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"Epigallocatechin-3-gallate combined with cognitive training in young adults with Down syndrome Phase II clinical trial: Important considerations for treatment- efficacy evaluation on cognitive and functional improvement."

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Tesis Doctoral- 2016

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DEPARTAMENTO DE FARMACOLOGÍA, DE TERAPÉUTICA Y DE TOXICOLOGÍA

UNIVERSIDAD AUTÓNOMA DE BARCELONA (UAB)

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Memoria presentada por Laura del Hoyo Soriano para optar al título de doctora por la Universidad Autónoma de Barcelona. Trabajo realizado bajo la dirección del Dr. Rafael de la Torre Fornell y del Dr. Magi Farré Albaladejo, en el grupo de Farmacología Integrada y Neurociencia de Sistemas (FINS) del IMIM-Institut Hospital del Mar d'Investigacions Mèdiques. Programa de Doctorado en Farmacología de la Universidad Autónoma de Barcelona.

Dr. Rafael de la Torre Fornell   Dr. Magi Farré Albaladejo   Laura del Hoyo Soriano
(Co-Director de tesis)   (Co-Director de tesis)   (Doctoranda)

A mis padres.

Por enseñarme a tener los pies en el suelo y la cabeza en las nubes,

A esperar siempre lo mejor y a estar preparada para lo peor.

Sin vosotros no existiría esta tesis.

Agradecimientos

Han sido más de cuatro años llenos de emoción: emoción por aprender, por descubrir, por imaginar, por soñar. Emoción por equivocarme, frustrarme, volver a empezar, que me corrijan y entonces aprender de nuevo.

Me siento la persona más afortunada del planeta por haberme embarcado en esta tesis doctoral y que eso pague mis facturas. Pero aunque la suerte no existe para nadie, estar en el sitio adecuado, en el momento adecuado, pero sobre todo con las personas adecuadas ha sido crucial:

En primer lugar querría agradecer al Dr. Magí Farré, por abrirme la puerta. Fuiste tú quien me propuso en aquella clase de Bellaterra doctorarme en el IMIM y algo que jamás me habría planteado fue cobrando forma poco a poco. Primero fueron las prácticas en el ensayo clínico NEUROTEGCG, y tras medio año llegó la oportunidad: tenía la beca de 4 años, no me lo podía creer. Cada vez que pienso en los inicios, pienso en ti, sin llaves no se abren puertas y tú confiaste en mí.

Al Dr. Rafael de la Torre, muy especialmente, por la codirección de esta tesis. Gracias por todas las oportunidades durante estos 4 años, gracias por hacer posible el ensayo TESDAD y por dejarme ser yo misma, por confiar en mí, por darme vía libre para indagar y molestar a todo el mundo, incluyéndote a ti. Pero sobre todo gracias por llevarme a París, tus tres ángeles de Charlie no olvidarán jamás aquel paseo en barco por el Sena. Ha sido un verdadero placer.

A la Dra. Mara Dierssen, por todo el tiempo que me has dedicado sin ser estudiante a tu cargo oficialmente. Me has enseñado muchísimo, sobre todo a ser crítica y a ser más dura. A ver las cosas desde otra perspectiva cuando yo sólo veía una. Amas lo que haces, sientes pasión y lo contagias. Gracias.

A Su (lo he cambiado), porqué gracias a ti conseguí despegar en su día. Me enseñaste a buscarme la vida, a ser independiente, a volar. Eres una gran profesional y persona, alguien en quien confiar. Si hago balance de estos cuatro años me quedo con todo lo positivo, no lo dudes. Mil Gracias.

A Aida (JLo), porqué tú me enseñaste a "batallar" en la vorágine diaria, siempre mano a mano. Has sido mi persona desde el momento en que llegué, siempre a mi lado: en lo bueno, en lo malo, siempre ahí, algunas veces en modo avión, pero siempre con cobertura. Hemos vivido grandes momentos, muchos inolvidables. Contigo he reído, llorado… pero sobretodo he disfrutado. He aprendido infinidad de cosas de ti en todas sus dimensiones posibles. Eres genial.

A la mitad de esta tesis, la grande e inminente doctora Laura Xicota. Eres increíble, una persona transparente en todos los aspectos. Contigo he compartido grandes momentos de risas y frustración, hemos llorado aunque nunca a la vez. Tenemos la gran capacidad de balancearnos la una a la otra: cuando tú estás mal yo estoy bien y viceversa, quizás ha sido una estrategia inconsciente de supervivencia, pero amiga mía ha sido efectiva. Me encantas y espero poder trabajar contigo en el futuro porqué ha sido mágico.

A Cris Llop por tu generosidad diaria. Eres de esas personas que cuesta encontrar, siempre tienes tiempo para los demás (aunque no lo tengas físicamente) siempre estás ahí, cuando alguien tiene un problema (aunque tu tengas ocho mil en ese momento), siempre estás ahí. Eres un ejemplo de trabajo en equipo, eres una gran compañera y amiga. Cada vez que pienso en ti, me acuerdo del momento chimenea en la Centraleta: tan cálida como tú.

A Joan Rodríguez por su inestimable trabajo en toda la coordinación del ensayo TESDAD, desde el reclutamiento de los voluntarios hasta la

programación de las visitas, las revisiones médicas y otras miles de millones de cosas, otro gran ejemplo de compañerismo y trabajo en equipo. Pero sobre todo gracias por dejarme conocerte mejor. Atlanta fue inolvidable aunque se nos fue la olla con las compulsiones en todos sus posibles variantes patológicas, qué duro fue dejar de ser VIP!

I would also like to thank Eli de Souza: the author of the wonderful cover of this thesis. It was a real pleasure to meet you and your stay at the IMIM brought an air of good cheer for everyone.

A Judit Sánchez por su trabajo desde la plataforma FESKITS, eres una crack.

A *Santa* Klaus porqué si un día llegas al IMIM y te encuentras un busto con tu cara y una placa conmemorativa con tu nombre, qué no te extrañe, somos muchos los que te estamos eternamente agradecidos.

A Gonzalo por ser tan paciente, te gusta enseñar y se nota. He aprendido mucho contigo. Gracias.

A todo el grupo de Farmacología Integrada y Neurociencias de Sistemas, porqué siempre estáis dispuestos demostrar vuestro apoyo en los momentos de cambio. Sois un gran equipo, siempre con una sonrisa.

A Laura Forcano por transmitir tanta paz.

A todo el equipo de ensayos clínicos porque de vosotros he aprendido la rigurosidad en el trabajo. En especial a Esther y Clara porqué siempre habéis estado para echar una mano con lo que fuese. A mi muy señor Julián, por hacerme reír cada vez que te veo, espero que la fuerza te acompañe haya donde vayas.

A todo el equipo de Neuroimagen, sobre todo a Laura Blanco, vales millones.

A Alessandro Principe por dejarme formar equipo contigo y por valorar mi trabajo, ojalá salga bien lo que tenemos "en la nube". De ti también he aprendido.

A Laia Roca porque me ayudaste muchísimo con la memoria para solicitar la beca (aún recuerdo tu cara cuando me dijiste que era mía), desde entonces nunca has dejado de ayudarme cada vez que lo he necesitado.

A la Dra. Vicky Puig por tus consejos y tutorías de cara al Post Doctorado, espero volver a pedirte ayuda pronto porqué significará que hay buenas noticias.

A todos y cada uno de los chicos que han participado en este estudio y a sus familias, sin duda ha sido de vosotros de quien más he aprendido durante estos cuatro años.

Y ya para terminar, a mi familia:

A mis padres, que me han hecho de cabeza fría y corazón caliente y a mi hermano. Tengo el mejor hermano del mundo.

Y a ti: mi trapecio, mi red, mi adiós y mi ven, mi manta y mi frio, mi resaca, mi lunes, mi hastío. Eres todo para mí. Gracias por quererme tanto, pero sobre todo gracias por quererme tan bien.

List of abbreviations

5-HT: Serotonin

5-HTT: Serotonin transporter

5-HTTLPR: Serotonin transporter gene-linked polymorphic region

ABAS-II: Adaptive Behavior Assessment System-II

AD: Alzheimer disease

ADHD: Attention deficit hyperactivity disorder

AEMPS: Agencia Española del Medicamento y Productos Sanitarios

ANCOVA: Analysis of covariance

ANOVA: Analysis of variance

APOE: Apolipoprotein E

APP: Amyloid beta precursor protein

Aβ: Amyloid beta

BACE2: Beta-secretase 2

BFCNs: Magnocellular cholinergic neurons of the basal forebrain region

BMI: Body mass index

BNT: Boston Naming Test

BR: Backward recall

CANTAB: Cambridge Neuropsychological Test Automated Battery

CEU: Utah residents with Northern and Western European ancestry

CH: Clinical history

CLU: Clusterin

CNVs: Copy number variations

COMT: catechol-O-methyltransferase

CRT: Cued Recall Test

CSF: Cerebrospinal fluid

CT: Cognitive Training

DA: Dopamine

DAT: Dementia of the Alzheimer's Type

DAT1: Dopamine transporter1

DMR: Dementia Questionnaire for Persons with Intellectual Disability

DNA: Deoxyribonucleic acid

DOPAC: 3,4-Dihydroxyphenylacetic acid

DRD4: Dopamine receptor D4

DS: Down syndrome

DSCR: Down syndrome Critical Region

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders IV

DYRK1A: Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A

EGCG: Epigallocatechin-3-gallate

EMA: European Medicine Agencies

FDA: Food and Drug Administration

fMRI: Functional Magnetic resonance imaging

FR: Forward recall

FT4: Free thyroxine

GABA: Gamma-Aminobutyric acid

GP: General population

HSA21: Homo sapiens (human) chromosome 21

HVA: Homovanillic acid

ID: Intellectual disability

IQ: Intelligence Quotient

K-BIT: Kaufman Brief Intelligence test

MAPT: Microtubule-associated protein tau

Met: Methionine

MOT: Motor Screening Test

OSAS: Obstructive sleep apnea syndrome

PAL: Paired Associates Learning

PFC: Prefrontal cortex

PRM: Pattern Recognition Memory

PSEN1: Presenilin-1

PSEN2: Presenilin-1

PSQI: Pittsburgh Sleep Quality Index

REM: Rapid eye movement

SCS: Sum of Cognitive Scores

SH: Subclinical Hypothyroidism

SNPs: Single nucleotide polymorphisms

SOD-1: Superoxide dismutase 1

SOS: Sum of Social Scores

SPSS: Statistical Package for the Social Sciences

SRT: Simple Reaction Time

SSP: Spatial Span

SVFP: Semantic verbal fluency pattern

SVFT: Semantic Verbal Fluency Task

T3: Triiodothyronine

T4: Thyroxine

TMA: tetramethylammonium

TMS: Transcranial magnetic stimulation

ToLDx: Tower of London-Drexel University

TSH: Thyroid-stimulating hormone

TSI: Tuscans from Italy

Val: Valine

VNTR: Variable number tandem repeat

WAIS-III: Wechsler Adult Intelligence Scale- III

WCFST: Weigl Color-Form Sort Test

WHO: World Health Organization

Abstract

Introduction: Down syndrome is a neurodevelopmental disorder caused by trisomy 21, which leads to cognitive and functional deficits, high risk for Alzheimer's disease and cognitive impairment at early ages. Due to the increased life expectancy and the existing large cognitive and functional variability in Down syndrome, it is essential to assess possible factors explaining these differences. This knowledge will contribute to a more efficient design of those clinical trials that aim to evaluate the efficacy of new therapies for cognitive and functional improvement in Down syndrome.

Objectives and methods: In order to detect possible confounding factors in the assessment of cognitive and functional improvements in treatment-efficacy clinical trials in Down syndrome, four original research cross-sectional studies were performed. We evaluate the interaction between biomarkers (biochemical and genetic) and sleep factors (sleep quality, apnea and snoring disorders), cognitive performance (psychomotor speed, attention, memory, executive function, and language) and functional state (adaptive behavior, quality of life, early symptoms of dementia). The following biomarkers were evaluated: **Study 1**: amyloidosis biomarkers (Aβ40 and Aβ42), **Study2**: thyroid function biomarkers (Thyroid Stimulating Hormone, free thyroxine and T-thyroxine dosage), **Study 3**: *COMTVal158Met*, *VNTR-DAT1* polymorphisms and **Study 4**: *5-HTTLPR* polymorphism.

Results: In **Study 1**, poorer semantic verbal fluency strategies, communication skills and a higher rate of early symptoms of dementia were associated with higher plasma $Aβ_{42}$ concentrations. In **Study 2** we observed that higher concentrations of free thyroxine (controlling for L-thyroxine dosage) were partially correlated with a better performance in memory,

executive function and attentional span**.** Hypothyroidism diagnosis was associated to a poorer visuospatial memory. ***Study 3*** showed that *COMTVal158Met Met* allele carriers and *VNTR-DAT1 10-repeat* allele homozygotes displayed an improved mental flexibility. *Met* allele carriers showed poorer adaptive behavior and a higher rate in early social symptoms of dementia than *Val* allele homozygotes. Finally in ***Study 4,*** we observed that a poorer quality of sleep was associated to 5-HTTLPR *Short*-allele homozygotes. Regarding cognitive performance, poor sleep quality and apnea were both associated to worse visual memory skills. Furthermore apnea and snoring were associated to a lower performance in adaptive behavior, while only snoring was associated to a higher rate of early dementia symptoms and a higher $A\beta42/A\beta40$ ratio in plasma. The 5-HTTLPR was neither associated to cognition, adaptive behavior nor early dementia symptoms.

Conclusions: Plasma Aβ concentrations, plasma free thyroxine concentrations, L-thyroxine dosage, sleep quality, apnea and snoring diagnosis and genetic polymorphisms of the dopamine (*COMTVal158Met* and *VNTR-DAT)* neurotransmission system are factors of interference with the cognitive, behavioral and functional DS phenotype. Finally, the genetic polymorphism of the serotonin (5-HTTLPR) neurotransmission system does not interfere with cognitive or behavioral aspects, but it does with sleep quality in this population. Thus, these factors, which explain part of the phenotype variability, need to be assessed in the screening visit in the context of a drug-efficacy clinical trial for cognitive, behavioral and/or functional enhancement. This knowledge will allow us to balance those factors associated with phenotype variability across groups of treatment, leading to a proper efficacy assessment.

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Preface

Cognitive impairments of genetic origin associated with intellectual disability (ID) syndromes were believed to be orphan syndromes, but recent progress in the understanding of the underlying mechanisms has resulted in the identification of potential treatment targets like acetylcholinesterase inhibitors (donezepil, rivastagmine, galantamine), *gamma*-Aminobutyric acid (GABA) antagonists (pentetrazol, basmisanil), and N-methyl-D-aspartate receptor antagonists (memantine), which efficacy has been evaluated in clinical trials with limited success as well as for a miscellaneous compounds such as vitamins, mineral supplements, piracetam, or growth hormone. None of the previous clinical trials had combined pharmacological treatment with Cognitive Training (CT) and none of them showed significant improvement in cognition or adaptive functionality (De la Torre et al., 2014) . While some positive outcomes were reported for individual participants, overall, these treatments were considered ineffective, and the trials were hampered by low power (Gardiner, 2015).

Epigallocatechin-3-gallate (EGCG), the major catechin in green tea leaves (about 40–50% of total catechins) has noncompetitive inhibitory properties on the kinase activity of the dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (DYRK1A), a serine-threonine kinase encoded by the *DYRK1A* gene, located in the DS critical region (DSCR) and thought to be a major contributor to cognitive phenotypes of DS. EGCG can cross the blood–brain barrier, and chronic administration of a green tea extract containing 45% EGCG promoted learning and memory in DS mouse models and was safe in young individuals with DS in our Phase 1 clinical trial (De la Torre et al., 2014).

Regarding CT, it is a used paradigm capable to improve learning, memory and behavioral skills in DS (Bennett, Holmes, & Buckley, 2013) and other intellectual disabilities (Kirk, Gray, Riby, & Cornish, 2015). CT is associated with significant changes in synaptic plasticity. Environmental conditions that facilitate enhanced sensory, cognitive, motor, and social stimulation normalizes DYRK1A kinase activity in the hippocampus of DS mouse models, suggesting that EGCG acts synergistically with cognitive stimulation. The long-term efficacy of CT has not been conclusively shown in individuals with DS.

In this context, our investigation team (the TESDAD Study group) proposed **a double-blind, placebo-controlled, Phase 2, single center clinical trial (TESDAD;** NCT01699711**)** encompassing the use of epigallocatechin-3-gallate (EGCG) in combination with cognitive CT for 12 months, as a treatment for cognitive and functional enhancement in DS young adults (followed up for 6 months after treatment discontinuation). The results have shown that EGCG and CT were significantly more effective than placebo and CT at improving visual recognition memory, inhibitory control, and adaptive behavior.

But when the TESDAD Study was planned, we aimed to know many things about DS clinical variables that were yet unknown. It was not only about the assessment of EGCG + CT on cognition and functionality; it was also about doing this assessment correctly. Thus, the use of adequate tools to assess efficacy would allow us to achieve robust results about the real efficacy of our new therapeutic approach. In this context we developed the TESDAD battery (de Sola et al., 2015) which permitted a quantitative assessment of cognitive predictors of functional change as end-point measures of therapeutic intervention for clinical trials: being the former a cognitive key target for therapeutic intervention, and the latter a primary functional outcome measure of clinical efficacy. Other tools of exploratory assessment

as functional connectivity to assess the brain functional status were also developed. Resting-state whole-brain connectivity degree maps were generated to identify sites showing anomalous synchrony with other areas. A subsequent region-of-interest mapping served to detail the anomalies and to assess the potential contribution to poor adaptive behavior (Pujol et al., 2014).

The tested efficacy of these and other tools of assessment in DS population (**i.e. genotyping of key genes in neurotransmission systems, plasma Amyloid beta (Aβ) measurements as a biomarker of amyloidosis, thyroid assessment, etc.**) helped us to better define the DS phenotype in baseline conditions in all the possible domains: cognitive, adaptive, neuro-functional, physiological, biochemical, etc. In a step further, we explored the relationship between all those parameters. We wondered for example, if genotypes could explain in part some of the phenotype cognitive features. Specifically we were interested on factors underlying the cognitive phenotype in DS and its variability. Since phenotypic variability in the population with DS may affect assessments of cognitive and functional evaluations associated with a new treatment, it was crucial to determine those associations.

The present thesis is interested at co-variables/confounding factors that may modulate/condition tools used to assess the efficacy of treatments for cognitive and adaptive enhancement in DS. We were also interested at biomarkers of efficacy which interact with cognitive variability.

This knowledge has contributed to a better design of the TESDAD clinical trial design, allowing us to determine the real efficacy of EGCG in combination with CT as a treatment for cognitive and functional enhancement in DS young adults and also to understand DS as a complex and multidimensional

condition that can vary among subjects as a function of biological, chemical, neurodevelopmental, environmental and genetic factors.

As neuropsychologist I has been involved in the evaluation of cognitive performance of participants in the TESDAD study and I co-author the main publication of the study (de la Torre et al., 2016). In addition I contributed to the study with the evaluation of the impact of co-variables and confounding factors modulating treatment efficacy.

1. Introduction

Down syndrome (DS) is an aneuploidy syndrome that is normally caused by extra copy of all or part of human chromosome 21 (HSA21) leading to a cognitive and behavioral phenotype characterized by psychomotor delay, cognitive and behavioral deficits, and high risk for early onset of Alzheimer Disease (AD)-like dementia (Grieco, Pulsifer, Seligsohn, Skotko, & Schwartz, 2015).

DS is the most common neurodevelopmental disorder of known genetic origin, with an incidence between 1:1000 to 1:1100 live births worldwide ("World Health Organization (WHO)," 2016).

The prognosis is variable, depending upon the possible complications like heart defects, susceptibility to infections and development of leukaemia. In the early 1900's DS live expectancy was less than 10 years. Now, about 80% of adult DS patients reach their 50th birthday and beyond.

1.1 Variability of Clinical Phenotypes in Individuals with Down syndrome

DS is characterized by phenotypic physical features as proportionally large tongue, flat nasal bridge, low muscle tone, short stature, among others. Associated conditions may include thyroid dysfunction, obstructive sleep apnea, periodontal disease, seizures, as well as visual and hearing problems. Nevertheless in the long run the main handicap is ID: Some comorbidities cited earlier would affect neurological and/or cognitive phenotype, and both, in turn, would feedback each other (I. T. Lott & Dierssen, 2010). Some key features of the cognitive profile include: (1) receptive language that

overcomes language production, (2) visual memory better than verbal memory, and (3) global processing that is superior to local processing (Liogier d'Ardhuy et al., 2015). The morphology of the brain in DS includes reduced brain weight with diminished proportions in the volumes of the frontal and temporal lobes. Adult DS brain is a 20% smaller than typically developing brain, this reduction appears in 4–5-month fetuses. Neuroimaging studies have shown a remarkable preservation of subcortical gray matter structures in the face of a generally diminished brain volume, suggesting that there may be a temporal disassociation in neural development between the cortical versus subcortical areas (I. Lott, 2012). In adulthood, DS presents accelerated aging and an increased risk of developing early onset of AD (Krinsky-McHale & Silverman, 2013).

1.1.1   Individual differences in Down syndrome genetics

The most common cause of DS is the additional copy of an entire chromosome 21, which means each cell in the body has three copies (**full trisomy**) of chromosome 21 instead of the usual two copies. In 88% of cases the extra copy is maternally derived, through an error in cell division called non-disjunction.

Less commonly, DS can also occur when only a segment of chromosome 21 has three copies (**partial trisomy**).

Mosaicism occurs in a very small percentage of people with DS (1.3-5 %), when the whole chromosome is triplicated but only a proportion of the cells are trisomic, having the extra copy of chromosome 21 in only some of the body's cells.

Translocation is another mechanism yielding DS (in 4% of cases), whereby part of chromosome 21 becomes attached to another chromosome during the formation of reproductive cells in a parent or very early in fetal

development. Here, two normal copies of chromosome 21 plus extra material from chromosome 21 attached to another chromosome, results in three copies of genetic material from chromosome 21. Some of the genetic material from chromosome 21, usually from the long arm, is moved to chromosome 14 or 22, or from the long to the short arm of chromosome 21.

These multiple origins of DS (trisomy, partial trisomy, mosaicism and translocation) need to be taken into account when considering differences between individuals with trisomy 21, affecting phenotype. Studies indicate that children with translocation DS do not differ cognitively or medically from those with trisomy 21. Nevertheless those with mosaic DS typically have Intelligence Quotient (IQ) scores 10–30 points higher and fewer medical complications than do subjects with translocation or full trisomy 21 (Batshaw & Roizen, 2002), perhaps because their trisomic cells are interspersed with normal cells. In addition, the degree of ID has been associated to the increased dosage of chromosome 21 specific genes, being partial trisomy associated to a mild DS phenotype (Papoulidis et al., 2014). Several studies on cases with partial trisomy 21 propose a chromosomal area as the 'pathogenetic' chromosomal region for DS called Down Syndrome Critical Region' (DSCR). The DSCR contains 25–50 genes, crucially contributing to the majority of the DS cognitive, behavioral and physical phenotype (Delabar et al., 1993; Lyle et al., 2009) (i.e. *DYRK1A* is a likely candidate for neurodevelopmental alterations, amyloid beta precursor protein (APP) for neurodegeneration, and superoxide dismutase 1 (SOD-1) for incremented oxidation (Chettouh et al., 1995; Møller et al., 2008)).

Additionally, individual differences exist on other chromosomes. The euploid population, free from chromosomal abnormalities, is genetically different from one another, due to copy number variations (CNVs), single nucleotide polymorphisms (SNPs), and *de novo* mutations. Such differences also apply

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to people with DS, who in addition to their extra copy of all or part of chromosome 21; also have many of these variants. In addition, the expression of specific genes can interact with other genes in or out the chromosome 21 (epistasis), and also can have more than one effect on the DS phenotype (pleiotropism) (Karmiloff-Smith et al., 2016).

1.1.2   Individual differences in Down syndrome cognition

Most of the studies trying to delineate a cognitive and behavioral profile in DS have reported and described data at the group level, downplaying individual variation, and normally comparing DS cognitive phenotype either to typical developing controls or to other neurodevelopmental disorders, treating DS as a homogeneous group.

Typically, an estimation of the strengths and weakness of DS cognitive phenotype, when comparing different populations is reported: The hallmark of the DS phenotype is ID (IQ < 70). The weaknesses are consistently associated with expressive language, attention, executive function and memory (see **Figure 1**).
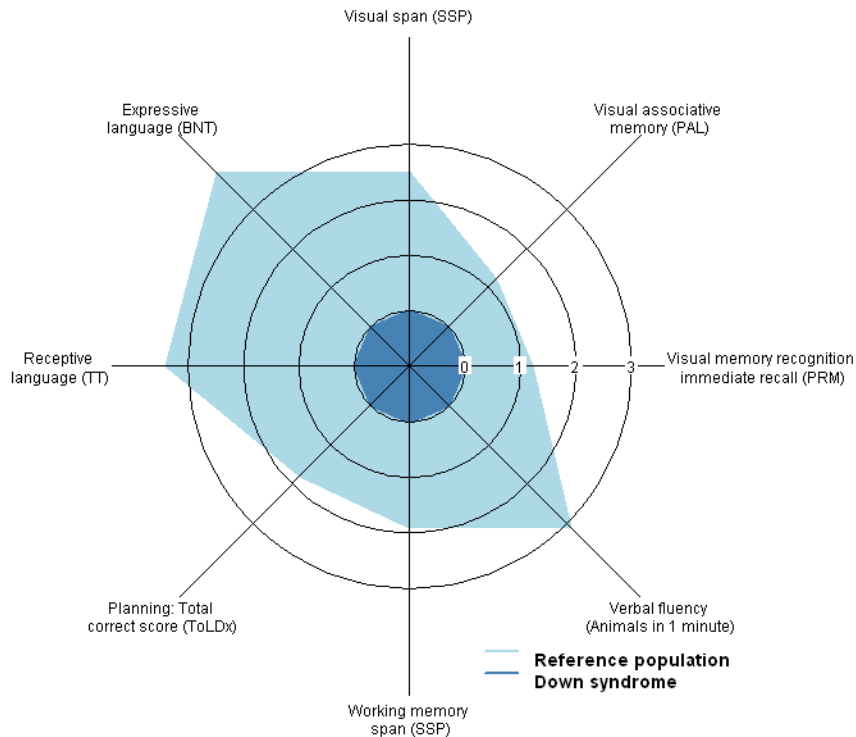
Figure 1: Radar plot representing the severity of cognitive impairment in Ds compared to age-matched typically developed adults on attention, memory, language and executive functioning components. Axis values indicate the absolute value of Cohen´s effect size (d) for the differences between both populations. For this purpose, the performance of the participants with DS has been standardized to 1 which is equivalent to an effect size of d=0. DS adults show a severe dysfunction of language capacity (|d|>3), a substantial deficit on attention span and executive functions (|d|>1.5) and a moderate deficit in episodic memory(|d|>1). Extracted from (de Sola et al., 2015)

These difficulties promote deficits in adaptive behavior, particularly in communication skills and abstract knowledge. Whereas relative strengths are associated with visuospatial skills (Liogier d'Ardhuy et al., 2015) promoting better skills in those cognitive, behavioral and functional areas which are non-verbal dependent. Furthermore, as the rate of cognitive development progressively becomes slower over years in relation to typically developing peers, a decline in general cognitive skill is also observed. Whereas, adaptive behavior uses to improve in the young adulthood (I. Lott, 2012).

Within these group data there are a wide individual differences, which are still hidden by floor or ceiling effects of some inappropriate tools and techniques of cognitive and functional assessment. In the present study, this limitation was observed in some variables of measure of given tests forming the TESDAD Battery (de Sola et al., 2015). Even though, those tests have a multiple set of measurements for the same outcome as: *time spent to perform the test, adjusted errors, reaction times, percentages of correct trials, etc.,* which are free from floor and ceiling effects. Thus, can still be considered suitable for DS young adult assessment. Furthermore, in some cases this tests provide an evaluation of high-order executive capacities (i.e. Weigl Color-Form Sorting Test), allowing us to detect those subjects with higher level performance in the framework of ID. This can be useful for many things, mainly in a clinical context. Whereas in the context of clinical trials the main scope is to compare treatments effects at group level, thus all tests included in the neuropsychological battery must be adequate for the maximum number of subjects, allowing in turn, the assessment of individual differences. This is the only way to ensure that treatment efficacy is properly assessed, and not being masked behind floor or ceiling effects. Using raw scores, instead of transforming into standardized scores, may also improve the characterization of cognitive functioning, avoiding floor effects in ID populations, especially for research applications (Hessl et al., 2009).

During the last years, the progress in the development of new neuropsychological tools has focused in detecting: (a) the full range of scores in DS, and (b) the individual differences between subjects. This has helped to stablish the current understanding of the DS cognitive/behavioral phenotype as follows:

IQ scores: In adults can range from severe ID with IQs at floor (IQ< 40) to mild-moderate dysfunction (IQ from 40 to 70), whereas a few have IQs in the

70s or above, usually linked to partial trisomy (Papoulidis et al., 2014). In fact, in our population study the only subject carrying partial trisomy of chromosome 21 had an IQ of 86 (assessed with the Kaufman Brief Intelligence test (K-BIT), (Kaufman & Kaufman, 1994)).

Speech and language: Language is the most impaired domain of functioning in DS and, perhaps, also the greatest barrier to independent meaningful inclusion in the community, the degree of impairment among DS individuals varies considerably as a function of IQ, because IQ tests partly measure language development, (Pennington, Moon, Edgin, Stedron, & Nadel, 2003). Relative to age-matched peers, language expressive delays are more severe than receptive language delays, persisting from childhood to adolescence and adulthood. Significant expressive language deficits on both syntactic and morphological abilities are appreciable across the sentence length, the number of different words, and the total number of words in both conversational and narrative contexts (Finestack & Abbeduto, 2010). Grammar weaknesses are also present, including the use of specific tense-related (e.g., past tense, third person singular, and modals) and non-tense-related (e.g., present progressive -ing, plural -s, and possessive -z) inflectional forms (Chapman, Seung, Schwartz, & Kay-Raining Bird, 1998; Eadie, Fey, Douglas, & Parsons, 2002). Furthermore, language and speech abilities can both be affected by common hearing and oral-motor structure problems in DS (Abbeduto, Warren, & Conners, 2007).

Executive function: Executive function at the lower level involves the regulatory components of behavior and cognition including aspects of attention, inhibition, and processing speed. Higher executive function level include higher ordered cognitive processes of information processing that include strategic planning, impulse control, organized search and flexibility of thought and action (Grieco et al., 2015). Executive function pattern varies

among age, being the latest ability to develop and the first to show early emerging decline in DS adulthood. Consequently, understanding the developmental course of executive function is important for understanding the DS cognitive phenotype from childhood to young adulthood as well as for identifying individuals with DS who are at higher risk for developing Alzheimer's disease.

Learning and memory: Certain aspects of learning are identified as strengths. Children with DS perform favorably in paradigms of observational learning, whereas, they have greater difficulty with instrumental learning, which requires manipulate the environment to meet their needs (Grieco et al., 2015). Regarding to memory, individuals with DS have primary deficits on explicit verbal and nonverbal long-term memory across life-span (Kogan et al., 2009), becoming more pronounced whit age.   Memory problems are believed to occur at the concentrations of encoding and retrieval and are impacted by attentional (Krinsky-McHale, Devenny, Kittler, & Silverman, 2008) and executive deficits (J Rowe, Lavender, & Turk, 2006) , and also by language dysfunction.   Instead, in DS subjects with Dementia of the Alzheimer's Type (DS-DAT) in their 50 's the episodic memory impairment is due to storage difficulties (Benejam, Fortea, Molina-López, & Videla, 2015) and not to other cognitive factors as attention deficits or difficulties in the proper use of retrieval strategies, which are in many cases, the typical problems underlying memory deficits in younger DS subjects which aren't diagnosed from DAT yet.

Behavioral skills: Individuals with Down's syndrome have more behavioral disturbances than typically developing, but fewer than other individuals with ID. A 17.6% of individuals with DS aged less than 20 years have a psychiatric disorder, most frequently a disruptive behavior disorder, such as attention deficit hyperactivity disorder (ADHD) (6.1%), conduct/oppositional disorder

(5.4%), or aggressive behavior (6.5%). The prevalence of psychiatric disorders grows in DS adulthood up to a 25.6%, the most common are: the major depressive disorder (6.1%), aggressive behavior (6.1%), and autism (7%), last one with great delays in diagnosis (Roizen & Patterson, 2003). In addition middle aged adults with DS develop neuropathological changes typical of Alzheimer's disease (AD) and as such, are at high risk for dementia. Behavioral symptoms of AD are noted in 75% of such individuals over 60 years of age, and are most frequently change in personality (46%), apathy (36%), loss of conversational skills (36%) and depression (Roizen & Patterson, 2003). Subsequent studies has been focused on the impact of dementia on trajectories of adaptive behavior thorough aging, and have reported substantially different patterns when individuals with dementia are included (i.e. higher concentrations of decline are seen with age) compared with when they are removed. In non-demented adults with DS age is also associated with a decrease in adaptive behavior, but these age-related declines are associated with changes in the adaptive behavioral range rather than intensity of adaptive ability (i.e. practical skills are maintained with age; however, social and conceptual skills are associated with declines, (Makary et al., 2015)).

1.1.3 Individual differences in Down syndrome sleep

Infants with DS exhibit smaller brains at birth and different morphometric relationships between individual bones of the craniofacial skeleton (Richtsmeier, Baxter, & Reeves, 2000) compared to general population (GP). These divergent craniofacial features impact breathing (Fung, Witmans, Ghosh, Cave, & El-Hakim, 2012). Specifically, tissue crowding produced by midface hypoplasia and realigned soft tissue (i.e., constriction of the pharynx and palate, posterior displacement of the tongue, enlarged tonsilsadenoids) curtails airflow through the upper respiratory tract (Pilcher, 1998; Shapiro,

Gorlin, Redman, & Bruhl, 1967). Propensity toward obesity and reduced muscle tone in DS put further strains on the upper airways (Rosen, 2011). As a result of all these issues (which can arise at different concentrations between DS subjects), people with DS can present symptoms associated with obstructive sleep apnea syndrome (OSAS) and sleep fragmentation (Capone, Aidikoff, Taylor, & Rykiel, 2013; Rosen, Lombardo, Skotko, & Davidson, 2011). The loss of sleep quality wrought by OSAS and its contribution to excessive daytime sleepiness, fragmented sleep, early awakening, and sleep maintenance insomnia (Robinson-Shelton & Malow, 2015) is predicted to modulate everyday intellectual difficulties experienced by people with DS, and the developmental pathways affected by an extra copy of HSA21 could interact at a macro-level to influence cognitive outcomes later in life (Fernandez & Edgin, 2013).

1.1.4   Individual differences in Down syndrome thyroid function

Thyroid disorders are more frequent among children and adults with DS than in the GP, hypothyroidism occurring most frequently (16% to 20%) (Van Cleve, Cannon, & Cohen, 2006). Hyperthyroidism could also be more prevalent than in GP although only sporadic cases have been reported in the literature (Goday-Arno et al., 2009). Subclinical hypothyroidism (SH) (defined as mild thyroid-stimulating hormone (TSH) elevation with normal free thyroxine (FT4) concentrations and no symptoms) is also common during the first few years of life in DS (Claret et al., 2013).

The thyroid gland responds to pituitary secretion of TSH by augmenting the synthesis and release of two chemicals, thyroxine (T4) and triiodothyronine (T3). T4 and T3 go on to regulate anabolic energy metabolism and hormone sensitivity throughout the body (Stenzel & Huttner, 2013), being the brain one of their primary sites of action (Kester et al., 2004).

