persons with an ID (DMR)[29, 30], a Dutch social competence rating scale for intellectually disabled people ("Sociale Redzaamheidsschaal") (SRZ))[31], and the Reisberg Functional Assessment Staging Tool[32].
To obtain information on past physical status and mental ability of the participant, first-line relatives were asked to provide information about the history of development. First-line relatives were also required to provide the family history of physical and psychiatric disorders.

Laboratory examination

The laboratory examination consisted of plasma screening for hematological, biochemical, and thyroid functions. DNA was isolated. Plasma and serum were stored at -80°C for future research.

Diagnosis of dementia

Baseline

At the baseline, the first-line relative, the primary carer and the general practitioner had to answer the question: has this patient been suffering from dementia for at least six months, yes or no? If only one interviewee did not respond affirmatively, the patient was not included as being a possible sufferer of prevalent dementia. For those who were included, the presence of dementia was subsequently determined by the research physician (A.C.), by means of a medical examination, according to a standardized protocol. Those participants who suffered from medical, psychiatric and neurological or laboratory conditions other than AD, potentially causing cognitive decline, were examined in detail to establish whether these conditions caused the deterioration. If this was the case, the participants were excluded from the group of demented persons.
The diagnosis is based on the ICD-10 Symptom Checklist for Mental Disorders[33], in particular, dementia in Alzheimer's disease[26] and the guidelines produced by an international consensus panel established under the auspices of the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID)[34, 35].

Follow up

All those participants who were demented (n = 85), as well as those who were not identified by interviewees as (possibly) being demented at the baseline, were monitored until they either died or censored (i.e. withdrawn from the study by their representatives, n = 3). The annual screening consisted of an extensive screening examination (DVZ, SRZ and clinical data). At present, there were 103 persons followed up for 1 year; 130 for 2 years; 160 for 3 years; 76 for 4 years; 30 for 5 years and 10 persons died within 1 year. Those who fulfilled the dementia criteria are followed more intensively at 6-month intervals. The examination for this group included a screening tool to assess functional, social and cognitive decline, as well as a clinical work-up, based on a medical examination to exclude other physical and psychiatric factors as the cause of decline. Finally, all patients were diagnosed according to the same protocol as the prevalent patients. All new diagno-

ses have been discussed in a diagnostic panel consisting of members of the reference group with expertise in the field of ID (A.M.W.C., G-J.V., F.E.V., H.M.E. and C.M.vD.).

Screening tools for the assessment of functional, social, and cognitive decline

Participants were followed longitudinally for clinical signs of dementia, using a number of screening tools. These included clinical data, the DMR, the social competence rating scale for intellectually disabled people (also known as SRZ), and the Reisberg Functional Assessment Staging Tool. The DMR[30] is a 50 item questionnaire, divided into two types of scores: cognitive and social. The cognitive scores relate to the following domains: short-term memory, long-term memory, and spatial and temporal orientation. The social scores relate to: speech, practical skills, mood, activity and interest, and behavioural disturbance. To screen for patients with dementia, we identified those with an increase in the cognitive score of $\geq$7 (SCS$\geq$7) and/or an increase in the social score of $\geq$5 (SOS$\geq$5). An increase in either or both scores was taken to indicate a possible deterioration in dementia (either deterioration in already established dementia or the first indication of dementia). The social competence rating scale for intellectually disabled people (SRZ)[31], which covers aspects such as: the ability to define and execute tasks for themselves, social skills, daily living skills and the effective use of language, was determined by 31 items (a maximum 4 per item). A decrease in the score of 25% was used to screen for those with a definitive decline.

Statistical analysis

Data were analysed using chi-square tests for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Logistic regression analysis was used to study the relation between mortality and age, sex and dementia status. Statistical significance was defined at a significance level of 5%.

RESULTS

Characteristics of the study population are presented in Table 1. The participants comprised 506 adults with DS, of whom 304 were men (60.1%) and 202 were women (39.9%). The mean age of the men was 51.9 years (standard deviation (SD) = 5.1, range: 45 to 77). The mean age of the women was 52.0 years (SD = 5.4, range: 45 to 77). Most of our participants, 319 (63%) lived in institutions or in homes connected with institutions, 11(2.2%) still lived with their parents, and 176 (34.8%) were living in (supervised) community units.

Table 1 General characteristics of the participants with and without dementia at baseline

		Dementia	
	All	absent	present
Population size	506	421((83.2%)	85(16.8%)
Gender			
Women	202	167(39.7%)	35(41.2%)
Men	304	254(60.3%)	50(58.8%)
Age (years)*	51.9(6.2)	51.4(5.0)	54.5(5.5)
Living			
Community	187	165(39.2%)	22(25.8%)
Institutionalised	319	256(60.8%)	63(74.2%)
Depression			
Past	115	84(19.9%)	31(36.4%)
Present	20	15(3.6%)	5(5.8%)
Antidepressive drug	46	30(7.1%)	16(18.8%)
Visual loss	277	224(53.2%)	53(62.3%)
Hearing loss	203	169(40.1%)	34(40%)
Use of psychotropic drug	76	51(12.1%)	25(29.4%)

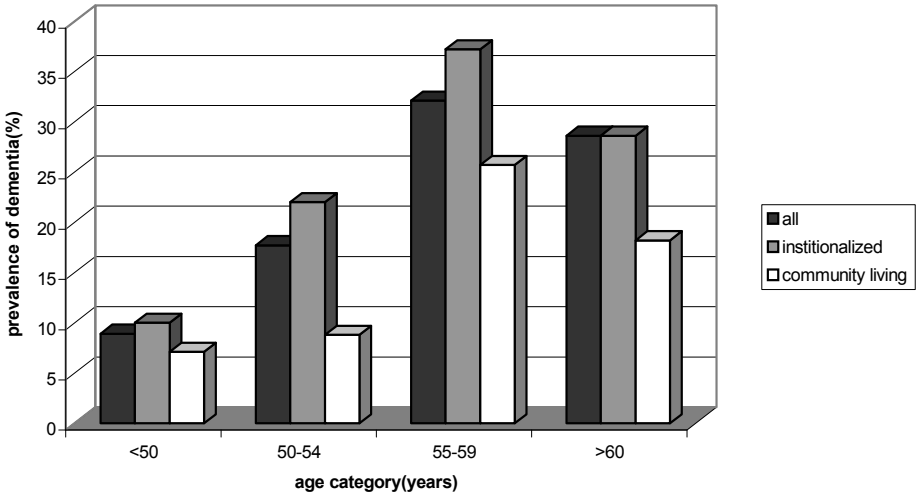
*values are means (standard deviation)

According to ICD-10 criteria, 150 of the participants (29.6%) had a severe to profound level of ID, 154 (30.4%) a moderate level, and 21 (4.2%) a mild level of ID. The severity of ID was not known for 181 participants (35.8%).

The overall prevalence of dementia was 16.8% (85 cases) (95% confidence interval (CI) = 16-23%). There was no significant difference between men (16.4%) and women (17.3%). The persons with dementia were significantly older, (ANOVA, F = 21.5, d.f. = 1, p<0.001), [n = 85, mean age 54.5 years ± 5.5 (SD)] than the non-demented [n = 421, mean age 51.4 years ± 5.0 (SD)]. There is no difference in the pre-morbid cognitive level between the persons with dementia and those without dementia. Persons with dementia were more often institutionalized (χ^2 = 5.37, d.f. = 1, p = 0.02), dementia may be a reason for institutionalization. The demented persons also were more frequently treated with anti-epileptic (χ^2 = 26.09, d.f. = 3, p<0.001) and anti-psychotic drugs (χ^2 = 19.34, d.f. = 3, p<0.001). Patients with depression and dementia were followed specially to exclude a misdiagnosis of dementia due to depression. There is a relation between a history of depression and dementia (χ^2 = 11.22, d.f. = 3, p = 0.01) and the use of anti-depressant drugs (χ^2 = 15.4, d.f. = 3, p = 0.002). Sensory problems were not associated with dementia in this study population.

Figure 1 shows that up to the age of 59 years, the prevalence of dementia doubled with each 5-year interval. Up to the age of 49, the prevalence is 8.9% (95% CI = 5-12%), from the age of 50-54, 17.7% (95%CI = 12-23%) and from the age of 55-59, it is 32.1% (95%CI = 22-42%). After the age of 60, we did not find a further increase in the prevalence of dementia 25.6% (95% CI = 12-40%).

Figure 1 Prevalence of dementia in 506 persons with Down's syndrome in the Netherlands by living situation



Up to the age of 59, the prevalence observed in this study is similar to that of the other studies. The study of Franceschi is an exception in that the prevalence of dementia is substantially higher at all ages. As can be seen in figure 2, the prevalence increases with age in all other studies. There are only two studies that included persons older than 65 year[19, 36]. In one (Visser et al.)[19], the prevalence increased to 100%, and in the other (Tyrell et al)[36], the prevalence peaks at 50%. In contrast with the findings of our study there is no levelling off after the age of 60 in these studies. However, other studies also show a deviation from the exponential increase in prevalence (Lai 1989, Schupf 1989, Haveman 1994), as can be seen in table 2 and figure 2.

There may be two explanations for the observed decrease in prevalence in our study. The incidence of dementia may decline in those aged 60 and above. Alternatively, the mortality rate may be increased in those with dementia after or before the age of 60 years. The possibility of a decrease in the incidence of dementia was evaluated by studying the incidence of dementia in each age group, per 100 person years, on the basis of follow-up screening of the non-demented group (followed up to 1 July 2005). The largest increase in incidence was seen in the age group of 60 and above (Table 3). The second possibility, an increased mortality in those with dementia, was studied using our follow-up data. After a follow-up with a mean time of 3.3 years (minimum 0.0; maximum 6.1), 81 (16%) participants had died, and 36 (44%) of these were diagnosed with dementia at the baseline. In a logistic regression analysis, mortality was significantly related to age and the dementia status ($p<0.001$). In all the age categories, through to 60, the mortality of those with dementia is more than twice that of those not suffering from dementia (figure 3).

Figure 2 literature overview of studies of the prevalence (%) of dementia

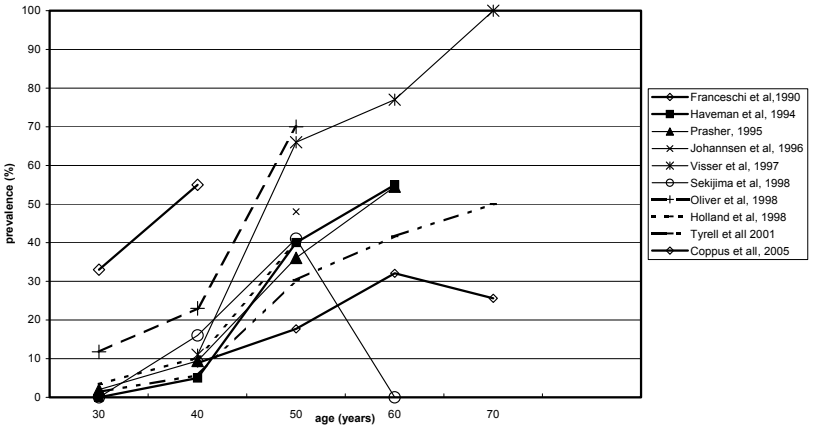


Table 2 Age-related prevalence of dementia in Down's syndrome

Authors	Sample	Residence	Criteria
Lai & Williams (1989)	53 DS	Institution	Functional decline
Schupf et al (1989)	99DS and controls	Institution Community	Regression
Franceschi, et al (1990)	50 DS	Community	NINCDS/ADRDA
Haveman et al (1994)	307 DS and 1274 controls	Institution Community	Diagnosis of dementia made by physician
Prasher (1995)	201 DS	Institution Community	DCR 10
Johannsen et al (1996)	72 DS	Community	Clinical criteria
Visser et al (1997)	307 DS	Institution	Clinical signs Cognitive performance EEG
Sekijima et al (1998)	106 DS	Institution	Clinical characteristics CT scanning
Oliver et al (1998)	57 DS	Community	Cognitive deterioration
Holland et al (1998)	75 DS	Community Institution	Camdex
Tyrell et al (2001)	285 DS	Community	DSMIV
This study	506 DS	Community	DSM IV ICD 10

CT, computed tomography; DCR, Diagnostic Criteria for Research; DSM, Diagnostic and Statistical Manual of Mental disorder; EEG, electroencephalogram; ICD, International Classification of Disease; NINCDS/ADRDA, National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association.

Figure 3 Mortality after 3.3 years (mean) of follow up in 506 persons with Down's syndrome in the Netherlands

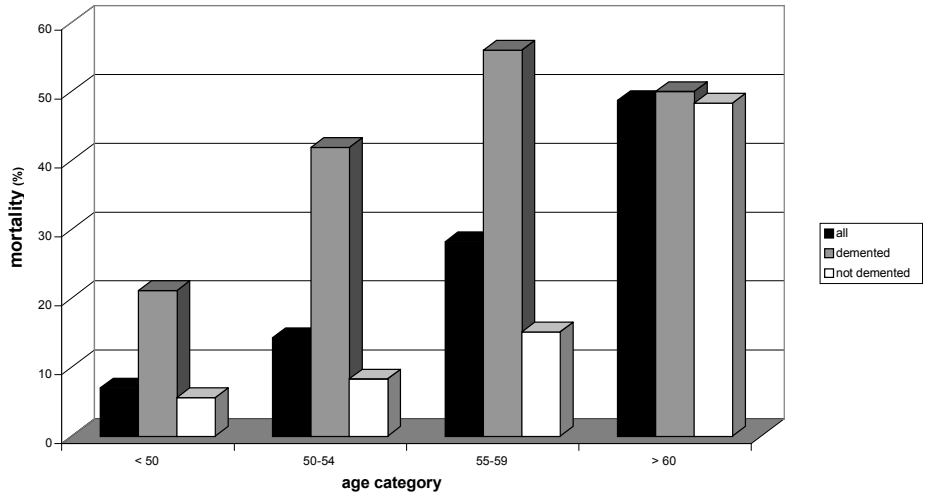


Table 3 Incidence of dementia per 100 person years in Down's syndrome

Age	Total follow-up years (01-07-2005)	Number incidence	Incidence per 100 Person years
< 50	709.39	18	2.53
50-54	495.79	14	2.82
55-59	163.73	8	4.88
>60	67.59	9	13.31

DISCUSSION

The prevalence of dementia in our population of persons with DS increased from 8.9% in the age category of 45-49 years to 32.1% in the age category of 55 to 59 years, but did not increase over the age of 60 years. In contrast, the incidence of dementia did increase, also after the age of 60 years. The incidence increased from 2.53 per 100 person years in the age category of 45-49 years, to 13.31 per 100 person years in the oldest age category.

Before interpreting the findings, an issue to be discussed is the validity of the diagnosis. Although there are now well-accepted criteria for the diagnosis of dementia in adults with ID[34, 35], which we have used, the diagnosis of dementia remains difficult, both in research setting and in clinical practice. For the present study, persons were systematically screened for dementia and examined in person. The demented patients in this study met the ICD-10 criteria and had an insidious and progressive course of the disease. Also, special attention was given to the exclusion criteria for

conditions such as depression, hypothyroid disease, infection, and visual and hearing loss. If there was no evidence from the history, physical examination, or special investigations for any other possible cause of dementia, the differential diagnosis of AD of the prevalent cases was made by a research physician (AMWC), with 15 years experience in the care of patients with ID.

Also related to the validity of the diagnosis is the problem that there is an overlap between the symptoms of depression and AD. Typical symptoms of depression in persons with DS, such as apathy and inactivity, loss of self-help skills and even loss of speech, can also be observed in AD[37, 38]. The overall reported prevalence rate of depression in DS varies widely from 2%[39] to 5%[40] to 11.3%[41]. In our study we found a prevalence of 22.7%, which concerned 115 DS persons. This history of depression is strongly associated with AD, as is also the use of anti-depressant drugs. The high prevalence of depression in our study and its association with dementia raises the question whether the demented patients with depression are misdiagnosed. However, we followed all patients with dementia for more than one year. It is extremely unlikely that patients with depression without a true dementia would not have improved during this period of follow-up. This makes it unlikely that misdiagnosis explains the high prevalence of depression in demented patients in our study.

Other factors that can contribute to the errors in the differential diagnosis in persons with DS include the high occurrence of sensory disabilities. We found a very high prevalence of visual and hearing problems. However, these were not associated with dementia after examination by the research physician.

Returning to the results of our study, we found there were 85 (16.8%, 95%CI = 16-23%) patients with dementia in a sample of 506 DS people. This figure is higher than the 4.4% reported by Devenny et al.[24] and the 13.3% by Tyrrell et al[36], but it is lower than the prevalence of 15-45% reported by Prasher et al.[42]. We found an increase in dementia cases up to the age of 59. These results were consistent with other population-based prevalence studies[10, 13, 17, 19, 20, 22, 23, 36, 43-45]. All studies showed an exponential increase in prevalence with age. There are important methodological differences that hamper direct comparison of studies. It is difficult to compare our study with those with only institutionalized persons as they may have been selected as a result of selection bias. Furthermore, the sample size of the previous listed studies is small. This concerns particularly the elderly DS persons. Despite these differences, most findings are similar, with one exception. In a community-based study of adult patients with mild ID, Franceschi et al.[13] reported very high prevalence rates, using the National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria. These are the criteria normally used in the general population, which may explain this study deviates from others and ours. The highest prevalence figures reported were found in the study of Visser et al.[19]. Including 307 institutionalized DS people; they found a prevalence of 100% in the age group of 70 years and

above. However, this finding is based on only two patients in this age category. Most studies included institutionalized people, like the study of Visser et al.[19] (see Table 2). Our population-based study clearly shows that the prevalence in those who are institutionalized is higher, suggesting hospitalized studies are susceptible to selection bias.

In our population-based study, we found that despite the exponential increase in prevalence with age, the prevalence of dementia decreases in the oldest group and did not exceed 28.6%. The observed decrease in prevalence cannot be explained by a decrease in incidence. In contrast, the largest increase in incidence of AD was seen in the age group of 60 and above (Table 3). Alternatively, differences in mortality between demented and non-demented persons with DS may explain the decrease in prevalence. When comparing the mortality between demented and non-demented persons, the largest differences were found before the age of 60, being not significant after 60. Therefore, our results suggest that the high mortality in persons with dementia, up to the age of 60, may explain partly our decrease in prevalence of dementia after the age of 60 years (see figure 3). However, other factors also may contribute to the low prevalence after this age, which remain to be unraveling. Also remarkable is the finding that after the age of 60, the mortality in those who are demented, as well as those who are not demented, at baseline, is almost the same. These results suggest, that somatic and physical pathology appears to become more important than dementia as a cause of death after the age of 60 years.

There is an ongoing debate on the percentage of DS persons that will eventually develop dementia in old age. Several studies, mostly neuropathological, suggested that all patients develop AD[7, 46-49]. Our study, including persons up to the age of 77, strongly argues against a full penetrance of AD. Further research is required to investigate the age-associated prevalence and incidence of dementia in persons with DS and the relation to morbidity and mortality. One of the major clinical implications of the fact that even with advanced age, not all persons with DS develop dementia, is the need to identify and treat reversible conditions. It will be interesting to see which factors predict the survival in this population-based study during a follow-up. Our study, among others, shows that dementia is a major problem in elderly persons with DS.

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Chapter 2.2

The impact of apolipoprotein E on dementia in persons with Down's syndrome

ABSTRACT

Apolipoprotein E (APOE) is consistently associated with dementia in the general population. Findings on the role of this gene in persons with Down's syndrome (DS) are inconclusive. We studied the effects of APOE on mortality and dementia in a longitudinal prospective study of a large population-based sample of persons with DS (n=425), demented and non-demented. There was evidence that APOEε4 is correlated with the rate of decline in the social competence rating scale (SRZ) (p=0.04). In our population we found overall a modest but not statistical significant effect on the prevalence of dementia: Odds Ratio (OR): 1.57, (95% CI = 0.87-2.82). We did observed a significant long-term effect on the incidence of dementia: Hazard Ratio (HR): 4.66, (95%CI = 1.35-16.14), but for those with a follow up less than 3 years the risk was not significantly increased: HR: 0.83, (95%CI = 0.35-1.94). When pooling our data in a meta-analysis, the APOEε4 allele shows a 1.59, (95%CI = 1.19-2.12) increase in risk of dementia in persons with DS. We conclude that APOE is influencing the risk of dementia in persons with DS.

INTRODUCTION

The increased risk of Alzheimer's disease (AD) in people with Down's syndrome (DS) is well known. In DS, AD is assumed to be caused by the triplication and over-expression of the gene for amyloid precursor protein (APP), located on chromosome 21 and leading to the accumulation of cerebral β-amyloid[1]. However, there is a wide variation in risks and age at onset of AD in persons with DS, suggesting there are other factors modifying the disease onset[2]. In the general population, the apolipoprotein E (apoE: lipoprotein; APOE: gene) on chromosome 19 modulates the risk of AD in some studies[3-5]. The ε4 allele of APOE is associated with earlier age at the onset and increased risk of AD. On the contrary the APOE ε2 allele may reduce the risk of dementia in heterozygous carriers.

In persons with DS, a large variation of the APOE allele frequencies has been found[6-8], most likely reflecting the different geographic origins. The relation between APOE ε4 and the risk and onset of AD has been controversial. Some studies showed an increased risk of AD associated with the APOE ε4 allele in persons with DS[2, 9-12], but others could not confirm this[7, 8, 13]. There is evidence that APOE ε2 is associated with increased longevity in some studies of DS[10, 14, 15], but a protective effect on AD[15-17] could not be confirmed by all[9]. The APOE ε4 allele has been associated further with early mortality[18], but a meta-analysis based on data from 538 persons with DS showed no significant evidence for an increased mortality of APOE ε4 homozygotes with DS[19]. From a biochemical perspective, one may assume that APOE ε4 is involved in dementia through the amyloid pathway[20, 21]. APOE ε4 may interact with the amyloid-β (Aβ) peptide to increase Aβ aggregation. However, one also may assume that having three copies of APP may overwhelm the effect of the APOE ε4 on Aβ metabolism, making the APOE genotype less relevant for persons with DS in comparison to the general population.

The aims of the present study are to investigate the effect of the APOE ε4 allele on cognitive and functional decline and the risk of developing dementia in people with DS.

We also compared our findings with those of the existing literature, focusing on the effect in elderly DS persons.

