Down & Alzheimer: Introduction to a Forced Marriage

& Dissertation Outline
The observations of John Down

In 1866, the British medical doctor John Langdon Haydon Down published his influential report *Observations on an Ethnic Classification of Idiots* (Down, 1866). Down was superintendent of the *Royal Earlswood Asylum for Idiots* in Redhill, a town south of London. His report was devoted to “the Mongolian type of idiocy” that he observed in more than ten percent of “the large number of idiots and imbeciles which come under my observation, both at Earlswood and the out-patient department of the [London] Hospital.” Down was interested in classifying the feeble-minded, and formulated a new ethnic or racial classification of idiocy.

“A very large number of congenital idiots are typical Mongols. So marked is this, that when placed side by side, it is difficult to believe that the specimens compared are not children of the same parents. The number of idiots who arrange themselves around the Mongolian type is so great, and they represent such a close resemblance to one another in mental power, (...) it is difficult to realize he is the child of Europeans, but so frequently are these characters presented, that there can be no doubt that these ethnic features are the result of degeneration.”

— J. L. H. Down, 1866

Down described various common physical and behavioural characteristics and portrayed the typical facial appearance, including straight and scanty hair, obliquely placed eyes, elasticity-deficient skin, and a long, thick and roughened tongue. Moreover, he elaborated on their sense of humour, imitation skills and ability to speak. He noted that the disability was present from birth (congenital), and rightfully recognized their learning capabilities: “The improvement which training effects in them is greatly in excess of what would be predicted if one did not know the characteristics of the type.”

Down’s ethnic classification would be regarded racist these days, and his wording would be considered abusive, if not offensive. However, Down’s report must be seen within the context of that time: mid-nineteenth century colonial times in which the evolution theory of Darwin came up (Darwin’s *On the Origin of Species* was published in 1859) and the descriptive terms of idiots and imbeciles were commonly used (Ward, 1999). Historian David Wright comprehensively described the history of Down syndrome and how the attitudes have changed over time in his book *Downs: The history of a Disability*. Indeed, he stated that “Down was clearly trying to bridge his own research on the mental diseases of children to Darwinian evolutionary theory, attempting to find a science of the mind that was relevant to specialists in idiocy” (Wright, 2011). In fact, Down was a great advocate for people with intellectual disabilities at the time: he argued that idiot asylums should be separate from lunatic institutions, since the latter were adapted for psychiatric patients rather than the intellectually disabled (Wright, 2011).

In his article, Down stated that he believed that tuberculosis was the hereditary origin of the Mongolian type of idiocy: “They are, for the most part, instances of
degeneracy arising from tuberculosis in the parents” (Down, 1866). Down was wrong, but it took almost a century before the real cause was elucidated. Whereas the Dutch geneticist Petrus J. Waardenburg already suggested in 1932 that mongolism was due to a chromosomal abnormality (Allen, 1974), its cause was not revealed until 1959. In that year the French geneticists Jérôme Lejeune and Marthe Gauthier discovered an additional third copy of chromosome 21 (trisomy 21) in mongoloid individuals, i.e. 47 chromosomes rather than the 46 chromosomes present in the general population (Lejeune et al., 1959).

From mongolism to Down syndrome
Since Down’s report in 1866, the term mongolism was increasingly being employed and by the beginning of the 20th century it was widely used. By the 1960s the term slowly fell into disfavour. The discovery of trisomy 21, the increasing participation of Asian researchers, and the new understanding that mongolism was not a racial condition, brought a group of renowned scientists to write a letter to the editor in The Lancet in which they urged to replace mongolism with another term (Allen et al., 1961; Ward, 1999):

“Some of the signers of this letter are inclined to replace the term ‘mongolism’ by such or designations as ‘Langdon-Down anomaly’, or ‘Down’s syndrome or anomaly’ or ‘congenital acromicria’. Several other signers believe that this is an appropriate time to introduce the term ‘trisomy 21 anomaly’ which would include cases of simple trisomy as well as translocations. It is hoped that agreement on a specific phrase will soon crystalize if once the term ‘mongolism’ has been abandoned.”

— G. Allen et al., 1961

Moreover, the Republic of Mongolia complained at the World Health Organization about the use of Mongolian idiot. Although the francophone countries favoured trisomy 21, the term Down syndrome came into fashion worldwide – a century after Down’s publication (Megarbane et al., 2009; Ward, 1999; Wright, 2011). From the French perspective, the use of Down syndrome was not satisfactory at all. It did not only ignore the more recent (French) discovery of trisomy 21 as the biological cause, but also gave credits to the Englishman Down for his pioneering work, while Jean-Etienne Esquirol, psychiatrist at the Salpêtrière hospital in Paris, already described the characteristic appearance long before Down had done. In his book Des maladies mentales considérées sous les rapports medical, hygiénique et medico-légal, published in 1838, Esquirol had devoted a special section to idiocy, including a phenotypical description of a group of patients that we would retrospectively refer to as having Down syndrome. A description that Edouard Séguin would elaborate on in greater detail in 1846, twenty years before the publication of Down (Megarbane et al., 2009; Roubertoux and Kerdelhué, 2006; Wright, 2011). Despite these historical and linguistic issues, Down syndrome – without the possessive apostrophe (Down’s) that is used in the British spelling – has become the international standard. Therefore, this dissertation will consistently use Down syndrome, abbreviated as DS.
Prevalence and incidence of Down syndrome

DS is present in approximately six million people worldwide (Ballard et al., 2016) and occurs in approximately 1 in 650-1000 live births (Bittles et al., 2007; Parker et al., 2010). Commissioned by the Dutch Ministry of Health, Welfare and Sport (VWS), the independent research organization TNO published a report on congenital deficits in The Netherlands based on the national perinatal registry by the Stichting Perinatale Registratie Nederland. The most recent prevalence data in that report concern the year 2013 with 13.5 DS per 10,000 births (i.e. 1 in 740 live births), which comes down to 230 children born with DS in that year (Schönbeck et al., 2015).