Thyroid dysfunction may be influenced by some of DS dysfunction as (i) decreased concentrations of selenium, which is required for thyroid hormone synthesis and metabolism (Nève, Sinet, Molle, & Nicole, 1983), (ii) impaired activity of phenylalanine hydroxylase, which converts the phenylalanine in tyrosine (Shaposhnikov, Khal'chitskiĭ, & Shvarts, 1979), and (iii) overexpression of DYRK1A kinase, which could reduce availability of tyrosine (Thiel & Fowkes, 2007).

Subclinical Hypothyroidism (SH) is usually a transitory disorder and there are factors (as the absence of goiter and thyroid autoantibodies) associated with spontaneous remission (in >70%) in children (Claret et al., 2013).

Hyperthyroidism is also more prevalent in patients with DS than in the GP. It has no gender predominance and is caused mainly by Graves' disease (a type of autoimmune thyroiditis). Anti-thyroid drugs are not effective in achieving remission and radioactive iodine, as a definitive treatment, is required (Goday-Arno et al., 2009).

Therefore, in the context of DS we can found around a 50 % of subjects whit any of those tree thyroid dysfunctions; most of cases will be diagnosed for hypothyroidism, other with SH (which can remise during childhood) and a few (but whit a higher prevalence than in GP) will be diagnosed for hyperthyroidism. Finally we can also find a high percentage of people that will not show biochemical markers of thyroid dysfunction across their live span. Thus, it means that thyroid functioning may differ between DS subjects and will evolve different across live span, showing a high variability which can influence neurocognitive development (we will focus on this in the following lines).

1.2 Factors underlying cognitive variability

The importance that the cognitive and behavioral variability plays in DS research is being increasingly recognized.

In order to understand DS development is crucial to study how individual differences constrain cognition and behavior. Cognitive and behavioral functioning evolves across the lifespan in different ways and rhythms between subjects. Those differences are modulated by several factors at multiple concentrations: which can go from genetic, cellular or brain functional and structural activity, to an environmental extent. And those factors can be associated to comorbid conditions (such as sensory impairments, seizures, thyroid dysfunction, sleep apnea, poor quality of sleep, or psychiatric disorders) related, in turn, whit cognitive processes.

Sociodemographic features like sex, age, years of education (see **Figure 2**) (de Sola et al., 2015), socio-economic status (Hunter et al., 2013) or day occupation (Foley et al., 2014) are also deemed to have an impact on cognitive and functional development.
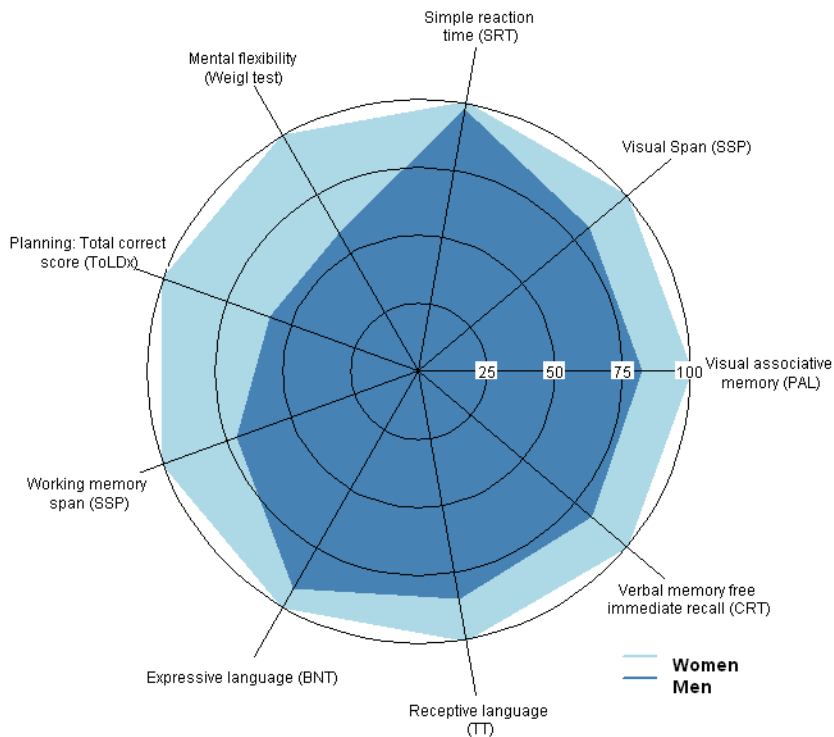
Figure 2: Radar plot representing the statistically significant differences in cognitive performance between men and women with DS on attention, memory, language and executive functioning components. Axis values indicate the performance in percentage relative to the women's performance, which has been set to 100%. Men with DS performed significantly poorer than women in all four cognitive domains. Extracted from (de Sola et al., 2015).

Regarding to the genetic context, the multiple origins of DS (Papoulidis et al., 2014) as well as individual differences in other chromosomes (i.e. CNVs, single nucleotide polymorphisms (SNPs), and the *novo* mutations) also deem to play a role in DS phenotypic variability (Mason, Spanó, & Edgin, 2015) since these genetic differences can influence on the activity of the corresponding neurotransmitter systems (i.e. dopaminergic , serotoninergic, cholinergic) which play a role in different cognitive and behavioral processes.

Concretely, acetylcholine is a primary neurotransmitter in the central nervous system. The magnocellular cholinergic neurons of the basal

forebrain region (BFCNs) project extensively to the hippocampus and cortex and provide direct synaptic inputs to both principal glutamatergic neurons and GABAergic interneurons in the hippocampus. Thus, BFCNs impose strong modulatory effects on the hippocampal neurons and, therefore cognitive function. Loss of BFCNs in DS devoid the modulatory effects of these cells on hippocampal neurons causing significant alterations in learning, memory and attention (Sanchez, Moghadam, Naik, Martin, & Salehi, 2011).

Serotonin (5-HT)-ergic neurotransmission regulates behavioral inhibition in response to adverse stimuli, mood, appetite and sleep. Thus, disruptions of 5-HT-ergic inputs to the hippocampus result in increased impulsivity and decreased avoidance behaviors, both common in people with DS. The overexpression of *SOD1* gene (a triplicated gene in DS) in cultured neurons has been shown to lead to significant failure in 5-HT uptake in these cells. Accordingly, decreased 5-HT concentrations and increased 5-HT metabolites have been reported in DS. Reduced 5-HT concentrations, presynaptic serotonin transporter proteins, and cell size of 5-HT-ergic neurons in the dorsal raphe nucleus have been reported in adults with DS. All these studies suggest that 5-HT-ergic system undergoes significant dysregulation in DS (Das et al., 2014)

Dopamine (DA)-ergic neurotransmission has since long been identified as having a central role in efficient motor functioning. However, several more recent lines of evidence (from patient, animal, electrophysiological, genetic, pharmacological, and neurocomputational studies) suggest that DA is also critically implicated in many higher-order cognitive abilities. And it is also very important to the reward/pleasure sensation. Several studies have demonstrated that there is an inverted-U curve between DAergic signaling and cognition/behavior, where too little or too much impairs cognitive/behavioral performance (Bäckman, Nyberg, Lindenberger, Li, &

Farde, 2006). Some DS human structural imaging studies evidence reductions in grey matter volumes in the frontal cortex and cingulate gyrus of DS adults (J Rowe et al., 2006). In addition, the response to DA-ergic stimulation is depressed in DS post-mortem cerebral cortex (Lumbreras et al., 2006), and reductions in DA and its acidic metabolites (3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)) have been detected in DS fetal and adult brains(Risser, Lubec, Cairns, & Herrera-Marschitz, 1997; Schneider et al., 1997; Whittle, Sartori, Dierssen, Lubec, & Singewald, 2007). Then, DA concentrations are decreased in some, though not all DS patients, maybe because variations in protein availability and enzyme level or activity have been associated with several polymorphisms identified in the DA receptor D4 (DRD4), DA transporter (DAT1) and catechol-O-methyltransferase (COMT) genes, which are out of the 21 chromosome (Bhowmik et al., 2011). Thus, less attention has been payed to them. The role of genetic polymorphism of the DA system on cognition and behavior in GP and DS is reviewed next.

1.2.1   Genetic polymorphism of the dopamine system

In the GP, polymorphisms known to influence DA concentrations in the Prefrontal cortex (PFC), such as variable number tandem repeat (VNTR) in the gene of DAT1 (responsible for DA reuptake) and Val158Met in the gene of the COMT (responsible for DA catabolism), are associated to executive function abilities (Caldú et al., 2007; Witte & Flöel, 2012). The COMTVal158Met polymorphism (rs4680) is a SNP (single nucleotide polymorphism) that causes the substitution of a Valine (Val) for a Methionine (Met) at position 158/108 (Lachman et al., 1996), resulting in tree possible genotypes (Val/ Val, Val/Met and Met/Met) and has been shown to affect COMT enzymatic activity, which is reduced in the Met allelic variant carriers. The DAT1 polymorphism is a VNTR of 40 nucleotides that can range from three to eleven repetitions and has been shown to affect expression of DAT1,

with the 10-repeat allele being associated with lower DAT1 concentrations than the 9-repeat allele (Shumay, Chen, Fowler, & Volkow, 2011; van de Giessen et al., 2009). Both the Met allelic variant and 10-repeat allele would result in greater availability of DA in the PFC and frontostriatal networks, which is linked to better ascribed cognitive skills as executive function (Barnett, Xu, Heron, Goldman, & Jones, 2011; Meyer-Lindenberg et al., 2005; Wonodi et al., 2009), whereas these same alleles had been associated with a disadvantage in adaptive behavior (Koutsilieri, Riederer, du Plessis, & Scheller, 2014; Mier, Kirsch, & Meyer-Lindenberg, 2009).

Although there is a lot of evidence about the role that those polymorphisms are playing in cognition and behavior in the GP, only two studies have explored the role of DA genetic polymorphisms in DS; A family-based analysis revealed significant over-transmission of a DRD4 VNTR- allele, which encodes for D4 DA receptor in DS (Das Bhowmik, Dutta, Sinha, Chattopadhyay, & Mukhopadhyay, 2008). In a second study, the 7-repeat allele of the DRD4 polymorphism was associated to behavioral and executive functions difficulties in children with DS (Mason et al., 2015), but there is still no evidence about the role of COMTVal158Met and DAT1 polymorphisms in DS.

1.2.2 Genetic polymorphism of the serotonin (5-HT) system

The involvement of 5-HT-ergic neurotransmission in cognition, behavior and sleep has been reported in GP (Barnett et al., 2011; Salminen et al., 2014). The 5-HT transporter (5-HTT) determines the duration of the action of serotonin by facilitating its reuptake from the synapse. A common 44-base pair deletion/insertion polymorphism in the promoter region of the 5HTT gene-linked polymorphic region (5-HTTLPR) has two frequent alleles, designated as Long and Short. The *Short*-allele (14-repeat) is associated with lower 5-HTT expression in cell lines, having less transcriptional activity and

lower serotonin uptake than the 16-repeat *Long* variant, with a final result of higher 5-HT concentrations (Goldman, Glei, Lin, & Weinstein, 2010; Heils et al., 1996). Sleep regulation was been related to 5-HT, being increased activity of serotoninergic neurons associated with wakefulness (Hartmann et al., 2014; Heils et al., 1996). In the same line, there is an association between carrying the *Short* allele of 5-HTTLPR and worse sleep quality, and the inhibition of 5-HTT function is associated with poorer sleep quality and less REM sleep (Gottesmann, 2004; Oberndorfer, Saletu-Zyhlarz, & Saletu, 2000).

Regarding the DS population a serotoninergic system deficit has been reported, and for the moment, the 5-HTTLPR role in DS phenotype has only been studied once, reporting a lack of association. (Das Bhowmik et al., 2008).

1.2.3 Thyroid function

The clinical significance of hypothyroidism on cognitive function and mental health has been studied in rats and humans. It affects a variety of cognitive domains, including decrements in general intelligence, attention/ concentration, memory, perceptual function, language, psychomotor function, and executive function (Constant et al., 2005; Correia et al., 2009; Davis & Tremont, 2007) , being memory the most affected domain , with specific deficits in verbal memory (Correia et al., 2009; Miller et al., 2007). L-T4 treatment has been effective in treating these cognitive deficits, although there may not be completely reversed (Blehaut et al., 2010; Davis & Tremont, 2007).

Imaging studies reported that hypothyroid patients showed altered brain structure and functioning , with decreased hippocampal volume, cerebral blood flow, and functional status globally and in several regions that mediate

attention, visuospatial processing, working memory, and motor speed (Bauer et al., 2009; He et al., 2011).

Since hypothyroidism affects cognitive function, thyroid status-related measurements (i.e. FT4, TSH) should be assessed in patients with impaired cognitive function. As it should be complicated to distinguish thyroid-related neurocognitive decrements from other related to DS, observation during L-thyroxine therapy may, in part, clarify these issues, as deficits due to hypothyroidism will partly decrease.

However, the role that thyroid dysfunction plays on cognition in DS has received less attention than in GP or in other pathologies. Maybe because cognitive consequences of hypothyroidism can be difficult to be differentiated from those found in the natural course of DS itself, getting overlapped.

In addition, the impact of congenital hypothyroidism on later adult cognition and behavior has received even less attention, maybe because cannot be adequately controlled for in assessments of the adult population and it isn't able to be screened and evaluated a posteriori if it wasn't done it the proper moment (before birth). Furthermore, how old they were when hypothyroidism diagnosis was done is sometimes difficult to assess since: (1) parents or legal guardians don't remember or (2) the thyroid dysfunction existed prior to the diagnosis.

1.2.4 Premature aging, Alzheimer Disease and Aβ

Most adults with DS will eventually show both clinical and neuropathological hallmarks of AD (Millan Sanchez et al., 2012).These age-associated changes are considered to be a result of the overexpression of genes localized in the DSCR of chromosome 21. The extra copy of the gene encoding amyloid precursor protein (APP) located on chromosome 21 appears to be the main

cause of the early onset of brain amyloidosis-β in patients with DS (Wegiel et al., 2008). Overexpression of APP has been associated with an increase of Aβ oligomers as early as during fetal development (I. Lott & Dierssen, 2010; Teller et al., 1996), the development of diffuse Aβ-positive plaques in almost all adults from 35-40 years of age (Zigman et al., 2008), Alzheimer-type pathology in the majority older than 40 years of age and an elevated risk of early onset of associated dementia. While early brain amyloidosis-β appears to be directly associated with the overexpressed APP gene, the mechanism leading to neurofibrillary degeneration in patients with DS seems to be related to the Mini-brain kinase DYRK1A gene, located in the critical region of chromosome 21 and also overexpressed in DS (Liu et al., 2008). DYRK1A may play a significant role not only in developmental brain defects associated with DS but also involved in tau protein phosphorylation in the human brain and potentially in neurofibrillary degeneration (Ferrer et al., 2005). Furthermore, other genes which are functionally linked to APP are also dysregulated in the DS brain (Beta-secretase 2 (BACE2), Apolipoprotein E (APOE), Clusterin (CLU), Presenilin-1 (PSEN1), Presenilin-2 (PSEN2), and microtubule-associated protein tau (MAPT)). Thus, although amyloid pathology is necessary, triplication of APP alone is not sufficient to cause AD. Therefore many people with DS will present dementia in their 30s, but some of them, even by their 80s and despite significant plaque pathology, will not show dementia symptoms. Therefore it is crucial to determine the mechanisms underlying early neurocognitive dysfunction progression versus later developing changes due to accumulating AD neuropathology. Findings suggest that early appearance of disease processes increase in impact and magnitude across age. To date AD conversion in aged DS subjects is mainly analyzed by measuring plasma and cerebrospinal fluid (CSF) Aβ concentrations. Several studies have shown increased plasma concentrations of both $Aβ_{40}$ and $Aβ_{42}$ in young DS compared to control population (Head et

al., 2011; Mehta et al., 2003) and most found higher concentrations of $A\beta_{42}$ in those DS individuals that were either demented or developed dementia at follow-up (Coppus et al., 2012; V. P. Prasher, Sajith, Mehta, Zigman, & Schupf, 2010; Nicole Schupf et al., 2007). Some correlations have also been found between high $A\beta_{40}$ plasma concentrations and dementia status, and between increases in $A\beta_{40}$ and decreases of $A\beta_{42}$ and risk of dementia (Coppus et al., 2012; Head et al., 2011; N Schupf et al., 2010; Nicole Schupf et al., 2007). Interestingly, most studies report no correlation between age and $A\beta_{42}$ concentrations (Head et al., 2011; V. P. Prasher et al., 2010). The increase in lifespan in the DS population has made the early detection of dementia of Alzheimer's type and its differentiation with ID symptoms a major objective of researchers and clinicians. In the GP impairments in semantic fluency exist prior to the clinical diagnosis of AD (Vogel, Gade, Stokholm, & Waldemar, 2005). Specifically, patients with AD exhibit important deficits in both semantic and phonemic fluency, being the former the most impaired (Canning, Leach, Stuss, Ngo, & Black, 2004; Cerhan et al., 2002; Henry, Crawford, & Phillips, 2004; Taylor, Salmon, Monsch, & Brugger, 2005). Thus, alterations in clustering and switching abilities during the performance of a semantic verbal fluency task are considered early predictors of the development of AD in the GP (Palmer, Bäckman, Winblad, & Fratiglioni, 2003; Romero et al., 2008). Thus, given the numbers of individuals with DS who will develop the disease, analyzing the association between changes among age in given biomarkers of AD (i.e. accumulation of $A\beta$ peptides, (Chartier-Harlin et al., 1991)) and cognitive (i.e. semantic fluency) and behavioral symptoms (i.e. social deterioration) of AD should help to identify risk factors against early onset of AD.

1.2.5 Sleep features cognition and Alzheimer disease.

In the euploid population, poor sleep quality, particularly sleep fragmentation, is also a strong predictor of lower academic performance (Fredriksen, Rhodes, Reddy, & Way), reduced attentional capacities (Ashworth, Hill, Karmiloff-Smith, & Dimitriou, 2013), poor executive function (Archbold, Giordani, Ruzicka, & Chervin, 2004), and challenging behaviors (Lewin, Rosen, England, & Dahl, 2002).

Individual differences in DS sleep patterns start early. There is an increased risk of sleep fragmentation in DS because of high prevalence of obstructive sleep apnea in this population (Diomedi, Placidi, Cupini, Bernardi, & Silvestrini, 1998; Edgin et al., 2015). Children with DS with obstructive sleep apnea account for impaired executive function as well as lower verbal IQs than those without apnea (Edgin et al., 2015). Concerning young adults with DS, those with disturbed sleep have poorer cognitive scores, lower adaptive behavior scores, and poorer verbal fluency than those without disturbances (Massand, Ball, & Erikkson, n.d.) in prep. The current work with infants and toddlers with DS is revealing correlations between increased sleep fragmentation (not duration) and decreased memory, language, and attention shifts (Massand et al., n.d.) in prep. Furthermore recent studies (Edgin, Pennington, & Mervis, 2010) have found that deficiencies in slow wave sleep are prevalent during childhood and adolescence in those with DS comorbid for OSAS, and that OSAS might explain some of the variability associated with verbal IQ, memory, and executive function in DS.

Furthermore, sleeping problems are linked to cognitive decline and Aβ deposition, assuming a cause-effect between sleep disturbance and higher concentrations of Aβ, supposing to be a risk factor for AD in both DS and GP (Fernandez & Edgin, 2013).

Sleep has a crucial function in ensuring metabolic homeostasis and the clearance of toxins like β-amyloid from the brain. Using real-time assessments of tetramethylammonium (TMA) diffusion and two-photon imaging in live mice, a recent investigation showed that deep sleep results in an increase in convective exchange of cerebrospinal fluid with interstitial fluid, and convective fluxes with interstitial fluid increase the rate of β-amyloid clearance during sleep (Xie et al., 2013). The restorative function of sleep may thus be a consequence of the enhanced removal of potentially neurotoxic waste products that accumulate in the central nervous system when awake. Therefore, if individuals with DS show differences in their sleep architecture, such β-amyloid clearance may be differentially compromised. If amyloid clearance is subject to wide individual differences in DS due to varying concentrations of sleep fragmentation, this may be one of the reasons why some individuals go on to present with dementia and others do not. It is therefore possible that individual differences in sleep patterns in the DS population across the lifespan, together with other factors, impact on risk and protective factors for AD.

1.3 Clinical trials in Down syndrome population

DS neurocognitive development is variably leaded by interactions between several factors, some of them previously named. These interactions translate into overlapping cognitive and functional outcomes. For example, in some subjects with congenital hypothyroidism, the consequent cognitive impairment will be compensated if bearing partial trisomy by moderating APP metabolism. Instead, a trisomic subgroup might have increased Aβ concentrations which at the same time would be augmented if suffering from obstructive sleep apnea or respiratory distress. Thus, despite the similar neuropsychological and functional pattern in DS at a group level, it is likely that a particular level of performance is achieved by different

developmental trajectories in each individual (D'Souza & Karmiloff-Smith, 2011). Thus, they might not be predicted to show the same performance responses to the same pharmacological treatment, therefore cannot be directly compared to one another, which is an important consideration for establishing drug efficacy in DS clinical trials.

1.3.1 Study Designs

Matching individuals who share factors that are highly likely to influence cognitive and functional parameters is an actual used strategy to minimize confounding factors interfering with drug efficacy interpretation following a **group-match design** (Boada et al., 2012). Due to the high number of comorbid conditions accompanying DS, almost all cases are followed up by physicians. Thus there is a huge amount of known medical information that can help us to determine several confounding factors to match individuals. Even doing this, it is obvious that not all possible confounds can be controlled. Sometimes remains unclear to caregivers the developmental trajectories of comorbid factors which are playing a crucial role in cognitive development (i.e. the exact age when the hypothyroidism diagnosis was done, on since when they are treated, apnea onset, Aβ concentrations among age…) and other genetic conditions out of the chromosome 21 as SNPs (i.e. *COMTVal158Met* and *VNTR-DAT1*) which are known to play a role in cognition and behavior in an euploid genetic context (Caldú et al., 2007) and other conditions. As far as some of this information remains unclear for caregivers, and we don't know if it will interact with treatment efficacy, one of our aims will be to explore those possible interacting factors at baseline visit. To do it, we will not only check if those factors (i.e. thyroid status, quality of sleep, Aβ concentrations, intellectual quotient, etc.) are in "normal concentrations" of what is expected in DS, but also will check if those values are linked to cognitive and behavioral outcomes. If we suspect that those

factors are linked to the primary variable of efficacy (cognitive and behavioral enhancement) then we will consider them for balancing subpopulations of subjects in terms of i.e. ID level or thyroid status in the different treatment arms. In addition these confounding factors will be controlled during the statistical analysis.

An alternative approach that reduces variance associated with individual differences is the **within-subjects design**; here those participating in the clinical trial serve as their own controls, being all under placebo and treatment conditions in all the different phases of the study. Because of this, the total number of people required for the trial is reduced in a half and statistical power is increased. But within-subjects design does not exclude some disadvantages. First, repeated testing of subjects might create learning effects that contribute to an improved performance as the trial advances, but this can be controlled among trend analysis, or by randomizing the order in which participants receive treatment (crossover strategies). Learning effects along time may also happen in a group matching longitudinal trial, but may be controlled with statistical trend analysis and choosing the appropriate neuropsychological tools for using alternative versions for every assessment.

The main problem of within-subject design is the time that participants and their families need to invest in the study, doubling or triplicating the time invested in a group matching trial.

Longitudinal group-matched clinical trials use to assess the effect of a drug over months or even years, finalizing whit an evaluation of the residual treatment effects after discontinuing it in the last months of the study. In a within- subject design every subject must apply for every condition, duplicating the period of the study if two arms of treatment, or triplicating if

tree arms, resulting in an increase of the study ongoing period of as many experimental conditions it has [i.e. placebo, different treatment combinations (non- pharmacological intervention, pharmacological intervention, both) or dosages combinations] needing of a wash out period in between each experimental condition period to avoid possible residual effects of the treatment.

In addition, because DS variability is very sensitive to individual development (Edgin, 2013; Ghezzo et al., 2014; Grieco et al., 2015), in just one year of follow up many cognitive and functional changes can happen hindering the application of a within-subject longitudinal study. If the ongoing period of a within-subject study is too long, an incorrect interpretation of results may be derived. Because we are comparing subjects with themselves but in a different neurodevelopmental stage, the later can act as a confounding factor by itself.

Furthermore, people with DS are known to have a hard time staying on task. Because fatigue effects can already be a problem in a group- matched study, it would obviously be a more complex handicap in a within- subjects design. To reduce it as much as possible it is necessary to break up testing sessions into shorter periods and to motivate continuing participation when subjects are at home in between visits (E. M. Berry-Kravis et al., 2012).

Taking into account all these considerations, the selection of the most ideal clinical trial design remains controversial. Although we can try to control as many confounding factors as possible, we will never control all of them in any of both design approaches. The election may depend on a set of scientific and ethical factors. Thinking about minimize confounding factors and also the time invested by participants and their families.

1.3.2 Evaluating efficacy

Determining whether an experimental drug can improve cognitive performance in a "laboratory setting" and whether cognitive gains are indicative of real-world functional efficacy is a very complex work, and also the main goal of a clinical trial for cognitive enhancement in the context of ID. The fine line which defines an improvement and whether these changes are associated to treatment, needs for a multivariable analysis, controlling for age, sex, IQ, years of education, socio-economic status, comorbid conditions (visual problems, seizures, sleep problems) genetic background encoded by HSA21 (trisomy, partial trisomy, translocation, mosaicism) and individual differences on other chromosomes in addition to their extra copy of all or part of chromosome 21. In addition is essential to analyze the baseline interaction between cognitive/functional, biochemical, neuroimaging and neurophysiological parameters and if those factors are coordinately modulated by the treatment across time.

The tools used to assess these outcomes and its good construct validity is essential to a high quality clinical trial. If the tools chosen do not measure what they claim to, it is not possible to validate any result of the study.

1.3.2.1 Primary end points: Neuropsychological outcomes

1.3.2.1.1 Cognitive outcome measures

In the last decade we gained substantial knowledge not only in understanding the general picture of the neurobiological bases of the cognitive disability in DS, but also in the underlying biochemical processes determining cognitive and behavioral skills that explain, in part, the variability observed. Parallel to this, accurate neuropsychological assessment has evolved over time.

Previous studies utilized global cognitive assessments (IQ and adaptive behavior) and did not focus on tasks which target specific abilities modulated by well-defined brain regions and the connectivity patterns affected in DS. In this line, a recent study carried by our study team (Pujol et al., 2014), has found that improved daily communication skills, an specific adaptive behavior outcome of functional intelligence assessed with the ABAS-II (Harrison & Oakland, 2003), was linked to functional connectivity patterns in DS young adult population (**Figure 3**). The general conclusion of this study was that measurable connectivity changes, as a marker of the brain functional anomaly, could have a role in the development of therapeutic strategies addressed to improve the quality of life in DS individuals. Thus, it could be considered as an exploratory efficacy outcome in the context of clinical trial for cognitive/functional enhancement, being resting-state functional connectivity and the subsequent generation of whole-brain "connectivity degree" maps a good tool for assessment.
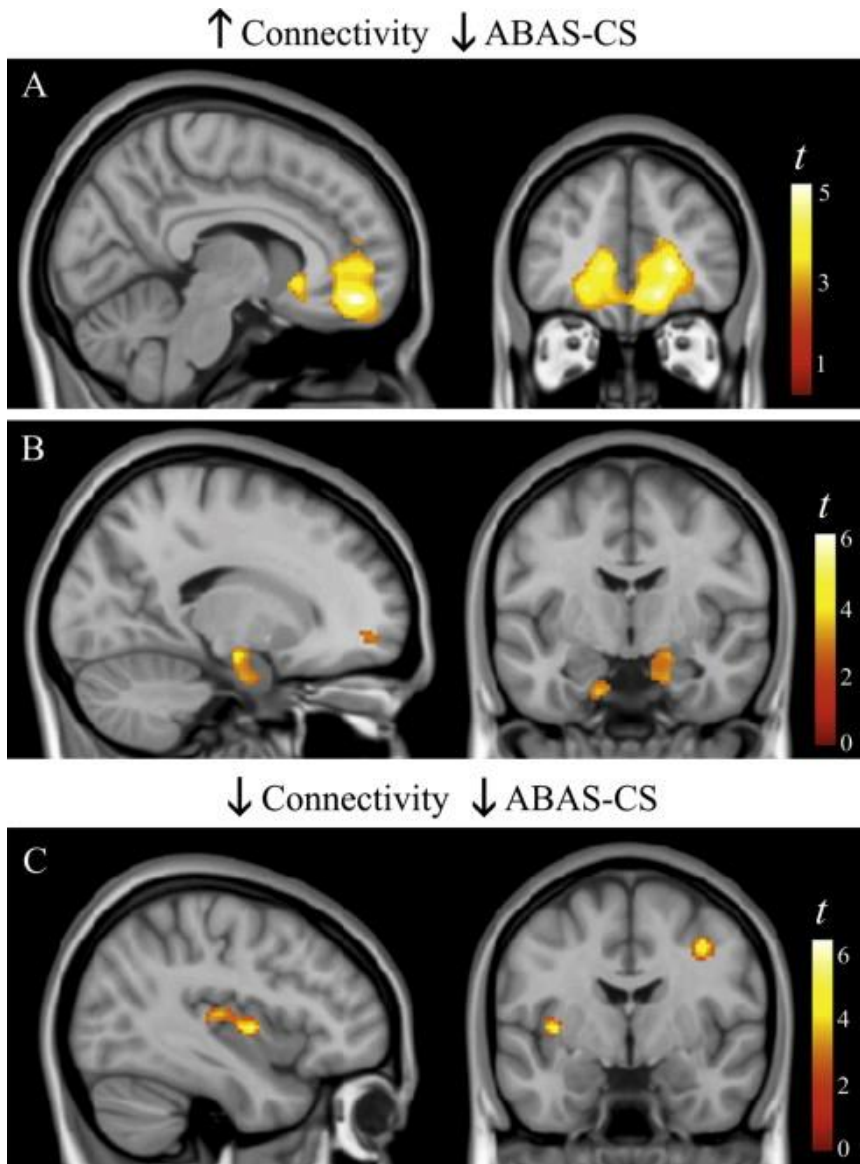
Figure 3. Higher functional connectivity in the ventral anterior cingulate seed map (A) and amygdala seed map (B) predicted lower communication skill scores (CS), whereas lower functional connectivity in the dorsal anterior cingulate seed map (C) predicted lower communication skill scores. The right hemisphere corresponds to the right side of coronal views, and the sagittal images show the right hemisphere in B and the left hemisphere in C. ABAS, Adaptive Behavior Assessment System (Pujol et al., 2014).

The ABAS-II is a multi-contextual parental report informing about adaptive behavior skills in different domains and situations. Thus it is included in those tools of functional assessment that target specific abilities. Instead,

composite measures of cognition, such as IQ, or general functional assessment can mask treatment effects in a clinical trial because the non-specific nature of the assessment. Nowadays tools for assessment should be specific and reflect cognitive strengths and weaknesses of trisomy 21 ,based in their underlying biologic processes (see **Figure 4**) (de Sola et al., 2015; Edgin, Mason, et al., 2010).
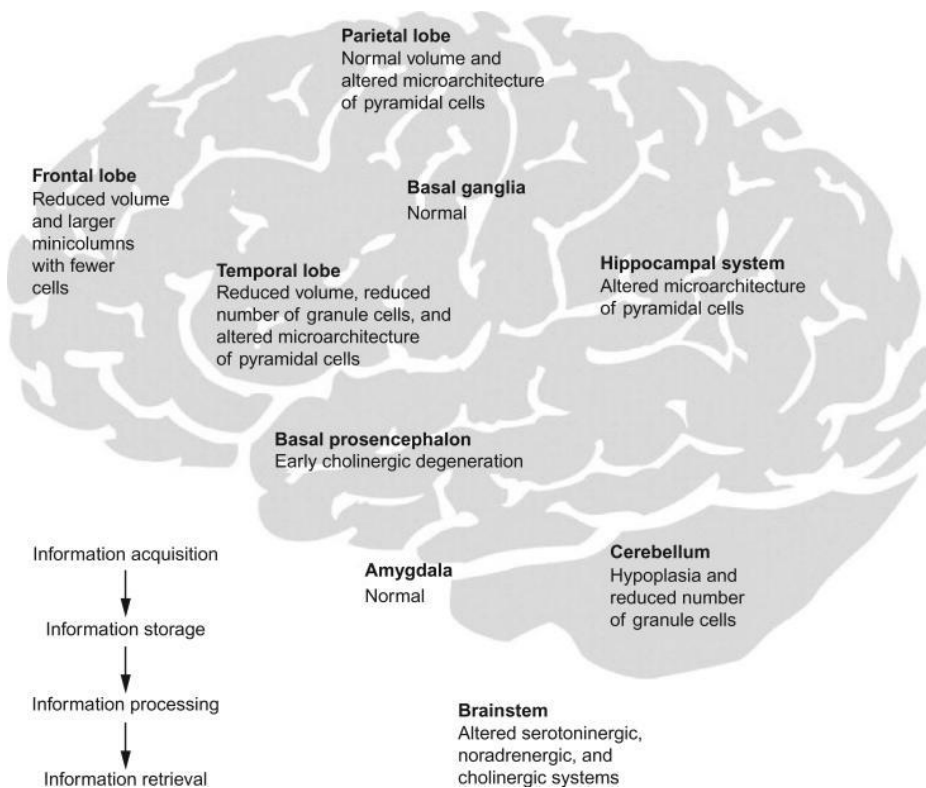
Figure 4. The learning circuit in DS. Structures involved in information acquisition, processing, or storage are affected in DS. The figure depicts some of the described changes that may lead to cognitive dysfunction in individuals with DS (I. Lott & Dierssen, 2010).

This perspective allows determining cognitive variability and subtle changes in performance associated to a treatment. But as mentioned before, how representative are these tools of their real-world performance is the key for determining treatment efficacy in a clinical trial. Some authors go a step further and postulate the use of more naturalistic tools for cognitive

assessment. One example is the Expressive Language Sampling (E. Berry-Kravis et al., 2013). Here, language samples are collected in highly structured, yet naturalistic, interactions with a clinician, reflecting a number of dimensions of language skill or atypical language behavior. These kinds of tools are more closely aligned with performance in real-world functional context.

Previous clinical trials in DS were sometimes limited by a lack of endpoints regarding cognitive and functional changes (Heller et al., 2006). Thus, it is essential to hypothesize which changes in specific cognitive and functional domains are expected as a function of the mechanism of action of the treatment. This will help us to determinate the primary efficacy endpoints. Assessing the suitability and reliability of tools that measure cognitive and functional changes prior to the clinical trial will help to detect which ones are the most effective in the context of a DS drug-efficacy longitudinal clinical trial.

Specifically, clinical trials require measures that can be repeatedly and reliably administered across different sites, to participants of a defined age range, and that do not exhibit large practice, floor, or ceiling effects (Liogier d'Ardhuy et al., 2015).

In conformity with previous studies (Edgin, Mason, et al., 2010), an adequate neuropsychological assessment for DS needs to:

1) Assess a wide range of skills including general skills (IQ, adaptive behavior) and those linked to particular neural systems. Control task (those not expected to be affected by an intervention or related to a particular set of genes) should also be included. Assessing strengths as well as weaknesses allow testing for the specificity of treatment effects.

2) Assess language abilities, but also choose a set of non-verbal tools to evaluate other cognitive skills to not confound the neuropsychological assessment with language demands.

3) Have distributional properties appropriate for research studies to identify genetic modifiers of variation.

4) Show sensitivity to within and between sample differences.

5) Have specific correlates with brain function and other biomarkers which are supposed to interfere in concrete cognitive and behavioral skills.

6) Be applicable to a wide age range and across contexts.

7) Be representative of the performance in their real-world functional context.