METHODS

Study population

Participants are persons with DS, aged 45 years and older, who were enrolled from 1 December 1999 until 1 December 2003 in a community-based study on DS and ageing in the Netherlands.

The sampling procedures for this study have been described in detail elsewhere[22]. In brief, we studied 506 people with DS of whom 304 were men (60.1%). For this study the diagnosis of DS was re-evaluated based on clinical characteristics according to the criteria described by Roizen et al[23]. In 283 cases (56%) the clinical diagnosis was confirmed by available cytogenetic characterisation. Of these persons 271 (53.6%) had complete trisomy, 5 (1%) had a translocation of chromosome 21 and 7 (1.4%) had a mosaic form. Results of (premorbid) intelligence tests and previous level of functioning were studied by reviewing medical records. All participants received a complete assessment at the baseline. Blood was taken from the participants, when possible. Plasma and serum were stored at -80°C and DNA was isolated. In the event of death, permission to perform an autopsy on the brain was requested.
Ethical approval for this study was obtained from the medical ethical committee of the Erasmus Medical Center and the ethical committees consisting of relatives of DS persons residing in local institutions and organisations. Written informed consent to participate, and to provide blood samples, was obtained from legal representatives (relatives and/or carers), after written information was provided. Written consent was also obtained from persons with DS who had the mental capacity to consent.

Clinical assessments

The persons in our study were assessed for Alzheimer's disease using the ICD-10[24] criteria and according to the guidelines produced by an international consensus panel established under the auspices of the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID)[25]. The diagnosis of dementia was supported using two observer-rated questionnaires, namely the Dementia Questionnaire for persons with an intellectual disability (DMR)[26, 27] and the Social Competence Rating Scale for persons with an intellectual disability (SRZ)[28]. Both questionnaires were used as screening instruments, to support a possible diagnosis of dementia, through detecting a decrease in function over time, as recommended by the Ageing Special Interest Group in 1997 (IASSID)[25].
The Dementia Questionnaire for persons with an intellectual disability (DMR) is a 50-item questionnaire, separated into two types of scores: those related to cognitive function and those related to social skills. The cognitive scores relate to the following domains: short-term memory, long-term memory, and spatial and temporal orientation. The social scores relate to: speech, practical skills, mood, activity and interest, and behavioural disturbance. To screen for persons with dementia, we used a cut off, a worsening of cognitive scores of ≥ 7 points and /or a worsening of social scores of ≥ 5 points to ascertain suspect patients. The SRZ, the social competence rating scale for persons with an intellectual disability[28], is a Dutch rating scale which covers aspects as social skills, daily

living skills and the effective use of language. A decrease in score of 10% and higher was used to screen for those who were suspect of developing dementia.

Baseline

At the baseline, the first line relative, the primary carer, and the general practitioner had to answer the question: "Has this patient been suffering from dementia for at least six months?" If this diagnosis was also confirmed by the research physician (A.M.W.C.), by means of a medical examination, according to a standardized protocol[22] based on ICD10 criteria, these patients were included in the group of demented persons at the baseline, as the prevalent cases.
The demented, as well as the non-demented, received a complete assessment at baseline that included interviews with relatives, carers and the general practitioner. It also included a general physical and neurological examination. The medical reports were reviewed to examine past or present disorders and the possible use of drugs.

Follow up

Participants that were not demented at baseline were screened annually. Those who fulfilled the dementia criteria, at baseline or during follow-up, were followed more intensively at 6-month intervals. All new diagnoses were based on screening tools to assess functional, social and cognitive decline, as well as on a clinical work up, which was based on a medical examination to exclude other physical and psychiatric factors as the possible cause of decline. The screening tools used are the DMR, the SRZ, an interview with the primary carer and the general practitioner. All new diagnoses were discussed in a diagnostic panel consisting of members of the reference group with expertise in the field of intellectual disability (authors A.M.W.C., G-J.V., F.E.V. and H.M.E.).

APOE genotyping

APOE genotypes were determined on 5ng/µl dry DNA samples using Taqman allelic discrimination technology[29] on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA). APOE genotyping was obtained blind to all clinical information.

Meta-analysis

For the meta-analysis of the APOE allele frequency in DS persons, with and without dementia, we performed a computer search in PUB-MED for the period 1995-up to, and including July, 2005

using 'APOE' and 'Down's syndrome' as search terms. We only included studies if the diagnostic criteria for AD were well described and met our criteria, and if the data presentation also made it possible to calculate the APOE allele frequencies for the demented as well as the non-demented persons. We try to include studies with a population age comparable with our study population. Our population has a relatively "old age" with subjects 45 years and older and with a mean age of 51.6 years. To broaden the number of studies available for analysis we included studies with a mean age of persons with DS of at least 40 years.

Statistics

Data were analysed using chi-square tests for categorical variables and one-way analysis of variance (ANOVA) for continuous variables, with Bonferroni correction for multiple testing, to detect if there was a difference in mean scores between the groups according to their level of intellectual disability. The independent *T*-test was used to compare the mean decrease in SRZ scores between the different genotypes. Odds ratios (OR) were using the ε3ε3 genotype as reference, with a 95% Confidence Interval (CI). Multiple logistic regression analysis was carried out to estimate the relative influence of risk factors as age, gender, level of intellectual disability and APOE on the development of AD. The Cox proportional hazards model was used for survival analysis, using age as the underlying time of the model. The analyses were done using the "counting" option implemented in the survival analysis method included in the S-Plus package (S-Plus, version 6.0). In the meta-analysis we used the Mantel Haenszel procedure, random effects model, (RevMan V. 4.2) to compute the odds-ratios.

RESULTS

Characteristics

From the 506 participants with DS, the APOE genotype was determined in 425 persons (84%), who consented to give blood. From the 425 persons 267 were men (62.8%). The mean age of men was 51.6 years (range 45-67), and for women 51.7 years (range 45-77). Severity of intellectual disability was classified using the International Classification of Diseases, 42.6% had a severe level (IQ<35); 50.0% had a moderate level (IQ 35-50) and 7.4% had a mild level of intellectual disability (IQ 50-70).

Of the total study population, 67 persons (15.8%) were diagnosed as having dementia at the baseline and 60 persons died during follow-up (reference date 1 December 2005). After a mean time of

Table 1 Baseline characteristics by APOE carrier ship

Age (years)	number	gender men/ women	ε4 carriers	ε4 noncarriers	ε2 carriers	ε2 noncarriers
< 50	189	119/70	52 (44.8)	137 (44.3)	35 (41.7)	154 (45.2)
50-54	147	91/56	41 (35.3)	106 (34.3)	33 (39.3)	114 (33.4)
55-59	63	43/20	20 (17.2)	43 (13.9)	12 (14.3)	51 (15.0)
> 60*	26	14/12	3 (2.6)	23 (7.4)	4 (4.8)	22 (6.5)
All	425	267/158	116	309	84	341

Values are n (%)

* p = 0.04

3.68 years of follow-up (minimum 0.08; maximum 6.48) there are 405 persons followed up for at least 1 year, 301 for 3 years, 182 for 4 years, and 8 for 6 years or more.

APOE allele frequencies were comparable with the general Dutch population[30]: ε2ε2 n = 7(1.6%), ε2ε3 n = 59 (13.9%), ε2ε4 n = 18 (4.2%), ε3ε3 n = 243 (57.2%), ε3ε4 n = 88 (20.7%), ε4ε4 n = 10 (2.4%). APOE genotypes and allele proportions were in Hardy Weinberg equilibrium. There is no significant difference in the mean follow-up time between the different allele groups: F (5) = 0.30, p = 0.91. Differences in allele frequency in ethnic groups may lead to spurious findings in genetic studies[31]. We have only two persons from non-Caucasian origin included in the study, both with ε3ε3 genotype, which makes it unlikely they will lead to biased findings.

Table 1 shows the proportion of APOE ε4 and APOE ε2 carriers by age and sex. The proportion of APOEε4 carriers decreased significantly (χ^2, p = 0.04) after the age of 60 years, suggesting that APOE ε4 carriers have an increased risk of mortality. We did not observe a significant difference by age category for the APOEε2 carriers. We also did not observe a significant difference when stratifying by gender.

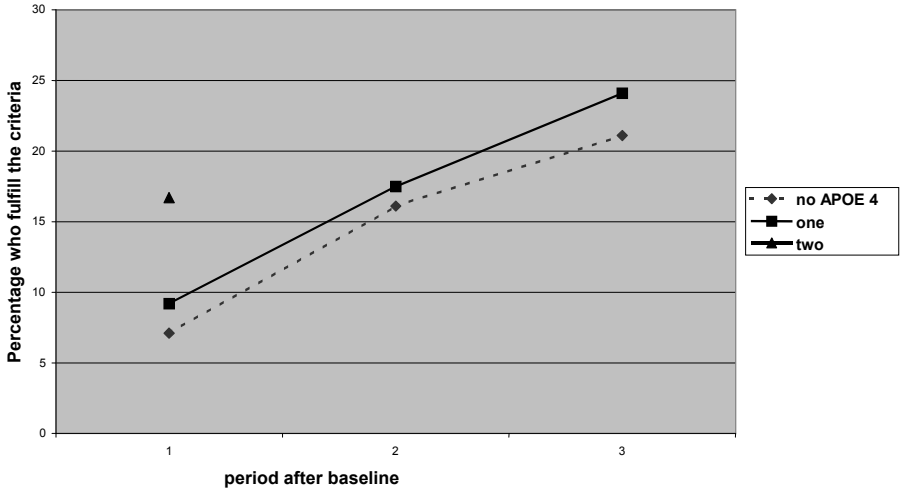
There was no significant difference in the mean age at the baseline as a function of the APOEε4 status: F (1) = 0.72, p = 0.39. Although the mean age at death for the APOEε4 carriers (55.8, SD 4.2) was younger than the mean age for the non-carriers (58.7, SD 6.4), the difference was not significant: F (1) = 3.02, p = 0.09. During the follow- up, there was no difference in mortality between the APOEε2 carriers and non–carriers: F (1) = 0.35, p = 0.55.

The association between APOE and cognitive and functional decline

We studied whether APOEε4 predicts cognitive and functional decline in the non-demented participants of the study (n = 358). We analysed the proportion of persons who met the screening criteria, suspect for dementia, for DMR and SRZ, after one, two, and three assessments separately, after the baseline measurement. The mean follow-up time is 3.68 years (SD 1.36). Not all participants take part in all assessments at the reference date; December 1, 2005.

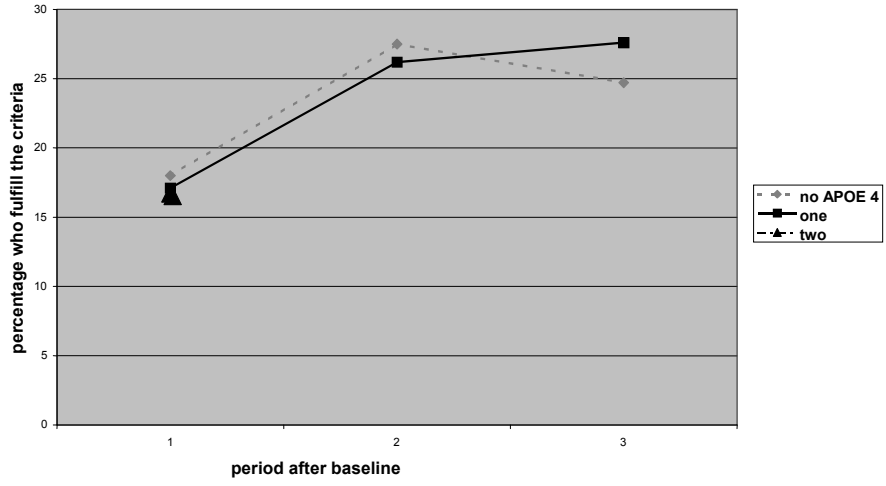
As expected, the proportion of persons who met the criteria increased with time. For the DMR, the risk of meeting the screening criteria, as mentioned before, was not significantly related to the presence of the APOEε4 allele (figure 1a and 1b), neither according to the cognitive scores (SCS) nor according to the social scores (SOS). There was only one person, with two ε4 alleles, who met

Figure 1a Decline according to the DMR/SCS criteria over time, by APOEε4 genotype



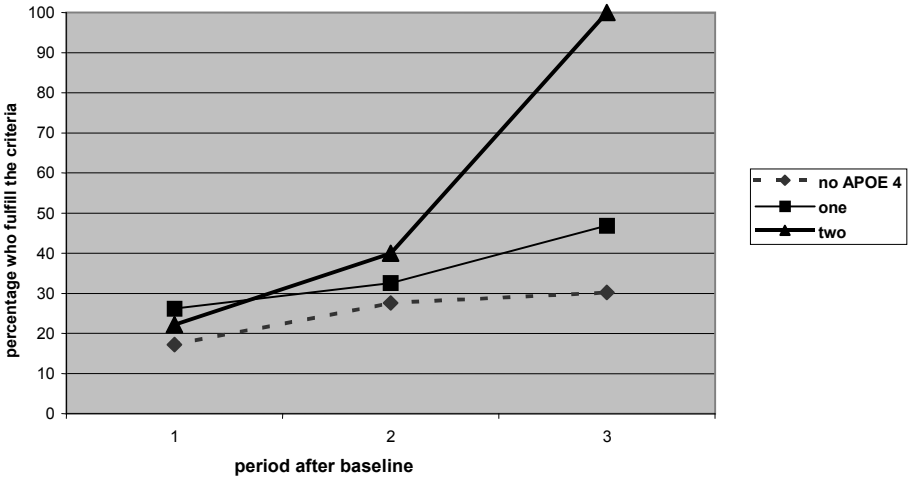
DMR: The Dementia Questionnaire for persons with an intellectual disability
SCS: sum of cognitive scores

Figure 1b Decline according to the DMR/SOS criteria over time, by APOEε4 genotype



DMR: The Dementia Questionnaire for persons with an intellectual disability
SOS: sum of social scores

Figure 2 Decline according to the SRZ criteria over time, by APOEε4 genotype



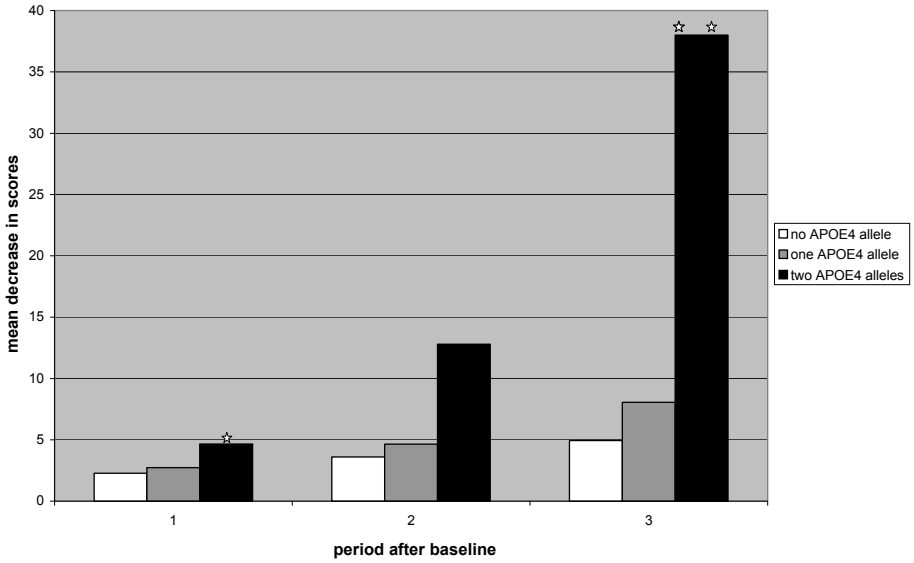
SRZ: The social competence rating scale for persons with an intellectual disability

the criteria, suspected of having dementia, after one assessment. This person passed away before the second assessment.

The SRZ scores show a strong correlation to the presence of the APOEε4 allele (see figure 2). After three assessment periods, following baseline, all the homozygotes (n = 7) for the ε4 allele met the screening criteria for dementia. We compared further the mean decrease in SRZ scores through the different assessments. Figure 3 shows that the decrease in mean scores increased overtime. Those homozygous for APOE ε4 had a significantly higher mean decrease than no carriers at the first and third assessment after baseline. The second assessment showed the same trend, though not statistically significant.

According to their level of premorbid intellectual disability, we divided our population in three different groups; mild, moderate and severe disabled. We used ANOVA, with Bonferroni correction for multiple testing, to detect, if there was a difference in the mean decrease in scores in SRZ and mean increase in scores in DMR after the first, second and third assessment period. We found no significant difference in mean decrease/increase between the three groups, according to their level of disability, after the different assessment periods. By interviewing the general practitioner we examine the possible use of drugs. None of our participants was using acetylcholinesterases or other pharmacological agents that may modify the SRZ or the DMR.

Figure 3 Mean decrease in SRZ scores over time by APOEε4 genotype



* Independent t-test p = 0.04

** Independent t-test p<0.0001

SRZ: The social competence rating scale for persons with an intellectual disability

APOE and the risk of dementia

We determined the odds ratios of being demented or not at the baseline, by the APOE genotypes with the ε3/ε3 as reference (see table 2). There was an increase in the odds ratios in the presence of one or two APOEε4 alleles, but the odds ratio was not significant, OR:1.57, (95% CI = 0.87-2.82). There was no detectable association in our study population between APOEε2 and AD.

Table 2 Effect of the APOE genotype on the prevalence of dementia

Genotype	demented	nondemented[1]	Odds Ratio[2]	95% Confidence Interval
ε2ε2	1	6	1.06	0.12-9.09
ε2ε3	10	49	1.29	0.59-2.81
ε2ε4	3	15	1.27	0.35-4.63
ε4ε3	18	70	1.63	0.86-3.08
ε4ε4	2	8	1.59	0.32-7.82
ε3ε3	33	210	reference	
ε4 present versus ε3ε3	23	93	1.57	0.87-2.82

[1] numbers demented/non-dement at baseline

[2] odds ratio against the reference ε3ε3

Table 3 APOE genotype and Alzheimer's disease in persons with Down's syndrome: a meta-analysis

		persons with DS with dementia allele frequency n (%)				persons with DS without dementia allele frequency n (%)			
Study	age mean(SD)	total alleles	ε2	ε3	ε4	total alleles	ε2	ε3	ε4
van Gool et al (1995)	51.9(6.2)	52	3 (5.8)	42(80.8)	7 (13.4)	52	6 (11.0)	41 (78.8)	5 (9.6)
Schupf et al (1996)	± 51.5	26	0	18(69.2)	8(30.8)	138	12(8.7)	109(79.0)	17(12.3)
Lambert et al (1996)	45.2(7.7)	16	1(6.2)	13(81.3)	2(12.5)	54	7(13)	40(74)	7(13)
Prasher et al (1997)	45.9(12.0)	34	4(11.8)	28(82.3)	2(5.9)	166	11(6.6)	135(81.3)	20(12.1)
Sekijima et al (1998)	42.3(6.8)	32	2(6.3)	24(74.9)	6(18.8)	174	4(2.3)	158(90.8)	12(6.9)
Tyrrell et al (1998)	47.1(8.2)	62	0(0)	51(82.3)	11(17.7)	120	10(8.3)	97(80.8)	13(10.9)
Lai et al (1999)	± 54	114	5(4)	89(78.0)	20(18.0)	86	11(13)	64(74)	11(13)
Rubinsztein et al (1999)	± 41.8	40	1(2.5)	32(80.0)	7(17.5)	50	3(6)	40(80)	7(14)
Deb et al (2000)	51(7.8)	48	0(0)	40(83.0)	8(17.0)	66	3(4.5)	57(86.5)	6(9.0)
Coppus et al (2006)	51.9(6.2)	134	15(11.2)	94(70.1)	25(18.7)	716	76(10.6)	539(75.3)	101(14.1)
Total		558	31(5.6)	431(77.2)	96(17.2)	1622	143(8.8)	1280(78.9)	199(12.3)

Weighted odds ratio (RevMan 4.2) random effects model:
APOEε4 versus rest: OR = 1.59,(95% CI = 1.19-2.12)
APOEε2 versus rest: OR = 0.67,(95% CI = 0.37-1.22)

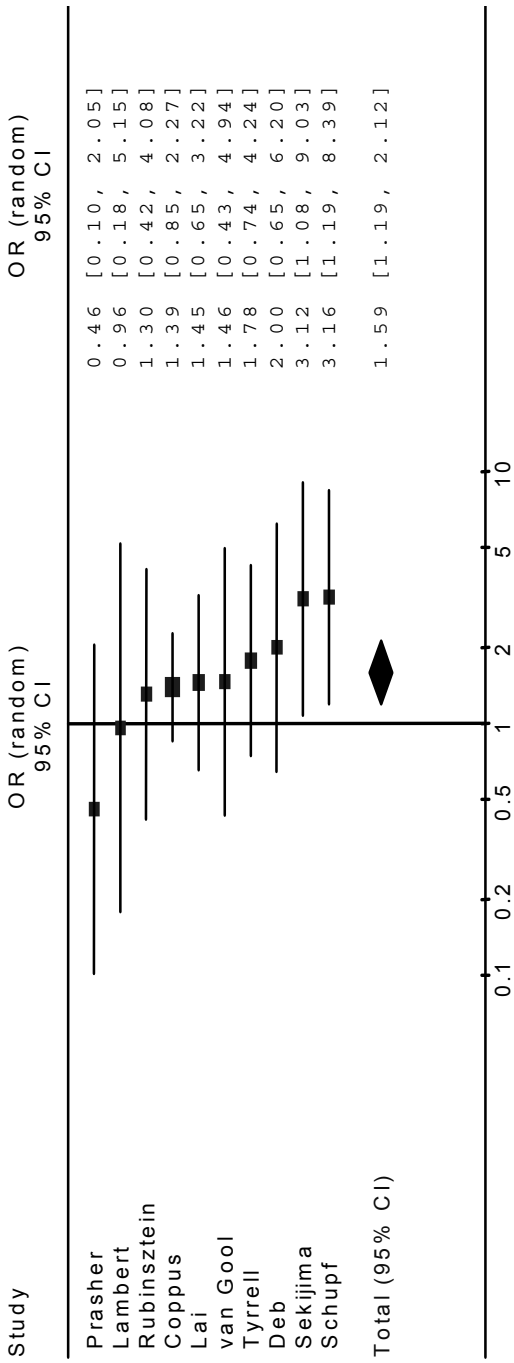


Figure 4 Meta-analysis total

We also studied the risk of developing AD for persons with DS who were not demented at the baseline. After an average of 3.68 years of follow up, 53 persons were detected as new cases of dementia (according to the criteria mentioned previously). For the initially non-demented APOEε4 carriers, the risk of developing dementia, after a follow up time of at least 3 years, was significantly increased: HR: 4.66, (95%CI = 1.35-16.14) adjusted for age at entry, sex and level of intellectual disability. For those with a follow-up less than 3 years the risk was not significantly increased: HR: 0.83, (95%CI = 0.35-1.94).

