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OBSESSIVE-COMPULSIVE DISORDER

NOVEL INSIGHTS ON EXECUTIVE FUNCTIONS, GUT MICROBIOME, AND GENETICS

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Obsessive-Compulsive Disorder
Novel insights on executive functions, gut microbiome, and genetics

Thesis for Doctoral Degree (Ph.D)

By

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Till mamma och Medicinareberget

Populärvetenskaplig sammanfattning

Tvångssyndrom, på engelska obsessive-compulsive disorder (OCD), är en funktionsnedsättande sjukdom som drabbar cirka 1–2% av befolkningen. Sjukdomen kännetecknas av tvångstankar som framkallar negativa känslor som ångest, tvivel, och äckel, vilket leder till repetitiva och tidskrävande tvångshandlingar eller undvikande handlingar. OCD ingår i kapitlet tvångssyndrom och relaterade tillstånd i DSM-5 tillsammans med diagnosen dysmorfofobi (BDD), där tvångssymtomen är relaterade till utseendet.

I denna avhandling undersökte vi biologiska faktorer som skulle kunna vara associerade med OCD.

Hur skiljer sig exekutiva funktioner hos patienter med OCD från BDD? Vilken roll spelar tarmfloran? Finns det ovanliga genetiska variationer hos patienter med svår och behandlingsresistent OCD?

I den **första studien** jämförde vi exekutiva funktioner mellan patienter med OCD, BDD och friska försökspersoner med hjälp av datoriserade och standardiserade neuropsykologiska tester. Vi fann inga signifikanta skillnader i prestation avseende förmåga till inhibition, kognitiv flexibilitet eller arbetsminne. Våra fynd stödjer inte ett tydligt samband mellan specifika exekutiva funktioner och OCD eller BDD.

I **studie två** undersökte vi skillnader i tarmflorans sammansättning mellan patienter med psykiatriska diagnoser och friska försökspersoner. Först genomförde vi en systematisk översikt över forskningsfältet. Efter genomgång av 4231 studier som publicerats fram till februari 2020 inkluderades 69 studier i sammanställningen. Fram tills sista sökdatumet hade inga studier publicerats om skillnader i tarmflora hos patienter med OCD och friska försökspersoner. Det fanns generellt få samstämmiga resultat mellan studierna, vilket delvis berodde på varierande studie-kvalitet och forskningsmetoder. Det mest reproducerade fyndet var minskad förekomst av butyrat-producerande bakterier hos patienter med psykiatriska diagnoser jämfört med friska försökspersoner.

I **studie tre** jämförde vi skillnader i tarmfloran mellan patienter med OCD och friska försökspersoner. Vi fann inga skillnader i mångfald, komposition eller individuella bakterier på art-nivå mellan patienter med OCD och friska försökspersoner. Dessutom analyserade vi gener i tarmfloran inblandade i metabolisering av ämnen som kan påverka hjärnans funktioner, till exempel bildning av serotonin eller metabolisering av butyrat. Vi såg inte någon skillnad i någon av de 56 funktionella profiler som undersöktes. Vidare undersökte vi skillnader i tarmfloran hos patienter med OCD före och efter kognitiv beteendeterapi. Trots minskade tvångshandlingar, till exempel upprepade handtvätt, var tarmfloran

oförändrad både avseende sammansättning och funktion. Slutsatsen från studie tre är att det i dagsläget inte finns tillräckligt med forskningsunderlag för att fastställa om det finns skillnader i tarmfloran mellan patienter med tvångssyndrom och friska försökspersoner.

I **studie fyra** sekvenserade vi de proteinkodande regionerna i arvsmassan hos patienter med svår och behandlingsresistent OCD som genomgått djup hjärnstimuleringsbehandling (DBS), en behandling där tunna elektroder kopplade till en stimulator opereras in i specifika områden i hjärnan. Vi inkluderade samtliga patienter i Sverige som genomfört detta ingrepp och som gett sitt tillstånd att delta i studien, totalt fem deltagare. Vi fann ovanliga och potentiellt genskadande genetiska variationer hos tre av dessa. Även om resultatet är lovande behövs det en större grupp deltagare för att vi ska kunna avgöra om frekvensen av ovanliga genetiska variationer är högre i denna patientgrupp, och om genetiska resultat skulle kunna vara en faktor av prediktivt värde för behandlingsutfall för DBS vid OCD.

Forskningen i den här avhandlingen understryker att patienter med OCD är en heterogen grupp och att det är svårt att identifiera enskilda biologiska faktorer som förklaringsmodell vid OCD. Däremot är genetisk forskning riktad mot svårt sjuka och behandlingsresistenta patienter med OCD lovande. Medicinsk forskning står inför en revolution när tillgängligheten ökar och kostnaderna minskar för nästa generations sekvenseringsteknik. Vi kan nu på en detaljerad nivå kartlägga tarmfloran och vårt DNA snabbt och träffsäkert. Detta öppnar upp nya möjligheter för att studera komplexa psykiatriska tillstånd.

ABSTRACT

Background: Obsessive-compulsive disorder (OCD) is characterized by intrusive thoughts and images, and repetitive, time-consuming compulsions. It causes disability and impaired quality of life. The neurobiological model of OCD revolves around dysfunctional brain circuits, referred to as the cortico-striato-thalamo-cortical (CSTC) loop.

Aim: This thesis sought to elucidate neurobiological factors associated with OCD.

Methods: In **study I**, we compared executive functions of patients with OCD (n=29), body dysmorphic disorder (BDD) (n=27), and healthy controls (n=28), using computerized and standardized neuropsychological tasks (CANTAB) for response inhibition (stop-signal task, SST), cognitive flexibility (intra-extra dimensional shift task, IED), and working memory (spatial working memory task, SWM). Correlation between task performance and symptom severity was assessed using linear regression models and effect sizes were measured with Pearson's *r*. In **study II**, we systematically reviewed gut microbiota studies of participants with psychiatric disorders, published up to February 2020, following the PRISMA guidelines and the pre-registered study-protocol at PROSPERO. In **study III**, we compared gut microbiome of patients with OCD (n=32) with healthy controls (n=32) using whole genome sequencing of stool samples. Moreover, we followed a subset of participants longitudinally to compare changes in gut microbiome within patients with OCD before and after exposure- and response prevention (ERP) therapy (n=15). In **study IV**, we used whole exome sequencing to explore presence of gene-disruptive rare variants (GDRVs) in patients with severe and treatment-resistant OCD (n=5) who have received deep brain stimulation (DBS).

Results: In **study I** we found no significant differences in test performance between individuals with OCD, BDD, and healthy controls. In **study II**, 69 studies were included in the systematic review. The majority of studies did not report any significant differences in gut microbiota α-diversity indices (44%), but different composition measured by β-diversity indices (67%) between patients with psychiatric disorders and healthy controls. However, the results were inconsistent regarding which genera are differentially abundant, except for some studies that reporter lower abundance of butyrate-producing microbes *Faecalibacterium prausnitzii* and *Roseburia species*. In **study III** we found no significant differences in α-diversity, β-diversity, or taxonomic dissimilarity at the species-level between patients with OCD and healthy controls, or within patients with OCD before and after ERP. Furthermore, functional analysis, based on gut-brain modules that are relevant for metabolizing neuroactive compounds, did not reveal any significant differences between patients with OCD and healthy controls, or within patients with

OCD before and after ERP. In **study IV**, we found three GDRVs, one of which was a missense variant in the ion transporter domain of KCNB1 (hg19 20-47991077-C-T). The patient with that missense variant was a responder to DBS treatment.

Conclusions:

Study I. Performance on neuropsychological tests suggest that patients with OCD have comparable executive functions with a related group of patients and healthy controls.

Study II and III. Despite the suggested importance of the microbiome-gut-brain axis in psychiatric disorders, evidence for altered gut microbiome in psychiatric disorders including OCD is inconclusive. Improved and consistent study design and methodology are crucial for future studies in this research field.

Study IV. Patients with severe and treatment-resistant OCD is a cohort fit for whole exome sequencing studies. Despite the small sample size, we found three GDRVs. More samples are required to evaluate if the frequency of GDRVs is higher in this cohort and if GDRVs could predict treatment outcome from DBS.

Patients with OCD represent a heterogeneous group, and individual biological factors cannot explain the pathophysiological underpinning of this disorder. Nevertheless, next-generation sequencing opens new opportunities to study complex psychiatric conditions. Genomic analysis of severe and treatment-resistant patients with OCD could increase our understanding of this complex disorder.

List of scientific papers

I. **Chen LL**, Flygare O, Wallert J, Enander J, Ivanov VZ, Rück C, Djurfeldt D. Executive functioning in body dysmorphic disorder and obsessive-compulsive disorder. CNS Spectr. 2021 Jul 27:1-18. doi: 10.1017/S1092852921000705.

II. **Chen LL***, Abbaspour A*, Mkoma GF, Bulik CM, Rück C, Djurfeldt D. Gut microbiota in psychiatric disorders: A systematic review. Psychosom Med. 2021 Sep 1;83(7):679-692. doi: 10.1097/PSY.0000000000000959. PMID: 34117156; PMCID: PMC8428865.
*Joint first authors

III. **Chen LL**, Abbaspour A, Aspvall K, Rück C, Bulik CM, Pascal D. (2023). Longitudinal study of gut microbiome in obsessive-compulsive disorder. (Submitted manuscript).

IV. **Chen LL**, Naesström M, Halvorsen M, Fytagoridis A, Mataix-Cols D, Rück C, Crowley J*, Pascal D*. Genomics of severe and treatment-resistant obsessive-compulsive disorder treated with deep brain stimulation: a preliminary investigation. medRxiv 2023.04.15.23288623; doi: https://doi.org/10.1101/2023.04.15.23288623
*These authors jointly supervised this work

CONTENT

List of abbreviations

ACC	Anterior cingulate cortex
ADHD	Attention deficit hyperactivity disorder
ARFID	Avoidance/restrictive food intake disorder
ALIC	Anterior limb of the internal capsule
ASD	Autism spectrum disorder
BDD	Body Dysmorphic Disorder
BNST	Bed nucleus of stria terminalis
CBT	Cognitive behavioral therapy
DBS	Deep brain stimulation
DSM	Diagnostic and statistical manual of mental disorders
ERP	Exposure and response prevention
GBM	Gut-brain modules
GDRV	Gene-disruptive rare variant
IED	Intra-extra dimensional set shifting
MADRS	Montgomery-Åsberg depression rating scale
MAF	Minor allele frequency
MGS	Metagenomic species
MINI	Mini international neuropsychiatric interview
NART-SWE	National adult reading test-estimated verbal IQ in Swedish
OCD	Obsessive-compulsive disorder
OCI-R	Obsessive-compulsive inventory revised
OFC	Orbitofrontal cortex
PANS	Pediatric acute onset neuropsychiatric syndrome
SCFA	Short-chain fatty acids
SCID	Structured clinical interview for DSM-5
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitors
SST	Stop-signal task

STN	Subthalamic nucleus
SWM	Spatial working memory
WES	Whole exome sequence
WGS	Whole genomse sequence
Y-BOCS	Yale Brown obsessive compulsive scale

1 INTRODUCTION

Without knowing it, several life events have steered me towards this thesis.