Thorough the TESDAD Study [ClinicalTrials.gov Identifier: NCT01699711, (de la Torre et al., 2016)] , the clinical trial in which this thesis has been developed, these factors have been taken into account to validate an adequate cognitive evaluation toolkit for DS, appropriate for clinical trials: The TESDAD Battery (de Sola et al., 2015). This battery, described in the "*Methodological approaches*" section, has been used in the current thesis to measure cognitive and functional parameters.

1.3.2.1.2   Functional outcome measures

In a clinical trial it is essential to achieve endpoints with external validity, such as adaptive behavior, which has a direct impact in quality of life. Thus, worldwide regulatory agencies as the Food and Drug Administration (FDA), La Agencia Española del Medicamento y Productos Sanitarios (AEMPS), and the European Medicine Agencies (EMA) have emphasized that regulatory

approval for a new drug must be accompanied by outcome measures reflecting a better functionality associated to the treated condition.

Adaptive behavior refers to the ways in which individuals meet their daily personal needs and cope with the natural and social demands in their environment. Thus adaptive behavior includes qualities that have a functional and pervasive impact on the quality of one's live, including the ability to function effectively and indecently at home, school, work, and the community. Thus, in many ways adaptive skills are the quintessential functional abilities in that they encompass key functional developmental tasks that typically are acquired first shortly after birth and continue throughout adulthood (Mpofu & Oakland, 2009).

Functional ability questionnaires aim to assess how they are doing in a daily basis approach, evaluating the ability to translate underlying competences into consistent daily habits that enhance autonomy. Thus, adaptive behavior questionnaires need to be administered to caregivers in every follow up visit as a part of the neuropsychological assessment. Parental- reports give extra information about participants' functional intelligence, which allows achieving some level of self-sufficiency by young adulthood. Tools assessing functional ability may also aim to capture the individual differences in how they develop and adapt to environments, and, as tools for cognitive assessment, they may don't comprise a uniform block. Thus, the instruments selected may assess the facing of daily intellectual challenges in different environments or domains which allow us a general vision of their strengths and weakness. For this purpose, it would be advisable to select general multidimensional scales as The Adaptive Behavior Scale (ABAS-II), (Harrison & Oakland, 2003), or the Vineland Adaptive Behavior Scale (Yang, Paynter, & Gilmore, 2016). Other tools assessing determinate daily skills as the Behavior Rating Inventory of Executive Function (Lee et al., 2015) or the Observer

Memory Questionnaire (Spanò & Edgin, 2016) must also be included as more specific tools when assessing functional ability in clinical trials.

Finally, the associations between raw scores on cognitive assessments to ratings on adaptive behavior scales allow the outcomes to be used as endpoints with external validity. Hence, it is essential to analyze how cognitive outcomes are related to parental-reports, this will help to detect if modest cognitive gains related to experimental treatments could imply a mild but clinically meaningful and significant impact on everyday life functioning in the DS population (de Sola et al., 2015).

1.3.2.2 Secondary end points: Biomarkers of efficacy

As mentioned before, to develop a clear picture of the biological pathways modified by an intervention to improve cognition, we need to understand if measures of brain function (i.e. neuroimaging and electrophysiological parameters, biochemical markers in plasma, genetic variants related to DA transmission) are reflected in neuropsychological outcomes prior to the intervention. This will help a proper selection of the efficacy outcomes in a clinical trial. Thereafter, it is essential to analyze how target biomarkers are changing across the clinical trial, if they are doing in the same direction as the neuropsychological outcomes and whether or not these changes are associated to the intervention.

Biomarkers of efficacy can interact with cognitive and functional outcomes, but also confounding factors (i.e. sex, age, IQ, comorbidities) can do, playing both a role in phenotype variability (see paragraph *1.1.3)*, even though they both are not the same concept. Here we may insist on differences and similarities between biomarkers of efficacy and confounding factors.

Biomarkers of efficacy can be targets in which the drug may have a direct impact through a mechanism of action. In DS some of these targets have a

direct link to the overexpression of one or more genes encoded by HSA21 (i.e. DYRK1A kinase activity, a major candidate for DS cognitive and neural plasticity phenotypes, which is supposed to be inhibited by EGCG compound, (De la Torre et al., 2014)). But surrogate biomarkers can also be modulated by the treatment, and other mechanisms of action should not be discarded (i.e. epigenetic, mitochondrial dysfunctions rescue, antioxidant and hypolipidemic effects).

Functional connectivity patterns (i.e. functional magnetic resonance imaging, fMRI) and/or neurophysiological activity (i.e. paired pulse transcranial magnetic stimulation, TMS) can also be modulated as a consequence of the mechanisms of action of the treatment. This modulation can be coordinated longitudinally with primary neuropsychological changes, as long as in basal conditions there has been observed a correlation between them.

Confounding factors are those which can interfere on primary or secondary efficacy endpoints. Confounding factors need to be controlled and balanced among groups of treatment, improving the clinical trial design and posterior analysis of the results (see section 1.2). But confounding factors can also be biomarkers of efficacy by themselves, as long as they were dynamic factors able to be modulated by a treatment. For example, thyroid status can be considered as a potential confounding factor as it plays a role in cognitive development (Samuels, 2014), but it also can be a biomarker of efficacy (free T4 concentrations) if the treatment for cognitive enhancement have an impact on thyroid system through a given mechanism of action (Blehaut et al., 2010). Other confounding factors as genetic variants which are expected to have an impact on cognition (Mason et al., 2015) can't be considered as efficacy biomarkers as they are static conditions that won't change directly or indirectly by the mechanism of action of a drug or a non-pharmacological therapy (i.e. cognitive or behavioral training). This static features are not

targets expected to be modifiable, considered as possible causes and not consequences of determinate cognitive/functional patterns.

1.4 Outline of the thesis

DS is the most common neurodevelopmental disorder of a known genetic cause: trisomy for HSA21, which leads to a cognitive and behavioral phenotype characterized by psychomotor delay, cognitive and behavioral deficits, and high risk for early onset of AD-like dementia (Grieco et al., 2015). Considering that trisomy 21 produces a set of "common" features compounding the typical DS phenotype, but yet underlying these group-level accounts there are large individual differences across DS at all concentrations—genetic, cellular, neural, cognitive, behavioral, and environmental, it is imperative to assess the potential factors underlying this phenotype variability to better understand this complex syndrome.

Furthermore, it is unclear how this variability may interfere in the design, analysis and interpretation of the results of a DS clinical trial for cognitive enhancement.

In order to overcome some of these limitations in current DS research and **to provide answers to the remaining questions concerning the potential confounding factors in DS clinical trials** and how they interact with the efficacy outcomes, **we designed four original research cross-sectional studies in young adults with DS**, drawn from the baseline visit of the TESDAD clinical trial (ClinicalTrials.gov Identifier: NCT01699711).

In **the first one** (n=50), we explored the association of semantic verbal fluency performance with early functional indicators of dementia, adaptive behavior and amyloidosis biomarkers (Aβ40 and Aβ42).

A **second study** (n=85) aim to analyze whether thyroid status (TSH, FT4 concentrations and hypothyroidism diagnosis) interfere on cognition in DS young adult population.

In **the third one** (n=69), we explored whether *COMTVal158Met* and *VNTR-DAT1* variants related with the DA-ergic neurotransmission system interact with the trisomic genetic background and how this in turn mediates DS phenotype variability regarding PFC-dependent cognition and behavior.

Finally, **in the fourth study** (n= 79) four main objectives were targeted: (1) To examine the association between allelic variation of 5-HTTLPR and sleeping features (2) To analyze the association between allelic variation of 5-HTTLPR and cognitive and functional features, (3) To study if sleeping features are associated to cognitive and functional state and also to early symptoms of AD, and (4) to elucidate a possible interaction between 5-HTTLPR polymorphism and sleep quality on cognitive and functional parameters among DS patients.

This thesis will contribute to understand the cognitive and behavioral phenotype variability, analyzing the role that selected biochemical parameters (as plasmatic concentrations of (1) Aβ40 and Aβ42 and (2) FT4 and TSH), genetic variability (as genetic polymorphisms out of the chromosome 21:*COMTVal158Met*, *VNTR-DAT1 and 5-HTTLPR)* and sleep quality play on DS cognitive and adaptive behavioral variability. Firstly, the study of the association between cognitive, behavioral, functional, and biochemical markers of AD would serve to find cognitive tools of assessment to be used as screening tests for early detection of early symptoms of dementia in DS. Secondly, the role that thyroid status plays on cognition, specifically, how low concentrations of FT4 in plasma (as a biomarker of hypothyroid activity) accounts (or not) to a worse performance in a set of cognitive tasks and if those concentrations of FT4 (which can vary as a function of L-thyroxine treatment) are more relevant that the hypothyroidism diagnosis. Moreover, the modulatory effects of DA on prefrontal cognition and behavior would also be studied, evaluating specific polymorphisms of the DA-ergic system (*COMTVal158Met* and *VNTR-DAT1) as*

well as the modulatory effects of serotonin on sleep quality, evaluating the 5-HTTLPR polymorphism of the serotonin system, and how this correlation in turn interferes on cognitive and functional impairment.

In the context of significant increases in DS life expectancy, the results of these studies, which are focused on individual differences in trisomy 21 at different concentrations -genetic, biochemical, cognitive, behavioral, and environmental-, constitute a potential approach to provide better scientific knowledge regarding genotype/phenotype interactions in DS and allowing the exploration of risk factors for early AD and deteriorated cognition. This knowledge must be considerate to improve the design of future clinical trials and personalize the therapeutic approaches.

2. Hypothesis and objectives

2.1 Hypothesis

Study 1

A poor semantic verbal fluency pattern (SVFP) is an early indicator of AD in GP. DS subjects are at high risk for early onset of AD-like dementia. We postulate that a poorer performance in semantic verbal fluency tasks will be associated to early indicators of dementia, worse adaptive behavior and higher plasma concentrations of amyloidosis biomarkers (Aβ40 and Aβ42).

Study 2

Thyroid dysfunction is common among subjects with DS. Hypothyroidism, the most frequent disorder, is associated with cognitive functioning. The evaluation of the thyroid status-related measurements (i.e. FT4, TSH) should be assessed in patients with impaired cognitive function as DS. We postulate that TSH and FT4 plasma concentrations, hypothyroidism diagnosis, and L-thyroxin treatment would interfere (in their own right) on cognition in DS. Thus, cognitive consequences of hypothyroidism can be discriminated from those found in the natural course of DS.

Study 3

Phenotypic consequences of genetic variants are modulated by the genetic background in which they occur. We hypothesize that DA related genetic polymorphisms *COMTVal158Met* and *VNTR-DAT1* variants will interact with the trisomic genetic background and this in turn may modulate PFC cognitive and behavioral skills in DS.

Study 4

The 5-HTTLPR has been associated with the quality of sleep. The later plays a role on cognition in GP and DS. We postulate that there will be an association between allelic variants of 5-HTTLPR and sleeping features as well as between the later and cognitive functioning and functional state. 5-HTTLPR variants will interact with the DS genetic background, the quality of sleep status and this in turn may modulate cognition and behavioral skills of DS individuals.

2.2 General Objective

The general objective of this thesis is to study biochemical and genetic factors interacting with neuropsychological performance of DS subjects. These findings will bring scientific knowledge to better assess drug-efficacy in DS clinical trials for cognitive and functional enhancement.

2.3 Specific objectives

Study 1

Two main objectives were targeted in this study: **(1)** to characterize the SVFP including clustering and switching abilities in adults with DS in comparison to age-matched GP (assessment will be performed by administering the Semantic Verbal Fluency Task (SVFT), which wasn't characterized yet in this population) and, **(2)** to explore the association of SVFP with early indicators of dementia (DMR), adaptive behavior (ABAS-II) and amyloidosis biomarkers (Aβ40 and Aβ42).

Study 2

The main objective is to analyze thyroid dysfunction (TSH, FT4, hypothyroidism diagnosis, age of onset and L-thyroxin dosage) and to evaluate whether it interferes on cognition in DS young adult population.

Study 3

The main objective is to explore whether polymorphisms known to influence DA concentrations in the PFC, (*VNTR* in the DA transporter-DAT1 and *Val158Met* in the COMT interact whit PFC-dependent executive cognitive functions (impulsivity, attention, working memory, mental flexibility, planning ability, inhibitory control) and adaptive behavioral skills in DS young adult population.

Study 4

Four main objectives were targeted in this study: (1) To examine the association between allelic variants of 5-HTTLPR genetic polymorphism and sleeping features (2) To analyze the association between allelic variants of 5-HTTLPR and cognitive and functional features, (3) To study if sleeping features are associated to cognitive and functional state and also to early symptoms of AD, and (4) To elucidate a possible interaction between 5-HTTLPR polymorphism and sleep quality on cognitive and functional parameters among DS patients.

3. Methodological Approaches

3.1 Participants

The samples of the four studies were drawn from the baseline visit of the TESDAD clinical trial (TESDAD Study ClinicalTrials.gov Identifier: NCT01699711).

3.1.1 Study 1: Plasma Aβ concentrations, cognition and behavior in DS

Participants enrolled in cross-sectional Study 1 (n = 50) were young adults (aged 17–34 years) of both genders with DS (complete trisomy 21, mosaic or translocation). Subjects with neurological disease other than DS (epilepsy, cerebral palsy, hemiplegia, central nervous system infection with neurological deficit), relevant medical disease, unstable co-morbid mental disorder (anxiety disorder, depression, obsessive compulsive disorder), or undergoing any treatment that could interfere with cognitive function or alter key biomarker analyzed were excluded from the study. Also, exclusion criteria included subjects with severe language deficit (significant speech and/or comprehension limitations), behavioral disturbances and/or poor level of collaboration during the assessment but no subjects were excluded from the analysis by this criterion.

To determine the gap in cognitive performance between DS subjects and healthy adults a comparison group, matched for age (mean age: 22.6 ± 3.8) was included 59 young healthy adults of both genders. These participants were assessed in previous neuropsychological studies (de Sola et al., 2008; Fagundo et al., 2010). Healthy volunteers were excluded if they had neurological or relevant medical diseases, or if they had been diagnosed with a psychiatric disorder following Diagnostic and Statistical Manual of Mental

Disorders IV (DSM-IV) criteria. Whilst prevailing methodology compares DS subjects to healthy subjects of the same "mental age" to provide an index of global level of mental functioning (Edgin, Mason, et al., 2010; Finestack & Abbeduto, 2010), other studies which are focused on specific cognitive capacities (i.e. language, memory) have used age-matched healthy population with standard norms (Næss, Lyster, Hulme, & Melby-Lervåg, 2011). Comparing mental-age matched subjects is based on the notion that the mental maturation rate in subjects with ID differs substantially from typically developed subjects of equal chronological age, but not significantly, or only in certain capacities, when matched for their "mental age". Even though, comparing with chronological-age matched allows determining the gap in cognitive performance between DS subjects and healthy adults, which is the cognitive target in clinical trials (de Sola et al., 2015).

3.1.2 Study 2: Thyroid function and cognition in DS

Participants enrolled in this cross-sectional **Study 2** ($n = 85$) were young adults (mean age 23.24, ± 4.19) of both genders (44 males and 41 females) with DS (complete trisomy 21, mosaic or translocation). Participants were excluded for same criteria than **Study 1,** but we here include subjects with speech limitations and we only exclude those with significant comprehension limitations, since the two subjects with speech limitations were balanced among groups of hypothyroidism diagnosis and a great number of the cognitive tasks (10/16) were non-speech dependent**.**

In the present study, the analysis of FT4 and TSH plasma concentrations was successfully performed in the whole eligible sample (n = 85). Only 15 subjects could give us the data about hypothyroidism onset and the total years under L-thyroxin treatment.

3.1.3 Study 3: Dopamine polymorphisms, prefrontal cognition and behavior in DS

Participants enrolled in cross-sectional **Study 3** (*n* = 69) were young adults (aged 18-37) of both genders with DS (complete trisomy 21, mosaic or translocation). Participants were excluded for same criteria than **Study 1.**

In the present study, the genotype analysis was performed in the whole eligible sample (n = 81), 10 subjects could not be genotyped because an insufficient amount of deoxyribonucleic acid (DNA) and 11–*repeat* allele carriers were excluded of the DAT1 polymorphism analysis since we only detected two subjects, resulting in a final sample of 69 participants. Subject disposition, withdrawals and the composition of the primary analysis population are shown in the CONSORT diagram (see **Figure 5**). The data of a sample of healthy control Spanish subjects genotyped for *COMTVal158Met* [n= 93, mean age = 22.8 (4.1), sex = 49 M, 44 F; (Cuyàs et al., 2011)]*,* and *VNTR-DAT1* [n = 57, mean age = 22.7 (2.83), sex = 57 M, 0 F; unpublished data] were drawn from a previous study (CEIC-IMAS 99/935/I 2001/1226/I), and were used to assess whether the genotype frequency in the TESDAD DS population was in equilibrium.

3.1.4 Study 4: Quality of sleep an cognition and its interaction with the serotonin transporter polymorphism

For this cross-sectional study a group of 87 young adults of both genders with DS was drawn from the baseline exploration of a clinical trial carried out by our research group (TESDAD Study ClinicalTrials.gov Identifier: NCT01699711). Participants were excluded for same criteria than **Study 1.** Four subjects were excluded for speech and language comprehension limitations. Subject disposition, withdrawals and the composition of the primary analysis population are shown in the CONSORT diagram (see **Figure 6**). The genotype analysis was performed in the whole eligible sample (n = 83), 4 subjects could not be genotyped (required DNA quantity unavailable), resulting in a final population of 79 participants of both genders (40 males

and 39 females) with any of the DS genetic variations (trisomy 21; n = 75, partial trisomy; n = 2, mosaic; n = 1 or translocation; n = 1), mean age of 23.6 (range =17-34, SD= 4.3) and median IQ of 41 (40.5% with IQ less than 40) and a maximum IQ of 86, whereas the mean K-BIT standardized score was 104.6 (SD: 17.2; range: 80–180).

A sample of 58 age-matched (mean age=23.2, SD=4.8) control Spanish subjects from a previous study (CEIC-IMAS 99/935/I 2001/1226/I) (Verdejo-García et al., 2013) was used to compare 5-HTTLPR genotypic frequencies.
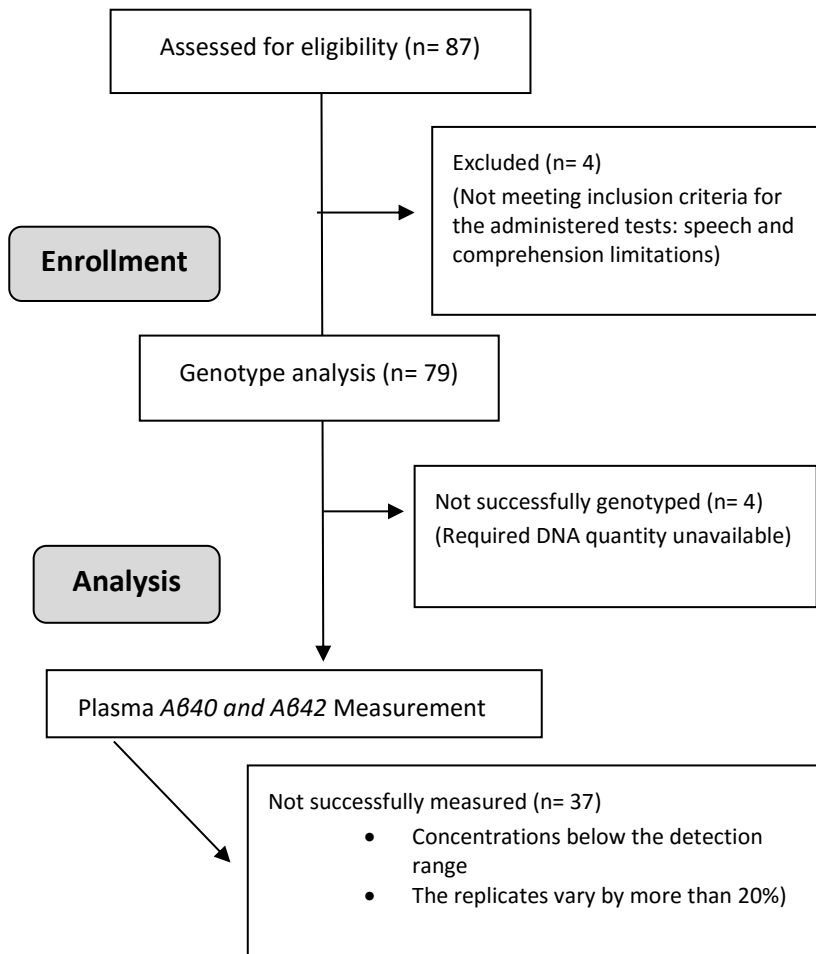
Figure 6. CONSORT diagram of Study 4 showing the flow of participants regarding subject disposition, withdrawals and the composition of the primary analysis population.

All **studies** were conducted in accordance with the Declaration of Helsinki and Spanish laws concerning data privacy. The protocol was approved by the Ethical Committee of the Parc de Salut Mar of Barcelona (CEIC-PSMAR). Upon arrival at the research center (Hospital del Mar Medical Research Institute-IMIM), participants, parents and legal guardians (in case of legal incapacitation) were informed of the ensuing protocol and they gave their written informed consent before participating.

3.2 Procedures

3.2.1 Cognitive assessment: The TESDAD Battery

In all studies the IQ estimation was assessed with The Kaufman Brief Intelligence Test (K-BIT) (Kaufman & Kaufman, 1994) to be controlled in the analysis as a possible confounding factor. This tool meets the need to evaluate the intellectual status of individuals covering a wide age range, from 4 to 90 years, and significantly correlates with the Wechsler Intelligence Scale for Children-III (Canivez, 2005). The IQ corresponds to the K-BIT standardized total score and was considered in the analyses to control for clinically relevant pre-existing differences in general ID among individuals.

The entire TESDAD battery (de Sola et al., 2015) was administered to all subjects, but only selected tests assessing specific cognitive and functional domains were included in the analysis of each study depending on the objectives for each one (i.e the role of DA polymorphisms on PFC- cognition).

Every test forming the TESDAD battery is described as follows:

Psychomotor speed

Motor Screening test ((MOT), from the Cambridge Neuropsychological Test Automated Battery (CANTAB)) (Cognition, 1996) Participants are instructed

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to touch a series of crosses that appear randomly on the screen. This task assesses psychomotor speed and accuracy. The measure of response latency (in milliseconds) was considered in the present study.

Attention

<u>Simple Reaction Time (SRT; from the CANTAB).</u> In this test, subjects must press the button on a press pad as soon as they see a square appearing in the middle of the screen. Intervals between the examinee's response and the onset of the next stimulus are variable during task performance. This task tests general alertness and motor speed. Measures of accuracy (number of correct trials; range: 0-100) and response latency (in milliseconds) were used for the analyses.

<u>Digit Span: forward recall (FR) (from the Wechsler Adult Intelligence Scale- III (WAIS-III), Spanish version)</u> (Wechsler, 1997) Subjects are required to listen to a series of numbers with randomized presentation, and then repeat them back. The length of the series increases with the subject's success. Forward recall score provides a good measure of verbal attention and memory span.

<u>Spatial Span forward recall (SSP-FR): (from the CANTAB).</u> This test constitutes an analogue of the Corsi block task (Milner, 1971). Subjects must memorize and reproduce a sequence of spatial locations demonstrated on the computer screen. Participants view a sequence of squares that change, one at a time, from white to a different color. The examinee has to touch on the screen the squares in the same order as they were presented. Length of the sequence increases with the subject's success. Forward recall is predominantly a measure of visual attention and memory span. Measures of span length and total number of errors were considered for the analyses.

Executive functions

Digits Span: backward recall (BR) (from the WAIS-III, Spanish version).
(Wechsler, 1997) Subjects are required to listen to a series of numbers with
randomized presentation, and then repeat them backwards. The length of
the series increases with the subject's success. Backward recall score is
predominantly a measure of verbal working memory span.

Spatial Span backward recall (SSP-BR): (from the CANTAB). Subjects are
required to memorize and inversely reproduce a sequence of spatial
locations that appear on the computer screen. The examinee has to
reproduce this sequence backwards by touching items in the inverse order
they were originally presented. Length of the sequence increases with the
subject's success. Backward recall is predominantly a measure of visual
working memory span. Measures of span length and total number of errors
were used for the analyses.

Semantic Verbal fluency Task (SVFT) (Benton, Hamsher, & Sivan, 1976)
Subjects are asked to generate as many words as possible in 1 minute
belonging to the specified category of "animals". High scores indicate greater
verbal fluency ability.

Tower of London-Drexel University (ToLDx) (Culbertson & Zillmer, 2005). For
this study we used the pediatric version (ages 7 to 15) in order to avoid floor
effects. Standard norms were used from this version from the upper age
range: 13 to 15 years old. This test requires the movement of three different
colored balls across three different sized pegs in order to replicate a goal
configuration. Movements follow strict rules. The first two problems were
training tasks, following which 10 problems of increasing complexity were
presented. The task ceased after the examinee failed to solve two
consecutive problems. For those subjects who failed to complete the entire

test, number of movements and problem-solving time were adjusted for the number of uncompleted trials to allow a feasible comparison with higher performers, assigning the maximum number of movements (20) and maximum problem-solving time (120 s) to each failed item. Scores for total problem-solving time (in seconds), total number of moves needed to complete the configuration, and total number of problems solved within the minimum number of moves were used for the analyses, providing a good measure of planning ability.

Weigl Color-Form Sort Test (WCFST) (Goldstein, Scheerer, & Hanfmann, 1953). This is a set-shifting task that assesses the ability to categorize across two dimensions: color and shape. Instructions for administration and scoring were taken from Strauss & Lewin, 1982 (Strauss & Lewin, 1982)Test material consists of 12 tokens: four circles, four triangles, and four squares, shapes are colored blue, red, yellow or green. The 12 tokens are displayed unsorted in front of the examinee. In the first trial the examinee is required to sort the tokens in a way that they go together (color or shape). After this first trial, the examinee is required to sort them again but using a different combination. The examinee receives prompting from the examiner if he or she is not able to change the sorting principle used in the first trail. Scores range from 0 to 5. A maximum score of 5 points is obtained when the subject is able to shift the initial category without a prompt, whereas 0 points are obtained if the subject fails despite prompting. A higher score indicates greater capacity for set-shifting and is considered a good estimation of cognitive flexibility or reversal learning in individuals with ID.

Cats & Dogs Test (Ball, Holland, Treppner, Watson, & Huppert, 2008) This is a Stroop-like task assessing response inhibition, based on the original Day–Night task (Gerstadt, Hong, & Diamond, 1994). In this test, a sequence of 16 pictures, 8 cats and 8 dogs arranged in a prefixed order, are presented to the

examinee on a single strip of card. The task consists of two trials with two different conditions: a control and an experimental-inhibition trial. In the control the examinee is required to point to each picture in turn and name the animal as quickly as possible. In the experimental-inhibition condition, the examinee is instructed to say 'dog' when he or she points to a cat and to say 'cat' when pointing to a dog. A practice trial is given to the examinee before the performance of both trial conditions. Measures of task accuracy in the experimental inhibition trial (total number of correct responses) and total time performance (in seconds) were included in the analyses.

Memory and learning

<u>Paired Associates Learning (PAL, from the CANTAB).</u> Participants are required to learn associations between an abstract visual pattern and its location. In this task the participant is presented with a number of white boxes, arranged in a circle around an empty central space in the screen. When the trial begins, each of the boxes "opens" in turn to reveal what is underneath. In some cases, the box is empty, while in others a unique abstract pattern is presented. Each of the boxes opens in a randomized order until the participant has looked under every one. Next, a single pattern is presented in the center of the screen and the subject is instructed to touch the box where that pattern has been shown during the presentation phase of the trial. This task increases in difficulty from 1 to 8 patterns. The number of stages correctly completed provides an index of visual episodic memory. Other measures included in the analyses were the total number of errors committed after being adjusted for the number of trials completed, and first trial memory score which measures the number of patterns correctly located after the first trial across all stages completed (ranging from 0 to 26).

Pattern Recognition Memory (PRM, from the CANTAB). Participants are shown a series of two blocks of 12 abstract visual patterns, presented sequentially in the center of the computer screen. Patterns are designed so that they cannot easily be given verbal labels. Each pattern is shown for 3 seconds. In each of the 12 recognition trials, two patterns are presented: one from the series that the participants have already seen and another novel pattern. The participant should recognize the pattern that he or she has already previously seen. This same procedure is repeated with a second block of 12 new patterns. The recognition trials, however, commence 20 minutes after the presentation of this second block in order to provide a measure of delayed recall. Measure of correct responses expressed as a percentage (total percent correct) was recorded in this recognition task for immediate and delayed recall.

Cued Recall Test (CRT); (Devenny, Zimmerli, Kittler, & Krinsky-McHale, 2002) This test is an analogue of the original list-learning Free and Cued Selective Reminding Test (Grober & Buschke, 1987) that was adapted for detecting memory dysfunction in DS related to preclinical and clinical stages of DAT. The test consists of a list of 12 items which should be verbally recalled by the examinee during 3 trials of free and cued recall. The test starts with a learning phase where the examinee is required to learn the list of 12 items using 12 images. Four pictures are presented at a time, one in each quadrant of a card. First, the examinee has to name each of the four pictures in the card, and secondly assign each picture according to a verbal category-cue given by the examiner. In order to assure that the items are correctly encoded, the card is removed and the examinee is asked to immediately recall the four test items by memory. Presentation of the cards during the learning phase cease when the examinee is able to recall all four items correctly from the three cards. The testing phase immediately follows and

consists of the three trials of free and cued recall. Before every free recall trial, a short interference task is performed which consists of counting up to 20 for 20 seconds. After this interference task, the examinee is requested to verbally recall as many items from the list, in any order, as he or she is able to remember. Next, the missed items are requested again with a verbal cue for each item provided. If the examinee fails to retrieve the item despite prompting, he or she is reminded of the missed item in order to allow learning for the next trial. The same procedure is applied for each of the 3 trials. After 20 minutes, a free recall of the list of words is requested, again followed by a cued recall for those words that were not recalled. Measures of immediate free total recall (in the three trials), total immediate recall (free and cued), free delayed recall (after 20 minutes), and total delayed recall (free and cued) were considered in the study as good estimations of verbal episodic. In this test, scores of immediate free total recall across the 3 trials and total immediate recall (free and cued) range from 0 to 36 in both cases, whereas scores of delayed free recall and total delayed recall (free and cued) range from 0 to 12.

Language

Boston Naming Test (BNT) (Kaplan, Goodglass, & Weintraub, 1983) For this study we used the 60 item version validated in Spanish young adults in the Neuronorma Project (Peña-Casanova et al., 2009). The test consists of 60 black and white pictures graded in naming difficulty. Each picture is presented individually. The examinee is asked to name each item, and when unable to do so spontaneously, the examiner provides semantic and/or phonemic cues. Correct identification of a picture without cueing or with a semantic cue is awarded one point, whereas errors or identification with phonemic cue earn 0 points. Testing begins with item 1 for all participants and ceases after 4 consecutive errors. No choice recognition task is

administered for failed items. Measure of total number of items correctly identified (spontaneously and after semantic cueing) was recorded and considered for the analyses.

Token Test (De Renzi & Faglioni, 1978)A shortened version (36 items) was used to evaluate verbal comprehension in this study. Instructions for administration and scoring were taken from the original publication. Test material consists of 20 tokens in two shapes (circles and rectangles), two sizes (big and small), and five colors (red, black, yellow, white, and green). The tokens are laid out according to a fixed configuration in front of the examinee. The test requires the examinee to touch the tokens according to the oral commands provided by the examiner. Thirty-six commands are divided into six stages of increasing complexity. In the first five stages, the examiner repeats the command if the examinee fails to perform the command correctly or does not respond for 5 seconds. A score of 1 point is given when the examinee succeeds in carrying out the command correctly at the first attempt, and 0.5 points are given if he or she succeeds at the second attempt. In the last stage, just one attempt is permitted to carry out the command correctly and a score of 1 or 0 points is given for each item. The task ceases after the examinee fails to correctly carry out five commands in the first stage or four consecutive commands in any of the remaining stages. Total score in the test was considered for the analyses as a good measure of verbal comprehension.

3.2.1.1 Study 1 and 3: Assessment of the semantic verbal fluency pattern

Semantic Verbal Fluency Task (Benton et al., 1976) We here used the SVFT as a measure of semantic memory but also as a measure of executive functioning. Three outcome variables were obtained: (i) the total number of

correctly generated words in 60 s, and the percentage of words generated every 15 s; (ii) errors committed including intrusions (words not belonging to the specified semantic category), perseverations and repetitions (same words or same words with different endings); and (iii) clustering and switching measures that were obtained to determine the strategies used to perform the task. Mean cluster size was the main dependent variable for clustering, whereas number of switches was the main dependent variable for switching (Troyer, Moscovitch, & Winocur, 1997; Troyer, 2000). A cluster was defined as any series of two or more successively produced words belonging to the same semantic subcategory, determined a priori (Fagundo et al., 2010). Cluster size was computed by adding up series of words from the same subcategory starting from the second word within each cluster (i.e., a three-word cluster has a size of two). The number of switches was defined and computed as the number of times the participant changed from one cluster to another. Two clusters may also be overlapping, for example, from "farm animals" to "birds" in "cow–pig–chicken–pigeon–eagle." Here, one switch is made between the cluster "cow–pig–chicken" and "chicken–pigeon–eagle." The computation of number of switches included single-word clusters. An inter-rater reliability analysis was performed and the reliability studied by means of the intra-class correlation coefficient (ICC) was high with values ranging from 0.89–0.98.

3.2.1.2 Study 2 and Study 4: Global cognitive assessment

All the tests from the TESDAD battery were fitted in the analysis of both studies in order to analyze the possible relation between biochemical (Study 2) and genetic (Study 4) parameters with cognitive performance through the individual assessment of the following cognitive domains: psychomotor speed, attention, memory, executive function, and language.

3.2.1.3 Study 3: Assessment of Prefrontal dependent Cognition (components of executive function)

We focused on PFC-dependent cognition, exploring components of executive functioning, specifically the following domains: impulsivity, attention, working memory, mental flexibility, planning ability, inhibitory control and adaptive behavior.

Impulsivity was measured analyzing the relation between response times and errors in a set of tests from the non-verbal CANTAB: MOT, SRT, and SSP-FR.

To assess the *attentional span* the Digits Span-FR from WAIS-III and the SSP-FR (CANTAB) were administered.

Working memory for visual and verbal information was evaluated with the SSP-BR from the CANTAB and the Digits Span-BR from WAIS-III, respectively.

Mental flexibility was measured rating *switching ability* and *reversal learning*. Switching ability (times subject change from one semantic category to a new one) was assessed with the SVFT (animals in one minute), and reversal learning (disengagement from inappropriate strategies when reinforcement contingencies change) with the WCFST.

Planning ability was measured using the ToLDX. Finally the Cats & Dogs test was used to assess inhibitory control.

3.2.2 Functional assessment

The TESDAD battery included the assessment of a set of daily life domains reported to be relevant in DS functionality: adaptive behavior and quality of life. The normalized neuropsychological tools administered to parents: the adult version of the Adaptive Behavior Assessment System (ABAS-II) and the

parents' version of the Kidscreen-27. In addition, poor sleep quality, as reported by parents, was controlled by means of the Pittsburgh Sleep Quality Index (PSQI) for its possible negative impact on cognitive performance. Finally and although it was not included in the TESDAD battery, early symptoms of dementia in daily living were also evaluated with the Dementia Questionnaire for Persons with Intellectual Disability (DMR) (Evenhuis HM, Kengen MMF, 2006) as a part of the functional assessment since it was relevant for two of the studies performed in this thesis (Study 1 and Study 4).