The effect of the APOEε4 allele on the age of onset was determined using the Cox Proportional Hazard model with age as the underlying time. There was no significant effect in our study population of APOEε4 on the age at onset of AD: HR: 1.09, (95%CI = 0.56-2.12). Although the mean age of onset of AD among the APOE ε4 carriers was younger, 54.9 years (SD 3.6), compared to the non-carriers, 56.1 years (SD 5.5), this difference was not statistically significant.

Finally we added our data to the previous studies[7, 9-11, 15-17, 32, 33], see table 3 and figure 4. This was only possible for the prevalent patients as all previous studies were based on prevalence. The meta-analysis showed a statistically significant increase of the APOE ε4 allele in persons with DS and dementia compared with those without dementia. The odds ratio of APOE ε4 was: OR: 1.59, (95% CI = 1.19-2.12) using a random effects model. Our meta-analysis did not show a significant decrease of the APOE ε2 allele frequencies in the demented persons compared with the non-demented persons. The odds ratio of APOE ε2 was: OR: 0.67, (95%CI = 0.37-1.22).

We divided the meta-analysis in a " younger group" e.g. younger than 50 years: (Prasher[22] , Lambert[32], Rubinstein[17], Tyrrell[15] and Sekijima[33]) and in an "older age group" (Coppus[34], Lai[16], van Gool[7], Deb[9], and Schupf[11]). The effect of APOE ε4 in the younger age group was not significant: OR: 1.49, (95%CI = 0.84-2.65). Only in the older age group was there a significant relation between the presence of an APOE ε4 allele and dementia (OR: 1.62, 95%CI = 1.15-2.29).

DISCUSSION

This is the largest study of APOE genotype and dementia in persons with DS that combines both cross-sectional and prospective data. When combining our cross-sectional data with those of other studies in a meta-analysis, we found APOEε4 was significantly associated to the prevalence of dementia in persons with DS, while our own prospective data showed an increase in incidence of dementia in APOE ε4 carriers when following participants more than 3 years.

We found no robust effect of APOE ε4 on mortality in our study, although the allele frequency of APOEε4 decreased significantly after the age of 60 years. Since dementia is the major cause of mortality in persons with DS aged 45 years and older, an effect of APOEε4 on dementia is in

line with the reduction of APOEε4 allele frequencies after the age of 60 years. A problem of the interpretation of the mortality data in the current phase of the study is that the analysis is based on only 60 deaths and therewith suffers from a low statistical power. It remains to be determined in the future whether APOEε4 predicts mortality after a longer follow-up.

There was significant evidence of an APOE ε4 effect on the rate of decline of social, and daily living skills in our study. In particular DS persons who were homozygous for APOEε4 showed a higher decrease in function when assessing decline using the SRZ, independent of their pre-morbid level of functioning. The SRZ appears to be a more sensitive screening instrument to pick up differences than the DMR. The latter instrument did not show this trend in cognitive function (DMR/SCS), nor did it do so in the scores related to the social skills (DMR/SOS). Both instruments are informant-based questionnaires, which may be susceptible to recall bias. Although this could be a potential limitation for the DMR, this problem also concerns the SRZ. Since the SRZ appears to be useful in this study population this explanation seems less likely. Moreover, the errors in the questionnaire are expected to be independent of the APOE genotype of the person with DS.

For the initially non-demented APOEε4 carriers, the risk for developing dementia during the follow-up is not significantly related to APOE ε4 when considering a short follow up. The relative risk estimate as the hazard ratio of developing AD within 3 years after inclusion was: 0.83, (95%CI = 0.35-1.94). However in the long term the risk of AD was significantly increased: HR: 4.66, (95%CI = 1.35-16.14).

We found no significant evidence for an increased risk on the prevalence of dementia in our population. After pooling our prevalent patients with those of others, APOE ε4 was associated with a significant increased risk of AD in persons with DS: OR: 1.59, (95%CI = 1.19-2.12). When analysing our prevalent cases, the relative risk of 1.57, we found in our study, is extremely close to that we see in the meta analysis suggesting that the significant, 1.59-fold increase, in risk found, is the most reliable Bayesian estimate and that the absence of a significant association in our study may be due to power limitation.

Our study is the largest study of elderly persons with DS, who are examined clinically in person. The advantage of this single centre study is that we are able to screen our population for newly diagnosed patients annually. An important strength of the study is that all these patients were seen by the research physician making differences in diagnostic criteria across patients unlikely. We did not find an effect of APOEε4 on the age of onset of AD in our study population. This result may be influenced by the limited number of persons (53) who develop dementia during follow-up, which yields only a low statistical power. Schupf et al[11] found that the APOEε4 allele was associated with an earlier onset of dementia using survival analysis of a series of 13 persons with DS who developed dementia. The age at onset of dementia was not affected by the APOE genotype in a survival analysis done in a study including 49 cases by Margallo-Lana et al[21].

The findings of our meta analysis, show evidence that APOE ε4 determined the prevalence of AD, in accordance with the results of Prasher et al[10], but in contrast with the findings of Deb et al[9]. Using in the meta-analysis the same studies as Deb et al. did, supplied with our own study we found also evidence for an association: OR: 1.83, (95%CI = 1.23-2.72), suggesting that the findings of our meta-analysis and those of Prasher et al[10] are statistically robust. When dividing the meta-analysis in a 'younger' and 'older' age group, the effect of APOE ε4 only remains significant in the older persons.

In summary, our population based study and meta analyses show that the effect of APOE ε4, on the prevalence of dementia in persons with DS, is 1.6 times increased. After three years of follow-up there also was a strong, fourfold, increase in risk of dementia. Although we could not have the power to show an effect on mortality, the reduction in APOE ε4 frequency in persons with DS after the age of 60 years suggest there is an effect. This increased mortality may explain the difference between the odds ratio in prevalent patients and the risk in newly diagnosed ones. Due to these findings we can suggest that APOE ε4 is important in Aβ aggregation in persons with DS.

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Chapter 2.3

Survival in Elderly Persons with Down's Syndrome

ABSTRACT

The longer life expectancy now experienced by persons with Down's syndrome (DS) makes it necessary to know the factors influencing survival in older persons with this syndrome. In a prospective longitudinal cohort study of dementia and mortality, 506 persons with DS aged 45 and older were followed for a mean of 4.5 years (range 0.0-7.6 years). Cognitive and social functioning were tested at baseline and annual follow-up. The diagnosis of dementia was determined according to a standardized protocol. Cox proportional hazards modeling were used for survival analysis.
Relative preservation of cognitive and functional ability is associated with better survival in this study population. Clinically, the most important disorders in persons with DS that are related to mortality are dementia, mobility restrictions, visual impairment, and epilepsy but not cardiovascular diseases. Also level of intellectual disability and institutionalization are associated with mortality.

INTRODUCTION

In recent decades there has been a distinct trend toward longer survival in persons with Down's syndrome (DS)[1], increasing from 9 years in 1929 to 12 in 1949 and 49 years in 1997[2]. In developed countries recent estimates indicate a mean age of death of older than 50[3-7]. The change in mortality pattern raises the question of what the most important determinants of mortality are.

Despite major progress, persons with DS still show greater mortality rates early in life, as well as in later stages of life[2], suggesting that there still may be differences from the general population. Knowledge of factors influencing survival in older persons with DS is limited. Nearly 20% of the persons with DS aged 45 years and older suffer from dementia[8], and several studies have shown a greater risk of mortality for persons with DS and dementia or cognitive decline[9-11]. Cognitive decline has been shown to be associated with mortality in elderly people with and without dementia with and without DS[12, 13]. In addition to age, other factors that have been suggested might influence mortality risk in DS are the gene encoding for apolipoprotein E (APOE)[12, 14, 15] and co-morbidity[3].

The objective of this study was to assess the effect of cognitive and functional decline and physical comorbidity on mortality risk over time in a population-based cohort of persons with DS aged 45 and older.

METHODS

Study population

The design of this study, a prospective longitudinal study on dementia and mortality in persons with DS, has been described in detail elsewhere[16]. Briefly, 506 persons with DS, aged 45 years and older, were enrolled in the study from December 1, 1999 to December 1, 2003. All participants were monitored annually until they died (n = 109) or their representatives withdrew them from the study (n = 7), up to the reference date of January 1, 2007. At the time of study entry, each person received a complete assessment including interviews with relatives, caregivers, and their general practitioner. The medical records were reviewed to examine past or present disorders (e.g., cardiovascular risk factors, epilepsy and depression), mobility, and the possible use of drugs. All persons obtained a general physical and neurological examination and, if compliant, a venapuncture. The same questionnaires and interviews were used annually from 1999-2007.

Pre-morbid severity of intellectual disability, obtained from the medical records, was classified using the International Classification of Diseases, Tenth Revision (ICD-10)[17]. According to these criteria, 192 (38.5%) of the participants had a severe to profound level of intellectual disability,

and 250 (50.1%) had a moderate to mild level of intellectual disability. The severity of intellectual disability was not known for 57 (11.4%) participants.
The diagnosis of dementia was based on the ICD-10 Symptom Checklist for Mental Disorders, in particular, dementia and Alzheimer's disease[17], and according to the guidelines produced by an international consensus panel established under the auspices of the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID)[18]. Vital status was obtained using follow-up interviews and written correspondence. Information on causes of death was obtained from relatives or the primary caregivers of the deceased and was augmented with information from medical reports or autopsy records.
The medical ethical committee of the Erasmus Medical Center and the ethical committees of the local organizations approved the study protocol. Written informed consent to participate, and to provide blood samples, was obtained from legal representatives.
Written consent was also obtained from persons with DS who had the mental capacity to consent.

Diagnosis of dementia

Baseline

At baseline, the first line relative, the primary caregiver, and the general practitioner were asked to answer the question: "Has this patient been suffering from dementia for at least 6 months?" The research physician (A.M.W.C.) confirmed the diagnosis according to a standardized protocol, including a medical checklist and a physical examination. Participants fulfilling the criteria were included in the group of persons with dementia at the baseline, as prevalent cases.

Follow-up

Participants who did not have dementia at baseline were screened annually. Those who fulfilled the dementia criteria at baseline or during follow-up were followed more intensively at 6-month intervals. The diagnosis of dementia was supported using two observer-rated questionnaires, the Dementia Questionnaire for persons with an Intellectual Disability (DMR)[19, 20] and the Social Competence Rating Scale for persons with an Intellectual Disability (SRZ)[21]. The DMR is a 50-item questionnaire with two types of scores: those related to cognitive function (DMR/SCS; score range 0-44) and those related to social scores (DMR/SOS; score range 0-56). Higher DMR scores represent a higher frequency of behaviors that are considered to be indicative of dementia. The SRZ is a 31-item questionnaire (score range 0-124) and covers aspects such as social skills, daily living skills, and the effective use of language. Higher SRZ scores indicate better functioning. Diagnoses

of dementia were based on a clinical examination by the research physician (A.M.W.C.). Diagnostic examination was performed in patients who had a worsening of 7 points or more on the DMR/SCS scale, a worsening of 5 points or more on the DMR/SOS scale, or a 10% decrease of the SRZ score. All new diagnoses were discussed in a panel consisting of members of the reference group with expertise in the field of intellectual disability (A.M.W.C., G-J.V, F.E.V and H.M.E.). A final diagnosis was based on consensus of the panel.

Clinical assessments

The history of past or current disorders was ascertained using medical record review. Body Mass Index (BMI) was computed as weight in kilograms divided by height in square meters (kg/m^2). Visual impairment was assessed using a picture-graded test (the Teller acuity card) and hearing ability using a simple speech and whisper test. Screening for mobility restrictions took place during the personal physical examinations, using a standardized protocol[16].

APOE

Blood samples for determination of APOE were collected from participants who provided consent (n=425). APOE genotypes were determined using the Taqman allelic discrimination technology. APOE genotyping was obtained blind to all clinical information and coded dichotomously for analysis. Participant had one or two APOEε4 alleles or no APOEε4 alleles. APOE allele frequencies were comparable with the general Dutch population[15].

Statistics

The risk of mortality according to participant characteristics and clinical diagnoses was investigated using Cox proportional hazards models, adjusting for age at baseline as a covariate. Follow-up time was defined according to date at entry and date at death or January 1, 2007. The risks of mortality between groups were compared by estimating hazard ratios in the Cox models. Survival analyses were performed in persons for whom complete data were available (75.8%).

The relationship between rate of decline in DMR and SRZ and risk of mortality was investigated in persons without dementia using a mixed-model repeated-measures procedure[22]. This procedure takes into account that the same individual contributes information at different time points and allows the inclusion of persons with incomplete follow-up. Decline in functioning was investigated in patients with at least 5 years of follow-up (n = 483), totaling five measurements of cognitive, functional and social ability. Participants were categorized into three groups, according to their

DMR and SRZ scores at baseline. Mean DMR and SRZ scores were compared between tertiles and between survivors and decedents.

To further investigate the effect of age and birth cohort effects, the study population was divided into two birth cohorts based on the participant's age at the reference date. The birth cohorts distinguished patients who were aged 60 and older (born before 1947; n = 118) and those who were younger than 60 (born after 1947; n = 381). There were more persons with a profound or severe intellectual disability in those born before 1947 (31.3% versus 17.6%, p = 0.01). The cohorts did not differ according to sex or living situation. Follow-up time was defined according to date at entry into the study and date at death or January 1, 2007 as exit.

To establish whether there was a relationship between the presence of an APOEε4 allele and survival, the Cox models were additionally adjusted for sex, pre-morbid severity of intellectual disability, and living situation. To investigate whether the relationship between APOEε4 and survival was related to dementia status or birth cohort, the Cox analyses were repeated in subgroups stratified according to dementia status and in subgroups stratified according to birth cohort. In persons without dementia, the time to event variable was time since entry into the study, until incidence of dementia, until death or the reference date. Also in persons with dementia, the time to event variable was time since entry into the study, although in the incident persons with dementia, it was the time since onset of dementia until death or the reference date.

Finally, the Cox analysis was repeated including all covariates found to be related to mortality risk. To exclude that inclusion of patients in the final stages of dementia explained the relationship between poorer mobility and mortality, the analysis was repeated without persons known to be in the terminal phase of dementia[23].

RESULTS

A total of 506 persons with DS were studied, of whom 304 were men (60.1%). At baseline the mean age was 51.9 for men (range 45-70) and 52.0 for women (range 45-77). Seven persons, or their relatives, refused to participate in the study during the follow-up time, leaving 499 participants. The mean follow-up time is 4.5 years (range 0.0-7.6 years). At January 1, 2007, 78.2% of the original study population was alive. One hundred nine persons had died, yielding a mortality rate of 4.8 per 100 person years.

There was no significant difference between men and women in mean age at death (57.7 versus 58.4 years; p = 0.61), and there was no significant sex difference in survival (Chi-square = 0.11; p = 0.73). Mean age at death for persons without dementia (n = 29) was 56.6 and did not differ significantly (p = 0.12) from that of persons with dementia (58.5, n = 80).

Table 1 Vital Status at Follow-up and Risk of Mortality According to Baseline Characteristics

Characteristics	Survivors	Decedents	HR (95%CI)	p-value
All, n (%)	390 (78.2)	109 (21.8)		
Sex, n (%)				
Women	154 (39.5)	45 (41.3)		
Men	236 (60.5)	64 (58.7)	1.10 (0.75-1.63)†	0.62
Age start, years, mean (SD)	51.0 (4.5)	55.4 (6.2)	1.15 (1.12-1.19)	<0.001
Birth cohort				
Born before 1947	64 (16.4)	54 (49.5)		
Born after 1947	326 (83.6)	55 (50.5)	0.24 (0.16-0.35)	<0.001
Level of ID, n (%)				
Moderate to mild	209 (60.6)	41 (42.3)		
Severe to profound	136 (39.4)	56 (57.7)	1.84 (1.22-2.77)†	0.003
Living situation, n (%)				
Community living	154 (39.5)	29 (26.6)		
Institutionalised	236 (60.5)	80 (73.4)	1.67 (1.08-2.57)†	0.02
APOEε4 allele, n (%)				
Absent	254 (74.3)	55 (66.3)		
Present	88 (25.7)	28 (33.7)	1.58 (1.00-2.50) †	0.05

HR = Hazard Rate
CI = Confidence Interval
ID = Intellectual Disability
SD= Standard Deviation
† = Adjusting for age

Patients died of respiratory disease (n = 38), cardiac failure (n = 8), cerebrovascular disease (n = 9), carcinoma (n = 7), epilepsy (n = 2) and other diseases (frequency n = 1). The frequencies of causes of death were significantly different between persons with DS with and without dementia ($p < 0.01$). Physical health conditions and special respiratory problems were most frequently a cause of death in persons with dementia, and cardiac failure was the most frequent cause of death in persons without dementia. Cause of death was not known in one-third of those who had died.

The risk of mortality according to patient characteristics and different health conditions are described in Tables 1 and 2. Older persons at baseline, those with physical handicaps, those living in institutions, and those with a severe to profound level of intellectual disability were more likely to have died during follow-up (Table 1). Mortality risk was significantly related to morbidity (Table 2), except for depression and cardiovascular conditions and risk factors. The presence of a cardiovascular condition or risk factor was not associated with greater mortality risk.

A decrease in function over the annual assessment periods was observed in the DMR and SRZ scores in patients without dementia (figure1). Participants who had died after 5 years of follow-up showed significantly greater decline in functioning, than those who survived at all levels of performance ($p < 0.001$).

Table 2 Vital Status at Follow-up and Risk of Mortality According to Morbidity at Baseline

Characteristic	Survivors	Decedents n(%)	HR (95%CI)	p-value
Epilepsy	69 (20.6)	40 (44.4)	2.29 (1.50-3.48)	< 0.001
Depression	84 (25.7)	32 (36.8)	1.41 (0.91-2.19)	0.12
Prevalence of dementia at baseline	45 (11.5)	40 (44.4)	2.91 (1.94-4.36)	< 0.001
Incidence of dementia during follow-up	57 (14.6)	38 (34.9)	1.97 (1.32-2.94)	< 0.001
Vision < 30% at baseline	135 (37.3)	62 (63.9)	2.33 (1.54-3.55)	< 0.001
Hearing impairment at baseline	136 (46.5)	45 (61.6)	1.55 (0.94-2.56)	0.08
Mobility restriction n (%)	72 (19.5)	55 (51.9)	2.76 (1.85-4.13)	<0 .001
Cardiovascular risk factors, n (%)†	77 (19.7)	20 (18.5)	0.96 (0.59-1.57)	0 .88

HR = Hazard Rate
CI = Confidence Interval
SD = Standard Deviation
† Cardiovascular conditions and risk factors were rare. Therefore, we combined diabetes mellitus (n = 6), hypertension (n = 8), cerebrovascular disease (n = 12), known myocardial infarction (n = 2), and smoking (n = 72). 97 Participants had more than one condition/risk factor.

In persons with DS younger than 60, persons with dementia had a 6 times greater mortality risk than those without (Hazard rate (HR): 6.11, 95% confidence interval (CI) = 3.34-11.17). In persons aged 60 and older, the risk was 2.79 times as great (95%CI = 1.49-5.26), although the difference in HR between the two birth cohorts was not statistically significant (p = 0.46).

A significant 1.64 times greater risk of mortality (95%CI = 1.01-2.64; p = 0.04) was found for APOEε4 carriers. The presence of at least one APOEε4 allele was associated with greater mortality risk in persons with and without dementia, but these associations were not statistically significant (HR: 1.07, 95%CI = 0.58-1.95, and HR: 1.64, 95%CI = 0.88-3.01, respectively).

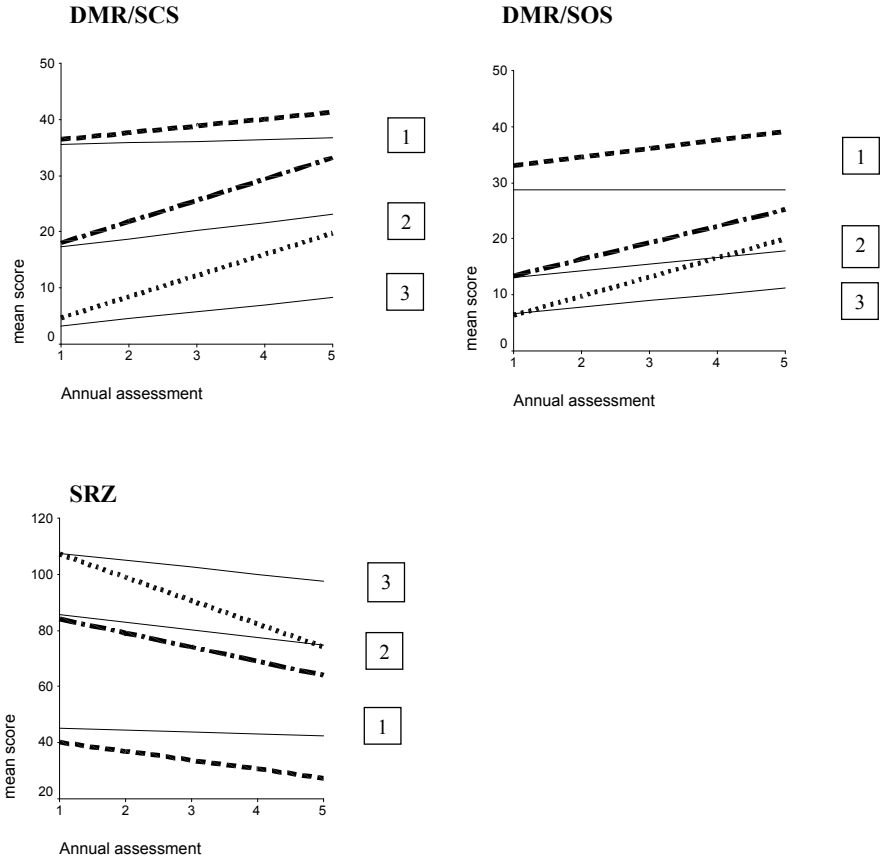
The association between APOEε4 and survival did not differ between the two birth cohorts.