Every day after school I would visit my mother who worked as a Ph.D. student at Annika Dahlströms lab at Sahlgrenska University, located on “Medicinareberget”. I played with Styrofoam from confocal microscopy packages, and I stained mouse brains with immunohistochemistry (I did not know that word then). My first book in Swedish, “Alla vi barn i Bullerbyn”, was a gift by Annika and she taught me how to ski. Science integrated me into the Swedish society.

As an intern (AT-läkare) I had the opportunity to see and treat patients with different psychiatric conditions. It was clear that patients suffered from brain disorders, but we could not explain why they were ill or why our treatments were effective or not. For the first time I started reading scientific reports out of curiosity. I knew from that moment that I had found my specialty.

After my internship I started my residency in Psychiatry at Psykiatri Nordväst. Diana was one of the first psychiatrists I had the opportunity to meet. She introduced me to deep brain stimulation for patients with OCD. Finding a treatment that targeted a specific part of the brain for a psychiatric disorder gave me hope to understand the biological underpinning of OCD and I decided to begin my journey towards Ph.D on this subject.

Seven years later, I have had the privilege to meet, diagnose, and treat many patients with OCD. As a doctor, I witnessed how debilitating OCD could be. At the same time, new treatments are emerging which is promising for those who are still struggling. I hope that I can continue to provide excellent care for them at OCD-programmet. I also hope that more psychiatrists will take an interest in patients with OCD and related disorders, because working with them is the most rewarding thing in the world.

陈龙喆

Duvbo 2023-04-19

2 LITERATURE REVIEW

2.1 Overview

2.1.1 What is OCD?

Obsessions are intrusive and unwanted thoughts or images that evoke anxiety or discomfort (1). Common themes include fear of harming someone or causing a disaster, contamination, unacceptable thoughts about sexuality, religion, violence, and order/symmetry. Obsessions can temporarily be neutralized by repetitive, and ritualistic compulsions, such as checking, cleaning, mental rituals, or asking for reinsurance. Obsessive-compulsive disorder (OCD) is characterized by time-consuming obsessions and/or compulsion (>1 hour per day), causing clinically significant distress or impairment in occupational, social, or other important areas of functioning (2).

OCD can resemble symptoms in other psychiatric diagnosis such as excessive worry in generalized anxiety disorder, inflexible behavior in autism, and ritualized eating behavior in eating disorders such as anorexia nervosa or avoidant/restrictive food intake disorder (ARFID). In some instances, the symptoms overlap (3, 4). Hallmarks for OCD are ego-dystonic obsessions, meaning that the intrusive thoughts are inconsistent with one's belief, and compulsions that cause distress due to their repetitive and unwanted nature. Nevertheless, patients with OCD may suffer from other psychiatric disorders as well, with the most common co-morbidities being major depressive disorder, anxiety disorders, and neurodevelopmental disorders (5, 6).

2.1.2 What do patients with OCD and BDD have in common?

In DSM-5, OCD was moved to a separate chapter from anxiety disorders, grouped together with related compulsive disorders, body dysmorphic disorder (BDD), hoarding, trichotillomania, dermatillomania, and olfactory reference syndrome. In BDD the obsessions and compulsions are focused on perceived flaws in one's appearance. OCD and BDD share similar symptomatology, demographic characteristics, clinical course, and treatment guidelines (7-9). Comorbidity between these two disorders is also common (10).

2.1.3 How does OCD affect patients' lives?

The prevalence of OCD is estimated to 1,3% in the general population, more common in women (1,5%) than men (1%) (11). However, age-of-onset is usually earlier for men, which is associated with more severe symptoms (12). OCD is among the most

disabling psychiatric disorders (1). It decreases educational attainment and causes labor market marginalization (13, 14). Furthermore, patients with OCD often involve relatives in their obsessions and compulsions, causing them distress as well (15).

Quality of life is significantly impaired in patients with OCD (16, 17). The decrease in quality of life is strongly correlated with the severity of symptoms (17, 18). A large population-based Swedish study reported a 4- to 10-fold higher risk for suicide in OCD (19). In addition, patients with OCD have twice the risk of premature death possibly explained by increased risk for metabolic and cardiovascular complications (20, 21).

Still OCD is underdiagnosed, and the mean time of delay from onset of disease to treatment is around seven years (22). Untreated, OCD is a chronic condition with a modest chance of spontaneous recovery (23).

2.2 Neurobiology

2.2.1 What emotions are associated with OCD?

Doubt, disgust, and feelings of incompleteness are negative emotions associated with OCD (24-26). Although universal for humans, negative affect caused by obsessions are evoked disproportionally in situations in which most people do not feel distressed or anxious. These obsessions are maintained when patients engage in compulsions to temporarily relieve the distress.

2.2.2 What role does the cortico-striato-thalamo-cortical network play in OCD?

Converging evidence supports a dysregulated cortico-striato-thalamo-cortical loop (CSTC) in patients with OCD. Chronic hyperactivity of orbitofrontal-subcortical pathways in mice using optogenetics induces aberrant grooming, a proxy for compulsive behaviors (27). In patients with OCD, dysfunctional connectivity in fronto-striatal networks is evident as early as 8-12 years age (28). The activity in these brain regions normalizes after successful treatment with either psychotherapy or medications in patients with OCD (29-31). Furthermore, specific connections between the prefrontal cortex, the subthalamic nuclei (STN) and the thalamus are associated with treatment response from deep brain stimulation (DBS) (32).

The brain regions associated with the CSTC networks are orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), bed nucleus of stria terminalis (BNST), insula, and the basal ganglia that are interconnected in the anterior limb of the internal capsule (ALIC) and the thalamus (33) (Figure 1).

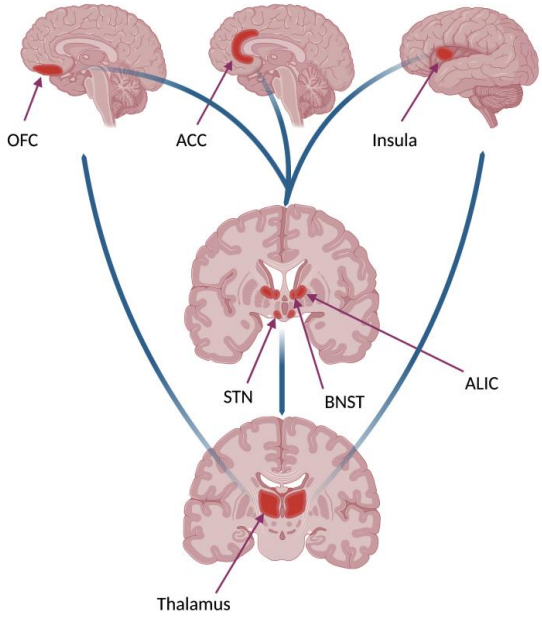


Figure 1. Schematic view of the brain regions involved in the cortico-striato-thalamo-cortical loop (CSTC). Neural impulses generated in the cortical regions of orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and insula, travel through the anterior limb of the internal capsule (ALIC) and terminate in the striatum and the subcortical regions which includes bed nucleus of stria terminalis (BNST) and the subthalamic nuclei (STN). The thalamus integrates the neuronal imputs that are relayed back to the cortical regions. Created with BioRender.com

OFC integrates cognitive and emotional information to guide goal-directed behavior (34, 35). ACC evaluates the reward- and punishment-value of a stimulus from the OFC to monitor the result of the outcome from an action (36). When the anticipated response conflicts with the outcome, an error signal is generated. Patients with OCD show deficits in action monitoring by decreased responsiveness to novel stimuli over time, which is thought to facilitate habit-formation (37). BNST receives input from the amygdala, indicating an important role for anxiety and threat regulation (38). Insula represents our body's interoceptive mirror, both physiologically and emotionally (39). In patients with OCD, insula is responsible for generating disgust when presented with OCD-stimuli (40). The basal ganglia have extensive connections with OFC and ACC, integrating various types of information to synchronize the execution of a decision. Electrophysiological recording of the STN in patients with OCD show that the neurons in the associative-limbic region activate during checking when subjects feel doubt, which suggest that doubt influences decision-making through modulation of neuronal activity in the STN (41).

In summary, the brain circuits associated with OCD could be responsible for exaggerated habit formation over goal-directed behavior when faced with negative emotions.

2.2.3 What are executive functions?

Executive functions are top-down mental processes that guide us away from automatic, instinctive, or habitual actions (42). Three core executive functions, inhibition, working memory, and cognitive flexibility, are fundamental for higher complex cognitive processes such as reasoning, problem solving and planning (43). These three core executive functions are separable and contribute differentially to performance on complex executive tasks (44).

Inhibition is key for all cognitive processes when you want to change decisions or deviate from habits or impulses. Working memory is required for holding and manipulating information for a limited period of time. Cognitive flexibility includes means of changing one's opinion or how one thinks of something, therefore crucial for problem solving. Importantly, the neuronal networks responsible for executive functions overlap with the CSTC loop implicated in OCD and the related diagnosis BDD (45-47).

2.2.4 How can executive functions be assessed with neuropsychological tests?

There is a wide array of neuropsychological tests for executive functions, including object alternation task, Stroop color-word test, Wisconsin card sorting test, Trail-making test, to mention a few (48). However, heterogeneity in how tests are administered, and the way underlying constructs have been operationalized have been different across studies. From a validity and reliability standpoint, automated computerized tests, such as Cambridge Neuropsychological Test Automated Battery (CANTAB), are preferable over the ones using paper and pencil (49).

Studies have shown that patients with OCD and BDD perform worse than control groups on neuropsychological tests that assess specific executive functions, stop-signal test (SST) for inhibition, spatial working memory (SWM) for working memory, and intra- and extra-dimensions set shifting (IED) for cognitive flexibility (50-53). However, direct comparisons of executive functions between patients with OCD and BDD has been limited (54, 55). Given the phenomenological similarities between the two related disorders, assessing performance on standardized and normed neuropsychological tests could differentiate the pathophysiology behind the separate conditions OCD and BDD.