Adaptive behavior in daily living

The Adaptive Behavior Assessment System-Second Edition (ABAS-II, adult version) (Harrison & Oakland, 2003). ABAS-II was designed, according to AAMS guidelines, for evaluating adaptive skills in people with mental disabilities of a wide age range and across multiple environments. The ABAS-II tool for adults (ages 16 to 89) includes 5 subscales which assess the individual's competence (in terms of behavior frequency) in 10 different skill areas: communication abilities, community use, functional academics, home living, health and safety, leisure, self-care, self-direction, social interaction, and working/labor skills. All answers to this questionnaire were self-reported by parents. Raw scores of the dimensions and total ABAS score provide a good index of adaptive behavior, higher scores corresponding to greater adaptive skills and independency in everyday living. For the sake of the study, those items rated/reported as guessed by parents were scored as zero in each subscale in order to avoid subjective judgments concerning functional changes. In addition, because most individuals in our sample were unemployed, scores in the work skill area were not included in the analyses and not considered when calculating the total ABAS score.

Quality of life (QoL)

Kidscreen-27 , parents version; (Ravens-Sieberer et al., 2007) This instrument assesses quality of life from the child and adolescent's perspective in terms of their physical, mental, and social well-being. The questionnaire measures five dimensions: physical well-being, psychological well-being, autonomy & parents, peers & social support, and school environment, by means of five graded subscales. Only the version for parents was used, and all answers to this tool were self-reported by them. Because a significant proportion of individuals in our sample did not regularly attend school or any educational program, scores in the school dimension were not included in the analyses and were not considered when calculating the total Kidscreen-27 score. Raw scores on the four mentioned dimensions (except school environment) and total score in the scale were included in the study, providing a good measure of quality of life.

Quality of sleep

Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) was used to assess sleep quality and patterns of sleep in each participant. The following domains are evaluated: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Parents self-reported all answers to each of the seven areas. Each component was weighed equally on a 0–3 scale, thus the PSQI score ranged from 0 to 21. A global PSQI score > 5 yielded a diagnostic of bad quality of sleep.

During the medical exploration other questions related to quality of sleep clinical history (CH) were made to caregivers as follows: *How is your child sleep quality? (Good/bad), how are your child postures during sleep time?*

(good/bad), does your child present snoring disorder? (Yes/no), is your child diagnosed for apnea? (Yes/no).

Early symptoms of Dementia in daily living

<u>Dementia Questionnaire for Persons with Intellectual Disability (DMR) (Evenhuis HM, Kengen MMF, 2006)</u>. The DMR is also a self-reported questionnaire about daily living abilities, but in this case it specifically measures the specific cognitive and social deterioration associated to dementia in people with ID. It consists of 50 items and eight subscales. Combined scores on the first three subscales (Short-term memory, Long-term memory and Orientation) are presented as the Sum of Cognitive Scores (SCS). Combined scores on subscales four through to eight (Speech, Practical skills, Mood, Activity and Interest, and Behavioral disturbance) are presented as the Sum of Social Scores (SOS).

Higher scores in DMR and PSQI reflect a worse state, while higher punctuations in ABAS-II and Kidscreen-27 reflect a better functionality.

All questionnaires were given to the caregivers for completion while participants completed the neuropsychological testing to minimize biasing error caused by subjective interviewer effects and to maximize efficiency (information vs time ratio) administration. We ensured they understood how to complete the questionnaires and solved all doubts before and after completion, and checked that all questions were filled.

3.2.3   Study 1 and Study 4: Plasma Aβ Measurement

Overnight fasting blood samples were collected on site by a qualified nurse, during the morning hours. The blood was drawn into 8 mL Heparin Lithium tubes (B&D, UK), centrifuged at 4°C for 15 min at 3000 rpm, and the plasma was distributed in aliquots and stored at −70°C until analysis. Samples (only

for DS subjects) were analyzed for plasma Aβ concentrations; using Inno-Bia Plasma Aβ forms (Aβ40 and Aβ42, truncated Aβ40 and Aβ42 not reported) assay (Innogenetics, Fujirebio) following the manufacturer instructions. The plaques were read in a Bio-Plex 200 Systems (Bio-Rad) instrument, and the standard curves were fitted using the provided software (Bioplex Manager 6.1).

3.2.4   Study 2: Thyroid Status assessment

Thyroid status was assessed among 3 primary and 3 secondary variables.

Thyroid Stimulating hormone (TSH) and FT4 plasma concentrations

 TSH and FT4 concentrations were analyzed by an external certified laboratory according to standard clinical analysis techniques, using ECLIA assays (Cobas, Roche, Switzerland) as specified by the manufacturer. Overnight fasting blood samples were collected in an 8mL Litium-Heparin tube (B&D, UK). Plasma was separated from the cellular fraction through centrifugation at 3,000 rpm for 15 minutes at 4ºC and subsequently distributed in aliquots and frozen at -80ºC until analysis.

Hypothyroidism diagnosis criteria (yes /no)

Participants were categorized in (1) subjects with non-previous thyroid dysfunction and (2) subjects with previous hypothyroidism if they were receiving L-thyroxin at time of enrolment.

Other variables of interest

Age of Hypothyroidism onset: Caregivers were asked for the age participants were when they were diagnosed for hypothyroidism.

Age of L-thyroxin treatment onset: Caregivers were asked for the age participants were when they started the L-thyroxin treatment.

Years under Hypothyroidism diagnosis: It was calculated by subtracting the current age to hypothyroidism onset age.

Years under L-thyroxin treatment: Caregivers were asked for the number of continued years participants were properly controlled by L-thyroxin treatment.

L-thyroxin dosage: Current L-thyroxin dosage.

3.2.5 Study 3 and 4: Genotyping

Genomic DNA was extracted from the peripheral blood leukocytes of all the participants using Flexi Gene DNA kit (Qiagen Iberia, S.L., Spain) according to the manufacturer instructions. COMTVal158Met allelic variants were determined as previously described (Verdejo-García et al., 2013). VNTR polymorphism of VNTR-DAT1 was evaluated by PCR using the following primers 5'TGTGGTGTAGGGAACGGCCTGAG-3' (forward) and 5'CTTCCTGGAGGTCACGGCTCAAGG-3' (reverse), the reaction conditions for this experiment were the following: 1X PCR Amplification buffer and 1X PCR Enhancer solution (Invitrogen, Carlsbad, CA), 1.5 mM MgSO4, 0.2 mM dNTPs, 0.2 µmol of each primer, 1.75 U of Taq DNA polymerase (Invitrogen), and approximately 50 ng of genomic DNA as template. The reaction was carried out on the following conditions: an initial denaturation of 5 minutes at 95ºC, followed by 50 cycles as follows: 30 seconds denaturation at 95ºC, 30 seconds annealing at 63ºC, and 45 seconds elongation at 68ºC, and a final elongation step at 72 degrees for 5 minutes. The PCR products were separated by electrophoresis on a 2% agarose gel, being the 9-allele 440bp, and the 10-allele 480bp.

In **Table 1** there is a summary of the variables of assessment (cognitive functional and biological) fitted for each study.

Table 1 Summary of the variables analyzed in each study.

Cognitive assessments in each study		Study1	Study2	Study3	Study4
IQ	K-BIT	x	x	x	x
Psychomotor speed	MOT		x		x
Attention	SRT		x		x
	Digit Span: FR		x		x
	SSP: FR		x		x
Executive functions	Digits Span: BR		x	x	x
	SSP: BR		x	x	x
	SVFT	x	x	x	x
	TOLDX		x	x	x
	WCFST		x	x	x
	Cats & Dogs Test		x	x	x
Memory and learning	PAL		x		x
	PRM		x		x
	CRT		x		x
Language	BNT		x		x
	Token Test		x		x
Functional assessments in each study					
Adaptive behavior in daily living (ABAS-II)		x		x	x
Early Dementia symptoms in daily living (DMR)		x		x	x
Quality of life (QoL) Kidscreen-27					x
Quality of sleep (QoS) PSQI & CH					x
Biological assessments in each study					
Plasma Aβ Measurement		x			x
Thyroid Status assessment			x		
COMTVal158Met &VNTR-DAT1 genotyping				x	
5-HTTLPR genotyping					x

3.2.6 Statistical analysis

In all studies the statistically significant associations were fixed at a significance level of 0.05, using the model-based estimated mean differences and the corresponding 95% confidence intervals. All statistical analyses were performed using the statistical software packages Statistical Package for the

Social Sciences (SPSS) (Version 18.0; SPSS Inc., Chicago, IL, USA) and R (Version 3.1.1; The R Foundation for Statistical Computing, Vienna, Austria).

3.2.6.1 Analysis in Study 1: Plasma Aβ concentrations, cognition and behavior in DS

Descriptive analyses were carried out for sociodemographic and clinical parameters of all the participants, providing measures of mean and standard deviation.

Regarding to inferential analysis, the results are described by means of measures of both central tendency (mean and median) and variability (standard deviation and range) for numeric variables, and absolute and relative frequencies for categorical variables. In the case of the IQ, only the median is reported because no distinction is made of values below 40. The differences between DS and GP groups with respect to semantic verbal fluency performance are quantified by means of the standardized mean difference (Cohen's d). The computation of all correlations of interest was done using Pearson's correlation coefficient. Analysis of covariance (ANCOVA) models were used to study the associations in DS between semantic verbal fluency outcomes, other cognitive and functional measures, and amyloidosis biomarkers, on one hand, and gender, IQ, and age, on the other hand. For these analyses, the IQ was categorized into two groups: mild/moderate (IQ ≥ 40) and severe (IQ < 40) within the range of ID level.

3.2.6.2 Analysis in Study 2: Thyroid status and general cognition

The first step consisted of a descriptive analysis of the sociodemographic and clinical parameters of all the participants. Descriptive analyses were also carried out for all neuropsychological variables, providing measures of mean and standard deviation. Cognitive performance in DS participants compared

to standard norms has been published in a previous work by our team (de Sola et al., 2015).

To study the association between hypothyroidism diagnosis as a categorical variable factor and cognitive outcomes as dependent variables, one-way Analysis of variance (ANOVA) models were fitted for all cognitive tests for both the entire sample and only in those subjects with normal concentrations of FT4 (0.73 to 1.95 ng/dl) and TSH (2 to 10 mu/ml). The computation of all correlations of interest (cognitive data, FT4 and TSH concentrations) was done using Spearman's correlation coefficient, and partial Spearman's correlation coefficient controlling for L-thyroxin dosage as an interfering factor in all the participants.

The association between cognitive variables and Age of Hypothyroidism onset, Years under L-thyroxin treatment and L-thyroxin dosage was computable only in the group diagnosed for hypothyroidism and was done using bivariate Pearson's correlation coefficient.

In addition, for those variables with statistically significant correlations (i.e., $p < 0.050$), a linear regression model was applied to study if the thyroid functioning outcomes (introduced in the model as independent variables) were good predictors of the cognitive outcomes (dependent variables).

3.2.6.3 Analysis in Study 3: Genetic polymorphism of the dopamine system, cognition and behavior in DS

Descriptive analyses were carried out for sociodemographic and clinical parameters of all the participants and for all neuropsychological variables, providing measures of mean and standard deviation.

The genotypic frequency of the different polymorphisms was tested by Hardy-Weinberg equilibrium.

To study the association between polymorphisms and cognition, two-way ANOVA models were fitted for each of the cognitive tests and questionnaires scores. The 2 genes were included in the models as independent factors and treated as binary variables, Val allele homozygotes vs. Met allele carriers (COMTVal158Met) and 10-repeat allele homozygotes vs. 9-repeat allele carriers (VNTR-DAT1), respectively. In addition, possible sources of epistatic effects on cognition were considered by assessing the interaction between COMTVal158Met and VNTR-DAT1 genotypes in each of the two-way ANOVA models.

3.2.6.4 Analysis in Study 4: Genetic polymorphism of the serotonergic system, sleep and cognition.

Four main analyses were carried out corresponding to the four main objectives of the present study. (1) To study the association between the serotonin transporter polymorphism (5-HTTLPR) and the quality-of-sleep variables, we applied tools to analyze the relation between two categorical variables. Contingency tables were used to represent the conditional distributions of the quality-of-sleep variables given the 5-HTTLPR genotypes. In addition, the N-1 chi-squared test (I. Campbell, 2007) was used for the homogeneity test of equal conditional distributions. For all the analyses, the genetic polymorphism 5-HTTLPR was treated as a binary variable classified into high/medium (Long- carriers) and low activity (Short homozygotes) groups. The quality-of-sleep variables were the following: Sleep quality (PSQI) (good/bad), and the rest PSQI ordinal subscales, which are Likert scales from 0 to 3 according to the periodicity the problem occurs: (0) never, (1) < once a week, (2) One or two times a week, (3) > Three times a week. We recoded them as follows to create binary variables: "The problem is present < once a week" vs "The problem is present > once a week". The chi-squared test was not applied whenever one of the expected frequencies of

the corresponding contingency table was less than 1. (2) To study the association between the polymorphism and the neuropsychological variables (cognition, functionality and risk for early AD development (DMR rates)) as well as Aβ plasma concentrations and (3) to assess the relation between the (dichotomized) sleep variables and these neuropsychological variables. Two-way ANOVA models were fitted for each cognitive and functional domain including the 5-HTTLPR genotype and the quality of sleep variables as independent factors. (4) The interaction between the 5-HTTLPR genotype and the quality of sleep on cognition, functionality and risk for early AD development was studied by including the interaction term in each of the previous 2-way ANOVA models and testing whether the model fit improved significantly.

3.3 Methodological schedules of each study: objectives, variables and participants.

STUDY 1
OBJECTIVES: • Characterize the semantic verbal fluency pattern (SVFP) • Explore the association of the SVFP with early symptoms of dementia, adaptive behavior and amyloidosis biomarker in DS young adult population.

Neuropsychological variables:	Biochemical variables:	Participants:
Semantic verbal fluency pattern (switching and clustering) Early symptoms of dementia Adaptive behavior	Aβ40 and Aβ42 plasma concentrations	*DS* *n* = 50 age =23.6±4.5 *GP* n=59 age =22.6± 3.8

STUDY 2

OBJECTIVES:

- Analyse thyroid dysfunction
- Evaluate whether it interferes on cognition in DS young adult population

Neuropsychological variables:	Biochemical variables:	Participants:
Psychomotor speed, attention, memory, executive function, and language.	TSH and FT4 plasma concentrations, hypothyroidism diagnosis, age of onset and L-thyroxin dosage.	**DS** *n* = 85 age= 23.2, ± 4.2

STUDY 3

OBJECTIVES:

Analyse whether polymorphisms known to influence DA concentrations (*VNTR* in the DA transporter-DAT 1 and *Val158Met* in the catechol-O-methyltransferase-COMT) interact whit executive function and adaptive behavioral skills in DS young adult population.

Neuropsychological variables:	Genetic variables:	Participants:
Executive functions: Impulsivity, attention, working memory, mental flexibility, planning ability, inhibitory control Adaptive behavior Early symptoms of dementia	Genotyping of COMTVal158Met and VNTR-DAT1	**DS** *n* = 69 Age= 25.29 ±4.46

STUDY 4

OBJECTIVES:

- Examine the association between allelic variants of 5-HTTLPR genetic polymorphism and sleeping features

- Analyse the association between allelic variants of 5-HTTLPR and cognitive and functional features

- Study if sleeping features are associated to cognitive and functional state and also to early symptoms of AD

- Elucidate a possible interaction between 5-HTTLPR polymorphism and sleep quality on cognitive and functional parameters among DS patients

Neuropsychological variables:

Psychomotor speed, attention, memory, executive function, and language.

Adaptive behavior

Quality of life

Early symptoms of dementia

Sleep quality variables:

Sleep quality, bad positions, daytime sleepiness, latency, duration, efficiency, alterations, diurnal dysfunction (PSQI)

Apnea Diagnosis

Snoring Disorder

Genetic variables:

Genotyping of 5-HTTLPR

Participants:

DS: n = 79, age= 23.6 ± 4.3

4.1 Study 1: Plasma Aβ concentrations, cognition and behavior in DS

4.1.1 Descriptive Demographic and Clinical Data of the Participants

In Study 1 our DS sample of 50 participants was formed by 24 male and 26 female with a mean age of 23.6 years (standard deviation (SD): 4.5 years; range: 17–34 years). The median IQ was 41 (38% with IQ less than 40) and a maximum IQ of 70, whereas the mean K-BIT standardized score was 103 (SD: 14.9; range: 80–151). In terms of gender, the median IQ among males was 40 (IQ less than 40: 37.5%; maximum: 66) and among females 41.5 (IQ less than 40: 38.5%; maximum: 70), whereas the mean K-BIT standardized scores were 101 (SD: 15.6; range: 80–144) and 105 (SD: 14.3; range: 80–151), respectively. Concerning the DS karyotypes, the sample showed the usual proportion for this population, with most individuals with full trisomy 21 (48 simple trisomies, one translocation, and one mosaic). Regarding Aβ plasma concentrations, the mean $Aβ_{40}$ concentration was 270.9 pg/mL [SD: 50.8; range: 174–439.3] and the mean $Aβ_{42}$ was 41 pg/mL [SD: 10; range: 21.5–60.9].

4.1.2 Semantic Verbal Fluency Performance in DS Individuals Compared to Standard Norms

Descriptive analyses, Cohen effect size differences (*d*), and confidence intervals (95% CI) of fluency task performance in DS individuals and age-matched standard norms are summarized in **Table 2.** Our results show that

in DS switching correlated more strongly than clustering with the total number of words generated (see **Table 3**).

Table 2. Cognitive performance in DS individuals compared to standard norms. The standardized mean differences are calculated using Cohen's d. Age range: DS: 17-34, Reference standard norms 18-33 Sample size: DS: n=51, Reference Standard norm: n=59.

	Down syndrome		Reference standard norms		Standardized Mean Differences	
Verbal Fluency	Mean (SD)	Range (min-max)	Mean (SD)	Range (min-max)	d	95% -CI
Number of correct words in 60'	9.4 (4.1)	1–20	25.1 (5.7)	11-38	-3.13	[-3.69, -2.57]
Percentage of correct words 0-15'	39.2 (16.6)	0-100	39.6 (7.9)	25-54	-0.03	[-0.46, 0.4]
Percentage of correct words 16-30	28.1 (12.3)	0-50	22.7 (6.5)	14-39	0.53	[0.09, 0.96]
Percentage of correct words 31-45	16.4 (12.4)	0-50	18.6 (5.8)	5-32	-0.22	[-0.65, 0.21]
Percentage of correct words 46-60	17.1 (12.0)	0-60	18.6 (9.5)	0-46	-0.14	[-0.56, 0.29]
Number of switches	4.3 (2.5)	0 – 13	7.4 (2.1)	3-11	-1.4	[-1.82, -0.97]
Mean cluster size	1.1 (0.8)	0 – 3.3	2.8 (0.9)	1.4-6.6	-1.93	[-2.39, -1.46]

Table 3. Correlation between fluency strategies and the total number of words produced (Pearson's correlation coefficient).

	Total correct words			
	Down syndrome		Reference standard norms	
	Correlation; 95%-CI	p-value	Correlation; 95%-CI	p-value
Number of switches	**0.73; [0.57, 0.84]**	< 0.001	0.17; [-0.1, 0.41]	0.244
Mean cluster size	**0.3; [0.02, 0.53]**	0.039	**0.49; [0.26, 0.67]**	< 0.001
Percentage of animals in the first 15 s	0.03; [-0.25, 0.31]	0.84	**-0.54; [-0.73, -0.25]**	0.001
Percentage of animals in the last 45 s	0.11; [-0.18, 0.38]	0.453	**0.51; [0.22, 0.72]**	0.001

We found no correlation between the percentage of words produced in the first 15 and last 45 seconds with the total number of words. The mean

percentage of words produced in the first 15 s was 37.8%. On the contrary, in age-matched healthy population there is a stronger correlation between clustering and the total number of generated words, while there is no correlation between switching and the total number of words produced. Besides, we found a negative correlation between the percentage of words produced in the first 15 s (mean *percentage* = 39.6) and the total number of words, and a positive correlation between the percentage of words produced during the last 45 s and the total number of words, indicating a more extensive lexicon in this population.

4.1.3 Correlation between Fluency Measures and Functional Outcomes

The total number of words produced in 1 min and switching have both a positive correlation with communication skills and a negative correlation with the DMR total score. Furthermore, the total number of words produced in 1 min is positively correlated with the ABAS total score, (see **Table 4**).

Table 4. Correlation between cognitive and functional variables measured using Pearson's correlation coefficient.

	DMR total		ABAS total		ABAS Communication skills	
	Correlation; 95%-CI	*p-value*	*Correlation; 95%-CI*	*p-value*	*Correlation; 95%-CI*	*p-value*
Number of correct words	**-0.5; [-0.68, -0.25]**	**< 0.001**	**0.32; [0.05, 0.55]**	**0.024**	**0.45; [0.2, 0.65]**	0.001
Number of switches	**-0.39; [-0.6, -0.12]**	**0.006**	0.21; [-0.07, 0.46]	0.144	**0.28; [0.01, 0.52]**	0.046
Mean cluster size	-0.14; [-0.41, 0.14]	0.328	0.13; [-0.15, 0.4]	0.36	0.22; [-0.06, 0.47]	0.127

4.1.4 Association between IQ, Gender and Age, and Semantic Verbal Fluency Outcomes in DS

ANCOVA models were applied to analyze the association between the IQ, gender, and age and the semantic verbal fluency performance of DS individuals. As shown in **Table 5**, no statistically significant associations were found between IQ, age and gender, and the verbal fluency pattern in the DS group.

Table 5. Association between the verbal fluency pattern and the intellectual quotient (IQ), sex and age in DS individuals. Parameter estimates, standard errors (SE), and p-values are obtained from ANCOVA models

Verbal fluency outcomes	IQ (< 40 vs. $\geq$ 40)		Sex (Women vs. men)		Age	
	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p
Number of correct words in 60 sec	-1.04 (1.18)	0.386	0. 45 (1.17)	0.704	0.18 (0.13)	0.190
Number of switches	0.25 (0.73)	0.736	0.44 (0.72)	0.547	0.04 (0.08)	0.663
Mean cluster size	-0.36 (0.23)	0.112	-0.07 (0.22)	0.750	0.02 (0.03)	0.488

4.1.5 Association between IQ, Gender, and Age and AD Biomarkers in DS

ANCOVA models were applied to analyze the association between IQ, gender, and age, on one hand, and $A\beta_{40}$, $A\beta_{42}$, $A\beta_{42/40}$ plasma concentrations of DS individuals, on the other hand. We found a statistically significant association between IQ and $A\beta_{40}$. The negative parameter estimate indicates lower $A\beta_{40}$ concentrations among DS individuals of the same age and sex with an *IQ* < 40 compared with those with an *IQ* $\geq$ 40 **(Table 6).**

Table 6. Association between Aβ concentrations and intellectual quotient (IQ), sex, and age in DS individuals. Parameter estimates, standard errors (SE), and p-values are obtained from ANCOVA models.

Aβ concentrations	IQ (< 40 vs. $\geq$ 40)		Sex (Women vs. men)		Age	
	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p
Aβ$_{42}$	-2.05 (2.97)	0.495	2.12 (2.94)	0.475	-0.21 (0.33)	0.527
Aβ$_{40}$	**-38.2 (14.6)**	**0.012**	19.9 (14.2)	0.167	-0.26 (1.59)	0.873
Aβ$_{40/42}$	0.007 (0.013)	0.564	0.004 (0.012)	0.758	-0.0002 (0.001)	0.893

4.1.6 Associations of Aβ$_{42}$ Concentration with Cognitive and Dementia Rating Functional Outcomes

ANCOVA models were applied to analyze the association between Aβ$_{42}$ concentration and both semantic verbal fluency outcomes and functional state among DS individuals. The models were adjusted for IQ, sex, and age **(Table 7).** Individuals with higher concentrations of Aβ$_{42}$ produced lower number of correct words and lower number of switches. Regarding adaptive behavior, subjects with higher Aβ$_{42}$ plasma concentrations had lower scores in the subscale "Communication skills" of the ABAS questionnaire. Concerning dementia rating, higher Aβ$_{42}$ plasma concentrations were associated with higher DMR total score; see **Figure 7** for graphical representations of the statistically significant associations.

Table 7. Association between Aβ42 concentration and cognitive and functional measures. Parameter estimates, standard errors (SE), and p-values are obtained from ANCOVA models adjusted for IQ, age, and sex.

	Estimate (SE)	p
Cognitive performance in SVFT		
Number of correct words in 60'	**-0.187 (0.052)**	**< 0.001**
Number of switches	**-0.085 (0.035)**	**0.018**
Mean cluster size	-0.006 (0.011)	0.582

Functional outcomes		
ABAS adaptive behavior: communication skills	**-0.532 (0.158)**	0.001
DMR total score	**0.366 (0.113)**	0.002
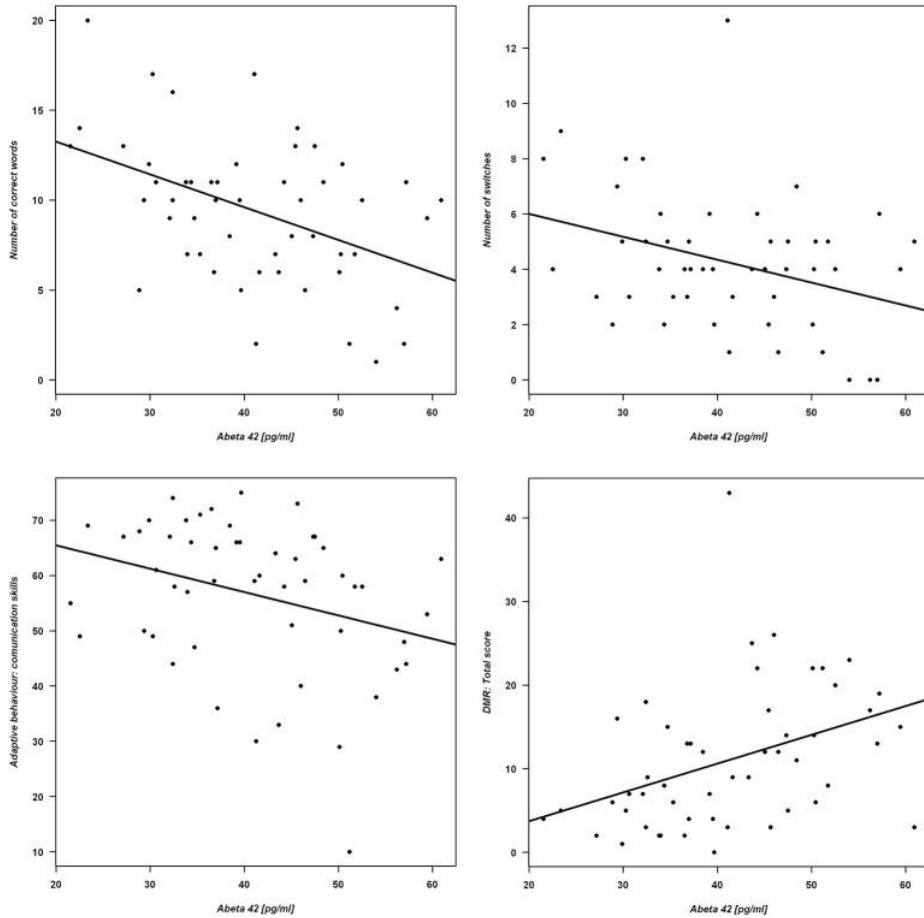
Figure 7. Verbal fluency and functional measures as a function of Aβ42 concentration. Correlations are shown for Aβ42 and Upper panel: number of correct words in the SVFT (left) and number of switches in the SVFT (right). Lower panel: ABAS adaptive behavior (left) and DMR total (right).

4.2 Study 2: Thyroid function and cognition

4.2.1 Descriptive Demographic Clinical and cognitive Data of the Participants

Socio-demographic, clinical and cognitive data for all the neuropsychological variables of the 81 participants are provided in **Table 8** and **Table 9**.

Table 8. Descriptive demographic and clinical data of the Participants

	Non-diagnosed 63.55%	*n*	*Hypothyroidism 36.45%*	*n*	*Total Group*	*n*
Sex (n)	29 M, 23 F	54	14 M, 17 F	31	44 M, 41 F	85
Age $_a$	22.3 (3.6)	54	24.8 (4.7)	31	23.2(4.2)	85
IQ $_b$	42 $_b$	54	41 $_b$	31	41 $_b$	85
K-BIT $_C$	104.8 (16.4)	54	106 (19.9)	31	105.3 (17.6)	85
TSH mU/mL	3.7 (2.3)	54	4.1 (5.2)	31	3.9(3.6)	85
Free T4 ng/dL	1.3 (0.3)	54	1.4 (0.3)	31	1.3(0.3)	85
Normal conc. of FT4 and TSH	41 (75.9%)	54	16 (51.6%)	31	57 (67%)	85
L-thyroxine dosage µg	0	54	84.5 (31.4)	31	30.6 (44.8)	85
Hypothyroidism onset $_a$	-	-	10.5 (6.5)	15	-	-
Hypothyroidis*m* duration $_a$	-	-	13 (5.5)	15	-	-

a: Expressed in years, b: Only the median is reported because values below 40 cannot be determinate exactly, c: standardized score

Table 9. Descriptive outcomes regarding cognitive performance.

COGNITIVE DOMAINS	*Mean (SD)*[A]	*Range (min-max)*	*n*
Attention			
SRT: Simple RT latency (ms)	*588.0 (220.0)*	*302 - 1430*	*85*
SRT: Simple RT(%) correct	*96.6 (5.7)*	*68 – 100*	*85*
SSP Visual Span	*3.2 (1. 5)*	*0 – 6*	*85*
Digit Span	*2.8 (0.8)*	*0 – 4*	*85*
Psychomotor Speed			
MOT: Mean latency (ms)	*1138.0 (391.0)*	*576 - 2645*	*85*
Visual Episodic Memory			
Visual Associative Memory			
PAL: Stages completed	*6.7 (1.8)*	*1 – 8*	*85*
PAL: First trial memory	*11.0 (4.8)*	*0 – 21*	*85*
PAL: Total errors adjusted	*70.1 (60.90)*	*6 – 213*	*85*
Visual Recognition			
PRM: (%) Immediate recall	*66.9 (19.3)*	*25 – 100*	*85*
PRM: (%) Delayed recall	*61.0 (18.6)*	*25 – 100*	*85*

Executive Functions			
Verbal Fluency			
Semantic Word Fluency	9.4 (4.3)	0 – 21	85
Working Memory			
SSP Visual Span Backwards	2.4 (1.6)	0 – 8	85
Digit Span Backwards	1.4 (1.2)	0 – 3	85
Planning			
ToLDx: Total correct Score	1.7(1.4)	0 – 5	82
ToLDx: Total Move Score	84.7 (39.2)	0 – 170	82
ToLDx: Probl-solving time (s)	763.0 (289.0)	0 - 1200	82
Language			
Comprehension			
Token Test: Total score	19.6 (6.5)	1 – 35	85
Naming			
BNT: Total score	24.0 (9.5)	0 – 53	82

Results are presented as mean (standard deviation)

IQ and gender were similarly distributed among both hypothyroidism and non-diagnosed groups. By contrast, an important difference was found regarding age, reporting that subjects with hypothyroidism diagnosis were significantly older than the non-diagnosed group (see **Table 8**). Due to this difference in the age distribution between groups we aimed to control our analysis by age as a factor. For this, we also analyzed whether age was playing a role in cognitive outcomes as a possible confounding factor. No statistically significant difference was found with respect to TSH, FT4 values, and IQ.

4.2.2 Hypothyroidism diagnosis and poor visuospatial episodic memory skills.

Hypothyroidism diagnosis was associated (p=0.022, F= 5.406) with a worse performance in the visuospatial episodic memory task: PAL, CANTAB (see **Figure 8**, **graph A**). Age could be excluded as a possible confounding factor, since it was not associated with PAL performance (p =0.20, F=0.15).

When excluding those subjects with abnormal concentrations of FT4 and TSH (see **Table 8** for distribution) from the analysis, stronger differences were found between groups: the hypothyroid group significantly performed worse

the same task PAL (p=0.013, f= 6.531; see **Figure 8**, **graph B**), and there was a trend to perform worse in the WCFST (p= 0.086, f=3.061) than the non-diagnosed group. No statistically significant differences between groups were found in the rest of the tests administered from the TESDAD Battery neither excluding nor including those subjects with abnormal concentrations of FT4 and TSH in the analysis.
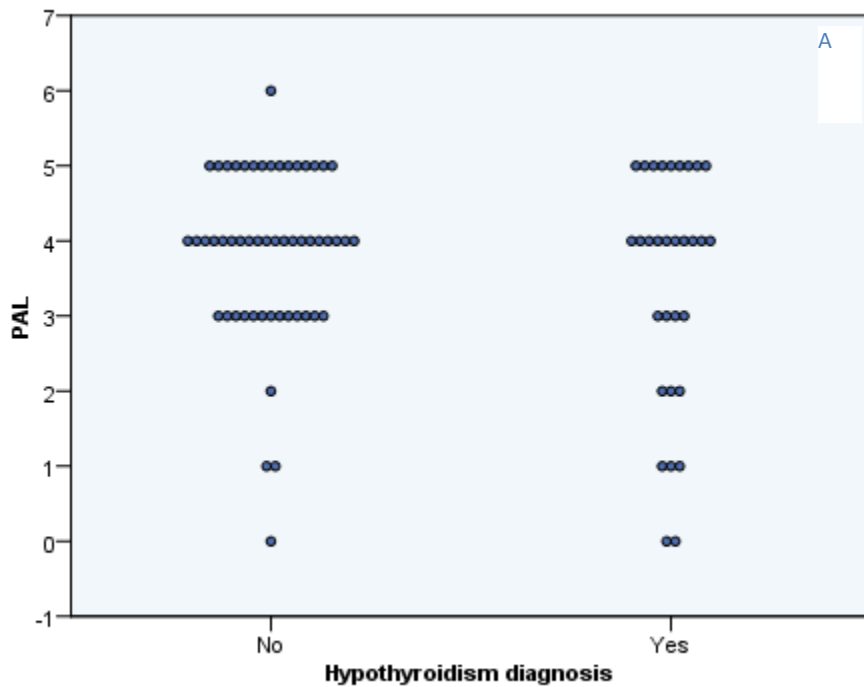
All subjects are included n=85
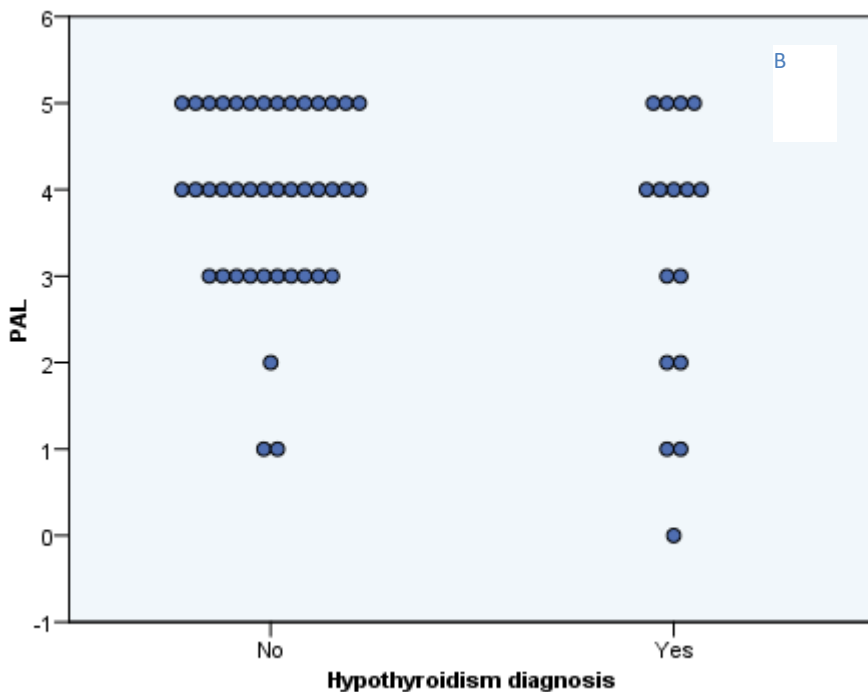
Only included those subjects with normal concentrations of FT4 (0.73 to 1.95 ng/dl) and TSH (2 to 10 Mu/ml) n=57

Figure 8. Hypothyroidism diagnosis and Visuoespatial Episodic Memory performance. Significant differences in the performance of the episodic spatial memory task (PAL, CANTAB) between hypothyroidism diagnoses groups. Analysis were done for the entire group of participants (A) (n 85, p=0.022), and (B) excluding subjects with abnormal FT4 and TSH concentrations (n 54, p=0.013).