Genetic origin

DS is the most common genetic cause of intellectual disability (Bittles et al., 2007). Trisomy 21 is caused by failed separation of chromosomes during meiosis (nondisjunction), and is predominantly of maternal origin: Antonarakis and colleagues have demonstrated that among 200 cases of trisomy 21, the nondisjunction was maternal in 188 cases, paternal in 9 cases and unknown in 3 cases (Antonarakis et al., 1992). Importantly, the chance of having a child with DS is strongly related to the age of the mother (Loane et al., 2013). Although 93-95% of DS individuals has a triplication of the entire chromosome 21 (whole chromosome trisomy 21), partial trisomies 21 occur as well. In particular, mosaicism (i.e. some, but not all cells have three copies of chromosome 21) is present in 1-3% of cases, whereas 3-4% of the cases concerns a translocation (i.e. two copies of chromosome 21, and a part of the third copy is attached to another chromosome, mostly chromosome 14) (Antonarakis, 1998; Bittles and Glasson, 2004). The dosage imbalance hypothesis subsequently states that certain triplicated chromosome 21-encoded genes are dosage sensitive, contributing to the DS phenotype, while other triplicated genes are not dosage sensitive and as such do not contribute to the phenotypical features of DS (Antonarakis et al., 2004).

Phenotype

DS individuals are characterized by a range of medical and physical features (Grieco et al., 2015). Their characteristic physical appearance is evident, including the short stature, broad hands with relatively short fingers, hypotonia, ligaments laxity, brachycephaly and craniofacial dysmorphic features (e.g. flattened nose, slanted eyes with epicantic folds and open mouth), most of which were already described in Down’s report in 1866 (Contestabile et al., 2010; Down, 1866; Zigman, 2013).

“The hair is not black, as in the real Mongol, but of a brownish colour, straight and scanty. The face is flat and broad, and destitute of prominence. The cheeks are roundish, and extended laterally. The eyes are obliquely placed, and the internal canthi more than normally distant from one another. The palpebral fissure is very narrow. The forehead is wrinkled transversely (...) The lips are large and thick with transverse fissures. The tongue is long, thick, and is much roughened. The
The skin has a slight dirty yellowish tinge, and is deficient in elasticity, giving the appearance of being too large for the body.”

— J. L. H. Down, 1866

Next to their distinctive facial appearance, people with DS are more prone to have congenital hearing loss, heart defects or ophthalmic impairments, and should thus be screened for this after birth (Roizen and Patterson, 2003; Tedeschi et al., 2015). Other phenotypical (medical) features of DS are the frequent occurrence of autism spectrum disorder, hypothyroidism, coeliac disease, obesity, haematological disorders and leukaemia, seizures (appearing during infancy (first peak) and advanced age (second peak)), obstructive sleep apnoea, and dementia due to Alzheimer’s disease (Grieco et al., 2015; Roizen and Patterson, 2003).

The most remarkable feature of DS concerns the reduced cognitive capacities, i.e. the intellectual disability. Various cognitive domains are affected, including learning, memory, language and executive function. IQ is strongly reduced in DS, ranging between 30 to 70 (Vicari et al., 2004). Learning skills are reduced for both short-term and long-term memory, in particular for explicit memory tasks. Verbal working memory and explicit long-term memory are generally impaired, while visuospatial short-term memory and implicit long-term memory are spared. Verbal learning is worse relative to visual learning. Deficits in information encoding and retrieval are thought to be central to the learning and memory problems, which is further aggravated by attentional problems in DS. Language deficits in articulation, use of multi-word phrases, morphosyntax, phonology and language comprehension have been reported as well (Contestabile et al., 2010; Grieco et al., 2015; Lott and Dierssen, 2010).

**Intellectual disability**

The term idiot, now regarded a pejorative term, dates back a long time. It is derived from Greek, meaning ‘layman’, and became widely used in the nineteenth century (Wright, 2011). After Binet and Simon had developed their intelligence test at the beginning of the twentieth century, a new measure (IQ score) could be used to categorize the ‘feeble-minded’. Those with the highest degree of mental deficiency were referred to as ‘idiots’, those with an intermediate degree as ‘imbeciles’ and those with a marginal degree as ‘morons’ (Dutch: debielen) (Detterman, 2010; Doll, 1957). These terms were commonly used in the twentieth century, but started to be misused as terms of abuse and were slowly replaced. It took until 2001, however, until ‘idiot’, ‘imbecile’ and ‘moron’ were also removed from official government documents in The Netherlands (Trouw redactie binnenland, 2001). This linguistic circle of specific terminology falling into disfavour and getting replaced by a new term, which again gets disfavoured after a number of years etc. is ongoing. For instance, feeble-minded became mental deficiency, which, in turn, was replaced by mental retardation, that has now been abandoned in favour of intellectual disability (Detterman, 2010).

Regardless of the terminology, how do we define an intellectual disability? IQ scores two or more standard deviations below the mean, i.e. IQ scores lower than 65-75,
are indicative of an intellectual disability, though clinical judgement is required to interpret the results from standardized, validated testing of intelligence performance (American Psychiatric Association, 2013). Intellectual disability, however, is much more than IQ alone. Currently, three definitions for intellectual disability are internationally used: the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) (World Health Organization, 2016), the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) (American Psychiatric Association, 2013), and the American Association on Intellectual and Developmental Disabilities (AAIDD) (The AAIDD Ad Hoc Committee on Terminology and Classification, 2010). The core of the three definitions is cited in Box 1.