2.3 Omics

2.3.1 How heritable is OCD?

Lifetime prevalence of OCD among first-degree relatives is estimated between 10% and 20% in most family studies of OCD, compared with 1-3% in the general population (56). Moreover, the risk for first-degree relatives is significantly higher than that for second- and third-degree relatives, indicating that the relative risk increases proportionally to the degree of genetic relatedness (57). Some studies suggest that the prevalence of OCD is higher in relatives of probands with early onset before adulthood (58-60). Thus, converging evidence supports the fact that OCD clusters in families.

Twin- and family studies have demonstrated that heritability of OCD is estimated to be in the range of 35-50% (57, 61). In a childhood onset sample, the heritability was even higher, 45-61% (62). Furthermore, Mataix-Cols et al., showed that familial risk for OCD was mostly attributed to additive genetic factors, with no significant effect of shared environment (57).

2.3.2 How is minor allele frequency used to determine common from rare genetic variants?

Minor allele frequency (MAF) is the proportion of alleles positive for the variant divided by the total number of alleles screened, in other words, the frequency at which the least common allele occurs in each population. MAF is used to separate common variants (MAF > 0.05), from low frequency variants (MAF 0.01 - 0.05), and rare variants (MAF < 0.01) (63).

Single nucleotide polymorphism (SNP) is a change in a single nucleotide at a specific location in the genome which is present in more than 1% of the population. Estimated 4-5 million SNPs are present in each genome compared with the reference genome (64). SNPs are common genetic variants (MAF > 0.05), and can be associated with a condition through genome-wide association studies (GWAS). Genetic variations identified by GWAS typically have small effect sizes, thus limited role by themselves (65). Psychiatric disorders are highly polygenic, as a result, the effects of a large number of SNPs converge to increase risk for that psychiatric disorder, also called polygenic risk (66).

Rare variants that have a functional impact on the protein expression, through missense, nonsense, frameshift, or truncation of the gene, can be called gene-disruptive rare variants (GDRVs). Studies have shown that the genetic variation of phenotypic consequence is likely to be rare in the human population, because these rare variants are subject to natural selection (67). This is explained by the high

frequency of rare variants that occur spontaneously (*de novo),* identified by sequencing parents and proband together (trios) (68).

2.3.3 Which common and rare genetic variants are associated with OCD?

The largest GWAS study on OCD to date found a significant SNP (rs2581789) on chromosome 3p21.1 (69). It is in a gene-rich region that has previously been associated with other psychiatric disorders including schizophrenia and anxiety disorders, and with psychological traits such as worry (70, 71). Based on experience from GWAS studies in other psychiatric disorders, more significant SNPs are expected to be identified when the number of cases in GWAS studies increase (72). GWAS studies across psychiatric disorders reveal a significantly shared common genetic risk between OCD with anorexia nervosa and Tourette's syndrome (73). Despite phenomenological similarities, OCD and autism spectrum disorder (ASD) are not genetically correlated regarding common variants (74, 75).

Although OCD is predominantly inherited by common variants, rare single nucleotide variants (SNVs) and insertions/deletions (indels) also contribute to the heritability of OCD (76). Whole exome studies on patients with OCD suggest that *de novo* variants are clustered in genes previously associated with neurodevelopmental disorders (77). The most excessive damaging variant in cases compared with healthy controls is SLITRK5, a gene that regulates synapse formation (78). Another identified risk gene is CHD8, a transcriptional regulator of neuronal development (78, 79). In addition, the prevalence of *de novo* rare copy number variation (CNV) has been estimated to between 1,4% and 2,3% in a cohort with OCD trios (80, 81). Again, neurodevelopmental genes, responsible for neuronal migration (ASTN2), synapse formation (PTPRD), and postsynaptic scaffolding (DLGAP1 and DLGAP2), were detected (81, 82). This shows that rare damaging variants associated with OCD are heterogeneous.

In summary, genetic heritability of OCD is polygenic, with contributions from both common and rare variants (83).

2.3.4 What is the microbiome-gut-brain axis?

The human microbes comprises about 1-3 % of our body mass (84). It is estimated that there are as many bacteria as the total number of human cells solely in the gut (85). Gut microbes have significant importance for nutrient metabolism, production of vitamins and prevention of pathogens from colonizing our intestine (86). Recently, microbiota in the gut has gained increasing interest due the extensive connection between the gut and the brain (87). The bidirectional communication through neural, hormonal and immunological signaling mediated by microbial metabolites and enzymatic processes in the gut forms the microbiome-gut-brain axis (88).

Mouse models have shown that brain function and behavior can be regulated by gut microbial composition. For example, mice isolated from exposure to microorganisms (germ-free mice) exhibit increased social avoidance and grooming, which can be reversed through colonization of gut bacteria from healthy mice (89). Transplantation of microbes from a mouse strain with a certain exploratory behavior can lead to the same exploratory behavior in the mice that have been transplanted (90). A recent article by Kelly et al showed that perturbed microbial signature from depressed patients transplanted to microbial-depleted mice induced anhedonia and anxiety like behavior (91). Nevertheless, results from animal studies on gut-microbiome are not automatically transferable to humans (92).

In humans, there are inconsistent results regarding differences in gut microbiota between patients with psychiatric disorders and healthy controls (93). Lately, systematic reviews and meta-analysis have found lower abundance of butyrate-producing genera in patients with psychiatric disorders (94, 95). However, lower abundance of specific microbes cannot directly be translated to changes in functional profiles of the gut microbiome. Therefore, Valles-Colomer et al., applied a module-based framework through assembling a catalogue of gut microbial metagenome with neuroactive potential (96). Using the gut-brain modules (GBM) they showed that the microbial synthesis potential of dopamine metabolite 3,4 -dihydroxyphenylacetic acid was positively correlated with mental quality of life in patients with depression. However, causal relationship between gut microbiota and psychiatric conditions is difficult to establish due to few longitudinal or interventional studies (97).

2.3.5 What implication could short-chain fatty acids (SCFAs) have in psychiatric disorders?

SCFAs are fatty acids with fewer than six carbon atoms that are mainly produced through fermentation of non-digestible food by gut microbes, such as dietary fiber. Butyrate is one of the most abundant SCFAs. It is a crucial energy source for colonocytes, maintains the barrier function of the gut, and has anti-inflammatory effects (98).

In mice, oral administration of sodium butyrate was associated with anti-depressive effects in forced swim and tail suspension tests (99). Additionally, supplementing sodium butyrate can attenuate social deficits and decrease repetitive behavior in mouse models (100). It has been suggested that butyrate could influence brain monoaminergic pathways by inhibition of the enzyme histone deacetylase, thus affecting gene-expression (101).

2.3.6 What are the associations between the microbiome-gut-brain axis and OCD?

Lately, there have been numerous hypotheses regarding the potential association between gut microbiome and OCD symptoms (102, 103). In mice, 5-HT 1A/1B

antagonist induced OCD-like behavior, which could be alleviated with probiotic treatment consisting of *Lactobacillus rhamnosus*, an effect comparable to fluoxetine treatment (104).

In humans, caesarean section is associated with perturbed gut microbiota in the newborn (105). Cesarean section is also a risk factor for OCD (106). However, it is unclear if method of delivery and OCD is mediated by disturbed gut microbiota in the newborn (107, 108). Patients with washing compulsions limit their exposure to the microbial environment we typically encounter every day. Moreover, other behaviors associated with OCD may also impact the diet, such as picky eating due to fear of contamination, and in extension the gut microbiome (109).

Autoimmune diseases are more prevalent among patients with OCD than in the general population (110). One form of obsessive-compulsive symptoms with acute onset is associated with infections, particularly group A streptococcus (111). Pediatric acute onset neuropsychiatric syndrome (PANS) is a syndrome where cross-reactive antibodies cause an autoimmune process resulting in a broad spectrum of psychiatric symptoms (112). Gut microbes play an essential role in training our immune system by presenting self and non-self-antigens and potentially harmful pathogens (113, 114). Consequently, a healthy gut microbiota during childhood might be protective for autoimmune diseases and OCD.

Patients with PANS between the ages of 4-8 have altered gut microbiota in comparison with healthy controls, most notably an increase in the phylum Bacteroidetes (115). A limitation in the study was that many patients with PANS took antibiotics. A recent study on gut microbiota in OCD found lower abundance of three butyrate-producing genera in patients with OCD, compared with healthy controls (116). Furthermore, oropharyngeal microbiota in patients with OCD showed a lower Fusobacteria to Actinobacteria ratio compared with healthy controls (117).

2.4 Treatment

2.4.1 What evidence-based treatments are there for OCD?

According to the NICE guidelines (https://www.nice.org.uk/guidance), first-line treatment for OCD in a stepped care model is cognitive behavioral therapy (CBT) that applies exposure- and response prevention (ERP) (118). In ERP, patients are encouraged to gradually expose themselves to obsessions, while at the same time refrain from relieving the distress by performing compulsions (119). The therapy typically includes psychoeducation, goal setting, planning and practicing ERP, relapse prevention and, most importantly, perform ERP as homework between the

sessions (120). The effect of ERP can be attributed to decreased level of anxiety through habituation, and increased tolerance for fear or anxiety (121).

Twenty-five randomized controlled studies have shown large effect sizes of ERP compared with waitlist or placebo (122). The long-term effect of ERP is long-standing (123). In addition, ERP is an effective treatment irrespective of mode of delivery. Several studies on both intensive ERP for four days and internet delivered ERP have shown efficacy, in parity with traditional ERP which consists of face-to-face psychologist-led therapy for 14 weekly 1-hour sessions (124-127).

Pharmacotherapy is effective for patients with OCD, alone or in combination with ERP. Selective serotonin reuptake inhibitors (SSRI) is the first-line option when both efficacy and side effects are weighted in (128). Gradually increasing the dosage to the maximal tolerable dose is an adequate strategy since high dosage is more effective in alleviating OCD symptoms (129). Augmentation with antipsychotic agents is efficacious in patients with OCD that do not respond to SSRI (130). Third line option is clomipramine, a non-selective serotonin reuptake inhibitor (131). If clomipramine is considered, starting with intravenous clomipramine is advantageous as it provides faster effect compared with increasing oral dosages gradually (132, 133).

Furthermore, it is essential to avoid on-demand medications such as benzodiazepines, particularly as these medications interfere with psychotherapy (134).

2.4.2 How many patients with OCD benefit from these evidence-based treatment?

Approximately 70% of patients with OCD improve after ERP therapy, but the treatment is demanding and only 38% of all patients who start ERP adhere to the full treatment (135). Patients with certain comorbidity, such as ASD, have poorer outcome and may require adapted ERP and increased number of sessions (136).