4.2.3 Correlations between Thyroid status parameters and cognitive outcomes

FT4 concentrations (normally distributed in the entire group; n= 85) were associated with a better performance in the following cognitive tasks: PAL errors (r=-0.247, p=0.023), (see **Figure 9**), WCFST (r= 0.236, p=0.015), (see **Figure 10**). No statistically significant correlation was found between TSH or FT4 and the rest of the tests administered.
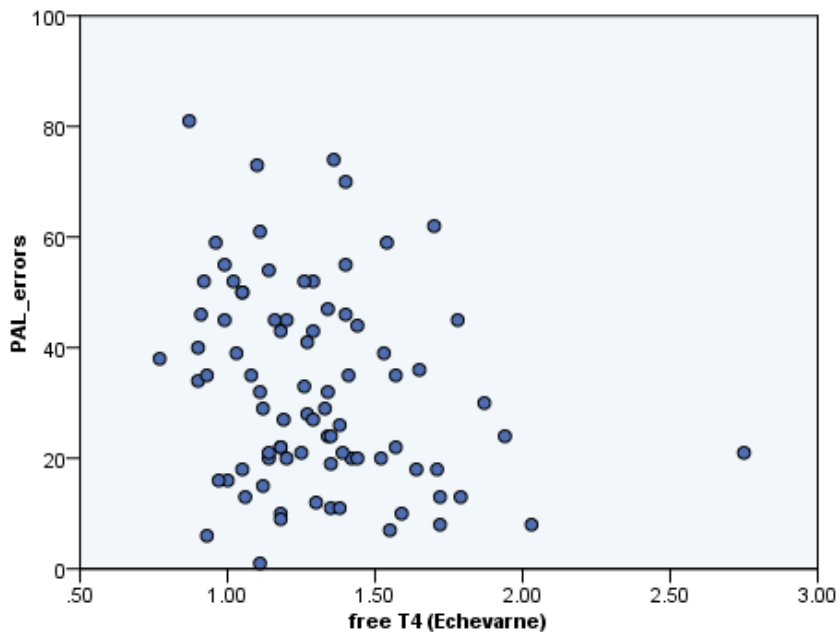
Figure 9. FT4 concentrations were associated with a better performance in the (A) Paired Associated Learning (PAL) committing a less number of errors (p= 0.023) (n=85)
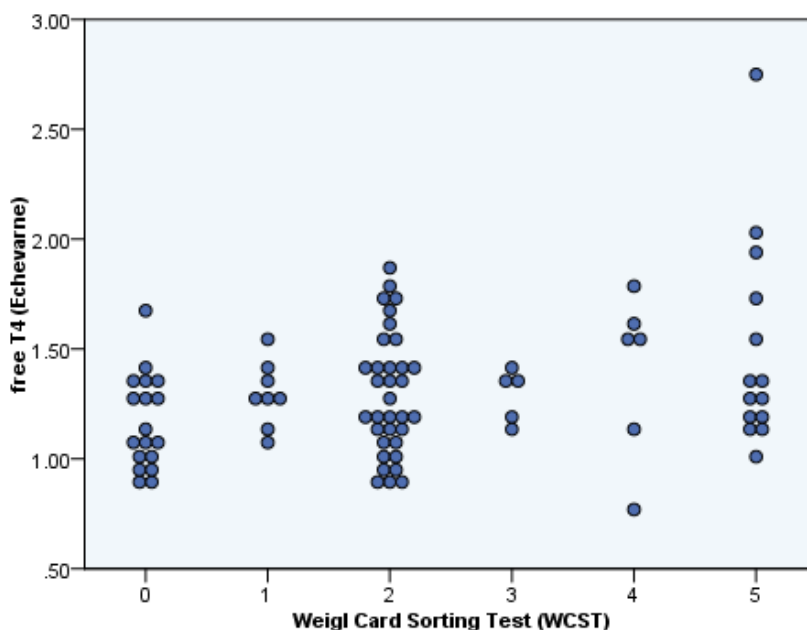
Figure 10. FT4 concentrations were associated with a better performance in the (A) Weigl Color-Form Sorting test (p=0.003) (n= 85)

When controlling for L-thyroxin dosage (ranged 0 to 150 µg) in the entire group, FT4 concentrations were positively partial correlated with a better performance in the cognitive tasks summarized in **Table 10**: PAL, Digits Span-BR , SSP-FR, WCFST, and free recall in BNT.

Table 10. Partial correlations between FT4 and cognitive data controlling for L-thyroxin dosage

Control Variable	FT4	PAL errors	SSP-FR	Dígits-BR	WCST	BNT
L-thyroxin dosage	Correlation	-.230	.261	.265	.386	.234
	Sig (2-tailed)	.041	.019	.017	.000	.037

Among the hypothyroid group (n= 31), L-thyroxin dosage (ranged 25 to 150µg) was not correlated with any cognitive domain. When doing the same analysis separately in those subjects with normal concentrations of FT4 and TSH (n=16) and those with abnormal concentrations (n= 15), no statistically

significant correlation between L-thyroxine dosage and any cognitive outcome were found neither.

The Number of years under L-thyroxin treatment, which was available in only 16 subjects, was associated with an improved performance in the PRM immediate (r= 0.671, p=0.006) and delayed recall (r =0.598, p=0.018) (see **Figure 11**).
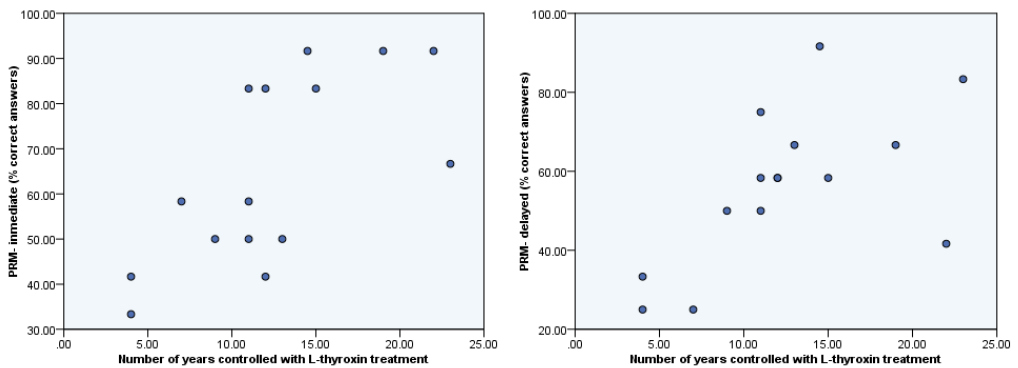
Figure 11 Regression model fits years under L-thyroxin treatment as a predictor of the performance in the Pattern Recognition Memory (PRM) (immediate: p= 0.006, and delayed: p=0.018) (n= 16).

4.3 Study 3: Dopamine polymorphisms, cognition and behavior in DS

4.3.1 Descriptive Demographic and Clinical Data of the Participants

Study 2 DS Socio-demographic data and cognitive parameters for all the neuropsychological variables of the 69 participants are provided in the **Table 11**.

Table 11. Descriptive, demographic and cognitive data of the participants

	Descriptive Statistics	
	Mean (SD)	N
		SOCIO-DEMOGRAPHIC DATA
Male		35 (50.7%)
Female		34 (49.3%)
Age	25.3 (4.5)	69
K-BIT standardized score	104.8 (17.8)	69
Median IQ	41 [a]	69
Education (Years)	14.3 (2.6) [b]	69
Trisomy		
Simple		65 (94.2%)
Translocation		2 (2.9%)
Mosaicism		1 (1.4%)
Partial trisomy		1 (1.4%)
		PEN AND PENCIL EXECUTIVE ATTENTIONAL TASKS
Digits forward Span length	3.1 (0.8)	69
Digits backward Span length	1.7 (1.1)	69
SVFT Total words	10.3 (4.0)	69
SVFT Switching	4.0 (2.1)	69
WCFST	3.2 (2.0)	69
TOL DX number of solved items (0-10)	7.4 (2.8)	69
Cats and dogs (total inhibition time)	20.7 (11.5) [c]	69
		CANTAB EXECUTIVE ATTENTIONAL TASKS
MOT Mean latency	917.3 (310.4) [d]	69
SSP Span length	3.7 (1.3)	69
SSP Mean time response	3309.1 (1065.5) [d]	69
SRT Mean correct latency	566.8 (229.3) [d]	69
SRT Total correct trial	98.2 (2.8)	69
SSP Span length [reverse]	2.8 (1.5)	69
SSP Mean time response [reverse]	3865.3 (1752.4) [d]	69
		FUNCTIONAL VARIABLES
ABAS Total Score	452.5 (100.8)	69
ABAS Self-direction	49.5 (14.4)	69
ABAS Social adaptive skills	52.4 (11.4)	69
DRM Total Score	11 (7.9)	69
DMR Cognitive Score	4.2 (5.1)	69
DMR Social Score	6.8 (4.1)	69

a: Only the median is reported because values below 40 cannot be determinate exactly b: Average years of school attendance in specialized or non-specialized educational centers, c: CANTAB mean times to response expressed in milliseconds, d: Cats and dogs mean time to execute the task expressed in seconds

4.3.2 Distribution of polymorphisms

In our study, the euploid Spanish population was used to obtain the expected frequencies for the Hardy-Weinberg equilibrium (see **Table 12**). The proportion of *COMTVal158Met* allelic frequencies among the Spanish reference population (n = 93) was similar to the reference CEU (Utah residents with Northern and Western European ancestry), and TSI (Tuscans from Italy) HapMap populations ("HapMap Project"); (see **Table 12**). No data from CEU and TSI HapMap reference populations was available for the proportion of VNTR-DAT1 allelic frequencies.

Table 12. Distribution of COMTVal158Met and VNTR-DAT1 allele frequencies in the DS and reference populations. Data represent the number of individuals bearing each genotype, and parenthesis account for genotypic frequencies (0-1).

		Hap Map CEU Population	Hap Map TSI Population	Spanish population	TESDAD DS population (Spanish)
COMTVal158Met	Met/Met (A/A)	28 (0.25)	20 (0.20)	21 (0.23)	6 (0.087)
	Val/Met (G/A)	52 (0.46)	52 (0.51)	45 (0.48)	38 (0.550)
	Val/Val (G/G)	33 (0.29)	30 (0.29)	27 (0.29)	25 (0.362)
VNTR-DAT1	10/10			30 (0.53)	35 (0.51)
	10/9			23 (0.40)	26 (0.38)
	9/9			4 (0.07)	8 (0.12)

Abbreviations: CEU (Utah residents with Northern and Western European ancestry), the TSI (Tuscans from Italy), DS (Down syndrome). Data represent the number of individuals bearing each genotype, and parenthesis account for genotypic frequencies (0-1**)**

In the TESDAD DS population, we found a Hardy–Weinberg disequilibrium ($\chi2$= 6.47, p<0.039) due to an under-representation of the *Met/Met* genotype in the *COMTVal158Met* polymorphism, as compared to the CEU, TSI and Spanish populations. Conversely, *VNTR-DAT1* allele frequency was in the expected Hardy–Weinberg equilibrium compared to the Spanish population (n = 57); (**Table 12**).

4.3.3 *VNTR-DAT1* and *COMTVal158Met* effects on cognitive and functional performance.

Table 13, **Figure 12** and **Figure 13** show the main effects in components of executive functioning and caregiver's rating scores between *VNTR-DAT1* and *COMTVal158Met* genotypes.

Table 13. VNTR-DAT1 and COMTVal158Met effects on cognitive and functional variables in the TESDAD DS population.

Test	Function assessed	Polymorphism	Figure	Estimate	Std.Error	t-value	p-value
SSP-FR (CANTAB)	Mean time to response	VNTR-DAT	F 1, G(a)	537	270	1.999	**0.05**
SRT (CANTAB)	Mean time to respond	VNTR-DAT	F 1, G(b)	117	48	2.424	**0.018**
MOT (CANTAB)	Mean Time to respond	VNTR-DAT		137	77	1.772	0.081
SVFT	Switching ability: MF	VNTR-DAT	F1, G(c)	-1.5	0.5	-3.265	**0.002**
WCFST	Reversal learning: MF	VNTR-DAT		-0.8	0.4	-1.921	0.059
SVFT	Switching ability: MF	COMTVal158Met	F 2, G(a)	-1.4	0.5	-2.935	**0.005**
ABAS Social Skills	Adaptive social behavior	COMTVal158Met	F 2, G(b)	6.99	2.9	2.453	**0.017**
ABAS Self-Direction	Capacity of regulate behavior to the demands of a situation.	COMTVal158Met	F 2, G(c)	7.65	3.7	2.08	**0.041**
DMR-SOS	Social Symptoms of Dementia	COMTVal158Met	F 2, G(d)	-2.1	1	-2.108	**0.039**

Estimate = estimated mean difference, Std.Error = standard error of the estimated mean difference, t-value= Value of the t-statistics, F=Figure, G= Graphic. Two-way ANOVA model was carried out to analyze main differences between allelic groups of each polymorphism regarding cognitive and functional outcomes

The VNTR-DAT1 *10-repeat* allele homozygotes showed shorter response times in the CANTAB Simple Reaction Time and SSP-FR tasks, not linked to impulsivity since they were not accompanied by a higher number of errors. *10-repeat* allele homozygotes also showed better performance of tasks requiring mental flexibility, as measured by the SVFT, but not by the WCFST. Taken together, these data indicate that in our DS population the *10-repeat* allele homozygotes are faster responders in attentional and psychomotor tasks and have better mental flexibility. No association was found between the *VNTR-DAT1* polymorphism and parental reports (ABAS-II and DMR).

Regarding *COMTVal158Met*, *Met* allele carriers showed no differences in response time in the CANTAB tasks but we detected the same profile as for *10-repeat* allele homozygotes regarding mental flexibility that was better as assessed by the SVFT, but not WCFST. In addition, *Met* allele carriers had worse parental reports referred to adaptive behavior, specifically in the social skills and self-direction subscales of ABAS-II, and higher early dementia rates in the DMR-SOS than *Val* allele homozygotes. Thus, in our population, the *Met* allele carriers have a greater mental flexibility but worse adaptive behavior, along with higher social deterioration.

No differences were found between *VNTR-DAT1* or *COMTVal158Met* genotypes with regards to attentional span (The Digits Span-FR, The SSP- FR Recall from CANTAB), working memory (the Digits Span BR from WAIS and the SSP BR from CANTAB), planning ability (ToLDx), or inhibitory control (the Cats & Dogs test).

Main interactions between genotypes were calculated for each cognitive and functional variable and no genotype interaction between *VNTR-DAT1* and *COMTVal158Met* polymorphisms for any cognitive or functional domain were found.
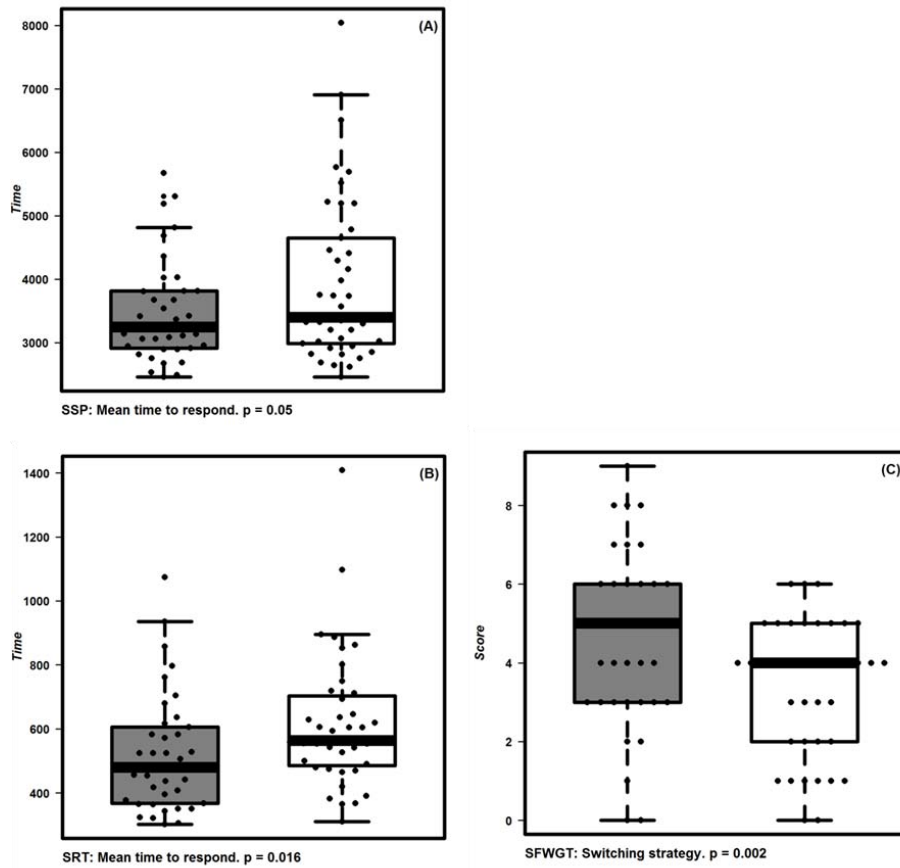
Figure 12. Cognitive performance associated with VNTR-DAT1 polymorphisms. Box plots represent the cognitive performance of allelic groups of VNTR-DAT1 polymorphism. 10-repeat homozygotes (grey boxes) displayed shorter response times in CANTAB (A) SSP ($p < 0.05$; two way ANOVA) and (B) SRT tasks ($p < 0.016$; two way ANOVA), and better mental flexibility measured by (C) SVFT (Switching strategy; $p < 0.016$; two way ANOVA) than 9-repeat carriers (white boxes). The boxplots show the median and interquartile range. Dots indicate individual values.
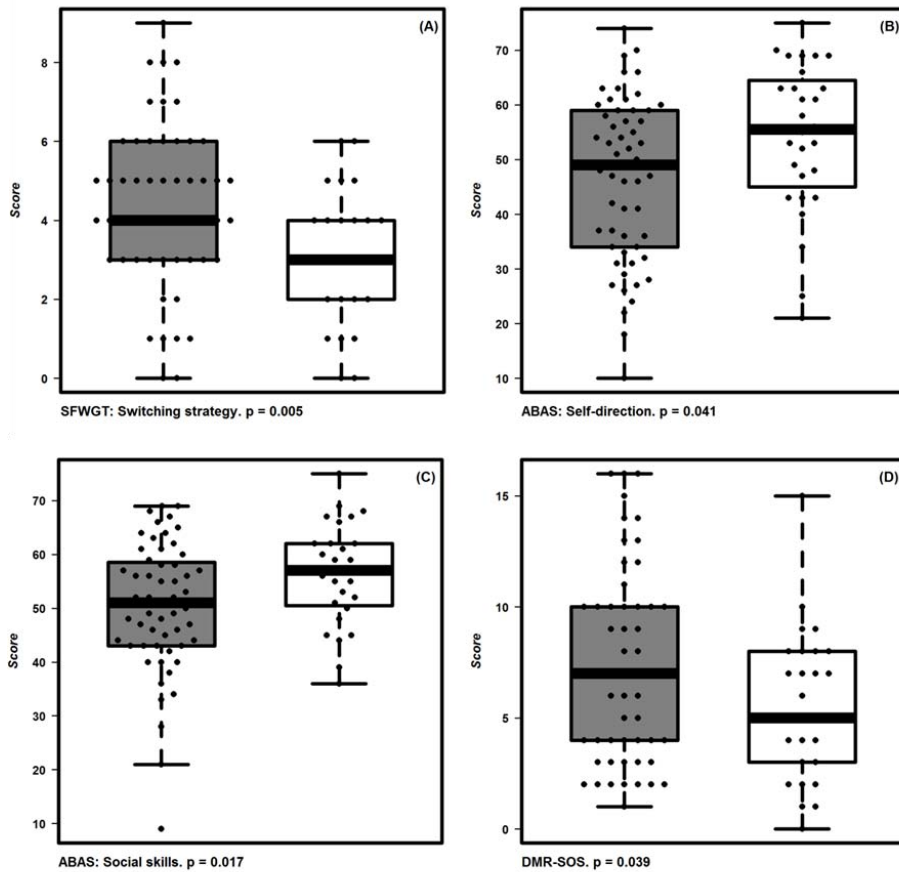
Figure 13. Cognitive and functional performance associated to COMTVal158Met polymorphisms. Box plots represent the behavioral and cognitive performance of allelic groups of COMTVal158Met polymorphism. Met carriers (grey boxes) performed better in mental flexibility measured by (A) SVFT (Switching strategy; p < 0.005; two way ANOVA) and had worse parental reports in two ABAS-II subscales: (B) ABAS-Self-Direction (p < 0.041; two way ANOVA) and (C) ABAS-Social Skills (p < 0.017; two way ANOVA), and also in the (D) social scale of the DMR; DMR-SOS (p < 0.039; two way ANOVA) than Val homozygotes (white boxes). The boxplots show the median as well as the interquartile range. Dots indicate individual values.

4.4 Study 4: Genetic polymorphism of the serotonin (5-HT) system, sleep and cognition.

4.4.1   Descriptive, demographic and clinical data of the participants

Socio-demographic data and clinical data of the 79 participants are provided in **Table 14**. Regarding to PSQI values we can observe that as a group they have a general good quality of sleep (PSQI 2.91 <5), the rest of the values for each PSQI domain are represented in **Table 14**. Regarding plasma Aβ concentrations, measurements for *Aβ42* and *Aβ40* were obtained in 42 subjects; the other 37 subjects either had concentrations below the detection range or the replicates varied by more than 20% and were, therefore, not reliable.

Table 14. Descriptive, demographic and clinical data of the participants

Down Syndrome Descriptive Statistics

	Mean (SD)	Frequency (%)	N
		SOCIO-DEMOGRAPHIC DATA	
Male		40 (50.6%)	
Female		39 (49.4%)	
Age	23.6 (4.3)		79
K-BIT standardized score	104.6 (17.2)		79
Median IQ	41 $_a$		
		CLINICAL DATA	
Apnea Diagnosis		9 (11.4%)	79
Snoring Disorder		30 (38 %)	79
Bad sleep quality		8 (10.1%)	79
Bad positions		10 (12.7%)	79
Daytime Sleepiness		10 (12.7%)	79
Sleep hours	8.8 (1.2)		79
Body Mass Index	25.8 (4.4)		79
Waist perimeter	80.5 (9.4)		79
Aβ 42 pgml	41.3 (10.1)		44
Aβ 40 pgml	264.0 (41. 0)		44
Ratio *Aβ* 42/40	0.16 (0.04)		44

		PSQI DATA
Subjective quality of sleep	0.6(0.7)	79
Subjective bad quality	6 (8.1%)	
Latency	0.7 (0.9)	79
>30 minutes	11 (16.4%)	
Duration	0.1 (0.4)	79
Less than 8 hours	2 (2.7%)	
Efficiency	0.1 (0.5)	79
Bad efficiency	2 (2.9%)	
Alterations	0.96 (0.8)	4 (5.5%)	79
Use of medication	0.1 (0.5)	2 (2.7%)	79
Diurnal dysfunction	0.5 (0.7)	6 (8.2%)	79
Global PSQI	2.9 (2.5)	79
PSQI <5	8 (10.1%)	

a: Only the median is reported because values below 40 cannot be determinate exactly.

4.4.2 Distribution of 5-HTTLPR polymorphism.

In the DS population the 5-HTTLPR polymorphism was in Hardy-Weinberg equilibrium (p=0.42) (see **Table 15**) when comparing with the reference population distribution. No data from CEU and TSI HapMap reference populations was available for the proportion of 5-HTTLPR genotypic frequencies.

Table 15. Genotypic 5-HTTLPR frequencies of the DS population

	Down Syndrome	Reference Population (Spanish)	p-value
L/L	18 (0.23)	16 (0.28)	0.42
S/S	18 (0.23)	11 (0.19)	
L/S	43 (0.54)	31 (0.53)	
Total	79	58	

Abbreviations: L/L= Long- allele Homozygotes, S/S= Short- allele Homozygotes, Long-Short-allele Heterozygotes. Data represent the number of individuals bearing each genotype, and parenthesis account for genotypic frequencies (0-1**).

4.4.3 Sex, age and IQ distribution among binary 5-HTTLPR grouping for the analysis (*Short*-allele homozygotes vs *Long*-allele carriers).

Sex, age, and IQ were similarly distributed among binary 5-HTTLPR groups (*Short*-allele homozygotes vs *Long*-allele carriers). Furthermore IQ and age were also similarly distributed among sexes (**Table 16**).

Table 16. Sex, age and IQ distribution among binary 5-HTTLPR groups.

		Sex				
		M (n)	W (n)	TOTAL (n)	K-BIT m(sd)	Age m(sd)
5-HTTLPR distribution	L*	29	32	61	104.9 (16.1)	23.4 (4.3)
	S/S	11	7	18	103.7 (21.2)	24.4(4.4)
K-BIT m (sd)		102.9(19.6)	106.4(19.3)	104.6(17.2)		
Age m (sd)		22.8 (3.9)	24.3 (4.6)	23.6 (4.3)		

Abbreviations: L*=Long-allele carriers, S/S= Short-allele homozygotes, M= man, W= woman, n= number of individuals bearing each genotype.

4.4.4 Associations between 5-HTTLPR genotype and quality of sleep.

The chi-squared tests showed that 5-HTTLPR *Short*-allele homozygote was linked to sleepiness during the activity and sleep disturbances (PSQI). Regarding to direct questions made to caregivers during the medical exploration, a higher percentage of subjects with bad sleep quality and bad postures while sleeping were found in the *Short*-allele homozygote group (see **Table 17** and **Figure 14**). Apnea and snoring did not show significant association neither with 5-HTTLPR, nor with BMI or waist perimeter. But snoring was linked to bad positions while sleeping (p=0.027, see **Table 18**).
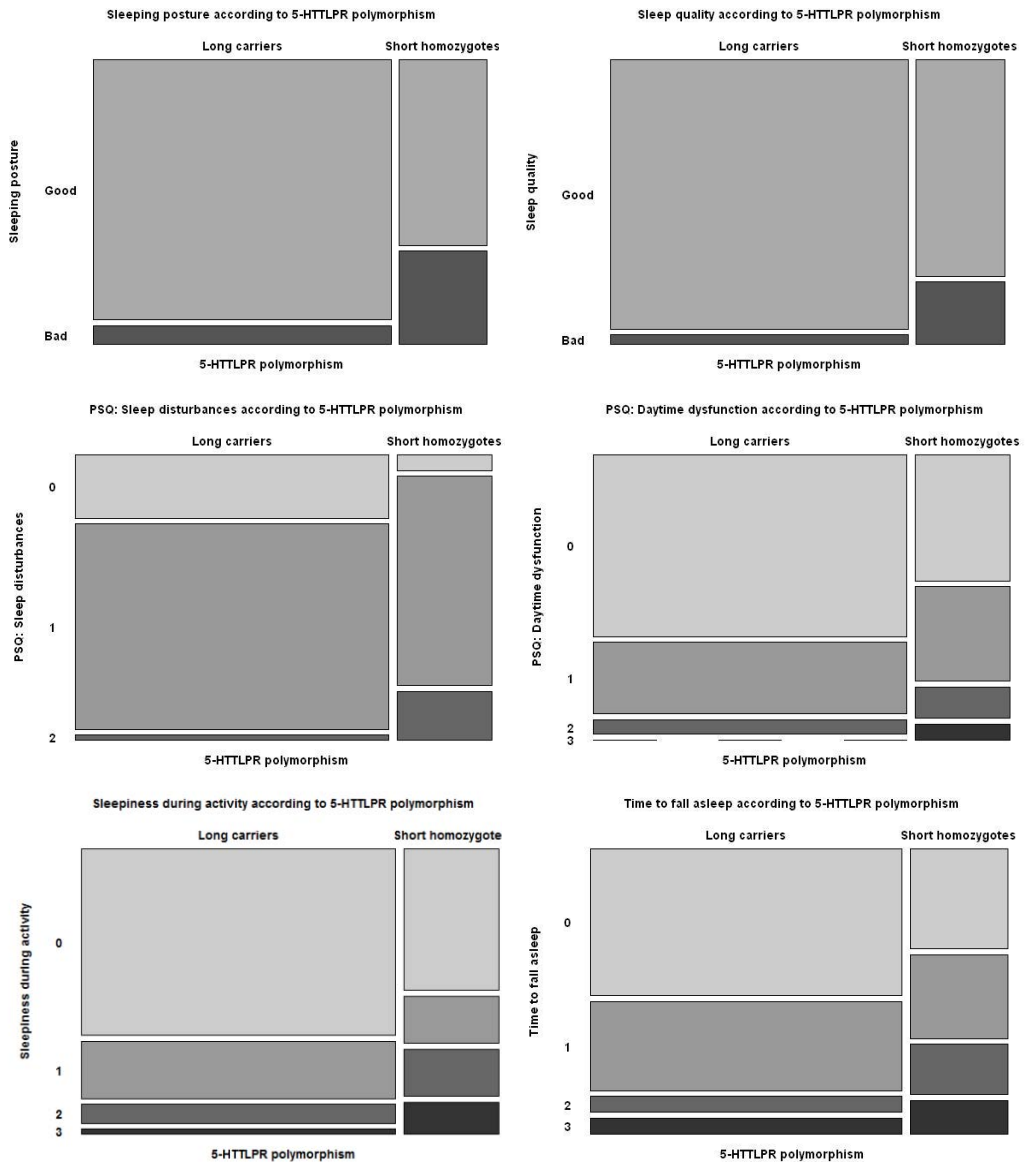
**Figure 14**. Differences in percentages related to quality of sleep variables between Long carriers and Short homozygotes. TOP-DOWN, LEFT-RIGHT: Differences between 5-HTTLPR groups in: Bad postures while sleeping, Sleep quality, Sleep disturbances, Daytime dysfunction, Sleepiness during activity and More than 30 minutes to fall asleep. Scores = 0 - 1 (< one time per week); 2 - 3 (> one time per week).

Table 17. Frequency of sleep disturbances regarding 5-HTTLPR distribution

Quality of sleep variables		5-HTTLPR distribution		Total	Pearson- Chi-Square Test		
		L C	S/S		Value	df	p- value
Somnolence while activity (PSQI)	< once a week	51	12	63	4.565	1	**0.033**
	> once a week	5	5	10			
Sleep disturbance (PSQI)*	< once a week	55	14	69	/	/	/
	> once a week	**1***	**3***	**4***			
> 30 minutes to fall asleep(PSQI)	< once a week	45	11	56	3.32	1	0.068
	> once a week	6	5	11			
Bad quality of sleep (PSQI)	good	57	14	71	3.539	1	0.06
	bad	4	4	8			
Bad postures while sleeping (CH)	good	57	12	69	8.899	1	**0.003**
	bad	4	6	10			
Bad quality of sleep (CH)	good	58	14	72	6.868	1	**0.009**
	bad	2	4	6			
Apnea (CH)	no	55	15	70	0.634	1	0.426
	yes	6	3	9			
Snoring Disorder (CH)	no	40	9	49	1.413	1	0.235
	yes	21	9	30			

The table represents the frequency of sleep disturbances regarding 5-HTTLPR distribution). Sleep quality variables were fitted as rows and 5-HTTLPR genotype (LC vs. S/S) as columns. Sleep quality variables were the following: *Quality of sleep* assessed with the PSQI (good/bad quality), *sleep disturbance, somnolence during activity*, and *>30 minutes to fall asleep* (less than once a week/ more than once a week). The following close questions to caregivers: *how do you consider his/her quality of sleep*? (good/bad), how are the *postures during sleeping*? (good/bad), *snoring* (yes/no) and, *apnea diagnosis* (yes/no). *Pearson Chi-Square test is not reliable due to the small size of the subgroups in *sleep disturbance* variable. Abbreviations: LC= Long-allele carriers, S/S = Short-allele Homozygotes, Value= Chi-Square values for Pearson Chi-Square test, df= degrees of freedom

Table 18. Frequency of snoring disorder regarding bad sleeping positions distribution.

CROSSTAB		Sleeping positions (CH)			Pearson- Chi-Square Test		
		good	bad	Total	Value	df	p- value
Snoring Disorder (CH)	no	46	3	49	4.922	1	0.027
	yes	23	7	30			
	Total	69	10	79			

Crosstab statistic for snoring disorder as rows, with sleeping positions (good/bad) as columns Chi-square test was selected to calculate the Pearson chi-square (2x2). Abbreviations: Value= Chi-Square values for Pearson Chi-Square test, df= degrees of freedom.

4.4.5   Associations   between   5-HTTLPR   genotype   and neuropsychological variables.

Two-way ANOVA analysis did not reveal any significant associations between the 5-HTTLPR genotype and cognitive or functional variables.

4.4.6   Associations between sleep quality features, cognition and adaptive behavior.

Regarding cognitive performance, Bad sleep quality (CH), as perceived by care-givers, was associated to a poorer performance in the PRM, and PAL and to a longer time to respond in the SSP-FR. In addition bad postures during sleep (CH) were only associated to longer response times in the PRM and in SSP-FR. Apnea diagnosis was also associated to a lower number of correct answers in the PRM. Regarding adaptive behavior, both Apnea diagnosis and Snoring were linked to poorer Communication Skills (ABAS-II Subscale). While only Snoring was associated to worse Academic Skills (ABAS-II Subscale) and general worse adaptive behavior (Total Score ABAS- II) (see **Table 19**).

Table 19. Associations between sleep quality features, cognition and functionality.

TEST	Cognitive/functional domain	Outcome exported	Sleep quality features distribution		p	F
			QUALITY OF SLEEP (CH)			
			Mean(sd)	Mean(sd)	p	F
			GOOD (n=72)	BAD (n=6)		
PRM (CANTAB)	Object memory	Total number of corrects	8.2 (2.3)	6.3 (2.5)	0.050	3.807
PRM (CANTAB)	Object memory	Time to response	3682.0 (2215.2)	6156.7 (5679.4)	0.028	5.034
SSP-F (CANTAB)	Visual attention	Time to response	3724.0 (1131.6)	4722.1 (1623.9)	0.048	4.028
PAL (CANTAB)	Spatial memory	Mean trials to success	3.0 (1.7)	4.7(3.8)	0.046	4.101
PAL (CANTAB)	Spatial memory	Stages completed on first trial	3.7 (1.3)	2.7 (1.9)	0.064	3.537
			BAD POSTURES (CH)			
			NO(n=69)	YES(n=10)		
PRM (CANTAB)	Object memory	Time to response	3619.6 (2143.4)	5461.3(4755)	0.039	4.395
SSP-F (CANTAB)	Visual attention	Time to response	3677.5 (1103.6)	4595.7 (4181.2)	0.021	5.529
			APNEAS (CH)			
			NO(n=70)	YES(n=9)		
PRM (CANTAB)	Object memory	Total number of corrects	8.4 (2.3)	5.9 (1.2)	0.002	10.19
ABAS-II	Communication Skills	Subscale score	55.9 (12.1)	47 (15.6)	0.047	4.069
			SNORING (CH)			
			NO(n =49)	YES(n=30)		
ABAS-II	Communication Skills	Subscale score	57.3 (12.6)	51.1 (12.2)	0.036	4.538
ABAS-II	Academic Skills	Subscale score	43.1 (18.6)	33.9 (15.0)	0.026	5.162
ABAS-II	Adaptive Behavior	Total score	467.9(104.2)	410.1(87.4)	0.013	6.453
DMR	Early Dementia Symptoms	Total score	9.1(6.4)	13.3(9.1)	0.021	5.575
			DIURNAL DYSFUNCTION (PSQI)			
			NO(n=67)	YES(n= 6)		
DMR	Early Dementia Symptoms	Total score	6.34 (4.0)	10.2 (4.5)	0.041	4.327

2-way ANOVA models, Abbreviations: sd= standard deviation, p= p-value (significance level), F= F statistic (variance analysis), CH (information extracted from Clinical History), PSQI (information extracted from the *Pittsburgh Sleep Quality Index.*

4.4.7 Associations between sleep quality features, DMR and $A\beta 42/$ $A\beta 40$ ratio in plasma.

Our results showed a significant association of both snoring (CH) and daytime dysfunction (PSQI) with higher rates in the DMR, (see **Table 19**). Furthermore the total score of the PSQI was associated (p=0.009, r=0.333) to higher rates in the DMR-SOS. The rest of sleep quality features did not show a statistically significant relation with the DMR rates.