**BOX 1: INTELLECTUAL DISABILITY**

**ICD-10: Mental retardation**
“A condition of arrested or incomplete development of the mind, which is especially characterized by impairment of skills manifested during the developmental period, skills which contribute to the overall level of intelligence, i.e. cognitive, language, motor, and social abilities. Retardation can occur with or without any other mental or physical condition.”
— World Health Organization, 2016

**DSM-5**
“Intellectual disability (intellectual developmental disorder) is a disorder with onset during the developmental period that includes both intellectual and adaptive functioning deficits in conceptual, social, and practical domains.”
— American Psychiatric Association, 2013

**AAIDD**
“Intellectual disability is characterized by significant limitations both in intellectual functioning and in adaptive behaviour as expressed in conceptual, social, and practical adaptive skills. This disability originates before age 18.
— The AAIDD Ad Hoc Committee on Terminology and Classification, 2010

The DSM-5 and AAIDD definition already refer to intellectual disability, whereas the ICD-10 (the original version dating back to the 1990s) still employs the term mental retardation. However, ICD-10 is due to be replaced by the new ICD-11 in 2018, and the World Health Organization has already provided a preliminary beta draft online version of the new ICD-11, which refers to ‘disorders of intellectual development’ (World Health Organization, 2017). The ICD and DSM define four levels intellectual disability: mild, moderate, severe and profound. The AAIDD definition focuses on an individuals’ need for support and does not distinguish these four categories of severity. In The Netherlands and
Europe, this categorization into four severity levels is commonly employed in health care institutions and clinical practice, and, therefore, also employed in this dissertation. In contrast to previous classifications based on IQ scores in the ICD-10 and DSM-IV-TR, severity is no longer defined by IQ in the new ICD-11 and DSM-5, which classify the severity based on someone’s level of adaptive functioning instead. Indeed, it is the adaptive functioning that determines the required level of support for an individual (American Psychiatric Association, 2013). Table 1.1 depicts these levels of severity, including the old terminology, as well as the IQ scores and their corresponding mental ages.

Table 1.1: Categorization of the severity of intellectual disability

<table>
<thead>
<tr>
<th>Severity level</th>
<th>Old terminology</th>
<th>IQ*</th>
<th>Mental age**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Moron</td>
<td>50/55 – 70</td>
<td>9 – 12 yr</td>
</tr>
<tr>
<td>Moderate</td>
<td>Imbecile</td>
<td>35/40 – 50/55</td>
<td>6 – 9 yr</td>
</tr>
<tr>
<td>Severe</td>
<td>Imbecile</td>
<td>20/25 – 35/40</td>
<td>3 – 6 yr</td>
</tr>
<tr>
<td>Profound</td>
<td>Idiot</td>
<td>&lt;20/25</td>
<td>&lt;3 yr</td>
</tr>
</tbody>
</table>

*Based on ICD-10 and DSM-IV-TR; **Based on ICD-10

The Netherlands Institute for Social Research (Sociaal en Cultureel Planbureau, SCP) has estimated a prevalence of intellectual disability (IQ<70) of 0.85% in the Netherlands, i.e. approximately 142,000 people (Woittiez et al., 2014), which is slightly less than the overall reported prevalence of intellectual disability of 1% worldwide (Maulik et al., 2011; Rijksinstituut voor Volksgezondheid en Milieu, 2017). Among the intellectually disabled population in developed countries, 12-15% concerns DS (Bittles and Glasson, 2004).

**Dementia**

In addition to the intellectual disability which is present from a young age onwards, there is an extremely high risk that intellectual and adaptive functioning of people with DS will deteriorate later in life as a consequence of dementia due to Alzheimer’s disease.

![Figure 1.1: Schematic illustration of the core clusters of dementia symptoms.](image)

The non-cognitive symptoms are jointly referred to as Behavioural and Psychological Symptoms of Dementia (BPSD).
Dementia is clinically diagnosed in 50-80% of DS individuals by the age of 60-70 (Wiseman et al., 2015; Zigman and Lott, 2007) compared to 11% in the general, non-intellectually disabled population aged over 65 years (Alzheimer’s Association, 2016). It is important to distinguish intellectual disability from dementia: intellectual disability concerns an individual’s baseline level of functioning, whereas dementia is a significant decline from that level.

Dementia is a clinical syndrome, i.e. a combination of various clinically recognizable signs and symptoms. In general, these symptoms can be subdivided into three clusters: decline in cognitive symptoms, non-cognitive symptoms and activities of daily living (Figure 1.1). Like criteria for intellectual disability, diagnostic criteria for dementia are provided in the DSM and ICD handbooks. Whereas DSM-IV-TR referred to dementia, the DSM-5 now refers to a major neurocognitive disorder. Box 2 cites the core description of dementia in ICD-10 (World Health Organization, 2016) and major neurocognitive disorder in DSM-V (American Psychiatric Association, 2013). In general, a loss of function in multiple cognitive domains from a previously higher level, should interfere with daily life and does not occur in the context of delirium or another mental disorder.

**BOX 2: DEMENTIA**

**ICD-10: Dementia**

“Dementia is a syndrome due to disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Consciousness is not clouded. The impairments of cognitive function are commonly accompanied, and occasionally preceded, by deterioration in emotional control, social behaviour, or motivation.”

— World Health Organization, 2016

**DSM-5: Major neurocognitive disorder**

A. “Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual-motor, or social cognition).

B. The cognitive deficits interfere with independence in everyday activities (i.e., at a minimum, requiring assistance with complex instrumental activities of daily living such as paying bills or managing medications).

C. The cognitive deficits do not occur exclusively in the context of a delirium.

D. The cognitive deficits are not better explained by another mental disorder (e.g., major depressive disorder, schizophrenia).”

— American Psychiatric Association, 2013
This overarching dementia syndrome may be caused by various underlying diseases (Alzheimer’s Association, 2016):

- Alzheimer’s disease (AD)
- Vascular dementia (also known as multi-infarct or post-stroke dementia)
- Dementia with Lewy bodies (DLB)
- Mixed dementia
- Frontotemporal lobar degeneration (frontotemporal dementia, FTD)
- Parkinson’s disease dementia
- Creutzfeldt-Jakob disease
- Normal pressure hydrocephalus

In 60-80% of the patients, AD is the underlying cause for the dementia. Mixed dementia is characterized by the presence of hallmarks from two or more types of dementia, most often AD + DLB, or AD + vascular dementia (Alzheimer’s Association, 2016). Importantly, dementia in DS is nearly always of the Alzheimer type (explained below).