For SSRIs the number needed to treat is one in five, and approximately 60% rate themselves as improved after taking recommended doses of SSRIs. However, a five-year follow up revealed that only 16,9% of patients with OCD are in full remission and 59% of the patients who remitted subsequently relapsed (137, 138). Despite add on treatment with antipsychotic augmentation and clomipramine, approximately 10% of patients with OCD are treatment resistant and experience severe symptoms (23, 139).

Early onset and long duration of illness are negative predictors of long-term outcome in OCD (22, 135, 140). Therefore, it is important to provide adequate diagnostics and evidence-based treatment without delay (141, 142). As the availability of therapists experienced in ERP is scarce in Sweden, complementary internet-based CBT can shorten the waiting-list for therapy (143). However, at the individual level, there are

currently no reliable predictors for treatment outcome for either psychotherapy or pharmacotherapy (144-146).

2.4.3 What is the definition for severe and treatment-resistant OCD?

Severity of OCD symptoms is assessed with Yale Brown obsessive compulsive scale (Y-BOCS) and a score above 30 out of 40 points is considered severe OCD (147, 148). Treatment resistance is defined as patients with OCD who do not respond to adequate trial of ERP, trials with two different SSRIs, augmentation with at least one antipsychotic agent, clomipramine at therapeutic dose based on the serum concentration of clomipramine, and illness duration more than five years (1, 149).

2.4.4 How effective is deep brain stimulation for patients with severe and treatment-resistant OCD?

Since 1999, DBS in OCD has been studied as a treatment alternative for a sub-group of treatment-resistant patients (150). In USA, the US Food and Drug Administration approved DBS therapy for OCD in 2009 under a Humanitarian Device Exemption for patients with chronic, severe and treatment-resistant OCD, as an alternative to capsulotomy (151). In Europe, DBS for patients with OCD has received the CE-mark and is used in clinical practice in the Netherlands.

Stimulation in several different areas have proven effective in case-control studies and RCTs, with bilateral stimulation of the anterior limb of the internal capsule (ALIC) and anterior region of STN being the most studied targets (152-154). Sixty percent of the patients with severe and treatment-resistant OCD responded to DBS treatment, defined as a >35 % decrease in Y-BOCS score (155). Furthermore, patients with OCD that received DBS continued to improve from CBT treatment. A meta-analysis of treatment response from DBS in patients with OCD found that early age-at-onset was a negative predictor of treatment response (155).

2.4.5 How safe is deep brain stimulation for OCD?

The largest prospective multi-center study on bilateral DBS in ALIC showed that the predominant adverse events were related to programming/stimulation, transient, and could be resolved by adjusting stimulation parameters (156). The most aggravating and frequent psychiatric side effect was hypomania, which was related to a rapid increase in stimulation voltage (157). Serious adverse events, including hemorrhage and infection related to implants was similar to those reported for DBS in movement disorders. Risk for intracerebral hemorrhage was approximately 2.2%, but clinically permanent neurological complications caused by intracerebral hemorrhage was only at 1% (158).

2.5 Knowledge gap

OCD and BDD are closely related disorders. Direct comparison in executive functions between OCD and BDD patients are scarce. Do these groups of patients perform comparably on executive functions?

Despite the increasing number of studies conducted over the past several years aiming to characterize differences in gut microbiota between patients with psychiatric disorders and healthy individuals, the results are inconsistent. Could we comprehensively summarize these results (159-161) and provide practical guidelines for improving the quality of future gut microbiota studies, thus increasing the comparability?

Omics research is currently undergoing rapid transformation. The cost for sequencing has dropped radically (162). For genomic and microbiome research, it is now economically justifiable to choose whole genome or exome sequencing instead of micro arrays or 16S rRNA sequencing. With better resolution we can also answer more complex questions. For example, does gut microbiome functionally differ between OCD cases and healthy controls? Do patients with severe and treatment resistant OCD carry gene-disruptive rare genetic variants?

3 RESEARCH AIMS

3.1 The overall aim of this thesis is to investigate the neurobiological underpinning of OCD

3.1.1 Study I. Compare executive functions between patients with OCD and BDD

The main hypothesis is that patients with OCD and BDD show similar impairment in performance on neuropsychological tests for executive functions in comparison to healthy controls. Secondary hypothesis is that performance on neuropsychological tests correlate with symptom severity.

3.1.2 Study II. Systematic review of gut microbiota in psychiatric disorders

The main aim is to review existing literature on differences in gut microbiota between patients with a major psychiatric disorder in DSM-5 and control groups. The secondary aim is to evaluate the quality of the studies included in the systematic review, and provide recommendations for how to improve study design and methodology in this research field.

3.1.3 Study III. Examine the gut microbiome of patients with OCD before and after ERP

The main hypothesis is that patients with OCD have different composition and functional profile of the gut microbiome compared with healthy controls. Furthermore, we aim to evaluate changes in gut microbiome before and after ERP-treatment.

3.1.4 Study IV. Whole exome sequencing of patients with severe and treatment-resistant OCD who have received DBS

The main hypothesis is that patients with severe and treatment-resistant OCD carry GDRVs. We also aim to investigate whether GDRVs could predict treatment response from DBS.

4 MATERIALS AND METHODS

4.1 Study design and protocol

Summary of study design for **study I-IV** are presented in Table 1.

Table 1. An overview of study design

Study	I	II	III	IV
Title	Executive functioning in body dysmorphic disorder and obsessive-compulsive disorder	Gut microbiota in psychiatric disorders: a systematic review	Longitudinal study of gut microbiome in obsessive-compulsive disorder	Genomics of severe and treatment-resistant obsessive-compulsive disorder treated with deep brain stimulation: a preliminary investigation
Study design category	Case-control	Systematic review	Case-control and cohort	Case series
Subjects	OCD (n=29), BDD (n=26), HC (n=28)	69 studies with 2880 participants	OCD pre-ERP (n=32), OCD post-ERP (n=15), HC (n=32)	OCD (n=5)
Data source	Specialist OCD clinics in Stockholm, clinical trials (163, 164) and online advertisement for HC	Medline, Embase, Cochrane, Web of science, Psychinfo	Specialist OCD clinics in Stockholm and online advertisement through Accindi.com for HC	Specialist OCD clinics in Stockholm and Umeå
Ethics (D.nr.)	2013/1773-31/4, 2014/1426-32, 2015/1088-32	_	2017/1711-31/1	2014/1897-31
Pre-registration of study design	_	PROSPERO CRD42019132642	ClinicalTrials.gov NCT03638791	_
Data sharing	_		SRA acc.nr PRJNA883179	_

Abbreviations: Obsessive-compulsive disorder (OCD), body dysmorphic disorder (BDD). Healthy controls (HC).

4.2 Methodology involving study participants

The inclusion and exclusion criteria for cases and controls respectively, are shown in Table 2. Beside main diagnosis of OCD for cases, and no psychiatric diagnosis for healthy controls, the criteria are adapted to suit the aims of each study.

All study participants in **study I, III,** and **IV**, underwent structured psychiatric interview with the mini international neuropsychiatric interview (MINI) and the structured clinical interview for DSM-5 (SCID). SCID was primarily used for diagnostics of obsessive-compulsive related disorders. During structured psychiatric interview eight healthy controls turned out to suffer from current psychiatric disorders in **study III**, and were subsequently excluded from participation.

In **study III**, ERP-treatment was administered as either face-to-face, or therapist-guided and internet-delivered. Adjustment in the study protocol was necessary due to the Covid-19 pandemic. A minimum of five ERP sessions or five completed modules on the internet-based psychotherapy were required for it to be considered a completed ERP-treatment.

In **study IV**, five patients with severe and treatment-resistant OCD received DBS in BNST (156, 165).

Table 2. An overview of study methodology of participants

Study	I	II	III	IV
Inclusion criteria for cases	Main diagnosis of OCD or BDD (DSM-IV)	Original observational studies with a control group	Main diagnosis of OCD (DSM-5)	Y-BOCS > 30 p, >5 years duration and treatment-resistant OCD with DBS
Exclusion criteria for cases	Medication changes <2 weeks prior to inclusion, CBT within 12 months, comorbid OCD or BDD, substance dependence or abuse, psychotic-, bipolar- or severe personality disorder, acute suicidal ideation	Animal studies, studies without a diagnosed psychiatric disorder, interventional studies, and studies not using high-throughput sequencing techniques	History of GI tract surgery, inflammatory bowel disease, irritable bowel syndrome, chronic or recurring bowel symptoms; antibiotic use < 3 months, probiotic use < 4 weeks, pregnancy, intellectual disability, substance abuse, autism spectrum disorder or psychotic disorders	_
Exclusion criteria for controls	Previous or present psychiatric disorder, psychotropic medication, or chronic somatic disease	_	Current psychiatric symptoms in addition to the exclusion criteria for cases	_
Diagnostics	Structured clinical interview	Mixed	Structured clinical interview	Structured clinical interview and clinical assessments
Intervention	_	_	ERP	DBS in BNST

Abbreviations: Diagnostic and statistical manual of mental disorders (DSM), Yale-Brown Obsessive-Compulsive Inventory Scale (Y-BOCS), deep brain stimulation (DBS), bed nucleus of stria terminalis (BNST), cognitive behavioral therapy (CBT), obsessive-compulsive disorder (OCD), body dysmorphic disorder (BDD), gastrointestinal (GI), exposure and response-prevention therapy (ERP).

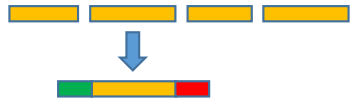
4.3 Sample collection, sequencing and bioinformatic analysis

In **study III**, study participants left stool samples at home in OMNI-Gut kits which contain solution for DNA stabilization, making it suitable for storage at room temperature. In **study IV**, participants left saliva samples in kits from Oragene. All biological samples were stored at Karolinska Institutet biobank at -80°C.

In **study III**, seventy-nine stool samples were sequenced at Clinical Microbiomics, Denmark, on an Illumina NovaSeq 6000 (Figure 2). Quality control of the DNA after extraction was carried out using Qubit 2.0 fluorometer quantitation. In **study IV**, five saliva samples were prepared and sequenced by the SNP&SEQ Technology Platform, hosted by Science for Life Laboratory in Uppsala, Sweden. Sequencing was carried out on an Illumina NovaSeq 6000.