Snoring was also associated to higher $A\beta 42/$ $A\beta 40$ ratio in plasma (p=0.027, t=2.254), (see **Figure 15**) but not to $A\beta 42$ or $A\beta 40$ concentrations. For the rest of the variables related to sleep features no significant association neither with the $A\beta 42$ or $A\beta 40$ concentrations nor with the $\beta 42/$ $A\beta 40$ ratio.
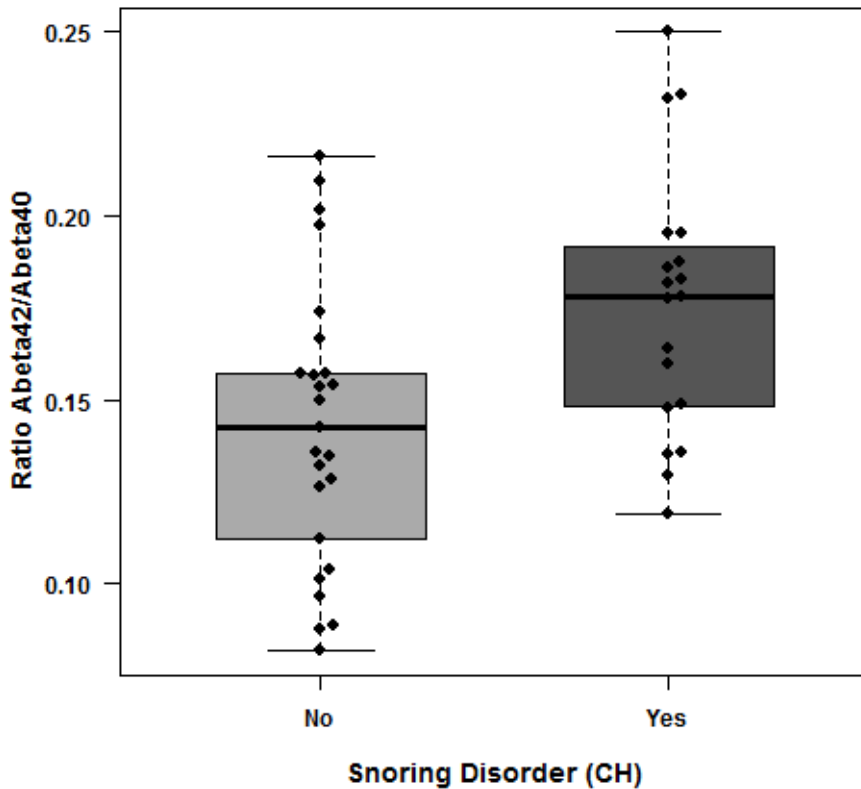
Figure 15. Differences in Aβ42/Aβ40 ratio in plasma associated to snoring disorders. Box plots represent the ratio between Aβ42 and Aβ40 in plasma (pg/ml.). Subjects with snoring disorder account for higher Aβ42/ Aβ40 ratio in plasma (p=0.006, r=8.464). The boxplots show the median and interquartile range. Dots indicate individual values.

4.4.8 Associations between interactive effects of 5-HTTLPR and sleep quality on cognition and functionality.

The analysis of the synergic effects between 5-HTTPLR polymorphism and Sleep Quality features on cognitive and functional parameters did not reveal any statistical reveals, since none of the interactions included in the two-way ANOVA models improved the model fit significantly.

In this final chapter, an individual discussion is provided for the findings of each study of the current thesis, followed by some methodological considerations, and ends by a general discussion of results with proposal of future research directions. This thesis wants to explore some of the underlying mechanisms of the cognitive and behavioral phenotype variability in individuals with DS, and the relevance of these considerations for efficacy assessment in DS clinical trials for cognitive and functional enhancement.

5.1 Study 1: Aβ Production and early signs of dementia in DS: the cognitive signature of premature aging.

In Study 1 we observed an association between the SVFP, dementia scores, and adaptive behavior related to communication skills in young adults with DS. Moreover, poorer semantic fluency, higher dementia rates, and poor adaptive behavior in communication skills were associated to higher plasma concentrations of an AD biomarker (Aβ42).

The observed associations between cognitive, functional, and biological parameters suggest that SVF assessment could be used as screening test for early detection of early symptoms of dementia in DS. Furthermore, our study shows for the first time clear differences in the SVFP between a DS young adult population compared to healthy age-matched individuals.

In the following lines we discuss point by point the results found for each objective of the Study 1.

5.1.1 Fluency Deficits in Young DS Adults

Impairment of verbal fluency, as estimated by lexical knowledge, is a feature of DS (John Rowe, Lavender, & Turk, 2006). Our results show a reduction of switching and cluster size as compared to the age-matched group, possibly

due to a poorer semantic knowledge. This profile is similar to the so called dysexecutive syndrome, described as a common pattern of dysfunction in executive functions such as planning, abstract thinking, flexibility, and behavioral control (Wilson, Evans, Emslie, Alderman, & Burgess, 1998). A dysexecutive syndrome has already been reported in DS (de Sola et al., 2015; Lanfranchi, Jerman, Dal Pont, Alberti, & Vianello, 2010; John Rowe et al., 2006), and has been related to the reduced volume of the PFC reported in neuroimaging studies (Carducci et al., 2013; Raz et al., 1995; White, Alkire, & Haier, 2003) in particular affecting the anterior cingulate gyrus, medial, and dorsolateral prefrontal cortices (Contestabile, Benfenati, & Gasparini, 2010; I. Lott & Dierssen, 2010). These areas actively contribute to mnemonic processing and executive control in euploid individuals (Blumenfeld, Parks, Yonelinas, & Ranganath, 2011; Braver, 2001; Wager & Smith, 2003), and, thus, the generalized impairment of high order frontal-dependent processes has a negative influence on SVFP which depends on both mnemonic and executive processes.

Similarly to what is observed in GP in our DS group the word production decreases significantly over time, although in the DS group we detected a wide interindividual variability. This can be explained according to the model of lexical organization (Crowe, 1996), which states that there are two types of storages, namely: (1) a long-term storage ("topicon") which is readily accessible and contains common words and (2) a more extensive "lexicon" which is searched after the "topicon" is exhausted. Thus, successful performance on a verbal fluency task seems to be subjected to the effectiveness of both automatic and controlled processing (Crowe, 1998; Hurks et al., 2006). In our DS sample, subjects are not differentiating between using automatic processing and instead, they access to the pool of frequently used words, but when this is exhausted, they fail in using

controlled attentional searching retrieval processes that involve executive strategies with high impact on total word production, such as switching. Word production in normative age matched population also decreases over time, paired with a high percentage of words produced in the first 15 s as reported in previous studies (Villodre et al., 2006). However, their topicon and lexicon are richer than the observed in DS subjects due to better semantic knowledge (clustering) and retrieval strategies (switching).

5.1.2 Aβ Plasma Concentrations in Young DS Group

Regarding the plasma concentrations of amyloid peptides, few studies have measured the concentrations of such biomarkers in young adults (Head et al., 2011; Mehta et al., 2003), and those were performed in older populations. Compared to them, we observed higher concentrations of Aβ42 (41 ± 10 pg/mL), possibly due to the sensitivity of method we used. However, another study performed in younger DS subjects (mean 7.2 ± 3.8 years old) reported concentrations (31.6 ± 8.2 pg/mL) that were closer to our mean values (Mehta, Capone, Jewell, & Freedland, 2007). Contrary to previous studies in older DS populations (Head et al., 2011; V. P. Prasher et al., 2010; N Schupf et al., 2010) that observed a correlation of Aβ42 with age, in ours this is not present suggesting that factors other than age are affecting the Aβ42 production, or maybe we cannot observe such age effect because the age-range (17-34) (median= 22, mean= 23.6 ± 4.5 years old) is too narrow. In light of our results, the impact of these biomarkers and their evolution pattern in DS should be studied longitudinally since young adulthood to elderly.

5.1.3 Association between Fluency Performance and Aβ Concentrations

A large subset of aged individuals with DS develop a neuropathology with clinical features of AD and some studies have suggested that deficits in executive function are the first to be compromised with the progressive development of Alzheimer-like neuropathology in people with DS (Ball et al., 2006; Holland, Hon, Huppert, & Stevens, 2000). In AD patients, disease progression was better predicted by the clustering ability in SVFP (Fagundo et al., 2008), while others (Raoux et al., 2008) found a significant decline in switching along the early phase until the clinical diagnosis of AD dementia. In our DS population, switching and the total number of words are the verbal fluency markers that better correlate with the plasma Aβ42 concentrations. This observation supports the hypothesis that impaired switching abilities could explain the early decline in semantic fluency performance in an early stage of AD. Moreover, the association between AD biomarkers and verbal fluency pattern is supported by the correlation that we found between Aβ concentrations, dementia ratings (DMR), and communication skills. We observed that higher concentrations of Aβ42 were associated to lower general adaptive behavior skills and communication skills (as a particular dimension of adaptive behavior) and higher DMR scores. Therefore DMR can be considered as a useful questionnaire for detecting early symptoms of AD in DS. These results would also be in agreement with previous studies relating higher Aβ42 plasma concentrations in elderly DS with dementia or its progression. Furthermore, DMR scores were inversely correlated with the SVFP. This observations is of interest because, in our study, positive correlations were found between communication skills, semantic verbal fluency, and switching, as discussed above. Communication abilities are a compilation of cognitive and social processes such as comprehension,

expression, and empathy. Semantic verbal fluency seems to be part of this compilation of abilities involved in verbal expression as forming part of communication skills. In our case, the DS subjects are not demented, but there is a clear correlation between higher Aβ42 and worse scores in functional variables that can be used to detect an early dementia state.

5.1 Study 2 Thyroid function and Cognition

In our study 2 we found an association between hypothyroidism diagnosis and a worse performance in a visuospatial associative memory task (PAL, CANTAB). This association was also observed when excluding from the analysis those subjects with abnormal FT4 and TSH concentrations. No differences in IQ, attention, psychomotor speed, executive function or language skills were found between diagnosis groups.

When analyzing the correlation between FT4 and TSH in the entire group, we found that a better performance in the same visuospatial episodic memory task (PAL) and a mental flexibility task (WCFST) were associated to higher concentrations of FT4. No correlations were found between FT4 or TSH concentrations and the rest of the cognitive outcomes. When controlling for L-thyroxine treatment we found that the same correlations became stronger, adding new positive correlations between FT4 concentrations, attentional span (SSP-FR), working memory (Digits-BR) and semantic memory (BNT). In addition in a subsample of 16 subjects with hypothyroidism diagnosis we found out that the number of years under L-thyroxine treatment was associated to an improved immediate and delayed visual recognition memory (PRM).

The observed associations between cognitive and biological parameters suggest that thyroid status plays a role in visual memory and mental flexibility in DS. In addition, other cognitive domains as semantic memory

(vocabulary), working memory and attentional spam are also influenced by when controlling for L-Thyroxine dosage.

Our study propose that thyroid assessment could be used as a screening test to detect possible factor of interference with the cognitive phenotype in the context of clinical trials for cognitive improvement in DS population and other associated pathologies with ID**.**

5.1.1   Hypothyroidism diagnosis, FT4 and TSH Plasma Concentrations in Young DS Adults

The association of DS with thyroid disorders has been known for decades. Thyroid dysfunction is highly prevalent in DS (Campos & Casado, 2015) being the more common rates being >20 per cent (V. Prasher, 1994). A previous study (Real de Asua, Quero, Moldenhauer, & Suarez, 2015) with DS age-matched (24 ± 4 years) Spanish population showed that a 43 % of subjects were diagnosed for hypothyroidism with TSH mean concentrations of 4.0 µIU/mL ± 2.1. In our study the 36.4% of the subjects are diagnosed and TSH concentrations are similar as in the previous study (3. 9 µIU/mL ±3.6) but reporting a bit more variability.

In patients with DS, the reported incidence of hypothyroidism is from 288 to 357 times greater than in the GP; in typical developing Catalan population (Lucas et al., 2010) only an 8.9 % are diagnosed. Our DS group TSH median concentrations (3.09 µIU/mL (0.03- 28.20) are 2.1 times higher than in GP (1.43 µIU/mL (0.38 – 4.59), with no significant trend towards a rise in TSH concentrations with increasing age in either sex in DS or GP.

5.1.2 Association between Cognitive skills and Thyroid function in Young DS Adults

The role that thyroid dysfunction plays on cognition has been extensively investigate in GP and results show that hypothyroidism (with elevated TSH and decreased FT4 concentrations) is associated with deficits in memory, psychomotor slowing, visuoperceptual and construction skills. A number of other factors as disease onset, severity, duration, as well as concentrations of L-thyroxine and TSH seem to contribute to the clinical manifestation of cognitive deficits. (Burmeister et al., 2001; Dugbartey, 1998; Karen J. Miller et al., 2007; Wheeler, McLelland, Sheard, McAndrews, & Rovet, 2015).

In our DS population, FT4 is the biomarker that correlates with the larger number of cognitive variables. It is positive associated to memory, attention and executive function skills when controlling for L-thyroxine dosage. The reason why FT4 is related to a smaller number of cognitive outcomes (visual episodic memory and mental flexibility) when we don't control for L-thyroxine dosage may be related to the role that L-thyroxin plays on cognition, which also interferes on FT4 concentrations. The results of a published clinical trial in DS with Folinic Acid shows that positive cognitive effects of the treatment were particularly strong in patients receiving concomitant thyroxin treatment (Blehaut et al., 2010).

On the contrary, TSH concentrations are not associated to the cognitive domains evaluated. Hypothyroidism diagnosis is implicated in poor visuospatial immediate memory (PAL) but is not associated with other memory and learning skills (visuospatial delayed memory, verbal episodic memory or semantic memory), or attention (attentional spam, simple reaction time), executive function (working memory, mental flexibility, planning ability) or psychomotor speed. Our results are in agreement with

the observed in GP and animal models. Lower concentrations of FT4 (associated with hypothyroid dysfunction) are partially linked (controlled for L-thyroxine dosage) to a poor performance in memory, attention and executive function skills. Hypothyroidism diagnosis also accounts for a significant worse performance than in the non-diagnosed group but just in a visuospatial memory task (PAL). This could mean that FT4 concentrations (controlling L-thyroxine dosage) is giving us more exhaustive information about thyroid status in DS than just the diagnosis or FT4 concentrations on its own right, being probably the reason why it is related to a large number of cognitive variables.

Cognitive symptoms of hypothyroidism can be difficult to discriminate from those found in the natural course of DS itself, getting overlapped in both DS and hypothyroidism. Cognitive dysfunction in DS is mainly due to triplicated chromosome 21 in which many genes accounting for brain development or neuronal loss are overexpressed. Trisomy 21 also has an influence on thyroid activity caused by the decreased concentrations of selenium, impaired activity of phenylalanine hydroxylase, and overexpression of DYRK1A kinase leading to a number of signs and symptoms including the cognitive dysfunction (Campos & Casado, 2015). Regarding DYRK1A effects on thyroid function, a recent study shows that its overexpression directly leads to thyroidal embryogenetic, functional and morphological impairment, concluding that young adult thyroid phenotype is probably a result of embryogenetic impairment which could be treated with DYRK1A inhibitors (i.e. EGCG) since embryonic stages, supporting the importance of the role that congenital hypothyroidism may play in DS cognitive phenotype (Kariyawasam et al., 2015).

Based in the importance of hypothyroidism onset and the years controlled by L-thyroxin treatment reported in DS literature, we wanted to explore how

this two factors influence cognitive skills. All subjects previously diagnosed with hypothyroidism who participated in this study were undergoing L-thyroxine treatment since they were diagnosed. This did not allow us to analyze if cognitive performance was influenced by the difference between the total years diagnosed (with Hypothyroidism) and the number of years controlled with L-thyroxine, just because this two variables are overlapping. We found a positive relation between years under L-thyroxine treatment (=years diagnosed) and immediate and delayed visual memory. But as the years treated are the same data as the years diagnosed, it is difficult to conclude what does this correlation could mean in terms of the benefits that continued L-Thyroxine treatment could bring to cognitive status in DS, which has been shown to increase mitochondria metabolism and thus production of reactive oxygen species (particularly in patients with hypothyroidism) and induce an antioxidant imbalance (Erdamar et al., 2008).

We also hypothesized that the age of diagnosis might be a key factor to better understand our results, affecting to cognitive statement (the earlier diagnosis the higher affection). But on the contrary we found that the longer years diagnosed the better visual memory skills. This should happen because all the subjects diagnosed were properly controlled under L-thyroxine therapy since the moment of the diagnosis, with non-critical periods of misadjusted T4 concentrations. No subjects reported congenital hypothyroidism. Even though data about: hypothyroidism onset, years under L-thyroxine treatment, and congenital hypothyroidism diagnosis was only available in 16 subjects of the 85 subjects.

The observed associations between cognitive and biological parameters suggest that thyroid status plays a role in some aspects of memory, attention and executive function in DS. This study propose that thyroid assessment could be used as a screening test to detect possible factors of interference

with the cognitive phenotype in the context of clinical trials for cognitive improvement in DS population and other pathologies that course with ID. Therefore thyroid function is as an important factor to be controlled in DS in this context. Further investigations with longitudinal studies are required to ascertain the mechanisms underlying our findings.

5.2 Study3: COMTVal158Met and VNTR-DAT1 polymorphisms contribution to cognitive and behavioral variability in DS.

Since it is known that the phenotypic consequences of genetic variants are modulated by the genetic background in which they occur, we here examined the association between DA related polymorphisms VNTR-DAT1 and COMTVal158Met and inter-individual differences in executive function, adaptive behavior and early symptoms of dementia in DS young adults. We show that genotypes conferring higher DA synaptic availability (Met allele carriers and 10-repeat allele homozygotes), result in improved performance in executive function tasks that require mental flexibility. We also observe worse social skills and self-direction and earlier social symptoms of dementia in Met allele carriers but not in 10-repeat allele homozygotes.

In the following sections we discuss point by point the results found for each objective of the Study 2.

5.2.1 Distribution of COMTVal158Met and VNTR-DAT1 polymorphisms in DS

In our DS population the genotype distribution for VNTR-DAT1 was in Hardy-Weinberg equilibrium, but the COMTVal158Met had a reduced frequency for Met allele homozygotes. Even though, there is a need of a more extensive analysis of the frequencies of the COMTVal158Met polymorphism in the DS population, and a triplet analysis would be required to confirm this

observation. The reduced Met frequency in our population could be explained as a case of natural selection against the Met allele homozygotes since in the GP it has been observed that women who are carriers of the Met allele have a higher risk of miscarriage (Khadzhieva, Lutcenko, Volodin, Morozova, & Salnikova, 2014).

5.2.2 *VNTR-DAT1* and *COMTVal158Met* effects on cognitive and functional performance.

We hypothesized that DA related polymorphisms would impact executive function in DS, although not necessarily in the same way as in euploid population since the genetic trisomic background and the underlying cognitive deficits may influence the variants' effects. Main differences in prefrontal dependent cognitive performance between DS and age-matched controls have already been reported in previous works (de Sola et al., 2015; Del Hoyo et al., 2015) with the baseline data from the TESDAD study population. Specifically, we found that after language (the strongest cognitive disturbance in young DS adults), attention and executive functions were the cognitive areas that differed more from standard norms. The verbal span capacity (Digits Span forward recall), semantic verbal fluency (SVFT: total word production) and mental flexibility (SVFT: switching ability) presenting the strongest deficiencies. In the present Study 2, in DS individuals (n = 69), the switching ability, which allow subjects to change from one semantic category to another, was also associated (p<0.001, r=0.689) to a higher word production leading to a better performance of the task.

We here found that 10-repeat allele homozygotes were faster respondents in attentional tasks (SRT and SSP-FR), and this same genotype and Met allele

carriers were associated to better mental flexibility showing better switching abilities (SVFT).

Previous studies reported that healthy subjects carrying the 10-repeat allele were more impulsive than 9-repeat allele carriers, being faster but committing more errors in the non-verbal attentional Continuous Performance Task from CANTAB (Caldú et al., 2007). Here, the faster responses of 10-repeat allele homozygotes as compared to the 9-repeat carriers could not be considered as a sign of impulsivity as there was no association between faster responses and the errors made. An association between 10-repeat allele and ADHD (Cornish et al., 2005), in which impulsivity is a core characteristic (Winstanley, Eagle, & Robbins, 2006), has also demonstrated, that the hyperactive/impulsive-ADHD-type phenotype was linked with the 10-repeat allele (Waldman et al., 1998). Although there is a high ADHD prevalence in DS population (Ekstein, Glick, Weill, Kay, & Berger, 2011), in our study there was no ADHD comorbidity nor signs of impulsivity at a group level, so we could not determine such association.

The association between Met allele carriers and better mental flexibility is in agreement with previous studies in GP in which subjects with the low-activity Met allele were associated to fewer perseverative errors in a mental flexibility task (Malhotra et al., 2002).

Taken together, our results indicate that 10-repeat allele homozygotes and Met allele carriers in our DS population have better mental flexibility. Interestingly, a reduction of the concentrations of DA in the frontal cortex of fetal DS brains has been observed (Whittle et al., 2007) that is associated with dysfunctional neuronal development in DS. Therefore, even though we have not directly measured PFC DA availability, Met allele carriers and 10-repeat allele homozygotes, which are supposed to have higher frontal DA

availability, could be compensating PFC dependent executive functions (John Rowe et al., 2006).

Since executive skills are strongly associated with adaptive behavior (ABAS-II), we explored how these polymorphisms were linked to adaptive behavior skills. Our results showed differences in parental-reports between COMTVal158Met genotypes but not between VNTR-DAT1 genotypes. Met allele carriers showed worse adaptive behavior, concretely worse social skills and self-direction. Self-direction is a subscale of the ABAS-II which measures the executive control in daily living. Social abilities are also mediated by executive functions, which helps the subject to adapt to different social situations regulating emotion (L. E. Campbell, McCabe, Melville, Strutt, & Schall, 2015). Previous studies in GP have also find a positive association between the Val allele and social skills (Walter, Markett, Montag, & Reuter, 2011). Our results show that the Met allele variant confers an advantage in cognition (mental flexibility) while penalizing adaptive behavior skills and observation already made in healthy and disease conditions in the euploid population (Mier et al., 2009).

Finally, individuals Met allele carriers displayed worse scores in the DMR-SOS than Val allele homozygotes. Conversely, some reports suggest that increased synaptic DA catabolism promotes the neurodegeneration within DA-innervated brain regions and thus, progressive cognitive and behavioral decline associated with AD (Gennatas et al., 2012). However, even though the Met allele (increasing DA availability) may confer resistance to AD, in our sample Met allele carriers did not show differences in the general DMR score as compared with other allelic variants. Thus, we interpret this result as an association between Met allele and social deterioration (DMR-SOS), instead of a link with early social dementia symptoms, since Met allele carriers also showed worse adaptive social behavior (ABAS-II).

In DS, one study has reported that the DRD4 7-repeat allele was related to behavioral and executive functions difficulties (Mason et al., 2015). We here did not explore this polymorphism and thus, possible interactions of our genes and the DRD4 allelic variants could also be contributing to the detected phenotypes. Other genotypes previously examined seem to little contribute (genetic polymorphisms in the DA-ergic and 5-HT-ergic systems, the DRD4 exon 3 VNTR and the 5-HTTLPR) to the cognitive dysfunctions observed in DS (Das Bhowmik et al., 2008).

5.3 Study 4: Serotonin transporter polymorphism, sleep features, cognition and behavior

This study has found a descriptive association between homozygotes for the *Short*-allele of the 5-HTTPLR polymorphism and bad sleep quality, bad sleep postures and sleepiness during activity in DS young adults. Secondly, bad sleep quality, bad sleep postures and apnea diagnosis are associated to worse visual memory skills (PRM and PAL), while only apnea is related to worse communication skills (ABAS-II). Finally, early dementia symptoms (DMR) are associated with higher PSQI scores, daytime dysfunction and snoring; but only snoring is related to higher $A\beta42/A\beta40$ ratio in plasma and to a general worse adaptive behavior (ABAS-II).

The observed associations suggest that on one hand the 5-HTTPLR polymorphism could be involved in some features of DS sleep quality, whereas it is not associated to other functional or cognitive skills as previous reports have shown (Das Bhowmik et al., 2008). On the other hand, bad sleep quality and some components of it as: Bad sleep postures, Apnea diagnosis and Snoring disturbance are interfering in memory, attention and adaptive behavior. In addition, snoring, a higher PSQI score, and daytime

dysfunction are linked to early symptoms of dementia (DMR), but only snoring is associated to higher *Aβ*42/ *Aβ*40 ratios in plasma.

5.3.1 Quality of sleep in DS

Literature shows that some sleeping anomalies are common in DS (Bassell, Phan, Leu, Kronk, & Visootsak, 2015; Breslin, Edgin, Bootzin, Goodwin, & Nadel, 2011; Stores & Stores, 2013). Sleep problems are present in around a 30% of youth with DS, and a 60% suffer daytime sleepiness, from a 50 to an 80% are affected by apnea (depending on diagnosis criteria) and a 50% report loud snoring (Angriman, Caravale, Novelli, Ferri, & Bruni, 2015; Breslin et al., 2011; Esbensen, 2015; Trois et al., 2009). Our results show a general good quality of sleep at group level (PSQI= 2.91 <5), with only a 10% of bad quality diagnoses. Other clinical conditions were reported in our DS group, but below the expected rates when comparing to literature, as follows: daytime sleepiness (12.7%), bad postures while sleeping (12.7%), sleep latency longer than 30 minutes (14%), snoring disturbances (38 %), and apnea diagnosis (11.4%). Parental reports can underestimate sleep disturbances, compared to objective methods of sleep assessment (Hoffmire, Magyar, Connolly, Fernandez, & van Wijngaarden, 2014; Maris, Verhulst, Wojciechowski, Van de Heyning, & Boudewyns, 2016). Concretely, many parents don't know if they child suffer from apnea since they don't get on an overnight sleep polysomnography. Our findings are in line with previous reports, suggesting that sleep problems may be important, but under-recognized in this population by both parents and treating physicians.

5.3.2 Association between 5-HTTLPR and Sleep quality features.

 As long as DS has been associated with defects in the serotonergic system (Whitaker-Azmitia, 2001), the role that 5-HT plays in DS is a controversial topic (Coyle, Oster-Granite, & Gearhart, 1986; Gardiner, 2015; Gulesserian,

Engidawork, Cairns, & Lubec, 2000; Lubec, Yoo, Dierssen, Balic, & Lubec, 2001). However, genetic polymorphisms in the transporter of this neurotransmitter and its relation with DS phenotype has only been studied once (Das Bhowmik et al., 2008), and concluded that the cognitive dysfunctions observed in DS patients were not contributed by this polymorphism. In our case we didn't find either any association between cognitive or functional skills and 5-HTTLPR in DS, but it seems that bad sleep quality, bad sleep postures and sleepiness during activity were linked to the *Short*-allele of the 5-HTTLPR polymorphism. Previous studies have evaluated the role that 5-HT plays in rapid eye movement (REM) rhythm, (Petre-Quadens & De Greef, 1971), and sleep disturbances (Tamasaki et al., 2016) in DS, but those studies have not compared sleeping features between the allelic variation in 5-HTTLPR.

The hypothesis behind our findings, may be the association between the 5-HTTLPR *Short*- allele and low 5-HTT expression in cell lines, having less transcriptional activity and lower serotonin uptake, which is finally translated into higher 5-HT concentrations. Higher 5-HT concentrations have been related to wakefulness promotion resulting in a worse quality of sleep in GP. Previous studies have shown that higher 5-HT concentrations and carrying twice the *Short*-allele of the 5-HTTLPR were two factors determining a worse quality of sleep in GP (Barnett et al., 2011; Brummett et al., 2007; Deuschle et al., 2010). Our results are in agreement with the previous research in GP, suggesting the possible effect of this gene on sleep quality, pointing to the *Short* allele involvement in DS poor sleep quality.

As expected, apnea and snoring anomalies were not related to 5-HTTLPR, we hypothesize both may be mediated by other variables, as previous studies demonstrate, such as anatomic anomalies (Guimaraes, Donnelly, Shott, Amin, & Kalra, 2008), Body mass index (BMI) (Shires et al., 2010) and bad

sleeping positions (Senthilvel & Krishna, 2011) which was found to be linked only with snoring in our DS group. Neither BMI nor waist perimeter were correlated to apnea or snoring.

5.3.3 Associations between Sleep Quality features, Cognitive and Functional state and Early Symptoms of Dementia.

Several interdependent factors have an influence on cognitive phenotype in DS and cause their eventual decline into AD. Previous reports (Chen, Spanò, & Edgin, 2013; Fernandez & Edgin, 2013) provide a hypothesis regarding the extent to which sleep disruption might alter cognitive development and contribute to pathological aging in DS. In this second part of the current study we aimed to study the relation between poor sleep quality variables, cognitive and functional skills, and early symptoms of dementia in a DS young adult population. Our results showed that bad sleep quality and some features of bad sleep quality as bad postures while sleeping and apnea diagnosis were associated to impaired visual memory. Moreover apneas and snoring were also associated to a worse adaptive behavior and communication skills. In addition, snoring disorders, daytime dysfunction, and a general worse punctuation in PSQI were also associated to early dementia rates (DMR) while only snoring disorders were also linked to higher $A\beta 42/A\beta 40$ ratios in plasma.

In GP the mechanisms underlying the relation between sleep and memory are based in different hypotheses such as the role of hormonal stress responses, the sleep loss impact on cellular mechanisms of learning and memory occurring during no -REM and REM, and the long-lasting changes in hippocampal synaptic efficacy which underlie memory formation (Brown, Basheer, McKenna, Strecker, & McCarley, 2012).

The association between snoring, daytime dysfunction and the total score of the PSQI with early symptoms of dementia (DMR) and snoring with higher $A\beta42/A\beta40$ ratios in plasma, point out several potential mechanisms that may relate to neurocognitive dysfunction across the lifespan, and is in agreement with previous studies showing that sleeping problems are linked to cognitive decline (Fernandez & Edgin, 2013) and Aβ deposition, assuming a cause-effect between sleep disturbance and higher concentrations of Aβ, supposing to be a risk factor for AD in both DS and GP (Fabian Fernandez and Jamie O Edgin, 2013). To date AD conversion in DS subjects is mainly analyzed by measuring plasma Aβ concentrations. Some correlations have been found between increases in $A\beta_{40}$ and decreases of $A\beta_{42}$ concentrations and risk of dementia (Coppus et al., 2012; N Schupf et al., 2010), but also between decreases in Aβ40 and increases in Aβ42 with increasing duration of dementia (V. P. Prasher et al., 2010). Plasma Aβ changes among age would not be linear in DS (Head et al., 2011) and how Aβ concentrations interfere with early symptoms of dementia in DS young adults was analyzed only once (Hoyo et al., 2015) showing that higher concentrations of $A\beta_{42}$ in plasma correlated with higher rates in the DMR, being both good tools to assess early risk of AD development in young adults with DS.

5.4 Limitations of the present studies

The four studies of this thesis have some methodological limitations.

First, plasma measurements of Aβ concentrations remain controversial. Their high variability and lack of correlation with the observations of amyloidosis in the brain are some of the reasons leading some researchers to perform their measurements in CSF. In our study, several peripheral tissues and cells, such as muscle and platelets, could be the source of peripheral Aβ (Toledo et al., 2014). However, in the context of clinical trials, as well as in clinical practice in general, it is worth improving the reliability of this blood measurement, as

it is much less invasive than CSF procurement, as well as exploring its correlations with early cognitive symptoms of AD or sleep disruptions. The analysis of these biomarkers in plasma exosomes could be in the future a promising alternative (Khan et al., 2016).

Second, we only had the possibility of comparing the cognitive variables, and genetic polymorphism distribution between the DS group and age-matched groups. The rest of assessments as dementia rates, adaptive behavior, sleep quality, Aβ concentrations and thyroid status are lacking of a comparative group. As an alternative approach we used reported data in the Spanish literature.

Regarding **study 4** only a 10% of the sample displayed bad sleep quality, which limits some of our conclusions. Due to the small size (n<5) of some subgroups of analysis, inferential statistical analyses are not reliable, even though descriptive statistics extracted from cross tabulations and graphs give us interesting information to be further discussed. In this study, the self-administered PSQI questionnaire and the questions made to caregivers during the clinical visit regarding apnea diagnosis, snoring disturbances and sleeping positions could be underestimating the prevalence of sleep disturbances in our population. Other objective methods of assessment as polysomnography would have robustly estimated the sleep pattern in our population. However, in the context of clinical trials, the use of good validity and reliability caregivers self-administered questionnaires in the screening visit is the most efficiency strategy used to detect possible daily living factors (i.e. sleep problems) interfering with cognition and behavior prior to the randomization for balancing subjects prior to the treatment allocation. However, prior to our analysis (DS sleep reports Vs DS cognition) we may have analyzed how the PSQI and sleep CH data correlate with objective methods of assessment as polysomnography in our population.

Finally, in all studies the high number of statistical tests carried out may increase the probability of Type-1 errors. Nonetheless, no correction to control a family-wise significance level of 0.05 has been applied in order not to increase the probability of Type-2 errors.

5.5 Implications and future directions

This thesis started with the hypothesis that general assumptions about DS are being modified by recent and ongoing research in the past years, pointing to a wide range of individual differences at every level of description. Hypothesizing that in the context of significant increases in DS life expectancy, a focus on individual differences in trisomy 21 at all concentrations—genetic, biochemical, cognitive, behavioral, and environmental— would constitute one of the best approaches for understanding DS and for exploring risk and protective factors for Alzheimer's disease in this high-risk population. The consequences that this assumption could have in the context of evaluating efficacy (cognitive and functional changes) in clinical trials are essential in the development of new therapies in the context on IDs. The tools, used to assess DS phenotypes in basal conditions and evaluate if the former is associated to a treatment condition in a clinical trial, need to be valid, reliable and adapted to the special needs of the DS population.

Consequently to these hypotheses we mainly focused on 5 possible factors that could explain DS cognitive and behavioral variability in basal conditions. Two of them were biochemical pointing to two general dysfunctions in DS: amyloidosis (Aβ plasma concentrations) and hypothyroidism (FT4, TSH plasma concentrations and L-thyroxin dosage). Two of them genetic, related to neurotransmission systems altered in DS: polymorphisms of DA and 5-HT related genes. Finally the last one was physiological: sleep quality. Obviously

there are additional factors that we have not analyzed that would need to be taken into account:

(i) Congenital heart disease occurs in nearly half of DS population (Mogra, Zidere, & Allan, 2011). Its surgical correction has been shown to account for some individual differences in expressive language delay (Visootsak, Hess, Bakeman, & Adamson, 2013), thus the relation between patterns of cardiovascular pathology and language or other cognitive domains would need to be further studied.