**Alzheimer’s disease**

In 1906, the German physician Alois Alzheimer gave a lecture in the city of Tübingen on a new form of dementia. He described Auguste D, a 51-year-old woman who had presented, amongst others, progressive cognitive decline, aphasia, disorientation, behavioural changes (including hallucinations and delusions) and psychosocial incompetence. After nearly five years of disease she died in April 1906. Alzheimer studied her brain for pathology and reported the histopathological findings which included ‘fibrils’ and ‘small miliary foci’. Emil Kraepelin, director of the Royal Psychiatric Clinic in Munich, where Alzheimer started working in 1903, subsequently coined the term ‘Alzheimer’s disease’ in his *Handbook of Psychiatry* in 1910. Although AD was initially used to refer to presenile dementia, it came into later use for senile dementia as well (Maurer et al., 1997). The revised diagnostic guidelines from the workgroup of the American National Institute of Aging and the Alzheimer’s Association, published in 2011 (McKhann et al., 2011), are commonly used to define dementia due to AD. Box 3 cites the specific criteria for dementia due to probable AD (in a shortened version).

Evidently, the patient needs to meet the criteria for dementia (all causes), as well as the specific AD criteria mentioned in Box 3. McKhann and colleagues defined the criteria for dementia (all causes) in their revised guidelines as well. Whereas these criteria strongly resemble the DSM-5 criteria (Box 2), they do not only focus on cognitive symptoms, but more specifically stress the importance of behavioural (neuropsychiatric) symptoms. Indeed, “changes in personality, behaviour, or comportment” are explicitly described: “symptoms include: uncharacteristic mood fluctuations such as agitation, impaired motivation, initiative, apathy, loss of drive, social withdrawal, decreased interest in previous activities, loss of empathy, compulsive or obsessive behaviours, socially unacceptable behaviours.” (McKhann et al., 2011).
BOX 3: PROBABLE ALZHEIMER'S DISEASE DEMENTIA

National Institute on Aging-Alzheimer’s Association diagnostic guidelines
“Probable AD dementia is diagnosed when the patient meets criteria for dementia and has the following characteristics:

A. Insidious onset. Symptoms have a gradual onset over months to years, not sudden over hours or days.
B. Clear-cut history of worsening of cognition by report or observation.
C. The initial and most prominent cognitive deficits are evident on history and examination in one of the following categories:
   a. Amnestic presentation
   b. Non-amnestic presentations:
      i. Language presentation
      ii. Visuospatial presentation
      iii. Executive dysfunction
D. The diagnosis of probable AD dementia should not be applied when there is evidence of (a) substantial concomitant cerebrovascular disease, defined by a history of a stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) core features of DLB other than dementia itself; or (c) prominent features of behavioural variant FTD; or (d) prominent features of semantic variant primary progressive aphasia or non-fluent/agrammatic variant primary progressive aphasia; or (e) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.”

— McKhann et al., 2011

Behavioural and psychological symptoms of dementia (BPSD)
It goes without saying that AD comprises more than memory loss only. Behavioural changes are common to the disease as well, generally referred to as Behavioural and Psychological Symptoms of Dementia (BPSD), or neuropsychiatric symptoms in a narrower sense. Sanford Finkel defined BPSD as “a heterogeneous range of psychological reactions, psychiatric symptoms, and behaviours resulting from the presence of dementia” (Finkel, 2001). Virtually all AD patients in the general population present one or more BPSD at some point (Gauthier et al., 2010). BPSD are burdensome, both for patients and their caregivers (Finkel, 2000), schematically illustrated in Figure 1.2.
Chapter 2 extensively introduces BPSD and comprehensively summarizes the available literature on BPSD in DS. Chapter 3 describes the development and first validation of the novel BPSD-DS scale in a large well-characterized DS cohort.

Prevalence and incidence of AD dementia

The impact of dementia worldwide is enormous. According to the World Alzheimer Report 2015, 46.8 million people presented dementia around the globe in 2015, a number expected to increase to an unimaginable 131.5 million by 2050 (Prince et al., 2015). Zooming in on AD, the most common cause of dementia, the Alzheimer’s Association provides annual figures on prevalence (existing cases) and incidence (new cases) for the USA, which may more closely resemble the situation in the Western world than global figures. In 2016, a prevalence of 5.4 million AD patients was reported (USA inhabitants: 325 million), among which 200,000 younger than 65 years of age. AD is present in respectively 11% of the population older than 65 and in 32% of the population older than 85 years. The incidence data show the epidemic nature of the disease even better: every minute (every 66 seconds) someone develops the disease in the USA (Alzheimer’s Association, 2016). Due to improved medical care, the population as a whole is ageing, and in particular the relatively large baby boom generation is growing old. Together, this causes the total number of elderly individuals to increase strongly. As age is the major risk factor for AD, the number of AD cases will rise strongly as well to an estimated 13.8 million by 2050 in the USA (Alzheimer’s Association, 2016).

In The Netherlands (17 million inhabitants), Alzheimer Nederland (2016) estimates that more than 270,000 individuals have dementia, of which 70% is due to AD. Every 15 minutes, someone develops the disease. 12,000 dementia patients are younger than 65 years of age. The ageing population causes the prevalence of dementia to double in the upcoming two decades, reaching an expected half million patients in 2040, and even increase further to a peak of 690,000 patients in 2055 (Alzheimer Nederland, 2016). Evidently, AD is reaching epidemic proportions.