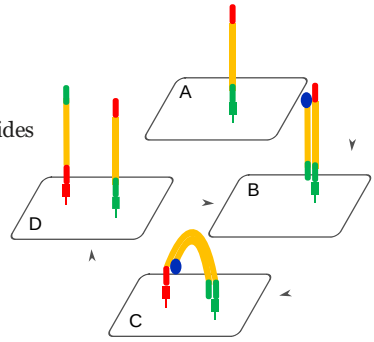
Sample preparation

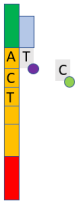
DNA is isolated, fragmented, and ligated with oligonucleotides that contain sequencing binding site and complementary sequence for hybridization to the flow cell.



DNA amplification by polymerase chain reaction (PCR)

DNA sequences are attached to the flow cell (A). The oligonucleotides are amplified, denatured, and washed away (B). Amplification through bridge building is repeated (C). Reverse or forward strand is denatured and washed away (D).





Sequencing by synthesis

Primers bind to the oligonucleotides. Sequencing is done with fluorescent-labeled nucleotides that are excited by laser, whereby the fluorescent signal is detected.

Bioinformatic analysis

The reads are overlayed and aligned.

ACTAAC
TAACGT
CGTGTAA
GTAAACA
ACTAACGTGTAAACA

Figure 2. Illustration of Illumina NovaSeq 6000 workflow.

Bioinformatic workflow in **study III** is summarized in the flowchart below (Figure 3).

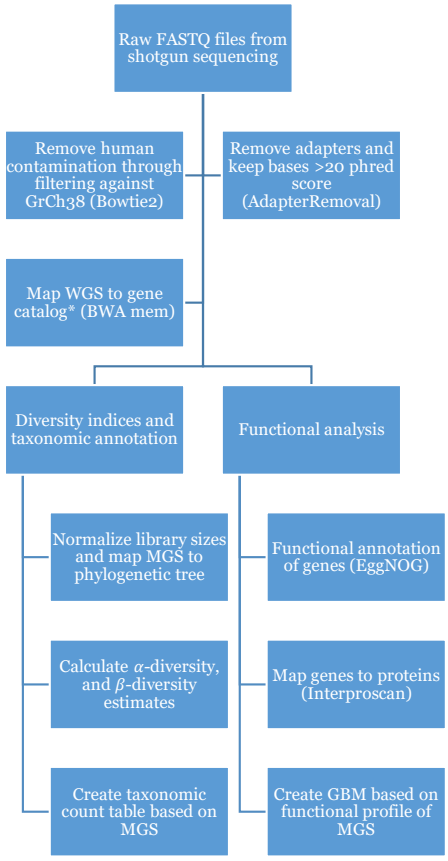


Figure 3. Bioinformatic workflow in study III. * Clinical Microbiomics in-house Human Gut microbiome gene catalog (HG04). Abbreviations: Whole genome sequence (WGS), metagenomic species (MGS), gut-brain modules (GBM).

High quality non-host reads were acquired from raw FASTQ files and mapped to the Clinical Microbiomics in-house Human Gut microbiome gene catalog (HG04). HG04 is a gene catalog based on human gut metagenomes from Clinical Microbiomics (12,170), publicly available data (9,428) (166), and genome assemblies from isolated microbial strains (3,567). It comes with the corresponding Clinical Microbiomics HGMGS version HG4.D.2 set of 2095 metagenomic species (MGS). To create the MGSs, they used a method where genes based on median abundance profile from a data-set were co-binned in co-abundance gene groups (CAGs), and CAGs with >700 genes were assigned as metagenomic species (MGS) (167).

An MGS is considered detected if reads from a sample uniquely mapped to at least three of its signature genes (specificity 99.6 %). Based on the MGS identified from the stool samples, counts tables were created for taxonomic annotation at the phylum, family, genus, and species-level. To account for differences in sequencing depth, normalization across samples was performed. A phylogenic species tree was created using the GTDB-toolkit based on single-copy bacterial and archeal marker genes from the Genome Taxonomy Database (GTDB) (168).

α-diversity indices refer to the species diversity in a site. It can be measured by richness, evenness, and phylogenetic diversity. β-diversity indices reflect dissimilarity of the microbiota between groups or sites. α-diversity and β-diversity estimates were calculated from rarefied abundance matrices and based on the phylogenetic tree when required (Faith's phylogenetic diversity and β-diversity indices).

The gut-brain modules (GBMs) are a set of 56 microbial pathways for metabolizing neuroactive compounds (molecules that have the potential to interact with the human nervous system). Each GBM corresponds to a single neuroactive compound synthesis or degradation process by members of the gut microbiota and is defined as a series of enzymatic steps represented by orthologue group identifiers (KEGG, TIGRFAM, and eggNOG version 3.0 orthology databases in order of preference) (96).

Bioinformatic workflow in **study IV** is summarized in Figure 4.

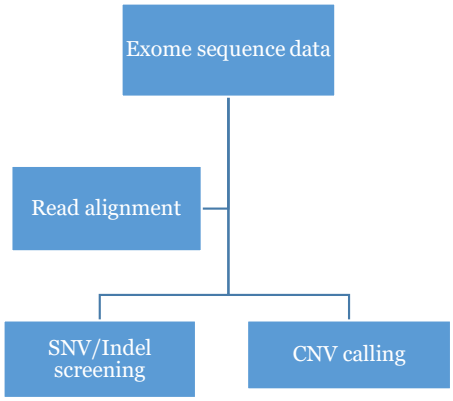


Figure 4. Overview of bioinformatic processes for study IV. Abbreviations: Single nucleotide variant (SNV), copy number variant (CNV).

First, exome sequencing data were aligned to the human reference genome hs37d5 using BWA v7.17. The read duplicates were removed with Picard v2.2.4, before indexing with samtools v1.8 (169). We next conducted indel realignment on sample-level data in GATK v3.7. As a final step in read alignment, we subjected sample-level alignments to base recalibration. Next, we assessed quality control metrics for the sequencing data. Sequences that passed quality metrics were retained.

For screening of single nucleotide variants (SNV)/indels and single variant (SV) calls, we required variants to be < 0.1% MAF in all gnomAD WES and WGS subpopulations. All copy number variant (CNV) calls were made using XHMM 1.0. Calling closely followed XHMM best practices (170). First, depth of sequencing coverage was calculated and then filtered to remove any samples and targets with outlier read depth values. Second, the exome data was normalized before discovery of CNV. Structural variants screened included deletions and duplications. We specifically focused on CNVs found in less than 1% of the population.

We defined a variant as 'gene-disruptive' if it met one of the following criteria (171, 172):

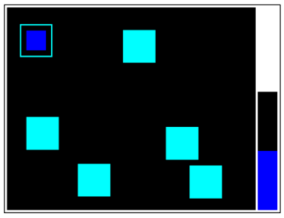
1) SNV or indel that is protein-truncating (stop-gain, splice donor/acceptor disrupting, or frameshift annotation) within a protein-coding gene.

2) Missense SNV with Missense badness, PolyPhen-2, and Constraint (MPC) > 3.

3) CNV deletion or duplication impacting at least one protein-coding base (gnomAD v2.1.1 pLI > 0.9).

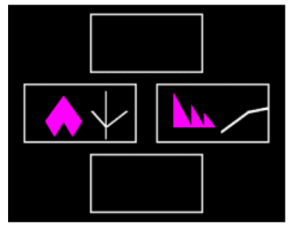
4.4 Outcome measures

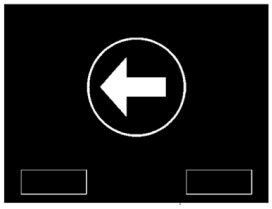
The neuropsychological tasks used in **study I** were spatial working memory (SWM), intra-extra dimensional set shifting (IED), and stop-signal task (SST) (Figure 5).



SWM: Participants search for blue tokens in the boxes. They are instructed not to return to a box where a token has previously been found. Between-search errors is the primary outcome. The strategy adopted is also assessed.

IED: Participants use feedback to learn a rule, and change that rule when feedback implies that the rule has changed. The rules involve two dimensions, pink shapes and white lines. At the critical extra-dimension shift (EDS) stage, participants must shift attention to the previously irrelevant dimension. Number of errors in the extra-dimensional shift is the main outcome.





SST: Participant press the button corresponding to the direction of the arrow (go trial). When they hear a tone, they must inhibit their response (no go-trial). Stop-signal reaction time is the mean time in milliseconds taken by the participant to suppress a prepotent response.

Figure 5. Cambridge neuropsychological test automated battery (CANTAB) for executive functions.

The clinical assessments used in **study I, III, and IV** are summaries for each study in Table 3.

Table 3. An overview of clinical assessments

Study	**I**	**III**	**IV**
Y-BOCS	✓	✓	✓
MADRS	✓		✓
BDD Y-BOCS	✓		
NART-SWE	✓		
OCI-R		✓	
EDE-Q		✓	
MMQ		✓	
Bristol stool scale		✓	
CGI-S			✓
CGI-I		✓	
EQ-5D-VAS			✓

Abbreviations: Yale-Brown Obsessive-Compulsive Inventory Scale (Y-BOCS), Montgomery-Åsberg depression rating scale (MADRS), Yale-Brown Obsessive-Compulsive Inventory Scale for Body Dysmorphic Disorder (BDD Y-BOCS), National Adult Reading Test-estimated verbal IQ in Swedish (NART-SWE), Obsessive-Compulsive Inventory-Revised (OCI-R), Eating Disorder Examination Revised (EDE-Q), MiniMeal Q questionnaire (MMQ), Clinical Global Impression Scale – Severity (CGI-S), Clinical Global Impression Scale – Improvement (CGI-I), Euro-Qol 5-dimensions Visual Rating Scale (EQ5-D-VAS).

In **study II**, articles included in the systematic review underwent structured evaluation. Two independent reviewers separately assessed the quality based on a modified Newcastle-Ottowa Scale for microbiome studies (MORS) (Table 4) (173). Primary outcome measures were α-diversity and β-diversity indices between cases and controls. Furthermore, we summarized the replicated dissimilarities at the genus-level in a heat-map divided by psychiatric disorders and weighted by sample size of each study. The same ecological outcome measures were used in **study III**, in addition to comparison of microbial dissimilarity at the species-level, and functional profiles.

Table 4. Criteria for modified Newcastle-Ottowa scale

Selection	Comparability	Sequencing and bioinformatic analysis
Diagnosis using DSM or ICD *	Control for age *	Samples stored -80°C without multiple freeze-thaw cycles *
Report comorbidities and use disorder specific assessment of symptom severity *	Control for ≥4 variables (BMI, sex, ethnicity, medication, antibiotic usage, pro-/synbiotics usage, diet, physical activity) *	Report of extraction kit used, amplification method and primers/target region *
Community controls *		Report sequencing platform *
Exclude controls with the specific psychiatric disorder *		i) Report of software package ii) description of quality-filtering iii) description of assignment of taxonomy ** (2/3 gives*)
		Accessible raw data *

A maximum of 12 stars can be given to an individual study. Abbreviations: Diagnostic and statistical manual of mental disorders (DSM), international classification of disease (ICD), body mass index (BMI).