(ii) The effect of APOE epsilon 4 genotype on the risk of dementia in Alzheimer's disease in DS (V. P. Prasher et al., 2008) would also need to be studied along with Aβ plasma concentrations. Thus, APOE status and secretion of amyloid-beta peptides from APP cleavage can be screened and evaluated for their effects on a treatment response (Fernandez & Reeves, 2015).

(iii) Neurological events such as epilepsy (Arya, Kabra, & Gulati, 2011) or sleep apnea (Trois et al., 2009) are also conspicuous factors that could interfere with the assessment of cognitive improvement, both have been taken into account in this thesis; being epilepsy an exclusion criteria (since those subjects with any other neurological disease different from DS were not selected to participate). Previous apnea diagnosis was collected at the baseline visit and further correlated with cognitive and behavioral outcomes in this thesis. Nevertheless the effect that personal history of events as infantile spasms (Eisermann et al., 2003) or sleep apnea onset could have on cognition or behavior wasn't analyzed since this information was not collected.

Personal history of these and other biological events (as congenital hypothyroidism, or early hypothyroidism onset and also the years that a person has been successfully controlled with L-thyroxine treatment) are

important data which need to be collected and taken into consideration with cognitive and functional basal outcomes. Its effects on later developmental trajectories need to be further studied, checking possible interactions, and balancing (if necessary) subject's features across groups of treatments in a drug-efficacy clinical trial.

This complex matrix of known and unknown features concealed in the average person with DS might interfere with drug efficacy assessment, creating a false sense of security in clinical trial settings where matched-group assignments are based only on already known variability factors. Thus it is essential to evolve in the investigation of DS at all concentrations of description and study the interaction between them and the contribution to cognitive and behavioral variability.

The results of the present studies provided evidence that individual differences in DS at genetics, biochemical concentrations of some biomarkers, and sleep quality are likely to be important contributors to determine differences in cognitive, behavioral and functional skills in DS, constituting an approach for understanding not only genotype/phenotype but also phenotype/phenotype relations.

Thus, our results may contribute to provide a better comprehension of this complex matrix of interacting features that underlie some of DS cognitive and behavioral differences. The studies demonstrate that **(1)** higher aβ42 concentrations are related to early cognitive and behavioral symptoms of dementia **(2)** differences on thyroid functioning account for differences in some aspects of memory, executive function and attentional spam **(3)** genetic polymorphisms of the DA system explain some of the differences in the executive pattern of DS, influencing in mental flexibility and adaptive behavior, **(4)** a genetic polymorphism of the 5-HT system contribute to some

sleep features (being these 3 polymorphisms out of the chromosome 21) and **(5)** bad sleep quality and apnea are associated to worse visual memory skills. Furthermore apnea and snoring are associated to a worse adaptive behavior, while only snoring is associated to higher rates in the Dementia Questionnaire for People with Intellectual Disabilities and higher *Aβ*42/ *Aβ*40 ratio in plasma.

However, more research is needed to gain further insight in the so-called factors that underlay phenotype variability in individuals with DS. Future efforts in DS research may focus on longitudinal and prospective studies instead of the retrospective investigation of the possible long-term effects that genetic, cellular, neural and environmental DS differences have in cognitive, behavioral and adaptive development.

Future efforts may also be focused on improving the tools used to assess drug efficacy in a clinical trial for DS cognitive enhancement, in which the last goal is to improve in their daily challenges, thus cognitive assessment need to be as much naturalistic as possible. If other exploratory efficacy outcomes as brain functional status (fMRI) or neurophysiological activity (TMS) are included in the clinical trial protocol, both will need to achieve some kind of correlation with the cognitive gains claimed, thus it would be essential to study how this and other secondary variables of efficacy are related to the primary goal in a clinical trial: translate underlying competencies into consistent daily habits that enable self-sufficiency.

We may don't forget that if a new drug shows significant benefits in some areas but it fails to perform better than the placebo on the primary goal of the study, the clinical trial will be halted; therefore the new drug won't be approved by any regulatory agencies (AEMPS, EMA or FDA). Unfortunately, treating an ID is not as treating high blood pressure. "How a primary

outcome measure withstands the phenotypic and response variability that exist between DS subjects?" Though there's no straightforward answer, understanding what's beyond this phenotypic and response variability will help us to get closer to a solution. But maybe finally, the regulatory practice, that authenticates the efficacy of an experimental drug through the lens of one and only one primary endpoint, will need to reconsider this statement in light of advances in the multidisciplinary science of DS and other genetic syndromes that course with intellectual or behavioral disability.

6. General conclusions

The main conclusions of this thesis, derived from study 1 (I, II, III), 2 (IV, V) 3 (VI) and 4 (VII, VIII) and a general conclusion for inferred the four studies (IX) can be summarized as follows:

I. The SVFT is a good screening test for early detection of dementia and amyloidosis in young adults with DS.

    a. Plasma Aβ42 concentrations is a good indicator of early symptoms of dementia.

    b. The total word production and the switching ability in the SVFT are good indicators of plasma Aβ42 concentrations.

II. Thyroid status interferes on cognitive performance in DS

    a. FT4 (controlled for L-thyroxine dosage), but not FT3 or TSH plasma concentrations is a good indicator of cognitive variability in memory, mental flexibility working memory and attentional spam.

    b. FT4 assessment could be used as a screening test associated with some aspects of the DS cognitive phenotype variability, while hypothyroidism diagnosis is less sensitive than FT4 concentrations to this cognitive variability as it is only related to worse visual memory skills.

III. Genetic variants of *COMTVal158Met* and *VNTR-DAT1* polymorphisms may contribute to PFC-dependent cognition, while only *COMTVal158Met* is involved in behavioral phenotypes of DS, similar to euploid population.

IV. Genetic variants of 5-HTTLPR may contribute to sleep features similar to euploid population, while only some sleep quality features but not variants of 5-HTTLPR have an influence on memory, adaptive

behavior and early symptoms of dementia in a DS young adult population.

V.   Apnea and Snoring disorders could be considered as risk factors to a worse adaptive behavior in DS. While only apnea is interfering in worse visual memory skills. Snoring disorder in DS could be a risk factor to early development of AD since it is associated to higher rates in the DMR and higher *Aβ*42/ *Aβ*40 ratio in plasma.

VI. Plasma Aβ concentrations, plasma FT4 concentrations, L-thyroxine dosage, sleep quality, apnea and snoring diagnosis and genetic polymorphisms of the DA (*COMTVal158Met* and *VNTR-DAT)* neurotransmission system are factors of interference with the cognitive, behavioral and functional DS phenotype. Finally, the genetic polymorphism of the 5-HT (5-HTTLPR) neurotransmission system does not interfere with cognitive or behavioral aspects, but it does with sleep quality in this population. Thus, these factors which explain part of the phenotype variability need to be assessed in the screening visit in the context of a drug-efficacy clinical trial for cognitive, behavioral and/or functional enhancement. This knowledge will allow us to balance those factors associated with phenotype variability across groups of treatment, leading to a proper efficacy assessment.

7. References

Abbeduto, L., Warren, S. F., & Conners, F. A. (2007). Language development in Down syndrome: from the prelinguistic period to the acquisition of literacy. *Mental Retardation and Developmental Disabilities Research Reviews*, *13*(3), 247–61. doi:10.1002/mrdd.20158

Angriman, M., Caravale, B., Novelli, L., Ferri, R., & Bruni, O. (2015). Sleep in Children with Neurodevelopmental Disabilities. *Neuropediatrics*, *46*(3), 199–210. doi:10.1055/s-0035-1550151

Archbold, K. H., Giordani, B., Ruzicka, D. L., & Chervin, R. D. (2004). Cognitive executive dysfunction in children with mild sleep-disordered breathing. *Biological Research for Nursing*, *5*(3), 168–76. doi:10.1177/1099800403260261

Arya, R., Kabra, M., & Gulati, S. (2011). Epilepsy in children with Down syndrome. *Epileptic Disorders : International Epilepsy Journal with Videotape*, *13*(1), 1–7. doi:10.1684/epd.2011.0415

Ashworth, A., Hill, C. M., Karmiloff-Smith, A., & Dimitriou, D. (2013). Cross syndrome comparison of sleep problems in children with Down syndrome and Williams syndrome. *Research in Developmental Disabilities*, *34*(5), 1572–80. doi:10.1016/j.ridd.2013.01.031

Bäckman, L., Nyberg, L., Lindenberger, U., Li, S.-C., & Farde, L. (2006). The correlative triad among aging, dopamine, and cognition: current status and future prospects. *Neuroscience and Biobehavioral Reviews*, *30*(6), 791–807. doi:10.1016/j.neubiorev.2006.06.005

Ball, S. L., Holland, A. J., Hon, J., Huppert, F. A., Treppner, P., & Watson, P. C. (2006). Personality and behaviour changes mark the early stages of Alzheimer's disease in adults with Down's syndrome: findings from a

prospective population-based study. *International Journal of Geriatric Psychiatry*, *21*(7), 661–73. doi:10.1002/gps.1545

Ball, S. L., Holland, A. J., Treppner, P., Watson, P. C., & Huppert, F. A. (2008). Executive dysfunction and its association with personality and behaviour changes in the development of Alzheimer's disease in adults with Down syndrome and mild to moderate learning disabilities. *The British Journal of Clinical Psychology / the British Psychological Society*, *47*(Pt 1), 1–29. doi:10.1348/014466507X230967

Barnett, J. H., Xu, K., Heron, J., Goldman, D., & Jones, P. B. (2011). Cognitive effects of genetic variation in monoamine neurotransmitter systems: a population-based study of COMT, MAOA, and 5HTTLPR. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *156*(2), 158–67. doi:10.1002/ajmg.b.31150

Bassell, J. L., Phan, H., Leu, R., Kronk, R., & Visootsak, J. (2015). Sleep profiles in children with Down syndrome. *American Journal of Medical Genetics. Part A*. doi:10.1002/ajmg.a.37096

Batshaw, M., & Roizen, N. (2002). *Children with Disabilities*. (G. Lotrecchiano, Ed.) (7th ed.). Batilmore, Maryland: Paul H. Brookes. Retrieved from https://books.google.com/books?id=P65sAAAAMAAJ&pgis=1

Bauer, M., Silverman, D. H. S., Schlagenhauf, F., London, E. D., Geist, C. L., van Herle, K., … Whybrow, P. C. (2009). Brain glucose metabolism in hypothyroidism: a positron emission tomography study before and after thyroid hormone replacement therapy. *The Journal of Clinical Endocrinology and Metabolism*, *94*(8), 2922–9. doi:10.1210/jc.2008-2235

Benejam, B., Fortea, J., Molina-López, R., & Videla, S. (2015). Patterns of Performance on the Modified Cued Recall Test in Spanish Adults With Down Syndrome With and Without Dementia. *American Journal on Intellectual and Developmental Disabilities*, *120*(6), 481–9. doi:10.1352/1944-7558-120.6.481

Bennett, S. J., Holmes, J., & Buckley, S. (2013). Computerized memory training leads to sustained improvement in visuospatial short-term memory skills in children with Down syndrome. *American Journal on Intellectual and Developmental Disabilities*, *118*(3), 179–92. doi:10.1352/1944-7558-118.3.179

Benton, A. L., Hamsher, K., & Sivan, A. (1976). *Multilingual aphasia exam*. (U. of I. Press, Ed.). Iowa City: Iowa City: University of Iowa.

Berry-Kravis, E., Doll, E., Sterling, A., Kover, S. T., Schroeder, S. M., Mathur, S., & Abbeduto, L. (2013). Development of an expressive language sampling procedure in fragile X syndrome: a pilot study. *Journal of Developmental and Behavioral Pediatrics : JDBP*, *34*(4), 245–51. doi:10.1097/DBP.0b013e31828742fc

Berry-Kravis, E. M., Hessl, D., Rathmell, B., Zarevics, P., Cherubini, M., Walton-Bowen, K., … Hagerman, R. J. (2012). Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. *Science Translational Medicine*, *4*(152), 152ra127. doi:10.1126/scitranslmed.3004214

Bhowmik, A. Das, Chaudhury, S., Dutta, S., Shaw, J., Chatterjee, A., Choudhury, A., … Mukhopadhyay, K. (2011). Role of functional dopaminergic gene polymorphisms in the etiology of idiopathic

intellectual disability. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*(7), 1714–22. doi:10.1016/j.pnpbp.2011.05.005

Blehaut, H., Mircher, C., Ravel, A., Conte, M., de Portzamparc, V., Poret, G., … Sturtz, F. G. (2010). Effect of leucovorin (folinic acid) on the developmental quotient of children with Down's syndrome (trisomy 21) and influence of thyroid status. *PloS One*, *5*(1), e8394. doi:10.1371/journal.pone.0008394

Blumenfeld, R. S., Parks, C. M., Yonelinas, A. P., & Ranganath, C. (2011). Putting the pieces together: the role of dorsolateral prefrontal cortex in relational memory encoding. *Journal of Cognitive Neuroscience*, *23*(1), 257–65. doi:10.1162/jocn.2010.21459

Boada, R., Hutaff-Lee, C., Schrader, A., Weitzenkamp, D., Benke, T. A., Goldson, E. J., & Costa, A. C. S. (2012). Antagonism of NMDA receptors as a potential treatment for Down syndrome: a pilot randomized controlled trial. *Translational Psychiatry*, *2*, e141. doi:10.1038/tp.2012.66

Braver, T. S. (2001). Anterior Cingulate Cortex and Response Conflict: Effects of Frequency, Inhibition and Errors. *Cerebral Cortex*, *11*(9), 825–836. doi:10.1093/cercor/11.9.825

Breslin, J. H., Edgin, J. O., Bootzin, R. R., Goodwin, J. L., & Nadel, L. (2011). Parental report of sleep problems in Down syndrome. *Journal of Intellectual Disability Research : JIDR*, *55*(11), 1086–91. doi:10.1111/j.1365-2788.2011.01435.x

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., & McCarley, R. W. (2012). Control of sleep and wakefulness. *Physiological Reviews*, *92*(3), 1087–187. doi:10.1152/physrev.00032.2011

Brummett, B. H., Krystal, A. D., Ashley-Koch, A., Kuhn, C. M., Züchner, S., Siegler, I. C., … Williams, R. B. (2007). Sleep quality varies as a function of 5-HTTLPR genotype and stress. *Psychosomatic Medicine*, *69*(7), 621–4. doi:10.1097/PSY.0b013e31814b8de6

Burmeister, L. A., Ganguli, M., Dodge, H. H., Toczek, T., Dekosky, S. T., & Nebes, R. D. (2001). Hypothyroidism and Cognition: Preliminary Evidence for a Specific Defect in Memory. *Thyroid*, *11*(12), 1177–1185. doi:10.1089/10507250152741037

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Research*, *28*(2), 193–213. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2748771

Caldú, X., Vendrell, P., Bartrés-Faz, D., Clemente, I., Bargalló, N., Jurado, M. A., … Junqué, C. (2007). Impact of the COMT Val108/158 Met and DAT genotypes on prefrontal function in healthy subjects. *NeuroImage*, *37*(4), 1437–44. doi:10.1016/j.neuroimage.2007.06.021

Campbell, I. (2007). Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations. *Statistics in Medicine*, *26*(19), 3661–75. doi:10.1002/sim.2832

Campbell, L. E., McCabe, K. L., Melville, J. L., Strutt, P. A., & Schall, U. (2015). Social cognition dysfunction in adolescents with 22q11.2 deletion syndrome (velo-cardio-facial syndrome): relationship with executive functioning and social competence/functioning. *Journal of Intellectual Disability Research : JIDR*. doi:10.1111/jir.12183

Campos, C., & Casado, Á. (2015). Oxidative stress, thyroid dysfunction & Down syndrome. *The Indian Journal of Medical Research*, *142*(2), 113–

9. doi:10.4103/0971-5916.164218

Canivez, G. L. (2005). Construct Validity of the Kaufman Brief Intelligence
  Test, Wechsler Intelligence Scale for Children-Third Edition, and
  Adjustment Scales for Children and Adolescents. *Journal of
  Psychoeducational Assessment*, *23*(1), 15–34.
  doi:10.1177/073428290502300102

Canning, S. J. D., Leach, L., Stuss, D., Ngo, L., & Black, S. E. (2004). Diagnostic
  utility of abbreviated fluency measures in Alzheimer disease and
  vascular dementia. *Neurology*, *62*(4), 556–562.
  doi:10.1212/WNL.62.4.556

Capone, G. T., Aidikoff, J. M., Taylor, K., & Rykiel, N. (2013). Adolescents and
  young adults with Down syndrome presenting to a medical clinic with
  depression: co-morbid obstructive sleep apnea. *American Journal of
  Medical Genetics. Part A*, *161A*(9), 2188–96. doi:10.1002/ajmg.a.36052

Carducci, F., Onorati, P., Condoluci, C., Di Gennaro, G., Quarato, P. P.,
  Pierallini, A., … Albertini, G. (2013). Whole-brain voxel-based
  morphometry study of children and adolescents with Down syndrome.
  Functional Neurology, *28*(1), 19–28. doi:10.11138/FNeur/2013.28.1.019

Cerhan, J. H., Ivnik, R. J., Smith, G. E., Tangalos, E. C., Petersen, R. C., &
  Boeve, B. F. (2002). Diagnostic utility of letter fluency, category fluency,
  and fluency difference scores in Alzheimer's disease. *The Clinical
  Neuropsychologist*, *16*(1), 35–42. doi:10.1076/clin.16.1.35.8326

Chapman, R. S., Seung, H. K., Schwartz, S. E., & Kay-Raining Bird, E. (1998).
  Language skills of children and adolescents with Down syndrome: II.
  Production deficits. *Journal of Speech, Language, and Hearing
  Research : JSLHR*, *41*(4), 861–73. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/9712133

Chartier-Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., … Hardy, J. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature*, *353*(6347), 844–6. doi:10.1038/353844a0

Chen, C.-C. J. J., Spanò, G., & Edgin, J. O. (2013). The impact of sleep disruption on executive function in Down syndrome. *Research in Developmental Disabilities*, *34*(6), 2033–9. doi:10.1016/j.ridd.2013.03.009

Chettouh, Z., Croquette, M. F., Delobel, B., Gilgenkrants, S., Leonard, C., Maunoury, C., … Chery, M. (1995). Molecular mapping of 21 features associated with partial monosomy 21: involvement of the APP-SOD1 region. *American Journal of Human Genetics*, *57*(1), 62–71. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1801243&tool=pmcentrez&rendertype=abstract

Claret, C., Goday, A., Benaiges, D., Chillarón, J. J., Flores, J. A., Hernandez, E., … Cano, J. F. (2013). Subclinical hypothyroidism in the first years of life in patients with Down syndrome. *Pediatric Research*, *73*(5), 674–8. doi:10.1038/pr.2013.26

Cognition, C. (1996). Cambridge Neuropsychological Test Automated Battery (CANTAB). Cambridge, England: Cambridge Cognition Limited.

Constant, E. L., Adam, S., Seron, X., Bruyer, R., Seghers, A., & Daumerie, C. (2005). Anxiety and depression, attention, and executive functions in hypothyroidism. *Journal of the International Neuropsychological Society : JINS*, *11*(5), 535–44. doi:10.1017/S1355617705050642

Contestabile, A., Benfenati, F., & Gasparini, L. (2010). Communication breaks-Down: from neurodevelopment defects to cognitive disabilities in Down syndrome. *Progress in Neurobiology*, *91*(1), 1–22. doi:10.1016/j.pneurobio.2010.01.003

Coppus, A. M. W., Schuur, M., Vergeer, J., Janssens, A. C. J. W., Oostra, B. A., Verbeek, M. M., & van Duijn, C. M. (2012). Plasma β amyloid and the risk of Alzheimer's disease in Down syndrome. *Neurobiology of Aging*, *33*(9), 1988–94. doi:10.1016/j.neurobiolaging.2011.08.007

Cornish, K. M., Manly, T., Savage, R., Swanson, J., Morisano, D., Butler, N., … Hollis, C. P. (2005). Association of the dopamine transporter (DAT1) 10/10-repeat genotype with ADHD symptoms and response inhibition in a general population sample. *Molecular Psychiatry*, *10*(7), 686–98. doi:10.1038/sj.mp.4001641

Correia, N., Mullally, S., Cooke, G., Tun, T. K., Phelan, N., Feeney, J., … Gibney, J. (2009). Evidence for a specific defect in hippocampal memory in overt and subclinical hypothyroidism. *The Journal of Clinical Endocrinology and Metabolism*, *94*(10), 3789–97. doi:10.1210/jc.2008-2702

Coyle, J. T., Oster-Granite, M. L., & Gearhart, J. D. (1986). The neurobiologic consequences of Down syndrome. *Brain Research Bulletin*, *16*(6), 773–87. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2875770

Crowe, S. F. (1996). The Performance of Schizophrenic and Depressed Subjects on Tests of Fluency: Support for a Compromise in Dorsolateral Prefrontal Functioning. *Australian Psychologist*, *31*(3), 204–209. doi:10.1080/00050069608260207

Crowe, S. F. (1998). Decrease in performance on the verbal fluency test as a function of time: evaluation in a young healthy sample. *Journal of*

Clinical and Experimental Neuropsychology, *20*(3), 391–401.
doi:10.1076/jcen.20.3.391.810

Culbertson, W. C., & Zillmer, E. A. (2005). *Tower of London Drexel University (TOL DX): Technical manual.* (2nd Editio.). New York: Multi-Health Systems Imc.

Cuyàs, E., Verdejo-García, A., Fagundo, A. B., Khymenets, O., Rodríguez, J., Cuenca, A., … de la Torre, R. (2011). The influence of genetic and environmental factors among MDMA users in cognitive performance. *PloS One*, *6*(11), e27206. doi:10.1371/journal.pone.0027206

D'Souza, D., & Karmiloff-Smith, A. (2011). When modularization fails to occur: a developmental perspective. *Cognitive Neuropsychology*, *28*(3-4), 276–87. doi:10.1080/02643294.2011.614939

Das Bhowmik, A., Dutta, S., Sinha, S., Chattopadhyay, A., & Mukhopadhyay, K. (2008). Lack of association between down syndrome and polymorphisms in dopamine receptor D4 and serotonin transporter genes. *Neurochemical Research*, *33*(7), 1286–91. doi:10.1007/s11064-007-9581-9

Das, D., Phillips, C., Hsieh, W., Sumanth, K., Dang, V., & Salehi, A. (2014). Neurotransmitter-based strategies for the treatment of cognitive dysfunction in Down syndrome. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *54*, 140–148. doi:10.1016/j.pnpbp.2014.05.004

Davis, J. D., & Tremont, G. (2007). Neuropsychiatric aspects of hypothyroidism and treatment reversibility. . *Minerva Endocrinologica*, *32*(1), 49–65. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17353866

de la Torre, R., de Sola, S., Hernandez, G., Farré, M., Pujol, J., Rodriguez, J., …
al., et. (2016). Safety and efficacy of cognitive training plus
epigallocatechin-3-gallate in young adults with Down's syndrome
(TESDAD): a double-blind, randomised, placebo-controlled, phase 2
trial. *The Lancet Neurology*, *15*(8), 801–810. doi:10.1016/S1474-
4422(16)30034-5

De la Torre, R., De Sola, S., Pons, M., Duchon, A., de Lagran, M. M., Farré, M.,
… Dierssen, M. (2014). Epigallocatechin-3-gallate, a DYRK1A inhibitor,
rescues cognitive deficits in Down syndrome mouse models and in
humans. *Molecular Nutrition & Food Research*, *58*(2), 278–88.
doi:10.1002/mnfr.201300325

De Renzi, E., & Faglioni, P. (1978). Normative data and screening power of a
shortened version of the Token Test. *Cortex; a Journal Devoted to the
Study of the Nervous System and Behavior*, *14*(1), 41–9. Retrieved from
http://www.ncbi.nlm.nih.gov/pubmed/16295108

de Sola, S., de la Torre, R., Sanchez-Benavides, G., Benejam, B., Cuenca-Royo,
A., del Hoyo, L., … TESDAD Study Group, the. (2015). A new cognitive
evaluation battery for Down syndrome and its relevance for clinical
trials. *Frontiers in Psychology*, *6*. doi:10.3389/fpsyg.2015.00708

de Sola, S., Tarancón, T., Peña-Casanova, J., Espadaler, J. M., Langohr, K.,
Poudevida, S., … de la Torre, R. (2008). Auditory event-related
potentials (P3) and cognitive performance in recreational ecstasy
polydrug users: evidence from a 12-month longitudinal study.
Psychopharmacology, *200*(3), 425–37. doi:10.1007/s00213-008-1217-5

Del Hoyo, L., Xicota, L., Sánchez, G., Cuenca, A. M., de Sola, S., Langohr, K., …
De La Torre, R. (2015). Semantic verbal fluency pattern, dementia rating

scores and adaptive behavior correlate with plasma Aβ42 concentrations in Down syndrome young adults. *Frontiers in Behavioral Neuroscience*, *9*. doi:10.3389/fnbeh.2015.00301

Delabar, J. M., Theophile, D., Rahmani, Z., Chettouh, Z., Blouin, J. L., Prieur, M., … Sinet, P. M. (1993). Molecular mapping of twenty-four features of Down syndrome on chromosome 21. *European Journal of Human Genetics : EJHG*, *1*(2), 114–24. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8055322

Deuschle, M., Schredl, M., Schilling, C., Wüst, S., Frank, J., Witt, S. H., … Schulze, T. G. (2010). Association between a serotonin transporter length polymorphism and primary insomnia. *Sleep*, *33*(3), 343–7. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2831428&tool=pmcentrez&rendertype=abstract

Devenny, D. A., Zimmerli, E. J., Kittler, P., & Krinsky-McHale, S. J. (2002). Cued recall in early-stage dementia in adults with Down's syndrome. *Journal of Intellectual Disability Research : JIDR*, *46*(Pt 6), 472–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12354318

Diomedi, M., Placidi, F., Cupini, L. M., Bernardi, G., & Silvestrini, M. (1998). Cerebral hemodynamic changes in sleep apnea syndrome and effect of continuous positive airway pressure treatment. *Neurology*, *51*(4), 1051–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9781528

Dugbartey, A. T. (1998). Neurocognitive Aspects of Hypothyroidism. *Archives of Internal Medicine*, *158*(13), 1413. doi:10.1001/archinte.158.13.1413

Eadie, P. A., Fey, M. E., Douglas, J. M., & Parsons, C. L. (2002). Profiles of

grammatical morphology and sentence imitation in children with specific language impairment and Down syndrome. *Journal of Speech, Language, and Hearing Research : JSLHR*, *45*(4), 720–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12199402

Edgin, J. O. (2013). Cognition in Down syndrome: a developmental cognitive neuroscience perspective. *Wiley Interdisciplinary Reviews: Cognitive Science*, *4*(3), 307–317. doi:10.1002/wcs.1221

Edgin, J. O., Mason, G. M., Allman, M. J., Capone, G. T., Deleon, I., Maslen, C., … Nadel, L. (2010). Development and validation of the Arizona Cognitive Test Battery for Down syndrome. *Journal of Neurodevelopmental Disorders*, *2*(3), 149–164. doi:10.1007/s11689-010-9054-3

Edgin, J. O., Pennington, B. F., & Mervis, C. B. (2010). Neuropsychological components of intellectual disability: the contributions of immediate, working, and associative memory. *Journal of Intellectual Disability Research : JIDR*, *54*(5), 406–17. doi:10.1111/j.1365-2788.2010.01278.x

Edgin, J. O., Tooley, U., Demara, B., Nyhuis, C., Anand, P., & Spanò, G. (2015). Sleep Disturbance and Expressive Language Development in Preschool-Age Children With Down Syndrome. *Child Development*, *86*(6), 1984–98. doi:10.1111/cdev.12443

Eisermann, M. M., DeLaRaillère, A., Dellatolas, G., Tozzi, E., Nabbout, R., Dulac, O., & Chiron, C. (2003). Infantile spasms in Down syndrome-- effects of delayed anticonvulsive treatment. *Epilepsy Research*, *55*(1-2), 21–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12948613

Ekstein, S., Glick, B., Weill, M., Kay, B., & Berger, I. (2011). Down syndrome and attention-deficit/hyperactivity disorder (ADHD). *Journal of Child Neurology*, *26*(10), 1290–5. doi:10.1177/0883073811405201

Erdamar, H., Demirci, H., Yaman, H., Erbil, M. K., Yakar, T., Sancak, B., … Yetkin, I. (2008). The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clinical Chemistry and Laboratory Medicine*, *46*(7), 1004–10. doi:10.1515/CCLM.2008.183

Esbensen, A. J. (2015). Sleep problems and associated comorbidities among adults with Down syndrome. *Journal of Intellectual Disability Research : JIDR*. doi:10.1111/jir.12236

Evenhuis HM, Kengen MMF, and E. H. (2006). *Dementia Questionnaire for People with Intellectual Disabilities (DMR).* Amsterdam: Harcourt Test Publishers.

Fabian Fernandez and Jamie O Edgin. (2013). Poor Sleep as a Precursor to Cognitive Decline in Down Syndrome : A Hypothesis. *Journal of Alzheimers Disease & Parkinsonism*. Retrieved from http://www.omicsonline.org/poor-sleep-as-a-precursor-to-cognitive-decline-in-down-syndrome-a-hypothesis-2161-0460.1000124.php&&aid=17433

Fagundo, A. B., Cuyàs, E., Verdejo-Garcia, A., Khymenets, O., Langohr, K., Martín-Santos, R., … de la Torre, R. (2010). The influence of 5-HTT and COMT genotypes on verbal fluency in ecstasy users. *Journal of Psychopharmacology (Oxford, England)*, *24*(9), 1381–93. doi:10.1177/0269881109354926

Fagundo, A. B., López, S., Romero, M., Guarch, J., Marcos, T., & Salamero, M. (2008). Clustering and switching in semantic fluency: predictors of the development of Alzheimer's disease. *International Journal of Geriatric Psychiatry*, *23*(10), 1007–13. doi:10.1002/gps.2025

Fernandez, F., & Edgin, J. O. (2013). Poor Sleep as a Precursor to Cognitive Decline in Down Syndrome : A Hypothesis. *Journal of Alzheimer's Disease & Parkinsonism*, *3*(2), 124. doi:10.4172/2161-0460.1000124

Fernandez, F., & Reeves, R. H. (2015). Assessing cognitive improvement in people with down syndrome: important considerations for drug-efficacy trials. *Handbook of Experimental Pharmacology*, *228*, 335–80. doi:10.1007/978-3-319-16522-6_12

Ferrer, I., Barrachina, M., Puig, B., Martínez de Lagrán, M., Martí, E., Avila, J., & Dierssen, M. (2005). Constitutive Dyrk1A is abnormally expressed in Alzheimer disease, Down syndrome, Pick disease, and related transgenic models. *Neurobiology of Disease*, *20*(2), 392–400. doi:10.1016/j.nbd.2005.03.020

Finestack, L. H., & Abbeduto, L. (2010). Expressive language profiles of verbally expressive adolescents and young adults with Down syndrome or fragile X syndrome. *Journal of Speech, Language, and Hearing Research : JSLHR*, *53*(5), 1334–48. doi:10.1044/1092-4388(2010/09-0125)

Foley, K.-R., Jacoby, P., Einfeld, S., Girdler, S., Bourke, J., Riches, V., & Leonard, H. (2014). Day occupation is associated with psychopathology for adolescents and young adults with Down syndrome. *BMC Psychiatry*, *14*, 266. doi:10.1186/s12888-014-0266-z

Fredriksen, K., Rhodes, J., Reddy, R., & Way, N. Sleepless in Chicago: tracking the effects of adolescent sleep loss during the middle school years. *Child Development*, *75*(1), 84–95. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15015676

Fung, E., Witmans, M., Ghosh, M., Cave, D., & El-Hakim, H. (2012). Upper

airway findings in children with Down syndrome on sleep nasopharyngoscopy: case-control study. *Journal of Otolaryngology - Head & Neck Surgery = Le Journal D'oto-Rhino-Laryngologie et de Chirurgie Cervico-Faciale*, *41*(2), 138–44. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22569015

Gardiner, K. J. (2015). Pharmacological approaches to improving cognitive function in Down syndrome: current status and considerations. *Drug Design, Development and Therapy*, *9*, 103–25. doi:10.2147/DDDT.S51476

Gennatas, E. D., Cholfin, J. A., Zhou, J., Crawford, R. K., Sasaki, D. A., Karydas, A., … Seeley, W. W. (2012). COMT Val158Met genotype influences neurodegeneration within dopamine-innervated brain structures. *Neurology*, *78*(21), 1663–9. doi:10.1212/WNL.0b013e3182574fa1

Gerstadt, C. L., Hong, Y. J., & Diamond, A. (1994). The relationship between cognition and action: performance of children 3 1/2-7 years old on a Stroop-like day-night test. *Cognition*, *53*(2), 129–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7805351

Ghezzo, A., Salvioli, S., Solimando, M. C., Palmieri, A., Chiostergi, C., Scurti, M., … Franceschi, C. (2014). Age-Related Changes of Adaptive and Neuropsychological Features in Persons with Down Syndrome. *PLoS ONE*, *9*(11), e113111. doi:10.1371/journal.pone.0113111

Goday-Arno, A., Cerda-Esteva, M., Flores-Le-Roux, J. A., Chillaron-Jordan, J. J., Corretger, J. M., & Cano-Pérez, J. F. (2009). Hyperthyroidism in a population with Down syndrome (DS). *Clinical Endocrinology*, *71*(1), 110–4. doi:10.1111/j.1365-2265.2008.03419.x

Goldman, N., Glei, D. A., Lin, Y.-H., & Weinstein, M. (2010). The serotonin

transporter polymorphism (5-HTTLPR): allelic variation and links with depressive symptoms. *Depression and Anxiety*, *27*(3), 260–9. doi:10.1002/da.20660

Goldstein, K., Scheerer, M., & Hanfmann, E. (1953). Tests of Abstract and Concrete Thinking. In *Contributions toward medical psychology: Theory and psychodiagnostic methods* (pp. 702–740). New York: Ronald Press Company. Retrieved from http://dx.doi.org/10.1037/11296-008

Gottesmann, C. (2004). Brain inhibitory mechanisms involved in basic and higher integrated sleep processes. *Brain Research Reviews*, *45*(3), 230–249. doi:10.1016/j.brainresrev.2004.04.003

Grieco, J., Pulsifer, M., Seligsohn, K., Skotko, B., & Schwartz, A. (2015). Down syndrome: Cognitive and behavioral functioning across the lifespan. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *169*(2), 135–49. doi:10.1002/ajmg.c.31439

Grober, E., & Buschke, H. (1987). Genuine memory deficits in dementia. *Developmental Neuropsychology*, *3*(1), 13–36. doi:10.1080/87565648709540361

Guimaraes, C. V. A., Donnelly, L. F., Shott, S. R., Amin, R. S., & Kalra, M. (2008). Relative rather than absolute macroglossia in patients with Down syndrome: implications for treatment of obstructive sleep apnea. *Pediatric Radiology*, *38*(10), 1062–7. doi:10.1007/s00247-008-0941-7

Gulesserian, T., Engidawork, E., Cairns, N., & Lubec, G. (2000). Increased protein levels of serotonin transporter in frontal cortex of patients with Down syndrome. *Neuroscience Letters*, *296*(1), 53–57. doi:10.1016/S0304-3940(00)01624-4

HapMap Project. (n.d.). Retrieved March 8, 2016, from

 http://hapmap.ncbi.nlm.nih.gov/

Harrison, P., & Oakland, T. (2003). *Adaptive Behavior Assessment System*

 (ABAS-II) (Second Edi.). Los Angeles: PEARSON. Retrieved from

 http://www.pearsonclinical.com/psychology/products/100000449/ada

 ptive-behavior-assessment-system-second-edition-abas-second-

 edition.html

Hartmann, J. A., Wichers, M., van Bemmel, A. L., Derom, C., Thiery, E.,

 Jacobs, N., … Simons, C. J. P. (2014). The serotonin transporter 5-

 HTTLPR polymorphism in the association between sleep quality and

 affect. *European Neuropsychopharmacology : The Journal of the*

 European College of Neuropsychopharmacology, *24*(7), 1086–90.

 doi:10.1016/j.euroneuro.2014.01.015

He, X.-S., Ma, N., Pan, Z.-L., Wang, Z.-X., Li, N., Zhang, X.-C., … Zhang, D.-R.