Neuropathology

Alois Alzheimer described ‘small miliary foci’ and ‘fibrils’ in the brain of Auguste D. Indeed, AD is characterized by two key neuropathological hallmarks: extracellular deposition of amyloid beta (Aβ) into plaques (“small miliary foci”), and intracellular neurofibrillary tangles (“fibrils”) (comprehensively reviewed in: Schellenberg and Montine, 2012; Serrano-Pozo et al., 2011; Van Dam et al., 2016). In short, Aβ peptides are generated by
cleavage of the transmembrane amyloid precursor protein (APP) mediated by secretase enzymes. α-secretase, cleaves APP in the middle of the Aβ sequence, generating non-amyloidogenic peptides. β- and γ-secretase (γ-secretase being a protein complex of nicastrin, Aph-1, Pen-2 and presenilin (PSEN) 1 or 2) cleave at the terminal sides of the Aβ sequence, yielding aggregation-prone Aβ peptides that finally accumulate into extracellular plaques. The predominant species are Aβ1-40 and Aβ1-42, the latter being the most aggregation-prone. Aβ aggregation conveys toxicity leading to synaptic damage and neurodegeneration (Van Dam et al., 2016). In addition to deposition in plaques in the brain parenchyma, Aβ also deposits in vessel walls. This so-called cerebral amyloid angiopathy (CAA) is present in approximately 80% of AD patients, and may weaken the vessel walls causing microbleeds or, if severe, haemorrhage (Serrano-Pozo et al., 2011). The most marked feature of neurofibrillary degeneration, on the other hand, are the so-called neurofibrillary tangles, composed of the paired helical filaments consisting of hyperphosphorylated microtubule-associated protein tau. Tau is physiologically involved in microtubule assembly and stability. In hyperphosphorylated state, however, tau affects stability, causing the microtubules to disintegrate, thereby impairing neuronal functioning (Schellenberg and Montine, 2012; Van Dam et al., 2016; Van Dam and De Deyn, 2006).

Both pathological hallmarks accumulate progressively in the brain in specific regional patterns. The spreading of Aβ plaques was described and classified into five phases in (Thal et al., 2002). Braak comprehensively described the sequence of neurofibrillary pathology into six stages (Braak et al., 2006; Braak and Braak, 1991). Neuritic plaques are a subset of senile plaques characterized by the presence of degenerated neuronal processes (tau-positive dystrophic neurites) – associated with neuronal injury. The Consortium to Establish a Registry for AD (CERAD) developed a protocol for the scoring of the density neuritic plaques: sparse, moderate, frequent (Mirra et al., 1991). In 2012, the National Institute on Aging and the Alzheimer’s Association published the new revised consensus guidelines for the neuropathologic evaluation of AD (Montine et al., 2012).

Table 1.2: Minimum recommended brain regions for ‘ABC scoring’ adapted from Montine et al. (2012).

<table>
<thead>
<tr>
<th>Region</th>
<th>A (Aβ plaques)</th>
<th>B (NFTs)</th>
<th>C (Neuritic plaques)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle frontal gyrus</td>
<td>1°</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Superior and middle temporal gyri</td>
<td>1°</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>1°</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Occipital cortex (BA 17/18)</td>
<td>Consider</td>
<td>Yes</td>
<td>Consider</td>
</tr>
<tr>
<td>Hippocampus and entorhinal cortex</td>
<td>2°: if 1° is +</td>
<td>Yes</td>
<td>Consider</td>
</tr>
<tr>
<td>Basal ganglia at level of anterior commissure</td>
<td>2°: if 1° is +</td>
<td>Consider</td>
<td></td>
</tr>
<tr>
<td>with basal nucleus of Meynert</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain including substantia nigra</td>
<td>3°: if 2° is +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar cortex and dentate nucleus</td>
<td>3°: if 2° is +</td>
<td></td>
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These guidelines comprise the so-called ‘ABC score’, which classifies the AD neuropathologic change in paraffin-embedded coupes along three parameters: Aβ plaque scores (Thal), Braak staging of neurofibrillary pathology and CERAD scores for neuritic plaques. In these new guidelines, a four-point scale is used, thus reducing the five Thal
phases and the six Braak stages to four for improved inter-rater reliability. The minimum recommended brain regions required for proper ‘ABC scoring’ are presented in Table 1.2. The obtained ‘ABC scores’ are finally converted into a ‘Not’, ‘Low’, ‘Intermediate’ or ‘High’ classification of AD neuropathologic change. ‘Intermediate’ and ‘High’ entail a diagnosis of AD (Montine et al., 2012).

In the general population, we distinguish two types of AD: early-onset familial AD (EOAD, presenile dementia) that originates before the age of 65 with a relatively rapid progressive decline, and late-onset sporadic AD (senile dementia) that originates after the age of 65 with a relatively slow progressive decline (World Health Organization, 2016). The familial form is an autosomal dominantly inherited form, caused by mutations in APP, PSEN1 or PSEN2 genes, in 7 out of 10 cases (Rao et al., 2013). An estimated 1% of the AD cases concerns this familial form. Inheriting such a mutation virtually guarantees someone to develop the disease (Alzheimer’s Association, 2016). In contrast, sporadic AD has no clear familial cause and develops later in life. Several risk factors have been implicated, such as ageing, cardiovascular risk factors, and polymorphisms of the apolipoprotein E (ApoE) gene. A person with one or two ε4 alleles has an increased risk compared to someone without an ε4 allele, while the ε2 allele is associated with a lower risk for AD (Alzheimer’s Association, 2016; Schellenberg and Montine, 2012).

In addition to amyloid and tau pathology, mounting evidence implicates neuroinflammation in AD, which has been comprehensively reviewed in (Heneka et al., 2015; Wilcock, 2012). Aβ, for instance, activates microglia, the resident immune cells in the brain, causing local upregulation of pro-inflammatory cytokines and chemokines (Heneka et al., 2015; Van Dam et al., 2016).