4.5 Statistical analysis

The statistical analysis in **study I, II** and **III** were done in R (version 4.0) and STATA version 15 (StataCorp, College Station, TX). No statistical analysis was conducted in **study IV**.

Less than 10% of the data-set in **study I** and **III** were missing and were assumed to be missing at random. Therefore, we applied random forest imputation. The statistical tests used in this thesis are presented in Table 5.

Table 5. An overview of statistical methods

Study	**I**	**II**	**III**
Mean (SD)	✓	✓	✓
Number (n) and percent (%)	✓	✓	✓
Differences between groups			
Independent samples t-test			✓
Chi-square test	✓		✓
Wilcoxon rank sum test			✓
ANOVA	✓		
PERMANOVA			✓
Linear regression	✓		✓
Calculation of effect sizes (Cohen's *d*)	✓		
Analysis of relationship			
Linear regression	✓		
Spearman's rank correlation		✓	
Calculation of effect sizes (Pearson's *r*)	✓		
Analysis of change within group			
Paired t-test			✓

Abbreviations: Analysis of variance (ANOVA), permutational analysis of variance (PERMANOVA).

Descriptive statistics were used for clinical characteristics and demographic data in **study I-III**.

In **study I**, we used analysis of variance (ANOVA) for continuous variables and chi-square for ordinal variables to test for differences between OCD, BDD and healthy control groups. Linear regression, adjusted for age, sex, intelligence, and depressive symptoms, were used for testing outcome from neuropsychological tasks. Linear regression was used for correlation between symptom severity and neuropsychological task performance. Effect sizes were estimated based on the linear regression-models. Significance threshold was set to $p<0.05$.

In **study II**, Spearman's rank correlation was used to examine the relationship between study quality and publication date.

In **study III**, we used t-test for continuous variables and chi-square test for ordinal variables on demographic and clinical data. Significance threshold was set to $p<0.05$. Linear regression adjusted for body mass index (BMI) was used for testing differences in micro- and macro-nutrient intake between patients with OCD and healthy controls. For testing differences in α-diversity indices between cases and controls we used non-parametric Wilcoxon rank sum test, and for β-diversity indices we used permutational analysis of variance (PERMANOVA). Differential abundance at the species-level was estimated with Dirichlet-multinomial model using a two-sample t-test (174). Lastly, linear regression models were applied on functional profiles based on GBM. Benjamin-Hochberg method was used to control the false discovery rate (FDR). Adjusted p-values were set to a significance threshold of $q<0.05$.

4.6 Ethical considerations

All studies included in this thesis have ethical permission, except for **study II** which is a systematic literature review. We ensured that all potential study participants went through structured diagnostic interview with experienced psychiatrists, both to make sure that they are eligible for the study and to rule out more urgent mental health conditions in need of treatment. Moreover, all participants in **study I, III, IV** gave their written informed consent prior to inclusion.

Performing neuropsychological tests, or providing biological samples, especially stool samples if the subjects are afraid of contamination due to OCD, can be very distressful. Therefore, patients eligible for the studies received both verbal and written information that participation is optional and that their decision would not affect the treatment they received. Moreover, all study participants were informed of their right to withdraw at any time without any explanation.

Personal integrity is another important ethical aspect. For all study participants, the data were pseudonymized, and national identity numbers and names were replaced

by study ID. In **study I** and **III**, all data were analyzed at group level, which further improved the anonymity of the study participants. To protect privacy in **study IV,** we removed personal information, such as age, sex, and educational attainment, through cell suppression. For data safety purposes, all sensitive data were kept at secure servers that require two-step authentication.

A major ethical dilemma revolves around if and how genetic information should be reported back to study participants. This is relevant for **study IV**. Should we provide participants information on unsolicited findings of clinical significance? How about variants of unknown significance? Genetic counseling has become increasingly difficult in the era of next generation sequencing due to the massive amount of data generated. We have decided to follow the rule "right not to know", considering the uncertainty of causality between genetic factors and OCD (175).

5 RESULTS

5.1 Summary of results

Study I

There were no significant differences in executive functions between patients with OCD, BDD, and healthy controls.

Study II

Lower abundance of butyrate-producing microbes, *Faecalibacterium prausnitzii* and *Roseburia species*, in patients with major psychiatric disorders was the most replicated finding. However, disparate methodology and study-quality hindered comparability between the studies.

Study III

There were no significant differences at the species-level or functional profiles of gut microbiome in patients with OCD compared with healthy controls. Furthermore, gut microbiome composition and functional profiles did not change after ERP treatment.

Study IV

Three GDRVs were identified from five cases with severe and treatment-resistant OCD using whole exome sequencing. One missense variant in the ion transporter domain of *KCNB1* was found.

5.2 Participants' characteristics with OCD in study I, III, and IV

Comparison of patients with OCD in **study I, III,** and **IV** is summarized in Table 6. There were no significant differences in the co-variates age, sex, or educational attainment between cases and controls in **study I, III,** and **IV**. In **Study IV,** patients with severe and treatment-resistant OCD had higher co-morbidity of neurodevelopmental disorders, attention deficit hyperactivity disorder (ADHD) (60%) and AST (20%).

In **study I** and **III**, participants with OCD scored higher on clinical assessments, including Y-BOCS, OCI-R, and MADRS. Moreover, patients with OCD had lower NART-SWE premorbid IQ in comparison to patients with BDD and healthy controls in **study I**. The proportion of participants on selective serotonin reuptake inhibitors was higher than healthy controls in **study I** compared with patients with BDD. In **study III**, Patients with OCD did not differ on eating habits, stool consistency or micro-, macronutrient intake except for significantly lower fiber consumption compared with healthy controls.

Table 6. Summary of study participants with OCD from study I, III, and IV

Study	I	III	IV
OCD cases (n)	29	32	5
Sex, female count (%)	15 (52%)	19 (59.4%)	2 (40%)
Age, mean (SD)	31.2 (9.2)	27.6 (6.4)	35 (14)
YBOCS, mean (SD)	24.9 (5.2)	23.6 (5.4)	33,8 (2,8)
Comorbid conditions, n (%)			
MDD	10 (37%)	6 (19%)	-
Social phobia	2 (7%)	4 (13%)	-
Panic disorder	2 (7%)	2 (6%)	-
GAD	2 (7%)	4 (13%)	-
BDD	-	1 (3%)	
Tourette's syndrome	3 (11%)	-	-
Trichotillomania/Dermatillomania	1 (4%)	1 (3%)	-
ADHD	-	1 (3%)	3 (60%)
AST	-	-	1 (20%)

Abbreviations: Yale-Brown Obsessive-Compulsive Inventory Scale (Y-BOCS), major depressive disorder (MDD), generalized anxiety disorder (GAD), body dysmorphic disorder (BDD), attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD).

5.3 Study I. Executive functioning in patients with OCD and BDD

Test performance on SWM with main outcome measure between-errors score was not significantly different between OCD, BDD, and the healthy controls. There was a weak positive correlation between number of errors and symptom severity among patients with OCD and BDD that did not reach statistical significance. Adopting a specific strategy, for example searching from one corner to the other, correlated positively with task performance.

Participants in the OCD and BDD groups committed more extra-dimensional shift errors compared with healthy controls, but the results were not significant. Fewer participants in the OCD group passed the extra-dimensional shift stage. We found a weak but not significant positive correlation between symptom severity and extra-dimensional shift errors.

On the main outcome measure SSRT, there were no significant differences between OCD, BDD, and the healthy controls. There was a significant inverse correlation between symptom severity in the BDD group and SSRT, but also between symptom severity and mean reaction time.

Group comparisons of effect sizes (Cohen's *d*) for each primary outcome measure is shown in Table 7. Higher age was associated with worse performance on SWM in terms of between-error score, and longer average reaction time on SST. Furthermore, sex was a significant covariate for extra-dimensional shift errors.

Table 7. Group comparisons of effect sizes using Cohen's *d* [95% confidence interval] on primary outcome measures for each neuropsychological task.

	BDD – HC	OCD - HC	OCD - BDD
SWM between errors	0.39 [-0.32 to 1.1]	0.51 [-0.33 to 1.35]	0.12 [-0.49 to 0.72]
IED extra-dimensional errors	0.14 [-0.57 to 0.85]	0.23 [-0.6 to 1.07]	0.1 [-0.51 to 0.7]
SST stop-signal reaction time	0.21 [-0.49 to 0.92]	0.06 [-0.78 to 0.9]	-0.15 [-0.76 to 0.45]

Abbreviations: Spatial working memory (SWM), intra-extra dimensional shift task (IED), stop-signal task (SST), healthy control (HC), obsessive-compulsive disorder (OCD), body dysmorphic disorder (BDD).

5.4 Study II. Gut microbiota in psychiatric disorders

We conducted a systematic review and searched for eligible studies up until February 13, 2020. Flow diagram according to PRISMA guidelines is depicted in Figure 6.

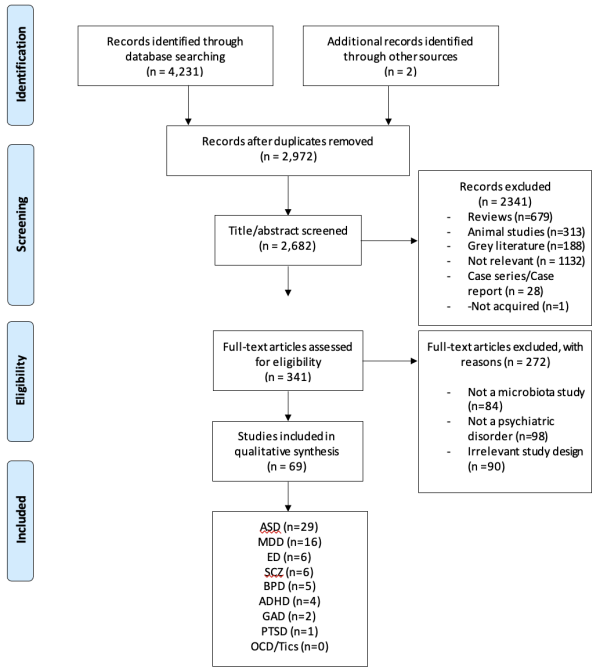


Figure 6. Prisma flow diagram. Abbreviations: Attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar disorder (BPD), eating disorders (ED), generalized anxiety disorder (GAD), major depressive disorder (MDD), tics or obsessive-compulsive disorders (OCD), post-traumatic stress disorder (PTSD), schizophrenia (SCZ).