 (2011). Functional magnetic resource imaging assessment of altered

 brain function in hypothyroidism during working memory processing.

 European Journal of Endocrinology / European Federation of Endocrine

 Societies, *164*(6), 951–9. doi:10.1530/EJE-11-0046

Head, E., Doran, E., Nistor, M., Hill, M., Schmitt, F. A., Haier, R. J., & Lott, I. T.

 (2011). Plasma amyloid-β as a function of age, level of intellectual

 disability, and presence of dementia in Down syndrome. *Journal of*

 Alzheimer's Disease : JAD, *23*(3), 399–409. doi:10.3233/JAD-2010-

 101335

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., & Lesch, K. P.

 (1996). Allelic variation of human serotonin transporter gene

 expression. *Journal of Neurochemistry*, *66*(6), 2621–4. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/8632190

Heller, J. H., Spiridigliozzi, G. A., Crissman, B. G., Sullivan-Saarela, J. A., Li, J. S., & Kishnani, P. S. (2006). Clinical trials in children with Down syndrome: issues from a cognitive research perspective. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *142C*(3), 187–95. doi:10.1002/ajmg.c.30103

Henry, J. D., Crawford, J. R., & Phillips, L. H. (2004). Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. *Neuropsychologia*, *42*(9), 1212–22. doi:10.1016/j.neuropsychologia.2004.02.001

Hessl, D., Nguyen, D. V, Green, C., Chavez, A., Tassone, F., Hagerman, R. J., … Hall, S. (2009). A solution to limitations of cognitive testing in children with intellectual disabilities: the case of fragile X syndrome. *Journal of Neurodevelopmental Disorders*, *1*(1), 33–45. doi:10.1007/s11689-008-9001-8

Hoffmire, C. A., Magyar, C. I., Connolly, H. V, Fernandez, I. D., & van Wijngaarden, E. (2014). High prevalence of sleep disorders and associated comorbidities in a community sample of children with Down syndrome. *Journal of Clinical Sleep Medicine : JCSM : Official Publication of the American Academy of Sleep Medicine*, *10*(4), 411–9. doi:10.5664/jcsm.3618

Holland, A. J., Hon, J., Huppert, F. A., & Stevens, F. (2000). Incidence and course of dementia in people with Down's syndrome: findings from a population-based study. *Journal of Intellectual Disability Research : JIDR*, *44 (Pt 2)*, 138–46. doi:10.1046/j.1365-2788.2000.00263.x

Hoyo, L. Del, Xicota, L., Sánchez-Benavides, G., Cuenca-Royo, A., de Sola, S.,

Langohr, K., … de la Torre, R. (2015). Semantic Verbal Fluency Pattern, Dementia Rating Scores and Adaptive Behavior Correlate With Plasma Aβ42 Concentrations in Down Syndrome Young Adults. *Frontiers in Behavioral Neuroscience*, *9*, 301. doi:10.3389/fnbeh.2015.00301

Hunter, J. E., Allen, E. G., Shin, M., Bean, L. J. H., Correa, A., Druschel, C., … Sherman, S. L. (2013). The association of low socioeconomic status and the risk of having a child with Down syndrome: a report from the National Down Syndrome Project. *Genetics in Medicine : Official Journal of the American College of Medical Genetics*, *15*(9), 698–705. doi:10.1038/gim.2013.34

Hurks, P. P. M., Vles, J. S. H., Hendriksen, J. G. M., Kalff, A. C., Feron, F. J. M., Kroes, M., … Jolles, J. (2006). Semantic category fluency versus initial letter fluency over 60 seconds as a measure of automatic and controlled processing in healthy school-aged children. *Journal of Clinical and Experimental Neuropsychology*, *28*(5), 684–95. doi:10.1080/13803390590954191

Kaplan, E., Goodglass, H., & Weintraub, S. (1983). *The Boston naming test.* (L. & Febiger, Ed.) (2nd ed.). Philadelphia.

Karen J. Miller, P. D., Thomas D. Parsons, M. A., Peter C. Whybrow, M. D., Katja Van Herle, M. D., Natalie Rasgon, M. D. . P. D., Andre Van Herle, M. D., … Michael Bauer, M. D. . P. D. (2007, April 1). Verbal Memory Retrieval Deficits Associated With Untreated Hypothyroidism. *The Journal of Neuropsychiatry and Clinical Neurosciences*. American Psychiatric Publishing, Inc. Retrieved from http://neuro.psychiatryonline.org/doi/abs/10.1176/jnp.2007.19.2.132?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub=pubmed

Kariyawasam, D., Rachdi, L., Carré, A., Martin, M., Houlier, M., Janel, N., … Polak, M. (2015). DYRK1A BAC transgenic mouse: a new model of thyroid dysgenesis in Down syndrome. *Endocrinology*, *156*(3), 1171–80. doi:10.1210/en.2014-1329

Karmiloff-Smith, A., Al-Janabi, T., D'Souza, H., Groet, J., Massand, E., Mok, K., … Strydom, A. (2016). The importance of understanding individual differences in Down syndrome. *F1000Research*, *5*. doi:10.12688/f1000research.7506.1

Kaufman, A. ., & Kaufman, A. L. (1994). KBIT: Test Breve de Inteligencia de Kaufman. Manual de interpretación. [KBIT: Kaufman's Brief Intelligence Test. Interpretation Manual]. *Madrid, Spain: TEA*.

Kester, M. H. A., Martinez de Mena, R., Obregon, M. J., Marinkovic, D., Howatson, A., Visser, T. J., … Morreale de Escobar, G. (2004). Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *The Journal of Clinical Endocrinology and Metabolism*, *89*(7), 3117–28. doi:10.1210/jc.2003-031832

Khadzhieva, M. B., Lutcenko, N. N., Volodin, I. V., Morozova, K. V., & Salnikova, L. E. (2014). Association of oxidative stress-related genes with idiopathic recurrent miscarriage. *Free Radical Research*, *48*(5), 534–541. doi:10.3109/10715762.2014.891735

Khan, M. B., Lang, M. J., Huang, M.-B., Raymond, A., Bond, V. C., Shiramizu, B., & Powell, M. D. (2016). Nef exosomes isolated from the plasma of individuals with HIV-associated dementia (HAD) can induce Aβ1-42 secretion in SH-SY5Y neural cells. *Journal of Neurovirology*, *22*(2), 179–90. doi:10.1007/s13365-015-0383-6

Kirk, H. E., Gray, K., Riby, D. M., & Cornish, K. M. (2015). Cognitive training as a resolution for early executive function difficulties in children with intellectual disabilities. *Research in Developmental Disabilities*, *38*, 145–60. doi:10.1016/j.ridd.2014.12.026

Kogan, C. S., Boutet, I., Cornish, K., Graham, G. E., Berry-Kravis, E., Drouin, A., & Milgram, N. W. (2009). A comparative neuropsychological test battery differentiates cognitive signatures of Fragile X and Down syndrome. *Journal of Intellectual Disability Research : JIDR*, *53*(2), 125–42. doi:10.1111/j.1365-2788.2008.01135.x

Koutsilieri, E., Riederer, P., du Plessis, S., & Scheller, C. (2014). A short review on the relation between the dopamine transporter 10/10-repeat allele and ADHD: implications for HIV infection. *Attention Deficit and Hyperactivity Disorders*, *6*(3), 203–9. doi:10.1007/s12402-014-0134-1

Krinsky-McHale, S. J., Devenny, D. A., Kittler, P., & Silverman, W. (2008). Selective attention deficits associated with mild cognitive impairment and early stage Alzheimer's disease in adults with Down syndrome. *American Journal of Mental Retardation : AJMR*, *113*(5), 369–86. doi:10.1352/2008.113:369-386

Krinsky-McHale, S. J., & Silverman, W. (2013). Dementia and mild cognitive impairment in adults with intellectual disability: issues of diagnosis. *Developmental Disabilities Research Reviews*, *18*(1), 31–42. doi:10.1002/ddrr.1126

Lanfranchi, S., Jerman, O., Dal Pont, E., Alberti, A., & Vianello, R. (2010). Executive function in adolescents with Down Syndrome. *Journal of Intellectual Disability Research : JIDR*, *54*(4), 308–319. doi:10.1111/j.1365-2788.2010.01262.x

Lee, N. R., Anand, P., Will, E., Adeyemi, E. I., Clasen, L. S., Blumenthal, J. D., … Edgin, J. O. (2015). Everyday executive functions in Down syndrome from early childhood to young adulthood: evidence for both unique and shared characteristics compared to youth with sex chromosome trisomy (XXX and XXY). *Frontiers in Behavioral Neuroscience*, *9*, 264. doi:10.3389/fnbeh.2015.00264

Lewin, D. S., Rosen, R. C., England, S. J., & Dahl, R. E. (2002). Preliminary evidence of behavioral and cognitive sequelae of obstructive sleep apnea in children. *Sleep Medicine*, *3*(1), 5–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14592247

Liogier d'Ardhuy, X., Edgin, J. O., Bouis, C., de Sola, S., Goeldner, C., Kishnani, P., … Khwaja, O. (2015). Assessment of Cognitive Scales to Examine Memory, Executive Function and Language in Individuals with Down Syndrome: Implications of a 6-month Observational Study. *Frontiers in Behavioral Neuroscience*, *9*, 300. doi:10.3389/fnbeh.2015.00300

Liu, F., Liang, Z., Wegiel, J., Hwang, Y.-W., Iqbal, K., Grundke-Iqbal, I., … Gong, C.-X. (2008). Overexpression of Dyrk1A contributes to neurofibrillary degeneration in Down syndrome. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *22*(9), 3224–33. doi:10.1096/fj.07-104539

Lott, I. (2012). Neurological phenotypes for Down syndrome across the life span. *Progress in Brain Research*, *197*, 101–21. doi:10.1016/B978-0-444-54299-1.00006-6

Lott, I., & Dierssen, M. (2010). Cognitive deficits and associated neurological complications in individuals with Down's syndrome. *The Lancet. Neurology*, *9*(6), 623–33. doi:10.1016/S1474-4422(10)70112-5

Lott, I. T., & Dierssen, M. (2010). Cognitive deficits and associated neurological complications in individuals with Down's syndrome. *Lancet Neurology*, *9*(6), 623–633. doi:10.1016/S1474-4422(10)70112-5

Lubec, B., Yoo, B. C., Dierssen, M., Balic, N., & Lubec, G. (2001). Down syndrome patients start early prenatal life with normal cholinergic, monoaminergic and serotoninergic innervation. *Journal of Neural Transmission. Supplementum*, (61), 303–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11771753

Lucas, A., Julián, M. T., Cantón, A., Castell, C., Casamitjana, R., Martínez-Cáceres, E. M., & Granada, M. L. (2010). Undiagnosed thyroid dysfunction, thyroid antibodies, and iodine excretion in a Mediterranean population. *Endocrine*, *38*(3), 391–6. doi:10.1007/s12020-010-9397-2

Lumbreras, M., Baamonde, C., Martínez-Cué, C., Lubec, G., Cairns, N., Sallés, J., … Flórez, J. (2006). Brain G protein-dependent signaling pathways in Down syndrome and Alzheimer's disease. *Amino Acids*, *31*(4), 449–56. doi:10.1007/s00726-005-0272-z

Lyle, R., Béna, F., Gagos, S., Gehrig, C., Lopez, G., Schinzel, A., … Antonarakis, S. E. (2009). Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. *European Journal of Human Genetics : EJHG*, *17*(4), 454–66. doi:10.1038/ejhg.2008.214

Makary, A. T., Testa, R., Tonge, B. J., Einfeld, S. L., Mohr, C., & Gray, K. M. (2015). Association between adaptive behaviour and age in adults with Down syndrome without dementia: examining the range and severity of adaptive behaviour problems. *Journal of Intellectual Disability*

Research : JIDR, *59*(8), 689–702. doi:10.1111/jir.12172

Malhotra, A. K., Kestler, L. J., Mazzanti, C., Bates, J. A., Goldberg, T., & Goldman, D. (2002). A Functional Polymorphism in the COMT Gene and Performance on a Test of Prefrontal Cognition. *American Journal of Psychiatry*, *159*(4), 652–654. doi:10.1176/appi.ajp.159.4.652

Maris, M., Verhulst, S., Wojciechowski, M., Van de Heyning, P., & Boudewyns, A. (2016). Sleep problems and obstructive sleep apnea in children with down syndrome, an overwiew. *International Journal of Pediatric Otorhinolaryngology*, *82*, 12–5. doi:10.1016/j.ijporl.2015.12.014

Mason, G. M., Spanó, G., & Edgin, J. (2015). Symptoms of attention-deficit/hyperactivity disorder in Down syndrome: effects of the dopamine receptor D4 gene. *American Journal on Intellectual and Developmental Disabilities*, *120*(1), 58–71. doi:10.1352/1944-7558-120.1.58

Massand, E., Ball, G., & Erikkson, M. (n.d.). Sleep fragmentation in infants/toddlers with Down syndrome: Relationships with language, motor abilities and attention. *Prep.*

Mehta, P. D., Capone, G., Jewell, A., & Freedland, R. L. (2007). Increased amyloid beta protein levels in children and adolescents with Down syndrome. *Journal of the Neurological Sciences*, *254*(1-2), 22–7. doi:10.1016/j.jns.2006.12.010

Mehta, P. D., Mehta, S. P., Fedor, B., Patrick, B. A., Emmerling, M., & Dalton, A. J. (2003). Plasma amyloid beta protein 1-42 levels are increased in old Down Syndrome but not in young Down Syndrome. *Neuroscience Letters*, *342*(3), 155–8. doi:10.1016/S0304-3940(03)00275-1

Meyer-Lindenberg, A., Kohn, P. D., Kolachana, B., Kippenhan, S., McInerney-Leo, A., Nussbaum, R., … Berman, K. F. (2005). Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. *Nature Neuroscience*, *8*(5), 594–6. doi:10.1038/nn1438

Mier, D., Kirsch, P., & Meyer-Lindenberg, A. (2009). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Molecular Psychiatry*, *15*(9), 918–927. doi:10.1038/mp.2009.36

Miller, K. J., Parsons, T. D., Whybrow, P. C., Van Herle, K., Rasgon, N., Van Herle, A., … Bauer, M. (2007). Verbal memory retrieval deficits associated with untreated hypothyroidism. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *19*(2), 132–6. doi:10.1176/jnp.2007.19.2.132

Milner, B. (1971). Interhemispheric differences in the localization of psychological processes in man. *British Medical Bulletin*, *27*(3), 272–7. Retrieved from http://europepmc.org/abstract/MED/4937273

Mogra, R., Zidere, V., & Allan, L. D. (2011). Prenatally detectable congenital heart defects in fetuses with Down syndrome. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *38*(3), 320–4. doi:10.1002/uog.8977

Møller, R. S., Kübart, S., Hoeltzenbein, M., Heye, B., Vogel, I., Hansen, C. P., … Kalscheuer, V. M. (2008). Truncation of the Down syndrome candidate gene DYRK1A in two unrelated patients with microcephaly. *American Journal of Human Genetics*, *82*(5), 1165–70. doi:10.1016/j.ajhg.2008.03.001

Mpofu, E., & Oakland, T. (2009). *Rehabilitation and Health Assessment:*

Applying ICF Guidelines. New York: Springer Publishing Company. Retrieved from https://books.google.com/books?id=iGNL--tb0WkC&pgis=1

Næss, K.-A. B., Lyster, S.-A. H., Hulme, C., & Melby-Lervåg, M. (2011). Language and verbal short-term memory skills in children with Down syndrome: a meta-analytic review. *Res Dev Disabil*, *32 (6)*, 2225–34. doi:doi: 10.1016/j.ridd.2011.05.014.

Nève, J., Sinet, P. M., Molle, L., & Nicole, A. (1983). Selenium, zinc and copper in Down's syndrome (trisomy 21): blood levels and relations with glutathione peroxidase and superoxide dismutase. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *133*(2), 209–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6226457

Oberndorfer, S., Saletu-Zyhlarz, G., & Saletu, B. (2000). Effects of selective serotonin reuptake inhibitors on objective and subjective sleep quality. *Neuropsychobiology*, *42*(2), 69–81. doi:26676

Palmer, K., Bäckman, L., Winblad, B., & Fratiglioni, L. (2003). Detection of Alzheimer's disease and dementia in the preclinical phase: population based cohort study. *BMJ (Clinical Research Ed.)*, *326*(7383), 245. doi:10.1136/bmj.326.7383.245

Papoulidis, I., Papageorgiou, E., Siomou, E., Oikonomidou, E., Thomaidis, L., Vetro, A., … Vassilis, P. (2014). A patient with partial trisomy 21 and 7q deletion expresses mild Down syndrome phenotype. *Gene*, *536*(2), 441–3. doi:10.1016/j.gene.2013.11.078

Pennington, B. F., Moon, J., Edgin, J., Stedron, J., & Nadel, L. (2003). The neuropsychology of Down syndrome: evidence for hippocampal dysfunction. *Child Development*, *74*(1), 75–93. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/12625437

Petre-Quadens, O., & De Greef, A. (1971). Effects of 5-HTP on sleep in
    mongol children. *Journal of the Neurological Sciences*, *13*(1), 115–119.
    doi:10.1016/0022-510X(71)90211-5

Pilcher, E. (1998). Dental Care for the Patient with Down Syndrome. *Down
    Syndrome Research and Practice*, *5*(3), 111–116.
    doi:10.3104/reviews.84

Prasher, V. (1994). Prevelance of thyroid dysfunction and autoimmunity in
    adults with Down syndrome. *Down Syndrome Research and Practice*,
    2(2), 67–70. doi:10.3104/reports.32

Prasher, V. P., Sajith, S. G., Mehta, P., Zigman, W. B., & Schupf, N. (2010).
    Plasma beta-amyloid and duration of Alzheimer's disease in adults with
    Down syndrome. *International Journal of Geriatric Psychiatry*, *25*(2),
    202–7. doi:10.1002/gps.2321

Prasher, V. P., Sajith, S. G., Rees, S. D., Patel, A., Tewari, S., Schupf, N., &
    Zigman, W. B. (2008). Significant effect of APOE epsilon 4 genotype on
    the risk of dementia in Alzheimer's disease and mortality in persons
    with Down syndrome. *International Journal of Geriatric Psychiatry*,
    23(11), 1134–40. doi:10.1002/gps.2039

Pujol, J., Del Hoyo, L., Blanco-Hinojo, L., de Sola, S., Macià, D., Martínez-
    Vilavella, G., … de la Torre, R. (2014). Anomalous brain functional
    connectivity contributing to poor adaptive behavior in Down syndrome.
    *Cortex; a Journal Devoted to the Study of the Nervous System and
    Behavior*, *64C*, 148–156. doi:10.1016/j.cortex.2014.10.012

Raoux, N., Amieva, H., Le Goff, M., Auriacombe, S., Carcaillon, L., Letenneur,

L., & Dartigues, J.-F. (2008). Clustering and switching processes in semantic verbal fluency in the course of Alzheimer's disease subjects: results from the PAQUID longitudinal study. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *44*(9), 1188–96. doi:10.1016/j.cortex.2007.08.019

Ravens-Sieberer, U., Auquier, P., Erhart, M., Gosch, A., Rajmil, L., Bruil, J., … Kilroe, J. (2007). The KIDSCREEN-27 quality of life measure for children and adolescents: psychometric results from a cross-cultural survey in 13 European countries. *Quality of Life Research : An International Journal of Quality of Life Aspects of Treatment, Care and Rehabilitation*, *16*(8), 1347–56. doi:10.1007/s11136-007-9240-2

Raz, N., Torres, I. J., Briggs, S. D., Spencer, W. D., Thornton, A. E., Loken, W. J., … Acker, J. D. (1995). Selective neuroanatomic abnormalities in Down's syndrome and their cognitive correlates: evidence from MRI morphometry. *Neurology*, *45*(2), 356–366. doi:10.1212/WNL.45.2.356

Real de Asua, D., Quero, M., Moldenhauer, F., & Suarez, C. (2015). Clinical profile and main comorbidities of Spanish adults with Down syndrome. *European Journal of Internal Medicine*, *26*(6), 385–91. doi:10.1016/j.ejim.2015.05.003

Richtsmeier, J. T., Baxter, L. L., & Reeves, R. H. (2000). Parallels of craniofacial maldevelopment in Down syndrome and Ts65Dn mice. *Developmental Dynamics : An Official Publication of the American Association of Anatomists*, *217*(2), 137–45. doi:10.1002/(SICI)1097-0177(200002)217:2<137::AID-DVDY1>3.0.CO;2-N

Risser, D., Lubec, G., Cairns, N., & Herrera-Marschitz, M. (1997). Excitatory amino acids and monoamines in parahippocampal gyrus and frontal

cortical pole of adults with Down syndrome. *Life Sciences*, *60*(15), 1231–7. doi:10.1016/S0024-3205(97)00067-2

Robinson-Shelton, A., & Malow, B. A. (2015). Sleep Disturbances in Neurodevelopmental Disorders. *Current Psychiatry Reports*, *18*(1), 6. doi:10.1007/s11920-015-0638-1

Roizen, N. J., & Patterson, D. (2003). Down's syndrome. *Lancet*, *361*(9365), 1281–9. doi:10.1016/S0140-6736(03)12987-X

Romero, M., Guarch, J., Fagundo, A. B., Lo, S., Marcos, T., & Salamero, M. (2008). Clustering and switching in semantic fluency : predictors of the development of Alzheimer ' s disease, (April), 1007–1013. doi:10.1002/gps

Rosen, D. (2011). Management of obstructive sleep apnea associated with Down syndrome and other craniofacial dysmorphologies. *Current Opinion in Pulmonary Medicine*, *17*(6), 431–6. doi:10.1097/MCP.0b013e32834ba9c0

Rosen, D., Lombardo, A., Skotko, B., & Davidson, E. J. (2011). Parental perceptions of sleep disturbances and sleep-disordered breathing in children with Down syndrome. *Clinical Pediatrics*, *50*(2), 121–5. doi:10.1177/0009922810384260

Rowe, J., Lavender, A., & Turk, V. (2006). Cognitive executive function in Down's syndrome. *The British Journal of Clinical Psychology / the British Psychological Society*, *45*(Pt 1), 5–17. doi:10.1348/014466505X29594

Rowe, J., Lavender, A., & Turk, V. (2006). Cognitive executive function in Down's syndrome. *The British Journal of Clinical Psychology / the British Psychological Society*, *45*(Pt 1), 5–17. doi:10.1348/014466505X29594

Salminen, L. E., Schofield, P. R., Pierce, K. D., Lane, E. M., Heaps, J. M., Bolzenius, J. D., … Paul, R. H. (2014). Triallelic relationships between the serotonin transporter polymorphism and cognition among healthy older adults. *The International Journal of Neuroscience*, *124*(5), 331–8. doi:10.3109/00207454.2013.845822

Samuels, M. H. (2014). Psychiatric and cognitive manifestations of hypothyroidism. *Current Opinion in Endocrinology, Diabetes, and Obesity*, *21*(5), 377–83. doi:10.1097/MED.0000000000000089

Sanchez, M. M., Moghadam, S., Naik, P., Martin, K. J., & Salehi, A. (2011). Hippocampal network alterations in Alzheimer's disease and Down syndrome: from structure to therapy. *Journal of Alzheimer's Disease : JAD*, *26 Suppl 3*(SUPPL. 3), 29–47. doi:10.3233/JAD-2011-0050

Schneider, C., Risser, D., Kirchner, L., Kitzmüller, E., Cairns, N., Prast, H., … Lubec, G. (1997). Similar deficits of central histaminergic system in patients with Down syndrome and Alzheimer disease. *Neuroscience Letters*, *222*(3), 183–6. doi:10.1016/S0304-3940(97)13379-1

Schupf, N., Patel, B., Pang, D., Zigman, W. B., Silverman, W., Mehta, P. D., & Mayeux, R. (2007). Elevated Plasma β-Amyloid Peptide Aβ42 Levels, Incident Dementia, and Mortality in Down Syndrome. *Archives of Neurology*, *64*(7), 1007. doi:10.1001/archneur.64.7.1007

Schupf, N., Zigman, W. B., Tang, M.-X., Pang, D., Mayeux, R., Mehta, P., & Silverman, W. (2010). Change in plasma Aß peptides and onset of dementia in adults with Down syndrome. *Neurology*, *75*(18), 1639–44. doi:10.1212/WNL.0b013e3181fb448b

Senthilvel, E., & Krishna, J. (2011). Body position and obstructive sleep apnea in children with Down syndrome. *Journal of Clinical Sleep Medicine :*

JCSM : Official Publication of the American Academy of Sleep Medicine,
7(2), 158–62. Retrieved from
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3077343&
tool=pmcentrez&rendertype=abstract

Shapiro, B. L., Gorlin, R. J., Redman, R. S., & Bruhl, H. H. (1967). The palate
and Down's syndrome. *The New England Journal of Medicine*, *276*(26),
1460–3. doi:10.1056/NEJM196706292762603

Shaposhnikov, A. M., Khal'chitskiĭ, S. E., & Shvarts, E. I. (1979). [Disorders of
phenylalanine and tyrosine metabolism in Down's syndrome]. *Voprosy
Meditsinskoĭ Khimii*, *25*(1), 15–9. Retrieved from
http://www.ncbi.nlm.nih.gov/pubmed/154771

Shires, C. B., Anold, S. L., Schoumacher, R. A., Dehoff, G. W., Donepudi, S. K.,
& Stocks, R. M. (2010). Body mass index as an indicator of obstructive
sleep apnea in pediatric Down syndrome. *International Journal of
Pediatric Otorhinolaryngology*, *74*(7), 768–72.
doi:10.1016/j.ijporl.2010.03.050

Spanò, G., & Edgin, J. O. (2016). Everyday memory in individuals with Down
syndrome: Validation of the Observer Memory Questionnaire - Parent
Form. *Child Neuropsychology : A Journal on Normal and Abnormal
Development in Childhood and Adolescence*, 1–13.
doi:10.1080/09297049.2016.1150446

Stenzel, D., & Huttner, W. B. (2013). Role of maternal thyroid hormones in
the developing neocortex and during human evolution. *Frontiers in
Neuroanatomy*, *7*, 19. doi:10.3389/fnana.2013.00019

Stores, G., & Stores, R. (2013). Sleep disorders and their clinical significance
in children with Down syndrome. *Developmental Medicine and Child*

Neurology, *55*(2), 126–30. doi:10.1111/j.1469-8749.2012.04422.x

Strauss, H., & Lewin, I. (1982). An empirical study of the weigl-goldstein-scheerer color-form test according to a developmental frame of reference. *Journal of Clinical Psychology*, *38*(2), 367–375. doi:10.1002/1097-4679(198204)38:2<367::AID-JCLP2270380226>3.0.CO;2-9

Tamasaki, A., Saito, Y., Ueda, R., Ohno, K., Yokoyama, K., Satake, T., … Maegaki, Y. (2016). Effects of donepezil and serotonin reuptake inhibitor on acute regression during adolescence in Down syndrome. *Brain & Development*, *38*(1), 113–7. doi:10.1016/j.braindev.2015.06.006

Taylor, K. I., Salmon, D. P., Monsch, A. U., & Brugger, P. (2005). Semantic and phonemic sequence effects in random word generation: a dissociation between Alzheimer's and Huntington's disease patients. *Journal of the International Neuropsychological Society : JINS*, *11*(3), 303–10. doi:10.1017/S1355617705050356

Teller, J. K., Russo, C., DeBusk, L. M., Angelini, G., Zaccheo, D., Dagna-Bricarelli, F., … Gambetti, P. (1996). Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. *Nature Medicine*, *2*(1), 93–5. doi:10.1038/nm0196-93

Thiel, R., & Fowkes, S. W. (2007). Down syndrome and thyroid dysfunction: should nutritional support be the first-line treatment? *Medical Hypotheses*, *69*(4), 809–15. doi:10.1016/j.mehy.2007.01.068

Toledo, J. B., Van Deerlin, V. M., Lee, E. B., Suh, E., Baek, Y., Robinson, J. L., … Trojanowski, J. Q. (2014). A platform for discovery: The University of Pennsylvania Integrated Neurodegenerative Disease Biobank.

Alzheimer's & Dementia : The Journal of the Alzheimer's Association,
10(4), 477–84.e1. doi:10.1016/j.jalz.2013.06.003

Trois, M. S., Capone, G. T., Lutz, J. A., Melendres, M. C., Schwartz, A. R.,
Collop, N. A., & Marcus, C. L. (2009). Obstructive sleep apnea in adults
with Down syndrome. *Journal of Clinical Sleep Medicine : JCSM : Official
Publication of the American Academy of Sleep Medicine*, *5*(4), 317–23.
Retrieved from
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2725249&
tool=pmcentrez&rendertype=abstract

Troyer, A. K. (2000). Normative data for clustering and switching on verbal
fluency tasks. *Journal of Clinical and Experimental Neuropsychology*,
22(3), 370–8. doi:10.1076/1380-3395(200006)22:3;1-V;FT370

Troyer, A. K., Moscovitch, M., & Winocur, G. (1997). Clustering and switching
as two components of verbal fluency: evidence from younger and older
healthy adults. *Neuropsychology*, *11*(1), 138–46. doi:10.1037/0894-
4105.11.1.138

Van Cleve, S. N., Cannon, S., & Cohen, W. I. (2006). Part II: Clinical Practice
Guidelines for adolescents and young adults with Down Syndrome: 12
to 21 Years. *Journal of Pediatric Health Care : Official Publication of
National Association of Pediatric Nurse Associates & Practitioners*,
20(3), 198–205. doi:10.1016/j.pedhc.2006.02.006

Verdejo-García, A., Fagundo, A. B., Cuenca, A., Rodriguez, J., Cuyás, E.,
Langohr, K., … de la Torre, R. (2013). COMT val158met and 5-HTTLPR
genetic polymorphisms moderate executive control in cannabis users.
*Neuropsychopharmacology : Official Publication of the American
College of Neuropsychopharmacology*, *38*(8), 1598–606.

doi:10.1038/npp.2013.59

Villodre, R., Sánchez-Alfonso, A., Brines, L., Núñez, A. B., Chirivella, J., Ferri, J., & Noé, E. (2006). [Verbal fluency tasks in a Spanish sample of young adults (20-49 years of age): normative data of clustering and switching strategies]. *Neurología (Barcelona, Spain)*, *21*(3), 124–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16575625

Visootsak, J., Hess, B., Bakeman, R., & Adamson, L. B. (2013). Effect of congenital heart defects on language development in toddlers with Down syndrome. *Journal of Intellectual Disability Research : JIDR*, *57*(9), 887–92. doi:10.1111/j.1365-2788.2012.01619.x

Vogel, A., Gade, A., Stokholm, J., & Waldemar, G. (2005). Semantic memory impairment in the earliest phases of Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders*, *19*(2-3), 75–81. doi:10.1159/000082352

Wager, T. D., & Smith, E. E. (2003). Neuroimaging studies of working memory: a meta-analysis. *Cognitive, Affective & Behavioral Neuroscience*, *3*(4), 255–274.

Waldman, I. D., Rowe, D. C., Abramowitz, A., Kozel, S. T., Mohr, J. H., Sherman, S. L., … Stever, C. (1998). Association and Linkage of the Dopamine Transporter Gene and Attention-Deficit Hyperactivity Disorder in Children: Heterogeneity owing to Diagnostic Subtype and Severity. *The American Journal of Human Genetics*, *63*(6), 1767–1776. doi:10.1086/302132

Walter, N. T., Markett, S. A., Montag, C., & Reuter, M. (2011). A genetic contribution to cooperation: dopamine-relevant genes are associated with social facilitation. *Social Neuroscience*, *6*(3), 289–301. doi:10.1080/17470919.2010.527169

Wechsler, D. (1997). *Wechsler Memory Scale (WMS-III)* (Third Edit.). San Antonio: TX: The Psychological Corporation.

Wegiel, J., Dowjat, K., Kaczmarski, W., Kuchna, I., Nowicki, K., Frackowiak, J., … Hwang, Y.-W. (2008). The role of overexpressed DYRK1A protein in the early onset of neurofibrillary degeneration in Down syndrome. *Acta Neuropathologica*, *116*(4), 391–407. doi:10.1007/s00401-008-0419-6

Wheeler, S. M., McLelland, V. C., Sheard, E., McAndrews, M. P., & Rovet, J. F. (2015). Hippocampal Functioning and Verbal Associative Memory in Adolescents with Congenital Hypothyroidism. *Frontiers in Endocrinology*, *6*, 163. doi:10.3389/fendo.2015.00163

Whitaker-Azmitia, P. M. (2001). Serotonin and brain development: role in human developmental diseases. *Brain Research Bulletin*, *56*(5), 479–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11750793

White, N. S., Alkire, M. T., & Haier, R. J. (2003). A voxel-based morphometric study of nondemented adults with Down Syndrome. *NeuroImage*, *20*(1), 393–403. doi:10.1016/S1053-8119(03)00273-8

Whittle, N., Sartori, S. B., Dierssen, M., Lubec, G., & Singewald, N. (2007). Fetal Down syndrome brains exhibit aberrant levels of neurotransmitters critical for normal brain development. *Pediatrics*, *120*(6), e1465–71. doi:10.1542/peds.2006-3448

Wilson, B. A., Evans, J. J., Emslie, H., Alderman, N., & Burgess, P. (1998). The Development of an Ecologically Valid Test for Assessing Patients with a Dysexecutive Syndrome. *Neuropsychological Rehabilitation*, *8*(3), 213–228. doi:10.1080/713755570

Winstanley, C. A., Eagle, D. M., & Robbins, T. W. (2006). Behavioral models of

impulsivity in relation to ADHD: translation between clinical and preclinical studies. *Clinical Psychology Review*, *26*(4), 379–95. doi:10.1016/j.cpr.2006.01.001

Wonodi, I., Hong, L. E., Stine, O. C., Mitchell, B. D., Elliott, A., Roberts, R. C., … Thaker, G. K. (2009). Dopamine transporter polymorphism modulates oculomotor function and DAT1 mRNA expression in schizophrenia. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *150B*(2), 282–9. doi:10.1002/ajmg.b.30811

World Health Organization (WHO). (2016). Retrieved April 11, 2016, from http://www.who.int/genomics/public/geneticdiseases/en/index1.html

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., … Nedergaard, M. (2013). Sleep drives metabolite clearance from the adult brain. *Science (New York, N.Y.)*, *342*(6156), 373–7. doi:10.1126/science.1241224

Yang, S., Paynter, J. M., & Gilmore, L. (2016). Vineland Adaptive Behavior Scales: II Profile of Young Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *46*(1), 64–73. doi:10.1007/s10803-015-2543-1

Zigman, W. B., Devenny, D. A., Krinsky-McHale, S. J., Jenkins, E. C., Urv, T. K., Wegiel, J., … Silverman, W. (2008). Alzheimer's Disease in Adults with Down Syndrome. *International Review of Research in Mental Retardation*, *36*, 103–145. doi:10.1016/S0074-7750(08)00004-9