In Chapter 6 the ABC scoring system has been applied for the neuropathological diagnosis of brain samples from DS individuals, EOAD patients and non-DS controls. Chapter 8 studied the pro-inflammatory marker Neutrophil Gelatinase-Associated Lipocalin (NGAL) in serum of DS individuals with/without dementia.

Alzheimer’s disease in Down syndrome: a forced marriage

To understand the high risk for AD in DS, we need to go back to the cause of DS: trisomy 21. Importantly, the APP gene is encoded on chromosome 21. The triplication of chromosome 21 (and thus of this gene) causes an overproduction of the APP protein in people with DS from birth onwards. Subsequently, APP is cleaved into Aβ peptides, thus also increasing the levels of ‘sticky’ Aβ peptides, which aggregate and eventually accumulate into plaques. The formation of amyloid plaques was found to start with deposition of Aβ1-42, already found in the brain of a 12-year-old child with DS. Plaques including Aβ1-40 were not found until approximately 30 years of age. At this age, the first neuritic plaques were observed as well (Lemere et al., 1996). Although tau is not encoded on chromosome 21, the presence of tau pathology becomes very evident in the third and fourth decade of life (Mann, 1988). Indeed, it appears that amyloid pathology triggers the pathological cascade in AD: Aβ is upstream of tau, and promotes the formation of the pathological, toxic form of tau (Bloom, 2014; Jack et al., 2010). Toxic tau, on the other hand, also influences Aβ and is – in part – required for Aβ toxicity in vivo (Bloom, 2014).
The essential role of APP and amyloid pathology for AD in DS is further emphasized by a case study of an elderly DS individual with a partial trisomy 21 lacking the third copy of the APP gene. The neuropathologic examination of this 78-year-old woman without evident clinical signs of dementia revealed hardly any AD-related neuropathology. Immunohistochemical staining for amyloid and tau was largely normal with only a limited number of immunoreactive tangles and senile plaques (Prasher et al., 1998). Therefore, the increased risk for AD in DS is generally attributed to the triplication of the APP gene. A vast majority of DS individuals has this third copy, thus inevitably facing progressive deposition of AD pathology: a forced marriage between DS and AD.

Importantly, post-mortem studies demonstrated that AD pathology is omnipresent in the brain of virtually all DS individuals from the age of 40 years onwards (Mann, 1988). However, the time window between the presence of pathology and the onset of clinical symptoms of dementia is highly variable (Zigman and Lott, 2007). As previously described, 50-80% of the DS population has developed dementia by the age of 60-70 years. In other words, a substantial number of DS individuals grows old free of dementia or dies before symptoms appear, despite extensive neuropathology being present for twenty to thirty years already. What makes the one DS individual more vulnerable, i.e. develop clinical symptoms already at young age, and what conveys protection or delays the onset of symptoms in the other? Understanding this inter-individual variability is like the Holy Grail in DS-AD research. This variability strongly complicates daily care for people with DS.

How can we differentiate between cognitive and behavioural changes related to normal ageing, dementia or other causes? Predicting and monitoring (the onset of) dementia in DS is of great importance for professional and familial caregivers to understand the change and adapt their daily caregiving. Moreover, early identification of those at risk allows for early therapeutic interventions. Although we can currently not prevent or cure AD, the study of novel drugs is ongoing and promising results have already been reported (Sevigny et al., 2016). This dissertation aims to contribute to predicting and monitoring dementia in DS by studying BPSD, as well as by investigating the potential of monoamine neurotransmitters associated with BPSD as biomarkers for AD in DS.

Life expectancy
Since the mid-twentieth century, life expectancy of people with DS has increased tremendously thanks to improved medical care. Particularly, respiratory infections are common in DS and the introduction of antibiotics in the 1950s has caused a reduction in infection-related mortality. Moreover, 48% of DS individuals presents congenital heart deficits, and the development of surgical correction of these deficits has strongly reduced mortality in the early years of life (Bittles and Glasson, 2004; Carfì et al., 2014). Consequently, DS life expectancy has increased from twelve years in 1949 (Penrose, 1949) and 35 years in 1982 (Thase, 1982) to an actual average life expectancy of 61.1 years for men and 57.8 years for women (Glasson et al., 2003). The lower life expectancy of females relates to early menopause and increased proneness to heart deficits (Coppus et al., 2010; Glasson et al., 2003). Given the fact that ageing is the major risk factor for dementia
(Alzheimer’s Association, 2016), ageing has thus caused dementia to become very evident in this population.

Dementia is now recognized as a major challenge in contemporary care for elderly individuals with DS. The diagnosis of dementia in this population is complex, and we are in need of tools that aid the diagnostic procedure. Surprisingly, despite billions of euros going to dementia research in the general population, only limited resources are available to study this devastating disease in DS. Due to the high risk to develop the disease, people with DS should occupy a privileged position in the context of dementia research. Nothing is further from the truth, unfortunately. The number of studies enlisted in the medical database PubMed serves as an indicator of this unequal distribution of scientific resources: a search for ‘Down syndrome + dementia’ yielded 58 hits in the database for the year 2016, while ‘dementia’ itself resulted in 9081 hits over that same year, demonstrating less than 1% of all dementia studies concern DS. Despite the extremely high risk to develop AD dementia in DS, provocatively many questions remain unanswered that require research. Understanding AD in DS, however, does not only contribute to the benefit of the DS population, but likely aids the understanding of (the role of amyloid in) AD in the general population as well since DS is a human amyloid overexpression ‘model’.

**Diagnosis of AD in DS**

The diagnosis of AD dementia in people with DS is complicated by the presence of a (variable degree of) intellectual disability, pre-existing behaviour and co-morbidities. To identify change, one needs to compare an individual’s premorbid or baseline level of functioning with the current level of functioning. People with DS, especially those with a more severe level of intellectual disability, are most often not able to describe their own internal state (emotions, feelings) and have limited or no understanding of relatively complex behavioural concepts, i.e. they will hardly/not be able to reflect on their own behaviour (Smiley and Cooper, 2003; Sturmey et al., 1991). Consequently, establishing someone’s baseline level of functioning is essential to know whether specific symptoms have always been characteristic for the person before any (dementia-related) change occurred. The Dutch guideline *Dementie in Beeld* urges to establish this baseline level before the age of 40 years in people with DS (Dautzenberg et al., 2005).