Note: the figure and figure legend are included in study I.

Mean number of cases was 42 per study, and average number of controls was less than one case per control. Mean age of the participants were 34.5 years and mean BMI was 23 kg/m^2.

There was a wide variety of methodological differences between the studies and the overall study quality has not improved over time.

A majority of studies (44%) did not find any significant differences in gut microbiota diversity between patients with psychiatric disorders and healthy controls in α-diversity indices.

Sixty-seven percent of the studies reported significant differences in gut microbiota composition between patients with psychiatric disorders and healthy controls on β-diversity indices. However, there were few replicated findings regarding which microbes at the genus-level that differentiated cases from controls (Figure 7). Lower abundance of *Faecalibacterium prausnitzii* and *Roseburia species*, and higher abundance of *Bifidobacterium*, was the most replicated findings. Eight studies measured levels of SCFA in stool samples, but the results were inconsistent.

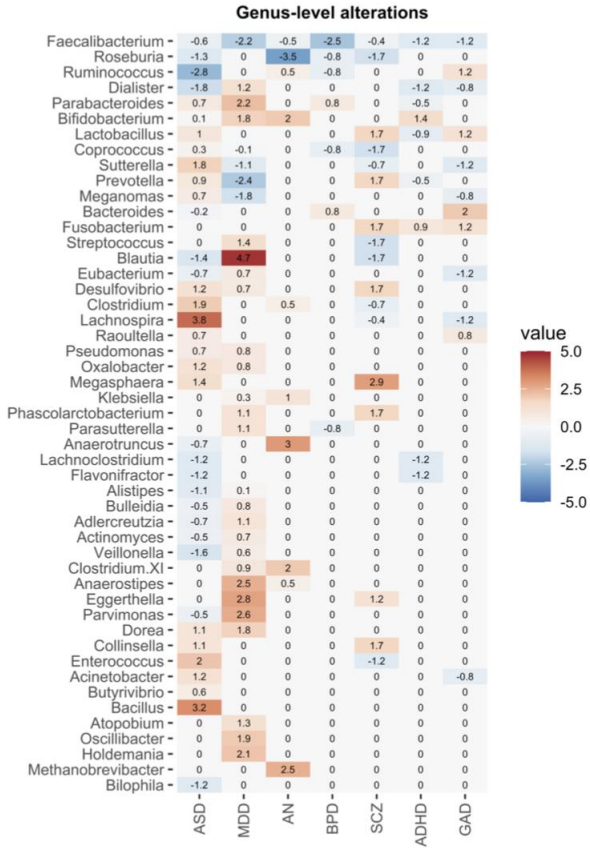


Figure 7. A heat-map over replicated findings of differences in abundance of genera across psychiatric disorders, weighted by sample size. The disorders are listed in order of number of studies included. Autism spectrum disorder (ASD), major depressive disorder (MDD), anorexia nervosa (AN), bipolar disorder (BPD), schizophrenia (SCZ), attention deficit hyperactivity disorder (ADHD), generalized anxiety disorder (GAD).

Note: the figure is included in study II.

5.5 Study III. Gut microbiome in OCD

Gut microbiome α-diversity indices waere not significantly different between OCD and healthy controls, and there were no significant changes after ERP-treatment in patients with OCD (Figure 8). The microbiome composition overlaps in the PCoA plots, and there were no significant differences in β-diversity indices between patients with OCD before ERP, after ERP, or healthy controls (Figure 9).

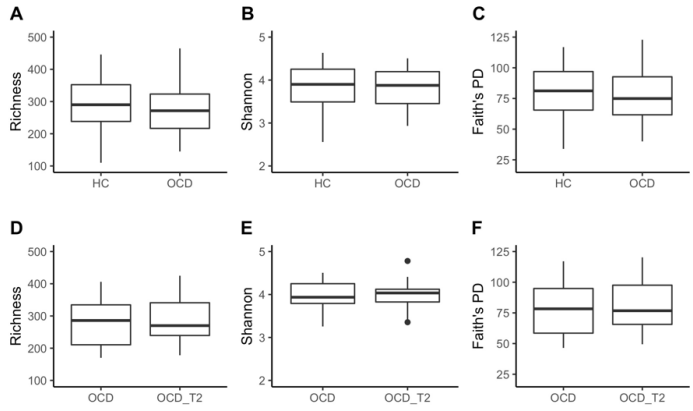


Figure 8. Comparison of alpha diversity indices (richness, Shannon index, Faith's phylogenetic diversity) between healthy controls and patients with OCD (A, B, C), and within patients with OCD before and after ERP treatment (D, E, F). *Note: the figure and figure legend are included in study III.*

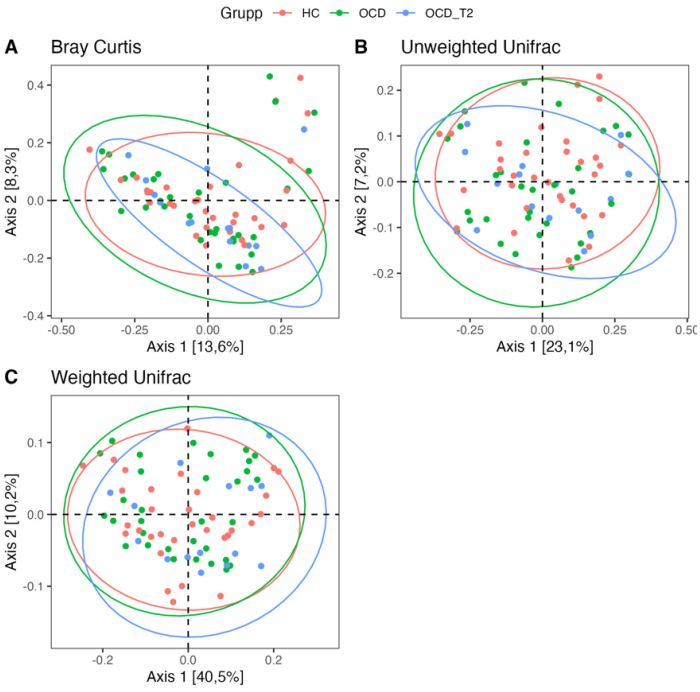


Figure 9. Principal coordinate analysis illustrating β-diversity indices, (A) Bray-Curtis, (B) unweighted UniFrac distances, and (C) weighted UniFrac distances. Samples from healthy controls are in red (n=32), samples from patients with OCD are in green (n=32), and samples from patients that have completed ERP-treatment one month prior are in blue (n=15). *Note: the figure and figure legend are included in study III.*

Dissimilarity analysis at the species-level revealed no significant differences between OCD and healthy controls, and changes within participants with OCD before and after ERP. Functional analysis grouped by GBM with neuroactive potential is shown in Figure 10. The differences in functionality of the gut microbiome between OCD and healthy controls, and within OCD participants before and after ERP-treatment was between -2.9 and 7.1 %. None of the results were significant after adjustment for multiple comparisons (FDR adjusted q-value < 0,05).

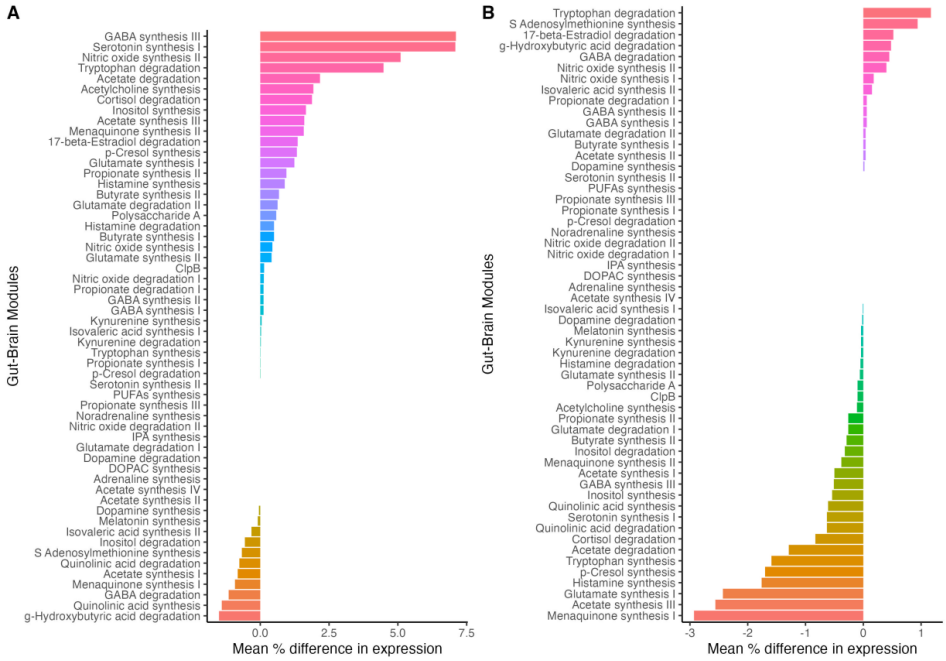


Figure 10. Differences in expression of gut-brain modules (GBM) in mean percentage between (A) OCD and healthy controls, and (B) OCD after ERP compared with before ERP.

Note: the figure and figure legend are included in study III.

5.6 Study IV. GDRVs in patients with severe and treatment-resistant OCD

GDRVs were found in three of the five cases. A deletion in 15q11.2 that disrupted four genes, of which two with pLI > 0.9. A CNV duplication in 15q26.1 that disrupted five genes, of which one with pLI > 0.9 (*ACAN*). Lastly, a missense variant in the ion transporter domain of the potassium voltage-gated channel subfamily B member 1 (KCNB1), hg19 20-47991077-C-T (Figure 11). This missense variant is located in a region highly depleted from missense variants and predicted to be deleterious (MPC score of 3.306). The patient carrying the missense variant was a responder to DBS with a decrease in Y-BOCS score >35%.