Finally, age at baseline, severity of intellectual disability, living situation, dementia status, epilepsy, mobility, visual impairment and APOEε4 carrier status were studied in a multivariate survival analysis. Only dementia (HR: 4.03, 95%CI = 2.16-7.49; p <0.001), age at baseline (HR: 1.11, 95%CI = 1.05-1.16; p <0.001) and a restricted mobility (HR: 1.92, 95%CI = 1.06-3.46; p = 0.03) remained significantly associated with mortality risk. Excluding patients in the terminal phase of dementia did not alter the results.

DISCUSSION

Age, presence of dementia and mobility restrictions are the most important predictors of mortality in this study cohort of almost 500 people with DS aged 45 and older. In contrast to the general population, impaired mobility, severity of intellectual disability, the presence of epilepsy and visual

Figure 1 Mean Scores of functioning in Persons without Dementia after 5 years of Follow-up



_____ Mean predicted values in those who are alive at the reference date, n = 345

--. --. -- All broken lines, mean predicted values in those who died after five years of follow-up (n = 69) before the reference date 01-01-2007

1: lowest tertile in performance at baseline

2: second tertile in performance at baseline

3: highest tertile in performance at baseline

DMR/SCS: Dementia Questionnaire for persons with an intellectual disability, Sum of the Cognitive Scores

DMR/SOS: Dementia Questionnaire for persons with an intellectual disability, Sum of the Social Scores

SRZ: Social Competence Rating Scale for persons with an intellectual disability

impairment, not cardiovascular risk factors or sex, predicted survival. From a population perspective, one of the most remarkable findings was that sex did not predict mortality. It is most likely that the fact that mortality from cardiovascular disease is not an important cause of death in this population (neither in absolute numbers nor as predictors of mortality) explain this.

Before interpreting the results of this study, three methodological issues need to be addressed. First, the study focused on elderly persons with DS and included persons aged 45 and older. No

information was available on persons who died before the age of 45. This means that several of the findings remain unexplained, such as the lower percentage of women in the study population. Second, some of the data were collected retrospectively from the medical records, which implies that the accuracy of the assessments such as premorbid level of intellectual disability could not be controlled, yet these assessments are relatively standard or part of usual care. Third, DMR and SRZ were informant-based questionnaires, completed by caregivers. Participants may have had different caregivers during follow-up, and their living situation may have changed. Reference standards may have differed and affected the observed performance of the participant, observation bias cannot be excluded. Nevertheless, all participants were followed longitudinally for clinical signs of dementia. A final diagnosis of dementia was based on consensus of a panel consisting of members of the reference group.

A gradual decrease in function in the participants without dementia in this study was documented (see figure1). Although they never met the criteria of dementia, these persons showed a decline in cognitive and functional skills during follow-up. More extensive longitudinal studies are required to identify whether it was likely that this decline was the result of the preclinical onset of AD or of normal aging in DS. One of the clinical challenges will be to discriminate between persons with faster and slower decline.

Even in participants not suffering from clinically manifest dementia, more rapid decline was associated with a greater risk of mortality. This finding is in line with those of others, in subjects with and without DS[12, 13, 24, 25], although the current study is the largest in persons with DS, with all participants examined in person and a clinical follow-up. The results of this study, using different screening instruments, show a decline, in participants without dementia, irrespective of the previous level of performance. Although, it is difficult to detect a decline in those with the lowest level of performance, this decline, monitored by screenings tests, was related to mortality even in this group. In addition, other studies[7, 26-28] have shown a significant negative association between severity of intellectual disability and survival, although this relationship did not remain significant in the multivariate analyses, including all predictors.

This study found an effect of the APOEε4 allele on mortality. Persons with one or two APOEε4 alleles have a 1.64 times greater mortality risk (95%CI = 1.01-2.64). However, this greater risk did not remain significant after adjusting for dementia, although in persons with dementia and in particular in persons with DS without dementia, the risk remained greater (HR: 1.64, 95%CI = 0.88-3.01). This estimate is lower than found in a previous study[14], which found that persons with at least one APOEε4 allele were approximately 5 times as likely to die (4 out of 27 APOEε4 carriers) in a 5- to 7 years follow-up study of persons with DS without dementia. In addition cognitive decline in persons without dementia is a predictor of mortality. Taken together with the findings

of APOEε4, these findings suggest that early pathology that does not express clinically as dementia is associated with mortality in persons with DS.

Because of the longer life expectancy, and their living in the community, persons with DS come to the attention of general practitioners and clinicians specializing in the care of elderly people. An important clinical implication of the data presented in this study is that these clinicians should focus not on cardiovascular risk factors but on impaired mobility and respiratory complications. Diseases of the respiratory system seem to be the most important cause of death in persons with DS with dementia.

In one-third of those who died, the cause of death could not be determined. This finding indicates the need for further clinical and pathological research. The limitation will be whether clinicians are willing to ask for autopsies.

Age and dementia have long been recognized as major predictors of mortality. This study demonstrates that other factors, such as underlying and associated disorders, mobility, morbidity, and social and functional skills also contribute to survival.

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Chapter 2.4

Early age at menopause determines dementia and mortality in a prospective study of women with Down's Syndrome

ABSTRACT

In a prospective longitudinal cohort study of dementia and mortality in persons with Down's syndrome (DS) aged 45 years and older, 85 postmenopausal women were followed for a mean follow-up time of 4.3 years (range 0.0-7.4 years). The effect of age at menopause on age at diagnosis of dementia and survival was estimated using correlation analysis and the Cox Proportional Hazard Model. We found a significant correlation between age at menopause and age at diagnosis of dementia ($\rho = 0.52$; $p < 0.001$), and between age at menopause and age at death ($\rho = 0.49$; $p = 0.01$). Early age at menopause, before 45 years, is associated with a 1.8 fold increased risk of dementia: Hazard Ratio (HR): 1.82 (95% Confidence Interval (CI) = 1.31-2.52) and with risk of death: HR: 2.05 (95%CI = 1.33-3.16). Our study suggests that age at menopause in women with DS is a determinant of age at onset of dementia and mortality.

INTRODUCTION

Whereas the general population shows a clear excess of women in the elderly, the population of elderly Down's syndrome (DS) patients is characterized by an excess of men, which is seen consistently across populations[1-4]. The origin of the excess males in elderly patients with DS is not clear. A small excess of males is seen at birth but this cannot explain the 3:2 ration of men to women in the elderly[5-7]. There also may be excess mortality early in life in females due to the increased prevalence of congenital heart diseases and auto-immune disorders in women[1,3]. However, this is not likely since most of these conditions are not lethal with the current standard of treatment. We have shown that the presence of dementia is one of the most important predictors of mortality in a study cohort of 506 persons with DS aged 45 years and older[7]. Although data are scarce, women with DS appear to have an earlier age at menopause than women in the general population[8-11].
We hypothesised that the earlier menopause, including the reduction in estrogens following menopause, contributes to the earlier onset and increased risk of Alzheimer's Disease (AD) in women with DS[12]. At present, there are no data on how age at menopause relates to age at mortality in women with DS.
We tested the hypothesis that an earlier age at menopause is associated with the age at onset as well as the risk of AD and mortality in elderly women with DS.

METHODS

Study population

Our study included 199 women with DS who were enrolled from December 1, 1999, to December 1, 2003 in a population-based study on DS and ageing in the Netherlands. The sampling procedures and inclusion criteria for this study have been described in detail elsewhere[7]. At the time of study entry, each person received a complete assessment of cognitive, functional and health status, including interviews with relatives, carers and their general practitioner. All participants obtained a general physical and neurological examination and if compliant a venapuncture. Participants that were not demented at baseline were screened annually, until they died, or up to the reference date of January 1, 2007. Those who fulfilled the dementia criteria, at baseline or during follow-up, were followed more intensively at 6-month intervals. These patients also received a clinical work-up, to exclude other physical and psychiatric factors as the possible cause of decline. Plasma and serum were stored at -80°C and DNA was isolated. The medical ethical committee of the Erasmus Medical Center and the ethical committees of the local organizations approved the study protocol.

Written informed consent to participate and to provide blood samples was obtained from legal representatives.

Diagnosis of dementia

Diagnosis of Alzheimer's disease was assessed using the International Classification of Diseases, Tenth Revision (ICD-10)[13] criteria and according to the guidelines produced by the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID)[14].

For the prevalent cases of dementia, age at entry was used to estimate the age at diagnosis of dementia. Age at diagnosis of patients with incident dementia was determined as the midpoint between the age of the person last known to be at risk for dementia and age at diagnosis. All new diagnoses were discussed in a panel consisting of members of the reference group with expertise in the field of intellectual disability (A.M.W.C., G-J.V., F.E.V. and H.M.E.).

Clinical assessments

Pre-morbid severity of intellectual disability (ID) was classified into two groups, using the International Classification of Diseases[13]: mild/moderate (IQ 35-70) and severe/profound (IQ <35). APOE genotype was determined as described in a previous study[15]. Participants were classified according to the presence or absence of at least one APOEε4 allele. The history of past or current hypothyroidism was ascertained by reviewing the medical records. The Body Mass Index (BMI) was computed as weight in kilograms divided by height in square meters (kg/m^2).

Age at menopause was ascertained by medical record review and interviews with carers and family members. Menopause was defined as cessation of menses for at least 12 months and age at menopause as the age of the last menstruation. A total of 11 women were pre-menopausal at baseline and at follow-up and for 33 the menopausal status was unknown. Of the 33 women with unknown status, 14 women still were using oral contraceptives and 6 women had a hysterectomy while for 13 there was no information available at all. Of the 155 women who were definitely post- or-perimenopausal at baseline or follow-up, for 85 (55%) women the exact age at menopause was known. All women had a natural menopause, except one who had an oophorectomy at the age of 38 years. None of the postmenopausal women was on Hormone Replacement Therapy during the analyses or before study entry. For each participant, we obtained the vital status by follow-up interviews and written correspondence.

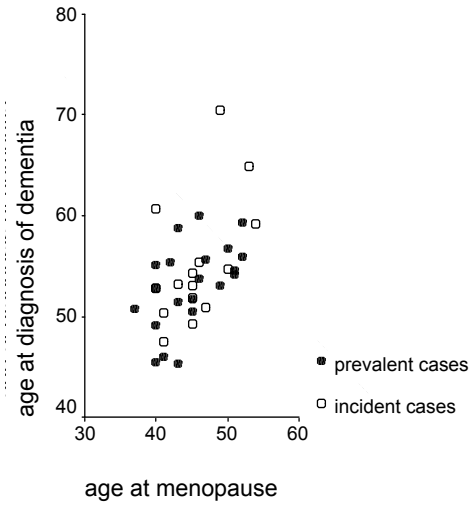
Statistical Analysis

To study the association between age at menopause and age at diagnosis of dementia, we used Pearson (ρ) correlation analysis. The median age of menopause (45 years) was used as cut-off point, to compare the groups, with an earlier age at menopause (before 45 years) to those with a later onset of menopause (after 45 years). The risk to develop dementia during follow-up by age at menopause was investigated using Cox proportional hazards models.
Entry time was defined as age at menopause. Censoring age was defined as age at diagnosis of dementia, age at death or age at the end of this study, January 1, 2007, whichever came first. Potential confounders, influencing the age at menopause, as level of intellectual disability, Body Mass Index (BMI), a history of past or current hypothyroidism and the presence of an APOEε4 allele were studied using correlation analysis.
We repeated all analysis to study the association between age at menopause and mortality. In the Cox proportional hazards model, entry time was defined, as age at menopause, censoring time was age at death or January 1, 2007.

RESULTS

Characteristics are shown in Table 1. The postmenopausal women with information of menopause (n = 85) did not differ significantly from those without information (n = 70). At the baseline the mean age of these 85 women was 51.7 years (range 45-67). The mean length of follow-up was 4.3 years (range 0.0-7.4 years). At January 1, 2007, 63 women (74%) of the original study population were alive. The mean age at menopause was 44.0 years (minimum 28-maximum 55 years); the median age was 45 years. Of these 85 women for whom age at menopause could be determined, 37 were demented (incident and prevalent), of whom 19 women died during follow-up (51.4%). We found no significant correlation between age at menopause and the presence of an APOEε4 allele ($p = 0.89$); level of intellectual disability ($p = 0.69$); BMI ($p = 0.85$) and the presence of hypothyroidism ($p = 0.06$).
Figure 1a shows that there is a strong and significant correlation between age at menopause and age at diagnosis of dementia (Pearson $\rho = 0.52$; $p = 0.001$). In line with these findings, women with an early onset of menopause (before45 years) had an earlier onset of dementia compared with women with a later (after 45 years) onset of menopause [52.5 years (SD3.5) versus 59.3 years (SD 7.2) respectively ($p = 0.02$)]. The Cox proportional Hazard model showed that the early age at menopause was significantly associated to the risk of dementia. Per 5 years interval, the hazard ratio (HR) was 1.82 fold (95%CI = 1.31-2.52) increased for those with an early menopause.

Figure 1a Age at onset of menopause and age at diagnosis of dementia, in women with Down's syndrome



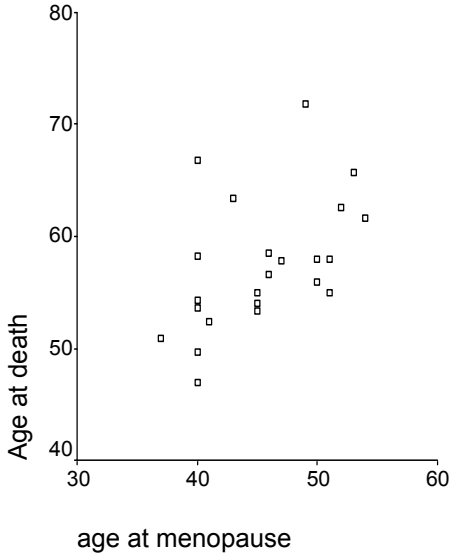
Age at onset of menopause and age at diagnosis of dementia, in women with Down's syndrome:
Prevalent cases: n=21
Incident cases : n= 16
Age at diagnosis of dementia: see methods

Table 1 General Characteristics of the study population

	Postmenopausal women Information available age at menopause			Premenopausal women	Unknown status menopause
	yes	no	p-value		
No. of participants	85	70		11	33
Age at entry	51.7 (4.8)	52.9 (5.8)	0.14	48.8 (3.9)	52.1 (6.2)
Intellectual Disability					
Severe to profound	19 (25)	21 (37)	0.27	1 (12.5)	13 (44.8)
Mild to moderate	56 (75)	36 (63)	0.07	7 (87.5)	16 (55.2)
APOEε4 positive	16 (22.9)	10 (18.5)	0.56	3 (30)	4 (16.7)
Body Mass Index	27.3 (4.6)	27.2 (4.6)	0.72	27.9 (6.7)	27.5 (4.7)
Age at menopause	44.0 (4.7)				
Menopause before 45 years	58 (68.2)				
Dementia status					
Prevalent demented	21 (25)	11 (16)	0.17	0	4 (12.1)
Age at diagnosis prevalent cases	53.1 (4.2)	55.3 (8.9)	0.30	/	49.8 (2.7)
Incident demented	16 (19)	17 (24)	0.40	3 (27.3)	4 (12.1)
Age at diagnosis incident cases	55.1 (6.0)	55.4 (4.4)	0.86	53.7 (5.8)	57.5 (5.6)
Mortality *	22 (25.8)	17 (24.3)	0.82	0	6 (18.2)
Age at death*	57.9 (6.5)	58.9 (6.5)	0.43	/	60.1 (8.3)

Data were reported as mean ± standard deviation (SD) or number and percentage
P-values: we used chi-square tests for categorical variables and Student's t-test and analysis of variance for continuous variables to compare the postmenopausal women with and without information about age at menopause.
For definitions see methods
* At reference date: January,1, 2007

Figure 1b Age at onset of menopause and age at death in women with Down's syndrome



Age at onset of menopause and age at death in women with Down's syndrome:
Number of persons who died during follow-up: n=22

To answer the question whether there is an association between age at menopause and mortality, we demonstrate in figure 1b that there also is a strong and significant correlation between age at menopause and age at death (Pearson $\rho = 0.49$; $p = 0.01$). The mortality of women with an earlier menopause is significantly earlier than that of the women with a later onset [54.8 years (SD5.6) versus 60.1 years (SD 5.0) ($p = 0.03$)]. Also, in a Cox proportional Hazard model, age at menopause was significantly associated with a greater risk of death, the HR being 2.05, (95%CI = 2.05 (1.33-3.16).

DISCUSSION

In this prospective study cohort of 85 postmenopausal women, we found that the mean age of menopause is relatively early (mean 44 years) compared to that seen in the general population (mean 51.4 years)[16]. We further found that age at menopause is significantly correlated with age at dementia diagnosis and age at death. Finally, our study showed that an early age at menopause is associated with an increased dementia ánd higher mortality risk.

In line with others[10], we demonstrated a correlation between age at menopause and age at dementia onset. Data of menopause in women with DS are very scarce and of low quality. However, the earlier menopause of women with Down syndrome has been recognized for long in clinical series. The origin of the early menopause in women with Down syndrome is not known but is not

unexpected as all patients show accelerated ageing of various organs including the brain and skin[17-19]. The early menopause may be related to either the aging of the brain, sex organs, or unrecognized genes over-expressed in the patients due to trisomy 21. Only one other group has studied the relationship with dementia[8, 10-12, 20, 21]. As the amyloid deposition, which is the origin of the dementia in DS patients, starts already in the second decade of patients with DS, it is unlikely that menopause or the withdrawal of estrogen itself after the menopause, initiates the deposition of beta-amyloid. More likely, menopause modifies the risk of dementia.

In our study, we also found a strong association of age at menopause and mortality. This is the first study in which a relation between age at menopause and mortality in women with DS is described. Age at menopause is strongly and significantly correlated to age at death ($\rho = 0.49$, $p=0.01$). Further, there is a two-fold increase in risk of mortality in women after menopause, which may contribute to the excess of men seen in elderly patients with Down syndrome. Unfortunately we do not have enough statistical power in this population, to study, in a multivariate analysis, the risk to develop dementia and the risk to die, adjusting for variables known to influence dementia and mortality. Age at menopause was not significantly correlated to the presence of an APOEε4 allele, level of intellectual disability, BMI and the presence of hypothyroidism, suggesting these factors are not likely confounders.

The strength of this study is its prospective design, allowing a very careful evaluation of the age of menopause. Many women with DS in the Netherlands are on oral contraceptives, making it difficult to determine age at natural menopause. We could determine the exact age of natural menopause in 55% of the women. Another potential problem is that selection bias could have occurred prior to study entry. Women with a very early menopause and women with an APOEε4 allele may have a higher risk to die before entry in the study. As a result, women with an early menopause that carry an APOEε4 allele who survived and entered the cohort may have been healthier than women with a later menopause and thus at lower risk of dementia. Similar to other studies, it is difficult for many women to pinpoint the age at menopause.

In sum, our study shows that women with Down syndrome have an earlier menopause. This most likely reflects the accelerated ageing process, either in the brain, sex organs or may be related to unrecognized genes overexpressed in the patients due to trisomy. More importantly from a clinical perspective, age at menopause is strongly correlated to age at dementia and age at death. Further age at menopause is a determinant of the risk of dementia and mortality. It remains to be determined what the mechanism is underlying the association. However, an important clinical implication of our findings is that women with DS may be screened for dementia more intensively after menopause. Finally, the association may explain the excess of men among elder DS patients.

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Chapter 3

Plasma amino acids and neopterin in persons with Down's syndrome

Chapter 3.1

Plasma amino acids and neopterin in healthy persons with Down's syndrome

ABSTRACT

In persons with Down's syndrome (DS) immunological abnormalities as well as hypothyroidism and Alzheimer type dementia are frequently observed. In addition, the activity of the enzyme cystathionine beta-synthase (CBS) is over-expressed which results in an altered homocysteine metabolism.

In the present study, 48 older healthy DS persons without signs of dementia, psychiatric or somatic comorbidity and free of medication were analysed for plasma levels of amino acids, neopterin and monoaminergic metabolites. Data were compared with those obtained from age and sex matched healthy controls.

It was found that the spectrum of amino acids showed widespread differences in that levels of nearly all essential amino acids were lower in DS patients as compared to healthy controls. In addition, a significantly lower methionine and higher taurine concentration were observed which is in accordance with a disturbed homocysteine metabolism. With respect to the monoamine metabolites, the concentration of 5-hydroxyindoleacetic acid was not altered whereas that of homovanillic acid was significantly increased. Finally, the concentration of the immune activation marker neopterin was increased in persons with DS.

It is concluded that healthy DS persons of older age show extensive biochemical abnormalities suggesting a compromised homocysteine metabolism, an activated cell-mediated immune response and an enhanced turnover of dopamine.

INTRODUCTION

Down's syndrome (DS) or trisomy 21 is the most common form of intellectual disability with an overall prevalence of 1,42/1000 that increases significantly with maternal age[1]. Immunological abnormalities like functional impairments in fagocytes with low chemotactic ability as well as the depressed production of cytokines are intrinsic to the chromosomal disorder and result in an increased morbidity and mortality from infectious diseases[2]. In addition, DS is associated with hypothyroidism in 20-40% of the patients and with neuropsychiatric disorders of which depression and Alzheimer-type dementia are the most frequent[3-5].

Since the activity of the enzyme cystathionine ß-synthase (CBS), which is encoded on chromosome 21 (21q22.3), is associated with the level of intelligence and with neuropsychiatric disorders[6, 7], the concentration of CBS was recently investigated in post-mortem brains of DS persons[8]. It was found that the concentration of CBS was three times higher as compared to brain levels of normal individuals. CBS catalyzes the conversion of homocysteine and serine into cystathionine. The latter dipeptide is further metabolized into the amino acids cysteine and eventually to taurine. In humans, the sole source of homocysteine is through dietary intake of the essential amino acid methionine[9, 10].

Thus, the over-expression of CBS in persons with DS may alter homocysteine metabolism resulting in a metabolic imbalance such that folate-dependent resynthesis of methionine is compromised, and may be in favour of the transsulfuration pathway, leading eventually to increased levels of taurine[11]. Due to the dosage effect of CBS, a decrease in plasma concentration of serine was found in DS persons. In addition, an increase in plasma lysine, which was explained by generalized premature aging in DS, was observed[12].