The diagnosis of dementia is in many regards still a diagnosis of exclusion. A variety of other disorders may mimic dementia symptoms, sometimes referred to as reversible dementias or pseudo-dementias, and thus need to be excluded in advance. Importantly, most of these disorders are reversible with treatment in contrast to the neurodegeneration underlying true AD dementia that cannot be prevented or stopped yet (Prasher, 2009). The list of conditions that need to be excluded within the context of a differential dementia diagnosis in DS includes:

- Cerebrovascular accident
- Delirium
- Depression – may also co-occur with dementia as BPSD
- Epilepsy
- Hearing impairment
• Hypothyroidism
• Medication intoxication
• Pain
• Sleep apnoea
• Visual impairment
• Vitamin B12 deficiency

For example, thyroid problems are common in DS with reported prevalences ranging from 35% to up to 73% (Carfì et al., 2014; Prasher, 1995). In the large majority of individuals it concerns hypothyroidism, which is associated with decreased motivation and energy (apathetic symptoms) and reduced cognitive functioning (Prasher, 2009). Hypothyroidism is relatively easy to test by determining the levels of thyroid hormones in a blood sample, and can be treated successfully with levothyroxine, thereby reversing the dementia-mimicking symptoms.

Unlike hypothyroidism that can be objectively tested, many other conditions are fairly complex to differentiate from possible dementia. Pain, for instance, is undertreated in DS due to difficulties recognizing it and the reduced tendency of DS individuals to complain about it (de Knegt, 2015). Likewise, depression is rather complex within the context of AD in DS. Depression and dementia may occur independent of each other, but can also occur together. Since both diseases have symptoms in common, and depression can negatively affect cognition, it is not unlikely that clinicians misdiagnose depression as dementia (Dekker et al., 2015b; Prasher, 2009).

To diagnose dementia, all these different causes of decline need to be ruled out first. A differential diagnostic procedure preferably adopts a multidisciplinary approach with expertise from different angles, including intellectual disability physicians (Dutch: arts voor verstandelijk gehandicapten, AVG), (neuro)psychologists (Dutch: orthopedagoog, GZ-psycholoog) and informants (professional caregivers, relatives), and, optionally, speech therapists, opticians/ophthalmologists, and medical specialists (neurologists, geriatricians, psychiatrists etc.).

Nevertheless, the differential diagnosis in DS remains difficult in numerous situations. Objective biomarkers for AD in DS would thus be of great added value for more sensitive and specific diagnoses. In the general population, the so-called ‘AD profile’ in CSF (low levels of Aβ42 and high levels of total tau (t-tau) and phosphorylated tau (p-tau)) strongly contributes to diagnosis (De Deyn, 2015). This CSF ‘AD profile’ has barely been investigated in DS, and the quest for alternative biomarkers continues. One promising avenue concerns altered monoaminergic neurotransmission, which is associated with BPSD.

Chapter 5 argues to investigate the clinical utility of the CSF ‘AD profile’ in the (differential) diagnosis of AD in DS.

**Monoamine neurotransmitters**
Alterations in neurotransmitter systems may, in part, be responsible for the cognitive and behavioural symptoms in AD (Lanari et al., 2006). The major neurotransmitters in the
central nervous system are categorized into three groups: cholinergic, monoaminergic and amino acid neurotransmitters. This dissertation focuses on the alterations in monoamine neurotransmitters since they have been frequently associated with BPSD (Herrmann et al., 2004; Lanari et al., 2006; Lacotot et al., 2001; Mitchell et al., 2011; Vermeiren et al., 2014a, 2014b). The monoaminergic system includes the three catecholamines dopamine (DA), noradrenaline (NA, also known as norepinephrine) and adrenaline (epinephrine), as well as the indolamine serotonin (5-HT). Mounting evidence links structural and functional changes in the monoaminergic system to AD pathophysiology (comprehensively reviewed in: Trillo et al., 2013).

Monoamines are predominantly produced by cells located in the brain stem, i.e. mesencephalon (midbrain), pons and medulla oblongata, and innervate a wide range of (sub)cortical structures. DA is primarily produced by cells of the substantia nigra and ventral tegmental area in the mesencephalon, and has been implicated in cognition, emotion, reward and the regulation of motor activity (Lanari et al., 2006; Nieoullon, 2002; Trillo et al., 2013). NA is produced from DA, and its major production site constitutes the locus coeruleus (LC) in the pons. Noradrenergic innervation of a large number of areas mediates amongst others, attention, arousal, and contextual memory (Aston-Jones and Cohen, 2005; Trillo et al., 2013; Vermeiren et al., 2016). Finally, the ascending serotonergic neurons predominantly arise from the dorsal and median raphe nuclei throughout the brainstem, and play a role in e.g. (behavioural) inhibition, and the regulation of aggression and mood (Lacotot et al., 2001).

Neurodegeneration in AD does not spare monoaminergic cells. Indeed, various studies have shown a degeneration or loss of monoaminergic cells, and altered concentrations of monoamines and metabolites in AD patients as compared to controls (reviewed in: Šimić et al., 2017; Trillo et al., 2013). The monoaminergic system is not only affected in AD, but also in DS. An increasing body of evidence suggests that people with DS present altered levels of one or more monoamines in serum/plasma (Coppus et al., 2007; Dekker et al., 2015a), CSF (Kay et al., 1987; Schapiro et al., 1987) and multiple brain regions (Godridge et al., 1987; Reynolds and Godridge, 1985; Risser et al., 1997; Whittle et al., 2007; Yates et al., 1981) as compared to non-DS controls.