Another amino acid abnormality is a decrease of systemic glutamate uptake as assessed by measuring glutamate uptake in platelets and fibroblasts from DS persons[13]. The authors suggest the use of this peripheral model as a biochemical ex vivo marker of a central glutamatergic dysfunction. Excessive glutamatergic stimulation as a consequence of glutamate uptake deficits could be responsible for neuronal suffering, excitoxicity and cell death, and may play a key role in the pathophysiology of neuropsychiatric disorders[14].

Apart from the abovementioned amino acid abnormalities, impaired immunological function in DS persons results in increased serum neopterin levels[15]. Neopterin is not only an immune activator for the cell-mediated immune response, but also induces or enhances cytotoxicity and exhibits antioxidant properties[16]. This observation is consistent with the increased susceptibility of DS persons to bacterial and viral infections[17].

No studies have been reported on the measurement of peripheral precursors or metabolites of the neurotransmitters serotonin and dopamine in DS. Since serotonin is involved in brain growth and

maturation, and experimentally induced high dopamine turnover may give rise to toxic metabolites and neurodegeneration[18-20], measurement of peripheral correlates of central serotonin and dopamine metabolism is also warranted. Since at least 40% of circulating homovanillic acid (HVA) originates from the brain, the plasma HVA concentration is thought to be a fairly good indicator of the changes in dopamine metabolism in the brain[21]. In addition, plasma 5-hydroxyindoleacetic acid (5-HIAA) is suggested to be an indicator of brain serotonergic activity[22].

The present study was designed to investigate peripheral parameters that reflect changes in homocysteine metabolism, immune function and monoaminergic neurotransmission in a group older non-demented healthy persons with DS. To this end, especially the sulphur containing amino acids methionine and taurine as well as serine, glycine, glutamate, lysine, tryptophan and the other large neutral amino acids (LNAA) were measured. The ratio of tryptophan to LNAA is a fairly good indicator of central serotonin synthesis. Concentrations of the dopamine and serotonin metabolites HVA and 5-HIAA were determined as well as the levels of the immune activation marker neopterin.

MATERIALS AND METHODS

Subjects

Over a period of 4 years a community based sample of 506 DS persons aged 45 year and older from the Southern and the Southwestern parts of the Netherlands was collected. The study protocol was approved by the Medical Ethical Committee of the Erasmus University Medical Centre in Rotterdam, The Netherlands (protocol number: MEC 185.974/1999/202). In addition, the ethical committee of the local institutions provided approval. Written informed consent was obtained from the legal representatives. Details of the study population and the used screening instruments are presented elsewhere[23].

For the present study persons were excluded in case of any relevant somatic comorbidity, including hypothyroidism and epilepsy, or the presence of a depressive disorder and signs of dementia. All persons were free of medication, including the use of folic acid, and did not smoke. The study group comprised 48 persons with DS (female: 11; male: 37; mean age: 50, Standard Deviation (SD) 4.8 years) of whom 2 had to be excluded because of laboratory problems, and was compared to a group of 48 age and sex matched healthy controls (female: 16; male: 32; mean age: 50.2, SD 9.1 years).

Biochemical analyses

Plasma amino acids are analyzed by high-performance liquid chromatography (HPLC) using pre-column derivatization with o-phthaldialdehyde[24]. The tryptophan-ratio (Trp-ratio) is calculated by dividing the total tryptophan level by the sum of the other large neutral amino acids (LNAA), i.e. valine, isoleucine, leucine, tyrosine and phenylalanine, which compete for the transport of tryptophan through the blood-brain barrier. The tyrosine-ratio (Tyr-ratio) is calculated in the same manner by substituting tryptophan for tyrosine. The concentrations of the monoamine metabolites 5-HIAA and HVA are analyzed by HPLC and electrochemical detection[25]. The concentration of neopterin in plasma is determined as previously described[26].

Statistical analyses

For statistical comparisons between persons with DS and controls, the unpaired *T*-test was used because all data sets showed a normal distribution. Normality of the distribution was tested with the Kolmogorov Smirnov test. A value of $p < 0.05$ was considered to be statistically significant. In case of finding statistically significant values for biochemical parameters, discriminant analysis was performed in order to assess the potential for further differentiating DS persons from controls, and to establish their relative contribution to the group difference.

RESULTS

As can be inferred from Table 1, the concentrations of methionine, glutamate and all LNAA are significantly decreased in the DS group as compared to the control group. Of the LNAA, tryptophan shows the lowest significance. The concentrations of taurine and glycine are significantly increased in DS persons as compared to controls, whereas the level of glutamate is significantly decreased. In addition, the Trp-ratio but not the Tyr-ratio, is significantly higher in the DS group compared to the controls.

With respect to neopterin, values in the DS group are significantly increased as compared to the control group. Furthermore, plasma HVA levels are significantly higher in the DS group, whereas no differences are found regarding 5-HIAA concentrations.

Finally, discriminant analysis (stepwise method) of the biochemical parameters with p-values <0.001 (valine, isoleucine, leucine, methionine, taurine and Trp-ratio) results in a discriminant function with valine, methionine and taurine as major predictors. Classification results are good:

Table 1 Plasma levels (mean ± S.D.) of biochemical parameters in persons with Down's syndrome and controls

Biochemical parameter	DS patients	Healthy Controls	p-value
Tryptophan (µmol/l)	47.1 ± 6.1 (46)	50.4 ± 9.0 (48)	0.040
Tyrosine (µmol/l)	61.9 ± 12.6 (46)	71.0 ± 17.3 (48)	0.004
Valine (µmol/l)	226.5 ± 42.8 (46)	286.2 ± 59.7 (48)	0.000
Phenylalanine (µmol/l)	58.0 ± 8.5 (46)	63.1 ± 10.0 (48)	0.010
Isoleucine (µmol/l)	61.2 ± 11.7 (46)	82.7 ± 24.1 (48)	<0.000
Leucine (µmol/l)	122.6 ± 20.9 (46)	148.5 ± 31.8 (48)	<0.000
Methionine (µmol/l)	25.6 ± 4.7 (46)	32.0 ± 6.5 (48)	<0.000
Taurine (µmol/l)	53.9 ± 11.8 (46)	42.9 ± 7.6 (48)	<0.000
Serine (µmol/l)	103.7 ± 17.3 (46)	109.8 ± 19.1 (48)	0.106
Lysine (µmol/l)	199.3 ± 31.8 (46)	186.3 ± 35.7 (48)	0.065
Glycine (µmol/l)	244.6 ± 48.7 (46)	213.0 ± 43.5 (48)	0.001
Glutamate (µmol/l)	37.4 ± 22.5 (46)	47.7 ± 20.3 (48)	0.021
Trp-ratio	9.0 ± 1.5 (46)	7.9 ± 1.5 (48)	<0.000
Tyr-ratio	12.2 ± 3.3 (46)	11.3 ± 2.1 (48)	0.113
Neopterin (nmol/l)	20.4 ± 5.8 (43)	17.4 ± 3.7(28)	0.008
HVA (nmol/l)[a]	64.1 ± 16.5 (45)	53.2 ± 12.9 (41)	0.001
5-HIAA (nmol/l)[b]	43.5 ± 9.0 (45)	41.5 ± 12.9 (42)	0.405

a: HVA: homovanillic acid. b: 5-HIAA: 5-hydroxyindoleacetic acid

84.8% of the DS persons were correctly classified, as were 83.3% of the controls. As a whole 16% was wrongly classified.

DISCUSSION

In the present study biochemical parameters related to neurotransmission and immunological function were investigated in a group of older persons with Down syndrome. It was found that the spectrum of amino acids shows widespread differences in the DS group as compared to controls. More specific, the amino acids valine, methionine and taurine were demonstrated to differentiate persons with DS from controls. Nearly all values of amino acids are lower in the DS group, which cannot be explained from dietary effects since all persons were provided with well-balanced meals. Besides, Ciaccio et al.[27] reported that dietary measures over one year have an only marginal influence on the plasma concentration of amino acids in children with DS.

With respect to the CBS-related parameters, plasma methionine was found to be significantly lower, while taurine was higher. In addition, plasma serine was slightly decreased in DS persons. These observations are in accordance with an over-expression of the CBS-gene in DS[28-30] and are also in line with results concerning serine and taurine plasma concentrations as reported by

Mircher et al.[12]. Although homocysteine was unfortunately not measured in this study, these data corroborate an altered homocysteine metabolism in persons with DS and are as such in line with the lowered plasma concentration of homocysteine reported by Progribna et al.[29]. Other investigators, however, reported increased concentrations of homocysteine[31 32]. As reviewed by Townsend et al.[33], the sulphur containing amino acids methionine, homocysteine and taurine are involved in the maintenance and integrity of cellular systems by influencing cellular redox state and cellular capacity to detoxify toxic compounds, free radicals and reactive oxygen species. An imbalance of these amino acids in DS persons may indicate neuropathological changes in this group.

The increased Trp-ratio in the DS group suggests an enhanced availability of tryptophan for the synthesis of serotonin in the central nervous system. Whether central serotonergic neurotransmission is increased in DS persons cannot be concluded from this study, especially since the plasma concentration of 5-HIAA is not changed. The latter parameter, however, is only a very weak and indirect measure of central serotonergic activity[34]. On the other hand, plasma HVA concentrations are significantly increased, which is an indication of a higher turnover of dopamine that was reported previously also in cerebrospinal fluid studies in persons with DS[35, 36].

Neopterin is increased in the DS group as compared to controls. This finding is in agreement with the observations by Metha et al.[15] who additionally reported that increase of neopterin is not correlated with age and sex. High concentrations of neopterin as seen in DS is consistent with the impaired immune function that was reported in persons with DS[37, 38].

In conclusion, in the present study of persons with DS without any signs of dementia, somatic and/or neuropsychiatric comorbidity and free of any medication, extensive biochemical abnormalities are found suggesting a compromised homocysteine metabolism, an activated cell-mediated immune response and an enhanced turnover of dopamine. Interestingly, a few peripheral biochemical parameters appeared to have a marked potential to differentiate between DS persons from controls.

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Chapter 3.2

Neopterin and the risk of dementia in persons with Down's syndrome

ABSTRACT

Persons with Down's syndrome show an altered immune response and an increased susceptibility to Alzheimer's disease. In a prospective study, we examined whether the plasma neopterin level, a marker for cell mediated immune activation and inflammation, is associated with an increased risk of dementia in persons with Down's syndrome.

Plasma concentrations of neopterin were determined in a population-based study of 506 persons with Down's syndrome, who were screened annually for dementia. We used Cox proportional hazards models to determine risk of dementia.

Demented persons with Down's syndrome have a significantly ($p = 0.05$) higher plasma neopterin concentration than the non-demented. In those without autoimmune disorders, or dementia at baseline ($n = 259$), those with a plasma level of neopterin above median, the risk to develop dementia increased to 1.83 (95% confidence interval = 1.04-3.20). High plasma neopterin level is an independent determinant of the risk of dementia in persons with Down's syndrome.

INTRODUCTION

Down's syndrome (DS) is a common genetic disorder affecting about 1 in 750 live births in the general population. It is caused by a complete, or occasionally partial, triplication of chromosome 21, which explains the increased risk of Alzheimer's disease (AD). Despite the relatively uniform cause of dementia in persons with DS, patients with dementia show a large spread in the onset age of dementia. This can vary from age 35 to 70 years which suggests other risk factors may modify the onset of disease[1]. Persons with DS also show an altered immune response and an increased susceptibility to infectious diseases and immune mediated and aging-associated diseases[2].
Among the various pathophysiological mechanisms involved in dementia in the general population, inflammation and immune system activation are considered to play an important role in the development and progression of dementia[3]. Increased concentrations of immune activation markers, including C-reactive protein (CRP), tumor necrosis factor-α and neopterin, were reported in the brain but also in the blood of patients with AD and other neurodegenerative disorders[4, 5]. While plasma levels of CRP may reflect an acute phase reaction, plasma neopterin levels are more likely to reflect cell mediated immune activation and inflammation[4]. In a previous study we found, in healthy persons with DS, aged 45 years and older, an increased plasma concentration of neopterin[6]. It is unknown whether plasma neopterin levels are associated with AD in persons with DS or with the onset of disease. The aim of this study is to investigate whether plasma neopterin levels in persons with DS are associated with the risk of dementia.

METHODS

Study population

We conducted a study of 506 persons with DS, aged 45 years and older, who were enrolled from December 1, 1999 to December 1, 2003 in a community-based study on DS and ageing in the Netherlands. Informed consent procedures and recruiting of subjects have been described in detail elsewhere[1]. All participants were assessed once yearly until they either died (n = 109), or were withdrawn from the study by their representatives (n = 7), up to the reference date of 1 January 2007. At the time of study entry, each person received a complete assessment including interviews with relatives, carers and the general practitioner. All medical records were reviewed to disclose past or present medical disorders, such as epilepsy and depression, and the possible use of drugs. The same questionnaires and interviews were used annually from 1999-2007. All persons underwent a general physical and neurological examination. Venous blood samples were taken fasting

in the morning. From the 506 participants in the total study of dementia and mortality in DS, plasma concentration of neopterin was measured in 394 (77.9%) persons who consented to give (sufficient) blood.

Clinical assessments

Pre-morbid severity of intellectual disability (ID), based on medical records, was classified using the International Classification of Diseases[7]. According to these criteria, 136 (34.5%) of the participants had a severe to profound level of ID, 211 (53.6%) had a moderate to mild level of ID. The severity of ID was not known for 47 (11.9%) participants. All persons were assessed for AD using the ICD-10[7] criteria and according to the guidelines produced by an international consensus panel established under the auspices of the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID)[8]. The diagnosis of dementia was supported using the Dementia Questionnaire for persons with an intellectual disability (DMR)[9, 10] and the Social Competence Rating Scale for persons with an intellectual disability (SRZ)[11]. Persons who were not demented at baseline were screened annually. Those who fulfilled the criteria for AD, at baseline or during follow-up, were followed more intensively at 6-month intervals. These patients also received a clinical work-up, to exclude other physical and psychiatric factors as the possible cause of deterioration.
The history of past or current disorders as epilepsy, thyroid disorders, celiac disease, diabetes type 1 and 2 and others were ascertained by reviewing the medical records. Information on causes of death was obtained from relatives or primary carers and supplemented with additional data from medical or autopsy records.

Measurements

Neopterin

Immediately after venapuncture, plasma was prepared by a 20-min centrifugation step at 2650 *g* and stored at −80°C. The concentration of neopterin in plasma was measured, blind to the dementia status, after acid oxidation as described earlier[12, 13]. In short, 0.1 ml 1 M trichloroacetic acid was added to 0.4 ml of plasma, followed by 0.05 ml iodine solution (0.5% I_2, 1% KI in 0.2 M trichloroacetic acid). After standing for 60 min under reduced light, excess iodine was reduced by the addition of 20 µl of 1% ascorbic acid solution and the mixture was centrifuged at 12 000 *g* for 15 min at 4°C. The supernatant (0.4 ml) was transferred to an amber glass vial, and 10 µl was injected onto a Hypersil C_{18} column (2.1×200 mm, 5 µm) using an HPLC system and fluorescence

detection (Agilent, Series 1100). The separation was achieved using an aqueous 15 mmol/l potassium phosphate buffer, pH 6.45, and a stepwise eluant gradient with methanol (from 1.5 to 2 min to 10%, from 4 to 5 min to 100%, and from 6 to 8.3 min to 0%). Total run time was 14 min, the flow rate was set at 0.4 ml/min, the column temperature was 50°C, and the excitation and emission wavelengths were 360 and 440 nm, respectively.

APOE

DNA was isolated, according to the salting out procedure as described by Miller et al.[14]. APOE genotypes were determined on 5ng/µl dry DNA samples using Taqman allelic discrimination technology[15] on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA). APOE genotyping was obtained blind to all clinical information.

Statistical analysis

In the descriptive analyses, data were reported as mean ± standard deviation (SD). Because not all of the data sets showed normal distribution, the non-parametric Mann-Whitney *U* test was used to compare subgroups and the Spearman rank correlation analyses (ρ) to assess correlations. Neopterin measurements took place in three separate runs and although the means of these runs did not differ significantly ($p>0.05$), we adjusted in all analyses for the runs in our risk calculations. As there is no standardized reference for a normal range of plasma neopterin in persons with DS, the median value of neopterin was used in the subgroup as a cut-off point for defining 'high' level of plasma neopterin ($\geq$ 21.20nM) versus 'low' level (< 21.20nM) of neopterin at baseline. To determine the cut-off, we used only Down persons without autoimmune disorders. The Kaplan-Meier life table methods and the Cox proportional hazards model was used to estimate the hazard ratio (HR) (with 95% confidence intervals (CI)) of dementia, adjusting for age at entry, gender and the presence of the APOEε4 allele. The time to event variable was follow-up time until reference date, death or the incidence of dementia. Statistical analysis was performed using the SPSS statistical package, version 11.0.

RESULTS

The mean concentration of plasma neopterin, in the total population, was 25.18nM (range 4.58-90.10nM). The median concentration was 22.61nM. Table 1 shows the general characteristics of this study population. Persons born before 1947, aged 60 years and above at the reference date,

Table 1 The association of plasma neopterin levels with general characteristics and clinical outcomes

	Total population			Without autoimmune disorders		
	Number	neopterin nM (SD)	p-value	number	neopterin nM (SD)	p-value
All	394			259		
Age						
Born before 1947	88	28.26 (11.4)*		54	27.99 (10.2)*	
Born after 1947	306	24.29 (10.7)	<0.001	205	22.29 (8.7)	<0.001
Gender						
Female	148	26.50 (12.6)		89	25.01 (8.2)	
Male	246	24.38 (9.8)	0.19	170	22.67 (8.2)	0.14
Intellectual Disability						
Severe/profound	136	25.98 (11.9)		87	24.50 (9.5)	
Mild/moderate	211	24.82 (10.3)	0.57	130	22.48 (8.4)	0.14
Dementia status						
Non-demented	256	24.37 (10.3)*		162	22.26 (8.6)*	
Demented	138	26.68 (12.1)	0.05	97	25.50 (10.1)	0.007
Prevalent dement	60	28.16 (14.3)		36	25.21 (10.3)	
Incident dement	78	25.55 (9.9)	0.50	61	25.67 (10.1)	0.61
Vital status						
Alive at reference date	315	24.77 (10.8)		208	22.84 (9.1)*	
Dead at reference date	79	26.81 (11.7)	0.09	51	26.07 (9.9)	0.03
APOEε4 allele						
Absent	281	25.20 (10.9)		185	23.74 (9.6)	
Present	110	24.95 (11.24)	0.09	71	22.46 (8.3)	0.35
Congenital heart disease						
Absent	300	24.27 (10.1)		231	23.35 (9.3)	
Present	36	27.09 (15.8)	0.76	26	24.91 (10.4)	0.65
Thyroid disorder						
Absent	247	23.29 (9.4)*		-		
Present	90	27.48 (11.9)	0.003	-		
Diabetes						
Absent	332	24.42 (10.3)		-		
Present	5	21.57 (6.2)	0.70	-		
Epilepsy						
Absent	249	24.47 (10.6)		194	23.64 (6.5)	
Present	86	25.0 (11.8)	0.99	63	23.06 (9.1)	0.62
Smoking						
Non-smoker	334	25.66 (11.5)*		210	24.09 (9.9)	
Smoker	60	22.49 (7.4)	0.05	43	20.64 (5.3)	0.06

Reference date: 01-01-2007

neopterin nM (SD): mean concentration neopterin at baseline (Standard Deviation)

* p-value: Mann Whitney U-test, significance at 0.05 level

1 January 2007, had significantly higher neopterin concentrations than the younger ones. Plasma neopterin concentrations were significantly higher in the demented persons (n = 138, 26.68 ± 12.06nM) compared with the non-demented persons (n = 256, 24.37 ± 10.32nM; *U* = 15614, p = 0.05). Further, neopterin levels were found to be significantly increased in non-smokers (n = 334, 25.66 ± 11.46) compared with the smokers (n = 60, 22.49 ± 7.45; *U* = 8441, p = 0.05). As was to be expected, plasma neopterin concentration was significantly higher in persons with a history of autoimmune thyroid disorders (n = 90, 27.48 ± 11.9nM) than in persons without (n = 247, 23.29 ± 9.4; *U* = 8784, p = 0.003).

There were 78 cases of incident dementia diagnosed during 4.5 years of follow-up (minimum 0.0-maximum 7.6 years). No significant differences in neopterin concentrations were found between the prevalent demented (n = 60, 28.2 ± 14.3nM) and those who developed dementia during follow up (n = 78, 25.6 ± 9.9nM; *U* = 2185, p = 0.50).

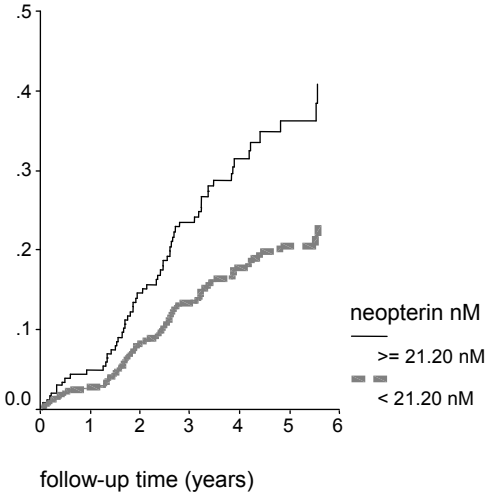
To correct for the influence of autoimmune disorders, we excluded in the analyses all persons (n = 135) known to have autoimmune disorders, including thyroid disorders (n = 90), diabetes (n = 5) and celiac disease (n = 3), and with unknown autoimmune status, leaving 259 persons in the analyses (Table 1). Due to the increased prevalence of auto-immune disorders in women, persons excluded from this selection were more frequently women (46.8 versus 34.3%, p = 0.05). The mean concentration of neopterin after exclusion of those with auto-immune disorders was 23.47nM (range 4.58-62.20); while the median concentration was 21.20nM. The difference between the demented and non-demented persons increased compared to that seen in the total study population (p = 0.007). There is a significant correlation between neopterin concentration and dementia in this subgroup: $\rho = 0.17$, p = 0.007, as well as between neopterin and age: $\rho = 0.22$, $p<0.001$, and the vital status: $\rho = 0.14$, p = 0.03.

When excluding in the analyses those persons with known autoimmune disorders, the risk of developing dementia was almost two times increased: 1.83, (95%CI = 1.04-3.20) in the subgroup with plasma neopterin levels above median (≥ 21.20 nM)(figure 1).

The absolute risk of disease increases from 17 % in those with a plasma neopterin levels < 21.20nM to 30% in those with a neopterin level ≥ 21.20nM. When excluding those with auto-immune disorders, mortality was also associated with increased neopterin levels (p = 0.03). This is expected as dementia is the main cause of death.

The Cox-analysis was repeated in the non-smoking persons only (n = 210), with 52 persons who developed dementia. The risk of developing dementia, in the group with the highest level of neopterin, adjusting for age, gender and the APOEε4 allele, increased to 2.10, (95%CI = 1.12-3.92).

Figure 1 Cumulative incidence (%) of dementia during follow-up in those with no autoimmune disorders



DISCUSSION

To the best of our knowledge, this is the first study in which a relation between plasma concentration of neopterin and (risk of) dementia in persons with DS is described. Compared with the persons with DS without dementia, the prevalent demented persons with DS, and those persons who were non-demented at baseline but who developed dementia during the follow-up, had significantly higher plasma neopterin concentrations at the start of the study. There was no significant difference in the concentrations between the incident patients who developed dementia during follow-up and the prevalent demented cases ($p = 0.50$). When excluding in the analyses those persons with known autoimmune disorders, the risk of developing dementia was almost two times increased: 1.83, (95%CI = 1.04-3.20) in the subgroup with plasma neopterin levels above median ($\geq$ 21.20nM).

Neopterin, produced by interferon-γ-activated monocyte-derived macrophages and dendritic cells, is a reliable and sensitive indicator of cell-mediated immune activation[16]. In our previous study increased neopterin concentrations were found in healthy persons with DS compared with controls in the general population[6].

The comorbidity associated with activation of the immune system is high in elderly persons and these disorders may explain part of the high plasma neopterin levels. The plasma neopterin concentration was higher in persons with thyroid dysfunction. There was indeed a significant difference between neopterin concentrations in the healthy group compared to those with hypo-or hyperthyroidism. We therefore evaluated the risk of developing dementia excluding those persons known to have autoimmune disorders. The association remained statistically significant. Smoking

had also a significant effect on the neopterin concentration, which is in agreement with earlier studies in healthy adults[17, 18]. While plasma CRP levels are significantly increased in smokers[19], plasma neopterin levels are decreased in our study of DS and others in the general population[17, 18]. This observation has been explained by a suppressive effect of tobacco products on the immune system, which may lead to lower neopterin production in smokers. Also when limiting the analysis to non-smokers, we found those with a high plasma neopterin level to have a significantly higher risk of developing dementia.

Although our study is relatively small, it is the largest longitudinal follow-up study of persons with DS in which participants were clinically examined in person. A major advantage of the longitudinal design is that the outcome of prevalent and incident demented patients can be compared. By limiting the analysis to incident patients, who are disease free at baseline, we can conduct an unbiased study of the risk of dementia for a determinant such as plasma neopterin, which may be altered by the disease status. Although the incident demented patients had a lower (25.55 ± 9.9nM) concentration of neopterin at baseline than the prevalent demented patients (28.16 ± 14.3nM), the groups did not differ significantly.

Persons with DS are at increased risk of dementia and AD. We have shown earlier in our population-based study that, from the age of 45 years onwards, the overall prevalence of AD was 16.8%[1]. The risk of dementia is an important question for relatives and carers. Here we show that the absolute risk is over 30% in those with a neopterin level $\geq$ 21.20nM. Our prospective follow-up study indicates that elevated plasma concentration of neopterin in non-demented persons with DS is an independent determinant of the risk dementia.

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Chapter 3.3

Plasma levels of nitric oxide related amino acids in demented persons with Down's syndrome are related to neopterin concentrations

ABSTRACT

Subjects with Down's syndrome (DS) have abnormalities in virtually all aspects of the immune system and almost all will be affected with Alzheimer's disease (AD). It is thought that nitric oxide (NO) is involved in the pathophysiology of AD. In addition, the immune activation marker neopterin is increased in AD patients.

In the present study including a total of 401 elderly DS subjects, the spectrum of plasma amino acids and neopterin was investigated and related to development of AD.

Concentrations of nearly all amino acids in DS subjects differed significantly from those of healthy controls. Neopterin was increased in DS subjects, especially in dementia. The production of NO as reflected by an increased citrulline/arginine ratio was enhanced during development of clinical dementia. Neopterin concentrations correlated to the NO production only in the group of demented subjects ($\rho = 0.48$, $p<0.000$).

The results of this study are suggestive for an increase in oxidative processes in DS subjects with AD.

INTRODUCTION

Down's syndrome (DS) is a common genetic disorder with a prevalence of 1:750 and is caused by a complete or occasionally partial triplication of chromosome 21. Its prevalence is highly dependent on maternal age at gestation. It is well known that individuals with DS have an increased rate of congenital and acquired medical complications including among others thyroid gland dysfunction. In addition, abnormalities have been reported in virtually all aspects of the immune system resulting in a greater susceptibility to infectious disease. Finally, they have a higher risk for haematological malignancies, particularly leukaemia's, and virtually all show the neuropathological changes of Alzheimer's disease (AD) by the age of 35 to 40 years. This may be due to an increased production of the amyloid precursor protein[1, 2]. Onset of clinical dementia is in general in the first half of the sixth decade. With respect to psychopathology, depression is frequently diagnosed and shows an age related increase[3].

A growing body of evidence indicates that there is an increased oxidative stress in AD brains and it is hypothesised that nitric oxide (NO) can be related to many of the pathophysiological mechanisms of AD[4, 5]. The presence of any stimulant that leads to an overproduction of NO will probably cause neuronal damage[6]. The biologically active molecule NO is formed by the conversion of L-arginine to L-citrulline, a reaction catalyzed by the enzyme nitric oxide synthase. In this process tetrahydrobiopterin (BH4) is a necessary cofactor. The ratio of the plasma concentrations of citrulline (Cit)-and arginine (Arg), the so-called Cit-Arg ratio, is regarded as an index of NO synthesis[7].

Brain inflammation is mainly caused by activation of glia cells that produce a variety of pro-inflammatory and neurotoxic factors, including free radicals such as NO[8, 9]. Inflammation and immune system activation are considered to play an important role in the development and progression of dementia[10]. Neopterin, as indicator for this activation process, is found to be increased in the brain and the plasma of patients with AD and other neurodegenerative disorders[11, 12].

It has become clear that amino acids are not only essential for various metabolic pathways such as the synthesis of the major neurotransmitters, but also for maintenance of cell structure and functionality of many organs. In addition, amino acids are involved in immune responses[13, 14]. Probably of special relevance are the co-called branched-chain amino acids (BCAA) that comprise the three essential amino acids: l-leucine, l-isoleucine and l-valine. In experimental conditions, administration of BCAA was found to improve immune function[15]. In a previous study with healthy DS individuals, wide spread differences in nearly all amino acids were found as compared to healthy control subjects with significant lower levels of the essential amino acids[16].

The present study was designed to assess the spectrum of amino acids and the role of NO and BCAA in a large cohort of DS individuals with and without dementia.

METHODS

Study population

Over a period of 4 years a community based sample of 401 individuals with DS, age 45 years and older, from the Southern and South-Western part of the Netherlands was composed. Recruitment procedures and the ethical protocol are described in detail elsewhere[17,18]. The sample comprises 151 women and 250 males with a mean age of 52 (SD 5.1) years (Table 1). In 232 subjects DS was confirmed by cytogenetic analysis. In the remaining, the diagnosis was based on the characteristic phenotype.

The study population was divided into two birth cohorts based on the subject's age at the reference date. The birth cohorts distinguishes patients who were 60 years or older (born before 1947; n = 88) and those who were younger than 60 years (born after 1947; n = 313). Separate analyses were performed comparing individuals who were demented at baseline (prevalent demented) with those who developed dementia at follow-up (incident demented).

Table 1 Characteristics of the study group

	All	Healthy[a]	Demented[b]	Depressive[c]	Epileptic[d]
All	401	130	84	38	34
Sex, n (%)					
women	151 (37.7)	41 (31.5)	32 (38.1)	19 (50)	11 (32.4)
men	250 (62.3)	89 (68.5)	52 (61.9)	19 (50)	23 (67.6)
Age start, mean (SD)	51.6 (5.1)	49.9 (3.9)	53.2 (5.7)	50.3 (3.3)	51.3 (4.5)
Level of intellectual disability, n (%)					
Severe to profound	138 (34.4)	40 (30.8)	34 (40.5)	15 (39.5)	13 (38.2)
Mild to moderate	216 (53.8)	68 (52.3)	46 (54.8)	18 (47.4)	17 (50)
Living situation, n (%)					
Institutionalised	247 (61.6)	79 (60.8)	50 (59.5)	32 (84.2)	22 (64.7)
Community Living	154 (38.4)	51 (39.2)	34 (40.5)	6 (15.8)	12 (35.3)
Dementia status, n (%)					
Prevalent demented	61 (15.2)		30 (35.7)		
Incident demented	79 (19.7)		54 (64.3)		

[a] healthy subjects without dementia or symptoms of depression and/or epilepsy
[b] demented subjects without symptoms of depression and/or epilepsy
[c] depressive subjects without symptoms of dementia and/or epilepsy
[d] subjects with epilepsy, without dementia and/or depressive symptoms

Clinical assessment

At baseline, assessment included an extensive interview with relatives, caregivers and the responsible physician in order to ascertain the medical history and daily functioning. In addition, all subjects had a thorough physical and neurological examination.
The level of premorbid intellectual disability (ID) was derived from the medical records and classified according to the ICD-10[19]. In addition, a diagnosis of AD was made based on the ICD-10 criteria and the guidelines of the IASSID[20]. All subjects were screened annually with a mean follow-up of 4.5 years. Patients, who met the diagnostic criteria of AD at baseline or during follow-up, were monitored every 6 months.
A probable diagnosis of depressive disorder was made by the responsible physician or the consultant psychiatrist. Epilepsy was diagnosed by a neurologist or the responsible physician.
Level of ID and living situation as an expression of general functioning are presented in Table 1.
The study population was divided into 4 subgroups according to the presence of only dementia (n = 84), only depression (n = 38) or only epilepsy (n = 34) and healthy DS individuals (n = 130).The ratio for this sub-classification was that the use of anti-epileptics and antidepressants might bias the results. At baseline, of the 401 DS subjects included in this study, 87 had a diagnosis of epilepsy and 38 of dementia resulting in a group of 276 non-demented subjects at the start of the study.

Biochemical analyses

Plasma amino acids were analyzed by high-performance liquid chromatography (HPLC) using pre-column derivatization with o-phthaldialdehyde phthaldialdehyde[17]. The tryptophan-ratio was calculated by dividing the total tryptophan level times 100 by the sum of the other large neutral amino acids (LNAA), i.e. valine, isoleucine, leucine, tyrosine (Tyr) and phenylalanine (Phe), which compete for the transport of tryptophan through the blood-brain barrier. The tyrosine-ratio was calculated in the same manner by substituting tryptophan for tyrosine. The value of aromatic amino acids (AAA) comprises the summed concentrations Phe, Tyr and tryptophan, and the value of branched chain amino acids (BCAA) is the sum of the concentrations of leucine, isoleucine and valine. The Phe-Tyr ratio was calculated to serve as an estimate of BH4 activity. Plasma concentration of neopterin was determined as described previously[18].
Amino acids could be measured in 401 subjects and neopterin in 394 subjects.

Statistical analysis

Univariate analyses of variance and chi-square analyses were used to determine whether missing data were associated with age, sex, and level of ID; no significant effects were found. To define syndrome specific differences, a subgroup of healthy subjects with DS (n = 130) was compared with a group of 48 age and sex matched healthy controls of the general population. For neopterin, data were available for 28 control subjects. In descriptive analyses, data were reported as means or numbers. Not all data showed normal distribution, and therefore statistical analyses were made using the nonparametric Mann-Whitney *U* test for comparison of groups. When analysed as continuous variables, as in the partial correlation analyses (ρ), variables were natural log transformed. *P* values were corrected for multiple comparisons.

RESULTS

During the follow-up period a total of 80 subjects died. As can be inferred from table 2, values of virtually all amino acids and their ratios as well as of neopterin are significantly different in the group of healthy DS (n = 130) and the demented group (incident and prevalent: n = 140) as compared to normal controls (n = 48). Since the effect of anti-epileptics are restricted to the levels of tryptophan and the tryptophan ratio only, subjects with epilepsy were not excluded from the demented group.

Plasma concentrations of most amino acids are not different in the 4 subgroups (data not shown). Significant differences ($p<0.05$) are found for taurine and phenylalanine in that the levels of both amino acids are increased in the demented group as compared to the healthy group (55.4 ± 12.3μmol/l versus 51.3 ± 12.0μmol/l; p = 0.02 and 62.5 ± 13.1μmol/l versus 58.7 ± 12.7μmol/l; p = 0.05). The increased level of taurine is present mainly in the incident demented group (incident demented: 56.6 ± 12.8μmol/l versus prevalent demented: 49.8 ± 11.8μmol/l; p = 0.003). In the epileptic subgroup only the tryptophan ratio is found to be decreased as compared to the other subgroups.

In the group of prevalent demented subjects, excluding those with epilepsy (n = 38; including 8 depressives), a significantly higher neopterin concentration 28.78 ± 14.4nmol/l versus 24.74 ± 10.3nmol/l (p = 0.05) and a lower Cit-Arg ratio 0.56 ± 0.13 versus 0.64 ± 0.2 (p = 0.006) are found as compared to the group of non-demented subjects (n = 276). In the group of incident demented subjects, a significantly higher Cit-Arg ratio is found as compared to the group of prevalent demented subjects (0.66 ± 0.2 versus 0.56 ± 0.1; p = .003). After adjusting for age, there is a high

Table 2 Values of biochemical parameters in healthy controls (n = 48), healthy persons with Down's syndrome (n = 130) and demented persons with Down's syndrome (n = 140)

parameter	Healthy controls	Healthy persons with Down's syndrome	Demented persons with Down's syndrome
Citrulline µM	34.23 (8.8)	45.89 (13.2)*	45.29 (13.9)*
Arginine µM	68.02 (24.3)	74.49 (21.1)	74.42 (19.3)
Taurine µM	42.88 (7.6)	51.28 (12.0)*	54.31 (13.5)*
Tyrosine µM	71.04 (17.3)	61.00 (15.2)*	62.95 (15.1)*
Valine µM	286.21 (59.7)	231.76 (60.6)*	235.78 (58.3)*
Methionine µM	32.04 (6.4)	24.99 (5.4)*	25.14 (6.7)*
Tryptophan µM	50.38 (9.1)	42.83 (9.4)*	40.38 (10.4)*
Phenylalanine µM	63.06 (9.9)	58.68 (12.7)	60.85 (13.3)
Isoleucine µM	82.69 (24.1)	63.87 (17.0)*	64.61 (16.6)*
Leucine µM	148.48 (31.8)	125.97 (28.4)*	124.31 (27.8)*
Tryptophan ratio	7.89 (1.5)	8.07 (1.4)	7.47 (1.6)#
Tyrosine ratio	11.33 (2.1)	11.82 (2.4)	12.16 (2.5)*
Phe/Tyr ratio	0.9 1 (0.1)	0.98 (0.1)*	0.98 (0.1)*
Cit/Arg ratio	0.55 (0.2)	0.63 (0.2)*	0.62 (0.2)*
Neopterin nM	17.3 (3.6)	23.40 (8.7)*	26.68 (12.1)*#
BCAA µM	517.37 (111.2)	421.60 (103.8)*	424.69 (99.9)*
AAA µM	184.48 (30.2)	162.51 (34.1)*	164.17 (34.8)*
LNAA µM	701.85 (135.2)	584.11 (131.6)*	588.87 (126.3)*

* $p<0.05$ (Mann-Whitney U test); Subjects with DS, healthy and demented, compared with healthy controls

# $p<0.05$ (Mann-Whitney U test); Demented subjects compared with healthy DS subjects

Phe/Tyr ratio is the phenylalanine to tyrosine ratio; Cit/Arg ratio is the citrulline to arginine ratio.

BCAA= Branched Chain Amino Acids (valine + isoleucine + leucine); LNAA=Large Neutral Amino Acids (phenylalanine + tyrosine + tryptophan + valine + isoleucine + leucine); AAA=Aromatic Amino Acids (phenylalanine + tyrosine + tryptophan).

correlation between neopterin and the Cit-Arg ratio in the group of demented subjects: $\rho = 0.48$; $p< 0.000$, but not in the non-demented: $\rho = 0.06$; $p = 0.2$.

From table 3, it can be inferred that there is a significant age effect, in that the concentrations of neopterin and the tyrosine ratio are increased whereas the levels of BCAA, taurine and tryptophan are decreased in the older group.

DISCUSSION

In the present study, including a large sample of DS subjects with or without dementia, plasma concentrations of nearly all biochemical parameters differ significantly from normal controls. The decreased tryptophan ratio is explained most likely by the frequent use of anti-epileptics. Concentrations of neopterin and the tyrosine ratio are increased with age whereas the levels of BCAA,

Table 3 Mean concentrations of amino acids in two birth cohorts of persons with Down's syndrome without epilepsy

parameter	Born before 1947 (n=63)	Born after 1947 (n= 251)	p-value
Citrulline µM	46.71 (16.1)	45.49 (12.7)	0.50
Arginine µM	72.59 (20.2)	74.73 (22.1)	0.62
Taurine µM	49.68 (12.4)	53.22 (12.5)	0.03*
Tyrosine µM	61.05 (19.2)	62.51 (15.2)	0.45
Valine µM	218.32 (75.6)	238.07 (56.4)	0.03*
Methionine µM	25.06 (8.4)	25.36 (5.5)	0.38
Tryptophan µM	39.54 (10.6)	43.70 (9.1)	0.01*
Phenylalanine µM	59.54 (17.3)	61.01 (12.4)	0.64
Isoleucine µM	60.14 (20.6)	65.36 (17.3)	0.04*
Leucine µM	118.68 (35.6)	127.87 (28.1)	0.03*
Tryptophan ratio	7.90 (1.6)	8.01 (1.4)	0.73
Tyrosine ratio	12.59 (2.8)	11.81 (2.4)	0.01*
Phe/Tyr ratio	0.98 (0.1)	0.99 (0.2)	0.82
Cit/Arg ratio	0.65 (0.2)	0.63 (0.2)	0.14
Neopterin nM	28.96 (12.2)	24.27 (10.2)	0.00*
BCAA µM	397.14 (130.1)	431.30 (99.1)	0.03*
AAA µM	159.45 (42.6)	164.78 (34.3)	0.16
LNAA µM	557.27 (166.0)	598.52 (125.7)	0.06

* $p<0.05$ (Mann-Whitney U test)
For abbrevations of Phe/Tyr ratio, Cit/Arg ratio, BCAA, LNAA and AAA see legend to Table 2

taurine and tryptophan are decreased in the older group. These findings are consistent with those reported previously in a small group of healthy DS subjects[16].

With respect to neopterin, a marked increase is found in the total group as compared to normal controls, that further increases in the demented group. In the group of prevalent dementia the enhanced concentration of neopterin is significantly correlated with a decreased Cit-Arg ratio. This finding is suggestive for an increase in oxidative, inflammatory or neurodegenerative processes in dementia. An explanation for a decreased Cit-Arg ratio in this condition may be a reduced bioavailablity of BH4, which is the cofactor for NO synthesis and also for the conversion of phenylalanine to tyrosine. Interestingly, the phenylalanine levels are also decreased in the demented DS subjects as compared to the healthy DS subjects. Possible mechanisms for the impaired bioavailability of BH4 are oxidation of the labile substance BH4 due to the oxidative stress and/or depletion of BH4 due to the higher neopterin synthesis at the expense of BH4[19, 20]. Since the Cit-Arg ratio is significantly higher in the incident demented cases and assuming that this ratio is a good reflection of NO synthesis indeed, it can be hypothesized that the production of NO is enhanced during the early development of dementia.

The plasma concentration of taurine is increased in the demented group especially in the subgroup of incident dementia. The finding of an increased level of taurine may be syndrome specific since similar observations have been reported previously[14, 16, 21]. It has been suggested that taurine may prevent neurotoxicity of beta-amyloid[22] and may decrease beta-amyloid aggregation[23]. It is, however, not clear whether an increase of amyloid precursor protein in DS is associated with increased taurine plasma concentrations.

The BCAA concentration is not different in the various subgroups but decreases with age. Since several decades it has been suggested from animal studies that a lower plasma level of BCAA may result in immune impairment[13, 24]. Whether the observation of diminished BCAA in the group of older DS subjects reflects a syndrome specific impaired immune function is not clear. This may also be the consequence of dietary factors albeit that both in the present study as in the previous one[18] a large difference between healthy controls and DS subjects was found in the BCAA levels.

In conclusion, the results of the present study demonstrate again that plasma levels of nearly all measured amino acids in DS subjects differ substantially from those in healthy controls. Moreover, in demented DS individuals an additional increase of taurine is observed, while during development of clinical dementia changes in levels of biochemical parameters related to NO metabolism and oxidative stress occur.

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Chapter 4

General Discussion

INTRODUCTION

The last decades have shown a distinct trend of improved survival in persons with Down's syndrome (DS)[1]. The life expectancy increased from 9 years of age in 1929 to 12 years in 1949, and 49 years in 1997[2]. In developed countries recent estimates indicate an average age of death of above 50 years[3-7]. Survival in the first year of life has shown particular improvement and for recently born cohorts at least 92% of the children with DS will live to 1 year of age and 90% up to 10 years[5, 8, 9]. Glasson (2002) found that in a follow-up cohort of 1332 persons with DS in Australia, of all ages, approximately 25% of all deaths occurred between the ages of 58 an 63 years[5]. Day et al.[10] investigated mortality and causes of death between 1988 and 1999 in 14781 persons with DS in California. During the study period mortality declined, especially for children with congenital heart defects, due to successful medical treatment[1, 2]. However, no significant decline was observed in adult mortality. The elderly persons with Down syndrome at present, are derived from cohorts, born between 1940 and 1950. This makes it difficult to predict whether the more recently born cohorts will also show an increased mortality of around the age of 60 and requires in depth research. Limited information is available on the timing and the consequence of the comorbidities affecting persons with DS as they age. Conditions as obesity, mobility restrictions, visual and hearing impairment, depression, hypothyroidism, cardiovascular diseases, epilepsy and Alzheimer's disease are known to become increasingly prevalent in later life[11-13], but as yet their effect on mortality risk is not well described. In this thesis we have focused on Alzheimer's disease (AD) as it is reported to be the main predictor of morbidity and mortality in elderly persons with DS.