The role of monoamine neurotransmitters in the pathophysiology of AD, and BPSD in specific, is further supported by the positive behavioural effect of various drugs acting on the monoaminergic system. For instance, risperidone, an atypical antipsychotic drug that has a balanced antagonistic effect on serotonergic (5-HT2) and dopaminergic receptors (D2) (Leysen et al., 1994) was found effective in reducing various BPSD in AD patients in the general population, including aggression, agitation and psychosis-associated symptoms (De Deyn et al., 2005, 1999; Rabinowitz et al., 2007; Sultzer et al., 2008).

Monoamine neurotransmitters and metabolites are studied in serum (Chapter 4), (paired) CSF/plasma samples and post-mortem brain tissue (Chapter 6) of DS individuals with/without dementia, as well as in DS mouse models (Chapter 7).
This dissertation adopts the behavioural perspective. BPSD, which may cause severe burden on patients and their environment, are at the center of the work presented here. In addition to the behavioural changes themselves, alterations in the monoamine neurotransmitter systems associated with BPSD are studied in detail.

Chapter 2 introduces BPSD, extensively reviews the existing literature on BPSD in DS and identifies gaps in knowledge. Moreover, we describe the (clinical) need for a comprehensive evaluation tool to systematically assess BPSD in DS, i.e. disentangling someone’s characteristic behaviour from behavioural changes possibly related to the presence of dementia.

Subsequently, Chapter 3 describes the multidisciplinary development and first validation of the novel BPSD-DS scale in one of the largest behaviourally characterized DS cohorts worldwide including 281 DS individuals with dementia (DS+AD), with questionable dementia (DS+Q) and without dementia (DS). In an interview setting, twelve behavioural sections in the BPSD-DS scale were completed with key informants of the DS individuals. Substantial changes in frequency and severity of various behavioural items were found. Overall, the proportion of individuals with a frequency or severity increase was highest in the DS+AD group, intermediate for DS+Q individuals and lowest in the DS subgroup. Which specific items changed most prominently? Chapter 3 provides the detailed results per behavioural section, as well as reports the first data on the validity and reliability of the scale.

Chapter 4 explores alterations in monoamine neurotransmitters and metabolites in serum of 151 demented (DS+AD), converted or non-demented DS individuals. By quantifying (nor)adrenergic, serotonergic and dopaminergic compounds, a series of monoaminergic alterations were found between the groups. Remarkably, serum MHPG levels were strongly decreased in DS+AD, but also in the converted DS individuals. This arose our interest in MHPG as potential biomarker to monitor and predict conversion to AD in DS. Following this exploratory study, we subsequently aimed to replicate and validate these results and understand the underlying causes.

Blood biomarkers are subject to (confounding) peripheral effects. In contrast, CSF is in direct contact with the extracellular space in the brain. Accordingly, CSF likely reflects biochemical changes in the brain better than blood. Whereas CSF studies are very common in AD in the general population, only few studies have been conducted in DS. Chapter 5 summarizes these results, discusses the clinical utility of the CSF ‘AD profile’ (low Aβ42, high t-tau and p-tau) in the diagnostic process, identifies issues hampering the implementation of lumbar punctures in DS, and makes a plea in favour of offering CSF analyses to this population at high risk for AD.

Would MHPG levels be altered in CSF? And in post-mortem brain samples? Chapter 6 studies regional monoaminergic differences in one of the largest collections of brain samples obtained from elderly DS cases with (n=17) and without (n=4) a neuropathologic diagnosis of AD, as well as EOAD patients in the general population and healthy non-DS controls. Moreover, (paired) CSF/plasma samples of the largest DS cohort
to have undergone CSF sampling are examined, comparing three diagnostic study groups: DS, DS+prodromal AD (resembling the aforementioned DS+Q classification) and DS+AD. Do the CSF/plasma results confirm the findings in serum (chapter 4) and in brain? Chapter 6 provides the answer.

In human material we cannot experimentally interfere in biological processes to further mechanistically study the altered monoaminergic system in DS. To that end, animals models are of great importance. Chapter 7 explores whether Ts65Dn mice, the most widely used mouse model for DS, also display the alterations found in the human situation. Monoamine changes in Ts65Dn mice versus their wild-type littermates are reported, specifically focusing on the effect of ageing in on monoaminergic neurotransmission these mice.

Chapter 8 addresses another core aspect of AD, neuroinflammation. Previously, the pro-inflammatory Neutrophil Gelatinase-Associated Lipocalin (NGAL) has been associated with AD in the general population, and serum levels were found to increase significantly with ageing in DS. Therefore, this chapter investigates whether NGAL levels differ between demented, converted or non-demented DS individuals using serum samples from the same Rotterdam cohort as described in Chapter 4.

In Chapter 9, we describe a promising avenue to normalize the effects of the third copy of the $APP$ gene, namely epigenetic editing. Since the discovery of trisomy 21, genetic studies have vastly increased our understanding of DS. However, it remains complex to explain the substantial inter-individual variability among DS individuals. Epigenetic mechanisms regulate gene expression, and may (in part) explain the large gene expression variation. Mounting evidence suggests aberrant epigenetic marks in DS, but comprehensive epigenetic studies in this population are still in its infancy. Interestingly, epigenetic marks are reversible, possibly offering new therapeutic strategies to, for example, target $APP$ overexpression, in DS. The technique of epigenetic editing is of great interest in this respect, as it enables specific epigenetic modification, e.g. repression, of a gene of interest. Currently, we have no means to dissolve the forced marriage between DS and AD. Normalizing the expression of $APP$ in DS (at a very early stage) through epigenetic editing might offer a novel way to realize this divorce.

Chapter 10 (summary, general discussion & future implications) recapitulates the core findings of this dissertation. Whereas the specific aspects of each individual study are contextualized and discussed in detail in the respective chapters, this last chapter focuses on overarching interconnections, addresses general strengths and limitations and describes future implications.

References