DEMENTIA

Prevalence of dementia

Alzheimer's disease (AD) is the most important cause of morbidity and mortality among elderly DS persons. The prevalence of clinical AD has been examined extensively[14-35]. Prevalence rates have varied from 4% in a community sample[11] to 88% in an institution-based sample[24]. In our longitudinal population based study, the overall prevalence of dementia was 16.8%. The prevalence of dementia increased from 8.9% in the 45 to 49-age category to 32.1% in the 55 to 59-age category, but dropped in the oldest group (60 years and older) to 25.6% (see chapter 2.1). These results were consistent with other population-based prevalence studies[22, 25, 29, 31, 32, 34-39]. All studies showed an exponential increase in prevalence with age, up to the age of 60 years.

Before interpreting the findings, a methodological issue, which needs to be discussed, is the validity of the diagnosis AD. Although there are now well-accepted criteria for the diagnosis of dementia in adults with intellectual disability[40, 41], which we have used, the diagnosis of dementia remains difficult, both in research setting as well as in clinical practice. For the present study, persons were systematically screened for dementia, annually during follow-up, and examined in person, making differences in diagnostic criteria across patients unlikely. The demented patients in this study met the ICD-10 criteria had an insidious progressive course of the disease. A diagnostic panel discussed all diagnoses. We further, followed all patients, to exclude misdiagnosis such as depression. This is in particular relevant for patients diagnosed with possible dementia. All persons except one progressed in time from a diagnosis of possible dementia to a definite dementia, which underscores the validity of our diagnosis. However, in only a few cases, the diagnosis was confirmed by autopsy.

An interesting finding in our population-based study is that we observed that, despite the exponential increase in prevalence with age, the prevalence of dementia decreases in the oldest group and does not exceed 25.6%. This is substantially lower than expected based on literature[31, 39]. Previous studies varied considerably in the populations studied, design of the study, and in the size of the population studied (see chapter 2.1). Studies conducted with institutionalised patients may have been biased in that they included a selection of more severely intellectually disabled or demented patients. Another major limitation of the previously listed studies is their small size, varying from 50 to 307 subjects. The most important problem is that previous studies only included a small number of elderly DS persons (aged 60 years and above), which limited the interpretation and analysis of the prevalence of AD in the oldest age group. Our population-based study included 118 persons with DS over 60 years, which were examined in person, making it one of the largest studies conducted to date.

There is an ongoing debate on the percentage of DS persons that will eventually develop dementia in old age. Several studies, mostly neuropathological, suggested that all patients develop AD[19, 42-45]. Our study, including persons up to the age of 77 years, strongly argues against a full penetrance of AD. The eldest, those in the age category of 60 and above, are the survivors that escaped dementia. Probably there are other factors, genetic and/or environmental, that protect them against developing dementia before this age. Further research is required to investigate the age-associated prevalence of dementia in persons with DS. Another issue to be examined further will be the incidence of Alzheimer's disease and the relation to morbidity and mortality. In our studies, the number of patients studied was too small to allow valid age specific incidence rates (see chapter 2.1, Table 3). This will be an important target for the near future in our research plan.

Predictors of dementia in Down's syndrome

APOE

In the general population, the apolipoprotein E (APOE) gene on chromosome 19 modulates the risk of AD in some studies[46-48]. The ε4 allele of APOE is associated with an earlier onset age and increased risk of AD. On the contrary the APOE ε2 allele may reduce the risk of dementia in heterozygous carriers. In persons with DS, a large variation of the APOE allele frequencies has been found[49-51], most likely reflecting the different geographic origins. The relationship between APOE ε4 and the risk and onset of AD in persons with DS has been controversial. Some studies showed an increased risk of AD associated with the APOE ε4 allele in persons with DS[52-57], but others could not confirm this[50, 51, 58]. There is evidence that APOE ε2 is associated with a protective effect on AD[33, 59, 60] but this could not be confirmed by all studies[52, 57].
In our study, in 425 persons with DS aged 45 years and older, we found overall a modest but not statistically significant effect of APOEε4 on the prevalence of dementia (Odds Ratio (OR): 1.57, 95% CI = 0.87-2.82). We did not find an effect of APOEε4 on the age of onset of AD. When pooling our data in a meta-analysis with all earlier studies of prevalent patients[35, 50, 52-54, 59-62], the APOEε4 allele shows a significant 1.59-fold, (95%CI = 1.19-2.12) increase in risk of dementia in persons with DS. We divided the meta-analysis up into a " younger age group"[33, 35, 53, 60, 61] and an "older age group"[50, 52, 54, 59, 63]. The effect of APOE ε4 in the younger age group (younger than 50 years) was not significant: OR: 1.49, (95%CI = 0.84-2.65). Only in the older age group there was a significant relation between the presence of the APOE ε4 allele and dementia: OR: 1.62, (95%CI = 1.15-2.29) (see chapter 2.2). In our study population, we did not find a detectable association between APOE ε2 and AD.

The findings of our meta analysis suggest that APOE ε4 determines the prevalence of AD in persons with DS. These findings are consistent with those of the general population[46, 47, 64] where a strong and consistent association between the presence of an APOE ε4 allele and risk for AD has been established. Our population based study and meta-analyses suggest that the effect of APOE ε4, increased the prevalence of dementia in persons with DS, by 1.6 times. APOE ε4 may interact with the amyloid-beta(Aβ) peptide to increase amyloid aggregation. The presence of an APOE ε4 allele was associated with increased plasma levels of Aβ1-42, in both nondemented and demented persons with DS, as described by Schupf et al.[65]. The effect of the APOE ε4 allele on Aβ1-42 levels may be related to acceleration of the rate of amyloid fibril formation.

Age at menopause

Although data are scarce, women with DS appear to reach menopause at an earlier age than women in the general population[56, 66-68]. The results in these studies suggest that the earlier menopause, including the reduction in estrogens following menopause, contributes to the earlier onset and increased risk of AD in women with DS[69-71]. In our prospective study cohort of 85 postmenopausal women, we found that the mean age of menopause is relatively early (44 years) compared to that seen in the general population (51.4 years)[72]. Age at menopause is significantly correlated with age at diagnosis of dementia ($\rho = 0.52$; $p< 0.001$), as well as with risk of dementia: Hazard Ratio (HR): 1.82 (95%CI = 1.31-2.52) (see chapter 2.4). Based on these findings we concluded that age at menopause in women with DS is a determinant of age at onset of dementia.

An important methodological problem is that many women with DS in the Netherlands use oral contraceptives, making it impossible to determine age at natural menopause. Similar to the problems encountered in other studies, it was difficult for many women in our study to pinpoint the age at menopause. Information bias cannot be excluded as we were able to determine the exact age of natural menopause in only 85 (55 %) of the postmenopausal women. It is also possible that selection bias has occurred prior to study entry, since women with a very early menopause and women with an APOEε4 allele may have a higher risk to develop dementia and to die before entry into the study. Nevertheless, our study showed that women with DS have an earlier menopause and that age at menopause is significantly associated with age at diagnosis of dementia.

The early menopause most likely reflects the accelerated ageing process seen in persons with Down syndrome, either in the brain, eye and inner ear, or sex organs and may be related to unrecognized genes overexpressed in the patients due to trisomy. If we use menopause as a read out of biologically determined accelerated ageing, a difference of 7.4 years is expected between women with Down syndrome and those of the general population if all other co-morbidity is treated successfully. The present difference in life expectancy between women with DS and women without DS is, however, much larger (in the Netherlands at present about 12-15 years). The underlying mechanism in the association between early menopause and the risk of dementia is still unclear. As the amyloid deposition, which is the origin of the dementia in DS patients, already starts in the second decade of patients with DS, it is unlikely that menopause or the withdrawal of estrogen itself initiates the deposition of beta-amyloid. More likely, menopause modifies the risk of dementia. There was no significant difference in age at diagnosis of dementia between men and women in the total study population. Both male and female persons with DS show accelerated aging. This raises the question whether there is a similar metabolic change in men that is related to aging. Nevertheless,

an important clinical implication of our findings is that women with DS should be screened for dementia more intensively after menopause, and research on the possibility of using hormone replacement therapy in the elderly women with DS is warranted.

Neopterin

Aging and dementia are accompanied by immune cell abnormalities, and in AD patients a correlation between interferon-γ secretion by mononuclear cells and the AD disease state was found. Immunoreactions in peripheral blood in AD reflect changes occurring in the brain, and may correlate with the clinical stage of the disease[73]. Interferon γ, produced by T-lymphocytes is also capable of inducing the secretion of neopterin from human activated monocyte/macrophages. Recent studies have reported increased concentrations of serum neopterin in AD patients[74-76]. Persons with DS show an altered immune response and an increased susceptibility to AD. Neopterin levels in serum were found to be higher in DS persons than in controls, and the levels were not correlated with age, sex, APOE phenotype, and amyloid β-40 or amyloid β-42[77-79]. We found not only higher neopterin concentrations in persons with DS having many comorbidities, but also in healthy persons with DS compared to controls from the general population[78](see chapter 3.1).
Our study is the first to show a relation between plasma concentration of neopterin and risk of dementia in persons with DS. The demented persons with DS had a significantly ($p = 0.05$) higher plasma neopterin concentration than the non-demented (see chapter 3.2). Compared with the DS without dementia, the prevalent demented persons with DS, and the incident patients who were non-demented at baseline but who developed dementia during the follow-up, had significantly higher plasma neopterin concentrations at the start of the study. After excluding those persons with known autoimmune disorders, the risk of developing dementia was increased by 1.83 times (95%CI = 1.04-3.20) in the subgroup with plasma neopterin levels above median (≥ 21.20nM). High plasma neopterin levels were shown to be an independent determinant of the risk of dementia in persons with DS.

Higher levels of neopterin have been found in patients with inflammatory diseases of the central nervous system, infectious disease and tumors[76, 80]. The higher neopterin levels seen in the DS persons compared with healthy controls[77, 78], as well as the higher neopterin levels seen in the demented persons with DS compared with the nondemented persons with DS are suggestive of a chronic state of cellular immune activation in DS and especially in those with dementia. This finding might have clinical relevance, in that it can be used to predict the risks of dementia in persons with DS or in that the findings can be used to initialize targeted preventive anti-inflammatory treatment of high risk DS persons. Although preventive trials in the general population have been

negative, the question whether this approach of preventive treatment works in persons with DS, who have an extreme high risk of dementia, still remains.

Nitric oxide related amino acids and neopterin concentration

Chronic inflammation has been implicated not only in diseases of the periphery, but also in neurodegenerative disorders such as AD[81]. Microglia have the capacity to secrete neurotoxic substances that directly contribute to neurodegeneration[75]; therefore suppression of microglia activation might be considered a therapeutic goal. As discussed in the previous paragraphs, neopterin is a marker of cell mediated immune activation and increased levels of this substance have been found in AD in the general population as well as in persons with DS. Neopterin is formed as a byproduct of tetrahydrobiopterin (BH4) synthesis and the latter compound is a cofactor for the enzyme nitric oxide synthase, which converts L-arginine into L-citrulline and NO. The ratio of the plasma concentrations of citrulline (Cit)-and arginine (Arg), the so-called Cit-Arg ratio, is regarded as an index of NO synthesis[82] (see chapter 1.1, figure 2).
In our study, plasma concentrations of almost all amino acids in persons with DS differed significantly from those of healthy controls[78]. The production of NO as reflected by an increased Cit-Arg ratio was enhanced during development of clinical dementia. The increased concentration of neopterin found in the group of prevalent dementia is significantly correlated with a decreased Cit-Arg ratio. This finding is suggestive for an increase in oxidative, inflammatory or neurodegenerative processes in dementia. An explanation for a decreased Cit-Arg ratio in this condition may be that there is a reduced bioavailablity of BH4, which is the cofactor for NO synthesis and also for the conversion of phenylalanine to tyrosine. Interestingly, the phenylalanine levels are also increased in the demented persons with DS as compared to those of the healthy persons. Possible mechanisms for the impaired bioavailability of BH4 are oxidation of the labile substance (BH4) due to the oxidative stress and/or depletion of BH4 due to the higher neopterin synthesis at the expense of BH4[83, 84]. Since the Cit-Arg ratio is significantly higher in the incident demented cases and assuming that this ratio is indeed a good reflection of NO synthesis, it can be hypothesized that the production of NO is enhanced during the development of dementia (see chapter 3.3).
Our first findings are promising. Further studies are required to refine and validate the optimal neopterin cut off values in relation to the Cit-Arg ratio, together with clinical assessments, to reach a prognosis of dementia in persons with DS.

PREDICTORS OF SURVIVAL IN PERSONS WITH DOWN'S SYNDROME

Survival in elderly persons with Down's syndrome

Our knowledge of factors influencing survival in older persons with DS is still very limited. Studies including persons with DS older than 60 years of age are rare. Nearly 20% of the persons with DS aged 45 and older suffer from dementia[13, 85] and several studies have shown an increased risk of mortality for persons with DS and dementia or cognitive decline[15, 86, 87]. Cognitive decline has been shown to be associated with mortality in demented and non-demented elderly with and without DS[88, 89]. In addition to age, other factors that have implied an influence on mortality risk in elderly persons with DS are the gene encoding for apolipoprotein E (APOE)[88, 90, 91] and co-morbidity[3].

Cognitive and functional decline

In our longitudinal study, we found, amongst the non-demented participants, that a relative preservation of cognitive and functional ability during follow-up to be associated with better survival, and more rapid decline to be associated with an increased risk of mortality. Although it is difficult to detect a decline in those with the lowest level of performance, this decline, monitored by screening tests even in this group, is related to mortality (see chapter 2.3). Mild Cognitive impairment (MCI) in the general population is defined as an intermediate stage between decline in cognition typical of brain aging and the decline characterizing dementia[92]. In a large number of patients, MCI progresses into AD[93]. MCI in persons with DS (MCI-DS), introduced as diagnostic identity by Jenkins et al[94], can only be ascertained by longitudinal assessments. In our study, in all the non-demented persons, independently of their premorbid intellectual disability, we observed a decrease in cognitive and social functions during the annual assessment periods.
More extensive longitudinal studies are required in order to identify whether this decline is likely to be the result of normal aging in DS, of MCI-DS, or the pre-clinical onset of AD. One of the clinical challenges will be to discriminate between persons with a fast and those with a slower decline and to predict mortality.

The impact of Apolipoprotein E on survival

In the general population, the APOEε4 allele has been related to early mortality in some studies, but the literature is inconsistent[95, 96]. The APOEε2 allele has been associated with increased longevity in some but not in all studies[95, 97, 98]. In persons with DS, an association between the APOEε4 allele and increased mortality in demented[49, 99] and non-demented[57, 90] has been reported, but a

meta-analysis based on data from 538 persons with DS showed no significant evidence for an increased mortality of APOE ε4 homozygotes with DS[100]. As in the general population, there is evidence that APOE ε2 is associated with increased longevity in some studies of DS[33, 53, 101].
Several studies have reported reduced frequencies of the APOE ε4 allele in elderly persons with DS[102, 103], suggesting that the ε4 allele may be associated with early mortality, notwithstanding the presence or absence of dementia. In our study cohort of elderly persons with DS, the frequency of APOEε4 decreased significantly after the age of 60 years (see chapter 2.2). We found an effect of the APOEε4 allele on mortality; the persons with one or two APOEε4 alleles have a 1.64-increased mortality risk (95%CI = 1.01-2.64). However, this increase did not remain significant when adjusting for dementia. The risk remained increased in demented persons as well as particularly in persons with DS without dementia. There was no detectable association between the presence of an APOEε2 allele and mortality (see chapter 2.3). Our results and those of others[57, 90] strongly support the hypothesis that the presence of an ε4 allele in persons with DS is an independent risk factor for early mortality.
Our study focused on elderly persons with DS and we included persons of 45 years and older. We have no information of persons who died before the age of 45 years. The differences in allele distribution by age (and sex) suggest that there may be early differential morbidity. In addition to dementia, APOEε4 is associated with other age related diseases such as cardiovascular diseases, inflammation and infection. The impact of the APOE ε4 allele on early mortality, independent of dementia, needs to be studied.

Co-morbidity and mortality

Many studies have focused on the relationship between dementia and mortality risk. However, only a small minority of these studies control for the confounding effects of comorbid health conditions and demographic characteristics[3, 6, 7, 104]. In our study we found, before the age of 60 years, dementia to be the most important cause of death. After the age of 60, the mortality in those who are demented, as well as those who are not demented, at baseline, is almost the same. These results suggest that, somatic and physical pathology seem to become more important than dementia as causes of death after the age of 60, (see chapter 2.1, figure 3).
In our study cohort, we compared the cause of death in the demented and non-demented separately. Respiratory problems have a significant higher frequency in the demented persons. Cardiac failure was a significant more frequently documented mortality cause in the non-demented persons (Fig.1a,1b, this paragraph). However in one third of those who died, the cause of death could not be determined. This finding requires further clinical and pathological research.

Figure 1a The slices represent the total number of the demented persons who died during follow-up according to their cause of death

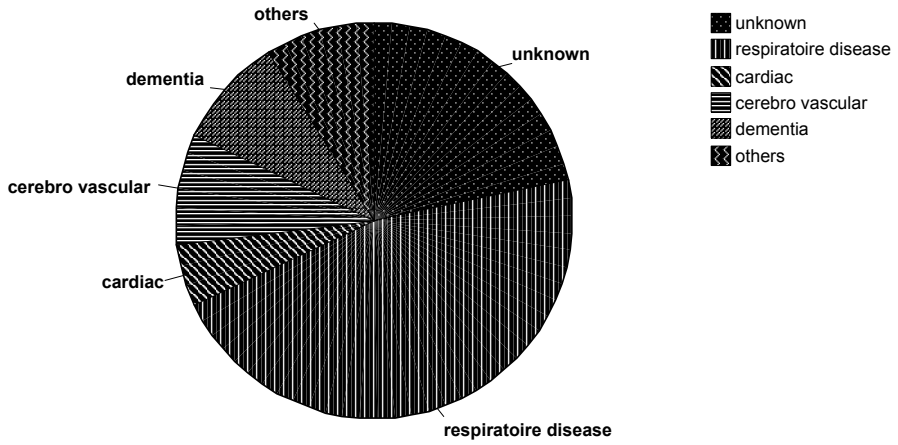
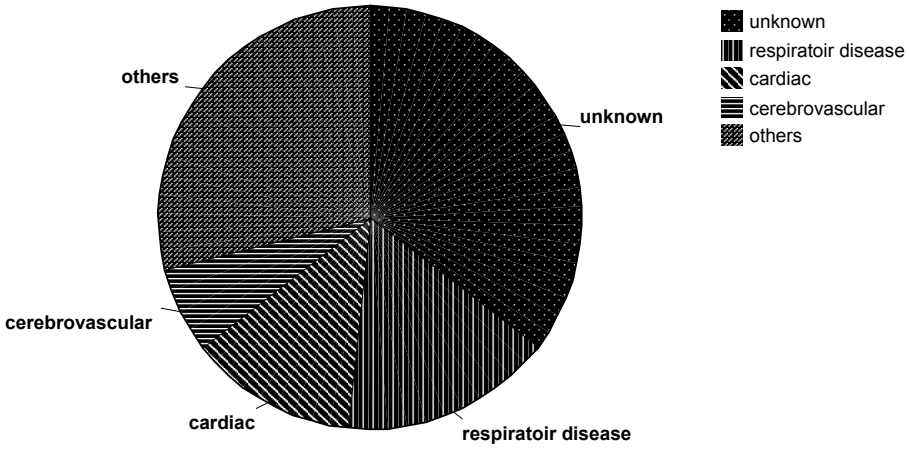


Figure 1b The slices represent the total number of the non-demented persons who died during follow-up according to their cause of death



We found a significant increase in mortality risk for those living in institutions, the more severe intellectual disabled, those with epilepsy and impairments (see chapter 2.3). In contrast to the general population, impaired mobility, severity of intellectual disability, the presence of epilepsy and visual impairment, not cardiovascular risk factors or sex, predicted survival. From a population perspective, one of the most remarkable findings was that sex did not predict mortality. It is most likely that the fact that mortality from cardiovascular disease is not an important cause of death in this population (neither in absolute numbers nor as predictors of mortality) explain this. Particular the finding that mobility is related to mortality opens the question whether physical

training programs are of benefit to patients. Of course, we have to exclude that immobility is not related to morbidity. Our findings, that mobility is significantly associated to survival when adjusting for comorbidity suggests this is not the case.
Clinical symptoms and signs of early disease are easily missed in persons with DS, because of their limited communicative skills. As a result, health problems often go undetected, increasing the morbidity and mortality[104-106]. Routine, systematic screening, using health watch programs for people with DS, is recommended and may minimize the morbidity and long-term complications, thus improving quality of life. Surprisingly little information is available on health care concerns of elderly persons with DS. Future research should be focused on comorbidities and mortality patterns that occur at later life stages.

Gender differences and survival

Whereas the general population shows a clear excess of women in the elderly, the population of elderly DS patients is characterized by an excess of men, which is seen consistently across populations [4, 5, 107, 108]. It is hypothesized that the earlier menopause, including the reduction in estrogens following menopause, contributes to the earlier onset and increased risk of AD and an increased mortality risk in women with DS.
In answer to the question whether there is an association between age at menopause and mortality, we demonstrated that there is a strong and significant correlation between age at menopause and age at death (Pearson $\rho = 0.49$; $p = 0.01$). Also, age at menopause was significantly associated with a 2.05 fold increase in risk of death (95%CI = 1.33-3.16)(see chapter 2.4). In our study it is difficult to determine whether the relation between menopause and mortality is independent of the relation of menopause to dementia. Further it is unlikely that age at menopause alone, contributes to the excess of men in this older age group. In our study, the mean age at death in men (57.7 ± 5.4 years) was not significantly different ($p = 0.61$) from the mean age at death in women (58.3 ± 6.4years). Gender does not predict survival in persons with DS. Most likely this is explained by the fact that mortality due to cardiovascular disease is not an important cause of death in this population, in contrast to the general population where the risk of vascular pathology is increased in men (see chapter 2.3). The APOE ε4 allele was significantly ($p = 0.02$) less frequent in women than in men (20.9 versus 31.1%). One could hypothesize that before study entry, APOE ε4 related diseases are more frequent in women, and increase mortality risk early in life. One may speculate that the early morbidity related to APOE may be related to risk and mortality of inflammation and infectious disorders which typically have a higher frequency in women. Further research will be necessary to understand the complex relationship between sex and life expectancy in this elderly population with DS.

SUGGESTIONS FOR FUTURE RESEARCH

Because of the major advances in care and medical treatment, there has been a steady improvement in the life expectancy and the quality of life for persons with Down's syndrome. However, increased life expectancy has given rise to additional medical and social concerns related to aging of persons with DS. A large number of persons with DS will develop Alzheimer's disease (AD) neuropathology in the brain, and comorbidity associated with accelerated aging. It is very important to understand the factors that contribute to this development. However, not all patients develop dementia and the challenge will be to unravel those factors that protect these patients.

As mentioned earlier, our study is one of the largest population-based studies to date. We found that despite the exponential increase in prevalence with age, the prevalence of dementia in the eldest persons with DS was not higher than 25.6%, which is substantially lower than expected based on literature (chapter 2.1). This finding opens the opportunities for research on, perhaps preventable causes of AD and mortality. Longitudinal measurements, in large population based multicentre cohorts is useful for predicting the early signs of dementia, and the comorbidity increasing the risk of developing dementia. One of the first clinical targets will be to explore the role of (treatment of) comorbidity and to determine the cause(s) of mortality. However, also other avenues exploiting the opportunities in genomics and proteomics should be explored.

There is consensus that the presence of the APOEε4 allele is associated with increased risk of dementia (chapter 2.2). Besides Amyloid Precursor Protein (APP) gene, other genes that are overexpressed by the triplication of chromosome 21, have received less attention in epidemiological studies. Nor has there been interest in genes regulated by chromosome 21 genes. Sequencing of human chromosome 21 and subsequently of its orthologues on mouse chromosome 16 has created an opportunity for exploring the relationship between various DS phenotypes and the extra copy of the genes on chromosome 21[109]. Another very important step will be the identification of the function of the proteins encoded by all the genes on chromosome 21. But also genome wide association studies and studies comparing RNA expression and proteomics profiles of persons that do and do not develop dementia will ultimately bring to light which are the key genes and proteins. As data from functional genomics and proteomics projects grow, correlations among datasets will emerge and may be expected to lead to helpful insights.

Relative preservation of cognitive and functional ability is associated with better survival in this study population and more rapid decline is associated with an increased risk of mortality. (chapter 2.3). Differential diagnostic procedures are needed to discriminate between Mild Cognitive

Impairment in DS (MCI-DS), and preclinical stages of dementia. The use of neuroimaging techniques may make it possible to link the neuropathology and clinical signs to determine which forms of pathology are significant for dementia and to establish diagnostic criteria.

There has been major improvement in the life expectancy of patients with DS. However, the determinants of morbidity and mortality late in life remain far from being understood. Age, dementia and mobility restrictions are the major predictors of survival in elderly persons with DS (chapter2.3). There is a need to study whether physical exercise programs, to promote mobility in the elderly persons with DS, not only may improve their mobility but also their quality of life.

Perhaps the most important and difficult questions from a clinical and ethical perspective, is whether the present available amyloid beta (β) treatment (A β-42 immunization) should be tested in a clinical trial in persons with DS. Although findings in the general population have been disappointing in that the amyloid β removal from the brain in patients with AD does not improve the cognitive function or survival[110]. It is widely acknowledged that a problem in the interpretation of the findings is that the treatment is likely to be effective only early in the disease process. In persons with DS, in whom the amyloid deposition starts already in the second decade, early preventive treatment, using amyloid β withdrawal is of interest. The high risk of patients, in particular in APOE ε4 carriers and the cause of dementia (overexpression of APP) make it likely that powerful trials can be conducted. However, so far ethical discussions have prevented these trials in the USA, this discussion should be opened in the Netherlands. Although there are obvious ethical arguments against using amyloid β treatment in persons with DS, due to known complications. One may also argue that it is not ethical to withhold treatment to this group who, on scientific grounds, may benefit from early treatment.

This thesis, rather serves as a starting point of new research, than as an endpoint. Understanding the factors that contribute to the longevity in DS will be the major challenge for clinical investigators in the coming decade.

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Chapter 5

Summary

Samenvatting

SUMMARY

Accelerated ageing and increased risk of Alzheimer's disease (AD) are characteristics of persons with Down's syndrome (DS). Due to improvements in medical treatment, life expectancy has increased enormously. In the Netherlands, estimates of life expectancy show that 44% of persons with DS will reach the age of 60. However, this has not changed the fact that there is still a lower life expectancy by 15 to 20 years compared with the general population. At present, AD appears to be the most important cause of morbidity and mortality among elderly persons with DS and is the focus of the studies in this thesis.

In **chapter 2.1** one of the largest population-based studies in elderly persons with DS to date is presented. The study was performed in the south and southwest of the Netherlands. In total, 506 persons with DS, aged 45 and above, were recruited between 1 December 1999 and 1 December 2003. All participants were monitored annually, up to the reference date of January 1, 2007. At the time of study entry, each person received a complete assessment including interviews with relatives, carers and the general practitioner. All persons obtained a general physical and neurological examination and, if compliant, a venapuncture. The diagnosis of dementia was based on the ICD-10 Symptom Checklist for Mental Disorders and according to the guidelines produced by the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID). The overall prevalence of dementia was 16.8% (85 cases). There was no significant difference in gender and in the pre-morbid cognitive level between the persons with dementia and those without dementia. Persons with dementia were more often institutionalized. The demented persons also were more frequently treated with anti-epileptic and anti-psychotic drugs. Sensory problems were not associated with dementia in this study population.
The prevalence of dementia increased from 8.9% in the 45 to 49-age category to 32.1% in the 55 to 59-age category, but did not increase above the age of 60, the prevalence of dementia in the eldest persons with DS was not higher than 25.6.

The apolipoprotein E gene (APOE), especially the APOEε4 allele, is consistently associated with dementia in the general population. In **chapter 2.2**, the effect of APOE on dementia in this study was described. There was significant evidence of an APOE ε4 effect on the rate of decline in social and daily living skills. In particular DS persons who were homozygous for APOEε4 showed a larger decrease in function using the SRZ, independent of their pre-morbid level of functioning. We found a modest but not statistical significant effect on the prevalence of dementia. We did observe a significant long-term effect on the incidence of dementia, in the presence of an APOEε4 allele the risk to develop dementia during follow-up was 4,66 times increased. When pooling these

data in a meta-analysis, the APOEε4 allele shows a significant 1.59-fold increase in risk of dementia, indicating that that APOE is influencing the risk of dementia in persons with DS.

Our knowledge of factors that influence survival in older persons with DS is still very limited. In **chapter 2.3**, the study of the impact of cognitive and functional decline and physical comorbidity on mortality risk over time is presented. After a mean follow-up time of 4.5 years (range 0.0-7.6 years), there was no significant difference in the mean age at death between men and women (57.7 versus 58.4 years). Older persons at baseline, those with physical handicaps, those living in the institutions, and those with a severe to profound level of intellectual disability were more likely to die during follow-up. Relative preservation of cognitive and functional ability, as measured using two screening instruments, DMR and SRZ, is associated with better survival in this study population. We found an effect of the APOEε4 allele on mortality. The persons with one or two APOEε4 alleles have a 1.64-increased mortality risk. However, this increase did not remain significant when adjusting for dementia. Finally, using all known risk factors in a multivariate survival analysis, only dementia, age at baseline and a restricted mobility, remained significantly associated with mortality risk.

In **chapter 2.4**, as an indication of the precocious aging in women with DS, age at menopause is described and related to survival and also to age at diagnosis of dementia. We determined the exact age of natural menopause in 85 (55%) of the postmenopausal women. The mean age of menopause is relatively early (44 years) compared to that seen in the general population (51.4 years). Age at menopause is significantly correlated with age at diagnosis of dementia, and is significantly associated with a 1.82-increased risk of dementia. There also is a strong and significant correlation between age at menopause and age at death. Age at menopause was significantly associated with a 2.05-increased risk of death.

Chapter 3 focuses on biochemical pathways involved in the development of dementia in persons with DS. In **chapter 3.1**, a study is described which focussed on peripheral biochemical parameters that reflect changes in homocysteine metabolism, immune function and monoaminergic neurotransmission. The study group comprised 48 healthy persons with DS and was compared with 48 age and sex matched healthy controls from the general population. It was found that nearly all values of amino acids are lower in the persons with DS as compared to controls. With respect to neopterin, values in the DS group are significantly increased as compared to the control group. Furthermore, plasma homovanillic acid (HVA) levels are significantly higher in the DS group, whereas no differences are found regarding 5-hydroxyindoleacetic acid (5-HIAA) concentrations.

In **chapter 3.2**, the relation between plasma concentration of neopterin and (risk of) dementia in persons with DS is presented. Compared with the DS persons without dementia, the prevalent demented persons with DS, and those persons who were non-demented at baseline but who developed dementia during the follow-up, had significantly higher plasma neopterin concentrations at the start of the study. When excluding from the analyses those persons with known autoimmune disorders, the risk of developing dementia was almost doubled in the subgroup with plasma neopterin levels above median ($\geq$ 21.20nM). Our findings suggest that elevated plasma concentration of neopterin in non-demented persons with DS is an independent determinant of the risk dementia.

In **chapter 3.3** we focus on the spectrum of amino acids, the concentration of neopterin, and the role of nitric oxide (NO). Special attention was given to those amino acids, involved in the oxidative processes and their relation to the development of Alzheimer's disease.
The production of NO, as reflected by an increased citrulline/arginine (Cit-Arg) ratio, was enhanced during development of clinical dementia. In the group of prevalent dementia the enhanced concentration of neopterin is significantly correlated with a decreased Cit-Arg ratio. This finding implies an increase in oxidative, inflammatory or neurodegenerative processes in dementia in persons with DS.

Finally, in **chapter 4**, all findings are put into perspective and future prospects and challenges are discussed.

SAMENVATTING

Kenmerkend voor het Down syndroom is het optreden van een vroegtijdige veroudering, en daarmee samenhangend een verhoogd risico op het krijgen van de ziekte van Alzheimer. De levensverwachting van mensen met dit syndroom is de laatste jaren enorm toegenomen, vooral door de verbetering van de medische zorg. In Nederland wordt 44% procent van de mensen inmiddels ouder dan 60 jaar. Desondanks is de gemiddelde levensverwachting 15 tot 20 jaar korter dan die van de mensen in de totale bevolking. In dit onderzoek hebben we ons met name geconcentreerd op de ziekte van Alzheimer omdat dit de belangrijkste oorzaak is van ziekte en sterfte bij ouderen met het Down syndroom.

In **hoofdstuk 2.1** wordt de uitvoering van het onderzoek beschreven.
Alle organisaties, in het zuiden en zuidoosten van Nederland, betrokken bij de zorg voor verstandelijk gehandicapten, werden uitgenodigd om deel te nemen aan dit longitudinale onderzoek. Tussen december 1999 en december 2003 werden in totaal 506 mensen met het Down syndroom bereid gevonden mee te doen in deze studie. Bij de start van het onderzoek kregen alle deelnemers een oriënterend lichamelijk, intern en neurologisch onderzoek en indien mogelijk een bloedonderzoek. Er werden van alle deelnemers uitgebreide gegevens verzameld. Tussen 1999 en 2007 werd jaarlijks contact opgenomen met de begeleiding om, middels vragenlijsten, het functioneren te kunnen volgen.
De diagnose dementie werd vastgesteld aan de hand van de ICD-10 criteria en de richtlijnen zoals die zijn opgesteld door de "the Ageing Special Interest Group of the International Association for the Scientific Study of Intellectual Disabilities (IASSID)". Volgens deze criteria is het belangrijk een achteruitgang in functioneren in de loop der tijd vast te stellen, ongeacht het niveau van functioneren. Deze achteruitgang moet groter zijn dan die welke op grond van leeftijd, en van normale veroudering, te verwachten is.
Bij de aanvang van het onderzoek werd bij 85 deelnemers dementie vastgesteld, dit betekent een prevalentie van 16.8%. Het prevalentie percentage was niet afhankelijk van geslacht en het niveau van functioneren. We stelden vast dat dementerende mensen vaker in een instituut wonen en opmerkelijk meer anti-epileptische- en antipsychotische medicatie krijgen. Er is geen relatie vastgesteld tussen het voorkomen van dementie en de aanwezigheid van zintuig stoornissen.
De prevalentie van dementie, nam toe per leeftijdscategorie; van 8.9% in de categorie 45 tot 49 jarigen, tot 32.1% in de leeftijds categorie 55 tot 59 jarigen. Ondanks deze exponentiele toename in prevalentie per leeftijdscategorie, stelden we een daling in de prevalentie in de leeftijdscategorie boven de 60 jaar vast. In tegenstelling tot wat men zou verwachten was de prevalentie in deze oudste categorie niet hoger dan 25.6%.

In de gewone bevolking wordt het Apolipoproteine E (APOE) gen en vooral het APOEε4 allel geassocieerd met een vier tot acht keer verhoogd risico op het optreden van dementie. In **hoofdstuk 2.2** wordt de studie naar de invloed van het APOE gen op het optreden van dementie bij mensen met het Down syndroom beschreven.
We stelden een duidelijk effect van de aanwezigheid van een APOEε4 allel vast op de mate van achteruitgang tijdens de onderzoek periode. Deelnemers, homozygoot voor het APOEε4 allel, lieten een significant grotere daling in functioneren zien, zoals vastgelegd met behulp van SRZ scores, onafhankelijk van hun intelligentie niveau. In onze onderzoekspopulatie stelden we een effect vast, zij het niet significant, van de aanwezigheid van het APOEε4 allel op de prevalentie van dementie. Na tenminste drie jaar follow-up, blijkt de aanwezigheid van tenminste een APOEε4 allel, een significant, 4.66 keer verhoogd risico te betekenen voor het ontwikkelen van dementie tijdens de onderzoek periode. Tevens wordt er een significant, 1.59 keer verhoogd risico op dementie gevonden, na het samenvoegen van onze data met die van anderen in een meta-analyse. We mogen concluderen dat het APOE gen, ook bij mensen met het Down syndroom, een invloed heeft op het ontwikkelen van dementie.

Alhoewel de levensverwachting van mensen met het Down syndroom enorm is toegenomen, weten we weinig van de factoren die deze levensverwachting beïnvloeden. In **hoofdstuk 2.3** worden de resultaten gepresenteerd van het onderzoek naar de invloed van cognitieve en functionele achteruitgang, alsook de invloed van co-morbiditeit op het percentage van de mensen die sterven tijdens de onderzoek periode. Na een gemiddelde onderzoekstijd van 4.5 jaar (minimum 0.0-maximum 7.5 jaar), werd er geen verschil in leeftijd vastgesteld waarop mannen (± 57.7 jaar) en vrouwen (± 58.4 jaar) overlijden. Mensen waarbij de cognitieve en functionele vaardigheden relatief intact bleven, habben een grotere kans om te overleven. Tevens stelden we vast dat het APOEε4 allel invloed heeft op het risico om te overlijden, de aanwezigheid van een APOEε4 allel, betekent een 1.64 verhoogd sterfterisico. Leeftijd, ernst van de verstandelijke handicap, de leefsituatie (geinstitutionaliseerd in tegenstelling tot wonen in de maatschappij), de dementie status, epilepsie, mobiliteit, visusstoornissen en de aanwezigheid van een APOEε4 allel zijn significant geassocieerd met het risico om te overlijden. Wanneer we in één model (multivariate survival) deze significante factoren tegelijkertijd bestuderen, zien we dat er slechts drie factoren overblijven, namelijk leeftijd, dementie, en een beperking in de mobiliteit, die van invloed zijn op een groter sterfte risico.

Een vroege menopauze zou men kunnen zien als een symptoom van een versnelde veroudering. In **hoofdstuk 2.4** beschrijven we de resultaten van een onderzoek naar de relatie tussen de leeftijd van menopauze en de leeftijd waarop dementie is gediagnosticeerd en de leeftijd waarop de vrouwen

met het Down syndroom in deze studie overlijden. Vrouwen met het Down syndroom komen eerder in de menopauze (gemiddeld op een leeftijd van 44 jaar) dan vrouwen in de gewone bevolking (gemiddeld 51.4 jaar). De leeftijd waarop de menopauze begint blijkt significant gecorreleerd te zijn met de leeftijd waarop de dementie werd vastgesteld en een vroege menopauze (jonger dan 45 jaar) is geassocieerd met een 1.82 verhoogd risico op het krijgen van dementie. De leeftijd waarop de vrouwen sterven is eveneens significant geassocieerd met de leeftijd waarop de menopauze begint en een vroege menopauze betekent een 2.05 verhoogd risico om te overlijden tijdens de onderzoeksperiode. Deze vroege menopauze zou veroorzaakt kunnen worden door een versnelde veroudering, ofwel in de hersenen, in de geslachtsorganen, dan wel door overexpressie van genen ten gevolge van de trisomie van chromosoom 21. Het is duidelijk dat een vroege menopauze samenhangt met een eerder optreden van dementie, een hoger risico op het krijgen van dementie en mogelijk hieraan gerelateerd een hoger sterfte risico.

Hoofdstuk 3 beschrijft een aantal biochemische processen die mogelijk een rol kunnen spelen bij het ontwikkelen van dementie bij mensen met het Down syndroom.
In **hoofdstuk 3.1** richten we ons op perifere biochemische parameters die betrokken zijn bij het homocysteine metabolisme, de immuun functie, en de monoaminerge neurotransmissie. Een groep van 48 gezonde mensen met het Down syndroom, werd vergeleken met een op leeftijd en geslacht gematchte controle groep, uit de algemene bevolking. Het spectrum van aminozuren in de Down syndroom groep laat significante afwijkingen zien in vergelijking met de controle groep. Het meest opvallend zijn de verhoging van de taurine en de verlaging van de methionine, wat kan wijzen op neurodegeneratieve processen. De concentratie van neopterine, een pteridine, die geassocieerd wordt met de cellulaire immunrespons, blijkt hoger te zijn in de groep van mensen met het syndroom van Down in vergelijking met de controle groep. Homovanilline zuur is verhoogd, wat zou kunnen samenhangen met een toegenomen dopamine turnover.

In **hoofdstuk 3.2** beschrijven we de relatie tussen de plasma concentratie van neopterine en het ontwikkelen van dementie tijdens de onderzoek periode. De prevalent demente deelnemers en degene die dementie ontwikkelden tijdens de onderzoek periode hebben een hogere plasma neopterine concentratie dan de niet demente deelnemers. Wanneer we de deelnemers met een autoimmuun aandoening buiten beschouwing laten, blijkt het risico op het ontwikkelen van dementie in de groep met een boven gemiddelde neopterine concentratie ($\geq$21.20nM) bijna te verdubbelen. Een hoge plasma concentratie van neopterine lijkt een marker te zijn voor een verhoogd risico op dementie.

In **hoofdstuk 3.3** richten we onze aandacht opnieuw op het spectrum van aminozuren, de concentratie van neopterine en tevens op de rol van nitriet oxide in relatie tot de ontwikkeling van dementie. We bestudeerden met name de aminozuren die betrokken zijn bij de oxidatieve processen in relatie tot de ontwikkeling van de ziekte van Alzheimer. De productie van nitriet oxide, weergegeven door de citrulline/arginine ratio, is toegenomen in de groep van de mensen die dementie ontwikkelden tijdens de onderzoek periode. In de groep van de prevalent dementen blijkt de verhoogde neopterine concentratie significant gecorreleerd aan een verlaagde citrulline/arginine ratio. Uit deze bevindingen mogen we concluderen dat er sprake is van toegenomen oxidatieve, inflammatoire en neurodegeneratieve processen gedurende het ontwikkelen van dementie bij mensen met het Down syndroom.

In **hoofdstuk 4** tenslotte, zijn alle bevindingen besproken en in perspectief geplaatst. Vooruitzichten voor klinisch onderzoek, onderzoek naar de co-morbiditeit en de mogelijke behandeling, worden beschreven.

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About the author

Tonnie Coppus, MD, is specialised in intellectual disability medicine.
She commenced her medical training at the University of Gent in Belgium and completed her studies at the University of Nijmegen, the Netherlands in 1985. During her study, she participated in a research project on Myotone Dystrophy initiated by the Department of Clinical Genetics. After her study, she worked in the practice of the renowned psychiatrist dr. Anna Terruwe. This was followed by a residency at Youth Health Care in Helmond. She subsequently was trained in Forensic Medicine and specialised in sexual abuse. In 1987, she started her training in General Practice at the Radboud University in Nijmegen and registered as a general practitioner in 1988.
Initially starting out as a general practitioner, she soon after accepted a position, which enabled her to work with the intellectually disabled in 'Maria Roepaan' in Ottersum. In 1992 she formed a team, to identify and to prevent sexual abuse among intellectual disabled persons. She participated in publications and lectures concerning sexuality and sexual abuse amongst the intellectually disabled. Currently, she is involved in several projects concerning this group and their wish to have children.
In 1999, she started the research described in this thesis at the Genetic Epidemiology Unit of the Department of Epidemiology & Biostatistics (Prof.dr.C.M. van Duijn), in close collaboration with the Department of General Practice, Intellectual Disability Medicine (Prof.dr.H.M. Evenhuis), the Department of Psychiatry (Prof.dr.W.M.A.Verhoeven) and the Department of Neuroscience (Dr.D. Fekkes) of the Erasmus Medical Center in Rotterdam. In 2005 she obtained a Master of Science degree in Genetic Epidemiology at the Dutch Institute of Health Sciences. Since 2007 her interest in the field of scientific research has led her to participate in the "Consortium VG Oost" at the University of Nijmegen.
In 2007 she initiated a specialised multi-disciplinal outpatient department, for persons with Down's syndrome, aged 18 years and older at the Elkerliek Hospital.
Meanwhile she continues her work as a medical doctor in an organisation specialised in the care of intellectually disabled persons in 'Dichterbij' in Gennep. She is also committed to training medical doctors in the field of intellectual disability medicine at the Erasmus University in Rotterdam.
Tonnie Coppus is married to Benoit de Leest and is mother of two children, David en Jozefien.

Echt waar

Ik ben een mongooltje
Heb spleetogen
Korte armen
Korte voetjes
Kleine handjes
Klein gezichtje
Een beetje dik
Een beetje slank
Ben heel lenig
Heb meestal
Altijd goeie zin
En soms niet

Dat is allemaal
Echt waar
Heel echt
allemaal

Marian Peters