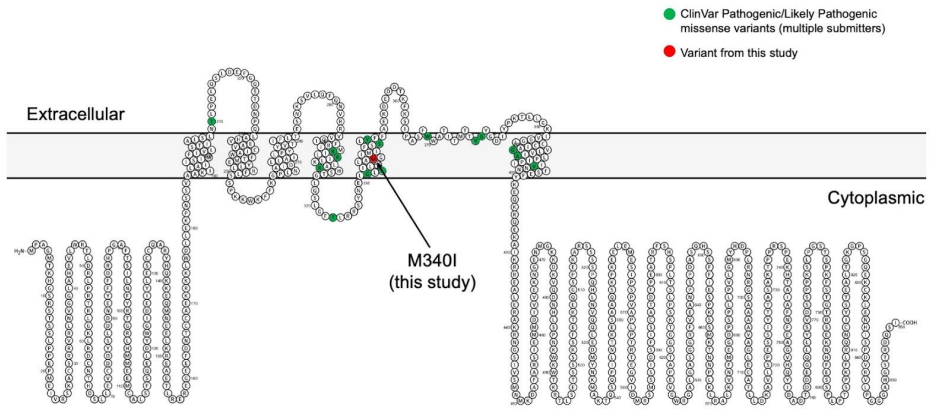


Figure 11. Illustration of missense variant detected in the potassium voltage-gated channel subfamily B member 1 (KCNB1) that leads to a substitution of amino acid from Methionine to Isoleucine in the ion transporter domain (S5) of the protein. Modified after de Covel et al., 2017 (176).

Note: the figure is included in study IV.

6 DISCUSSION

6.1 Executive functions are similar between individuals with OCD and BDD

We did not detect any significant differences in executive functions in patients with OCD or BDD compared with healthy controls. Although we estimated the number of participants required for adequate power based on results from previous studies, the non-significant results could be explained by the sample size. Previous meta-analysis reported small to moderate effect sizes on broad executive dysfunctions rather than specific impairment in patients with OCD (177, 178). In comparison, many psychiatric conditions, including patients with schizophrenia and depression, have been associated with these executive dysfunctions with similar or larger effect sizes compared to patients with OCD (179-181).

There was no significant correlation between symptom severity and neuropsychological task performance, except for a negative correlation between BDD symptom severity and SSRT. This is in line with results from studies on both patients with OCD and BDD where higher symptom severity did not correlate with poorer task performance (51, 53, 177).

Patients with OCD have reduced functional connectivity in resting-state imaging when performing tasks that require cognitive flexibility, and increased connectivity between anterior and posterior cortical regions during tasks assessing inhibitory control (182, 183). It is unclear if these dysfunctional connectivity's have a clinical impact on the symptomatology of OCD. In a multi-center study (*EQOLOC, Clinicaltrials.gov NCT02844049*) patients will be performing neuropsychological tests and symptom-provoking tasks during electrophysiological recording of the STN. The results could have clinical relevance for guiding the placement of electrodes, and in extension the treatment effect of DBS in the STN, as well as shed light on the association between OCD and executive functions.

6.2 Evidence for altered gut microbiota in patients with psychiatric disorders including OCD is inconclusive

We found similar gut microbiome diversity and composition between patients with OCD and healthy controls, which aligns with results from previous studies (117, 184). However, at the genus-level, the findings were inconsistent. While Turna et al., reported lower abundance of butyrate-producing genera, *Oscillibacter* and *Anaerostipes* (184), Domènech et al., found higher abundance of those genera in patients with OCD (117). Using whole genome sequencing, we analyzed taxonomic dissimilarities at the species-level. No significant differences in abundance of metagenomic species was detected. Furthermore, there were no significant differences in functional profiles between patients with OCD and healthy controls.

In perspective, our systematic review revealed little consensus on which microbes that are more or less abundant in patients with psychiatric disorders. The most replicated finding was lower abundance of certain butyrate-producing genera, *Faecalibacterium prausnitzii* and *Roseburia species*. Since dietary fiber is crucial for gut microbes to produce SCFA, lower fiber consumption could lead to decreased abundance of SCFA-producing microbes. Patients with OCD reported consuming less dietary fiber compared with healthy controls in **study III,** but we did not find any significant differences in the abundance of butyrate-producing species between patients with OCD and healthy controls, or any significant differences in functional profiles regarding GBM relevant for SCFA synthesis or degradation. Instead, a study found that increasing dietary fiber consumption among healthy adults leads to higher abundance of *Bifidobacterium* and *Lactobacillus* spp. (185).

Causality is another challenge when dissecting the relationship between the gut microbiome and psychiatric disorders. Differences in behavior could be the cause of altered gut microbiome rather than the other way around. For example, dietary preferences that relate to diagnostic features of children with ASD leads to reduced microbial taxonomic diversity (186). The gut microbiome is more stable during adulthood compared with the relatively volatile infant gut microbiome (86). Despite behavioral changes following ERP in patients with OCD, we could not detect any significantly alterations in the gut microbiome, taxonomically or functionally. However, the lost to follow up after ERP was approximately 30% among patients with OCD in **study III**. This affected the interpretation of the results from the longitudinal design that sought to evaluate changes in the gut microbiome. One way to address the causality issue is to collect samples from participants from birth, through childhood, and into adulthood, and simultaneously assess their psychiatric well-being (187). Despite being the ideal scenario, feasibility would be an issue given the relatively low prevalence of OCD in the general population.

6.3 Patients with severe and treatment-resistant OCD have earlier age-of-onset and neurodevelopmental comorbidities

Comparison of participants with OCD from **study I, III,** and **IV**, reveal that patients with severe and treatment-resistant OCD have an earlier age-of-onset and higher comorbidity with other neurodevelopmental disorders such as ADHD and ASD (Table 6).

early age-of-onset is to a higher degree associated with genetic influences, compared with nonshared environmental influences that increase in function with later age-of-onset (188). In patients with OCD, early age-of-onset is associated with higher symptom severity (189). Male OCD probands have earlier age-of-onset compared with females, and present with more severe symptoms (190). They also carry a higher load of damaging de novo coding single nucleotide variants (SNVs) and indels which indicates that early onset is impacted of rare variants (79).

Early-onset OCD has been associated with higher rates of comorbidity with neurodevelopmental disorders (191, 192). GDRVs that lead to loss-of-function in protein coding regions are enriched in neurodevelopmental disorders, such as intellectual disability and ASD (193). Studies have shown rare genetic variants associated with neurodevelopmental disorders are heterogeneous (194, 195). Importantly, Fu et al., found that genes expressed at earlier stages of cortical development, progenitor genes, are more often associated with general impairment and developmental delay, whereas genes involved in the maturation process of neurons are more linked to ASD (196). Consequently, OCD typically having an age-of-onset from early adolescence, is expected to be affected more by neuron-specific impairment, which could lead to dysfunctional connectivity in specific brain circuits that affect liability for habits and repetitive behavior (76).

In conclusion, OCD is a heterogeneous disorder in terms of clinical presentation and genetics, but patients with early-onset OCD may have a specific phenotypical and biological profile (197).

6.4 A model for genetic discovery in OCD

We identified three separate GDRVs in the five cases. The most deleterious variant we identified is located in the neurodevelopmental gene KCNB1. This potassium ion channel is mainly expressed in neurons located in the frontal cortex (GTEx, dbGaP Accession phs000424.v8.p2). Fifty-one distinct KCNB1 pathogenic variants have been reported in 74 unrelated patients with developmental and epileptic

encephalopathies (198). Depending on the exact location of the genetic variant and the impact on the functional profile of the protein, the severity and symptomatology varies. For example, pathogenic missense and loss of function variants in the outer membrane regions have lower risk for seizures, in addition to milder adaptive and behavioral deficiency (Figure 11) (199).

Three conclusions can be drawn from the current study and previous knowledge:

1. Patients with severe and treatment-resistant OCD is a cohort fit for genomic analysis of GDRVs.
2. GDRVs contribute to increased risk for broad neurodevelopmental disabilities with early age-of-onset, and large effect sizes compared with common variants.
3. The exact phenotype depends on the functional impairment of the genetic variant.

Based on a model for psychiatric genetics by Lappalainen et al., and conclusions listed above, a predictive model for genetic findings in OCD is proposed in Figure 12 (200).

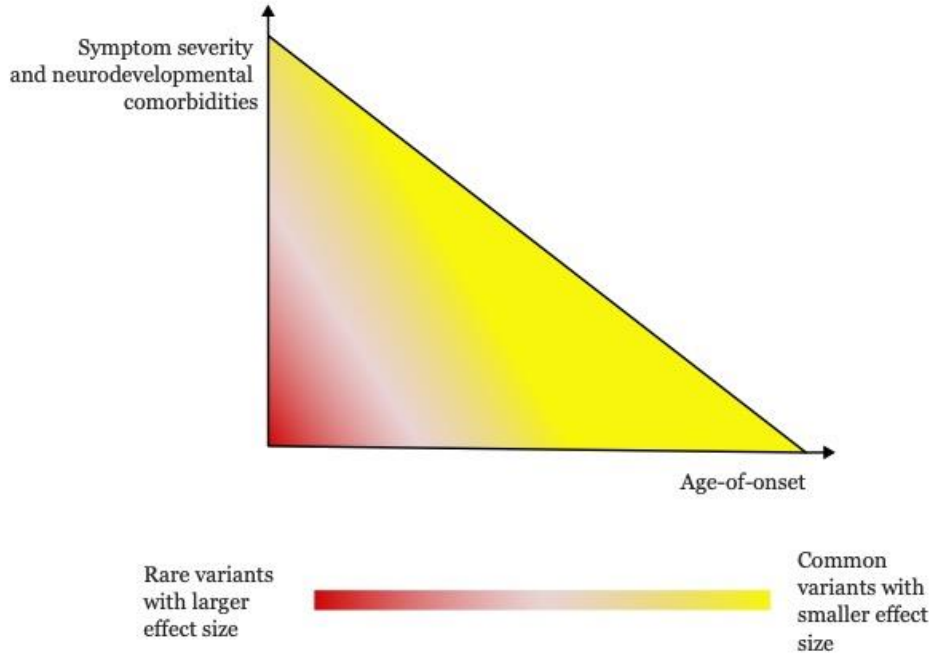


Figure 12. A predictive model for genetic discovery in patients with OCD. Patients with early age-of-onset have more severe symptoms and co-morbidities with neurodevelopmental disorders. Gene-disruptive rare variants could be more prevalent in this cohort. These GDRVs are damaging to proteins responsible for the brain development, thus imposing a larger effect size compared with common variants.

A power calculation recommended inclusion of 100 participants for this study, but it proved unfeasible as the total number of patients subject to DBS for OCD in Sweden was around fifteen. Therefore, the results should be interpreted with caution due to the small sample size. Statistical inference testing was not viable and based on single cases we cannot conclude if the genetic findings are associated with the OCD phenotype. A larger sample size is required to validate the proposed model, and if GDRVs could predict treatment outcome from DBS (201).

6.5 Methodological considerations

6.5.1 Who to study?

Structured psychiatric diagnostics of study participants is essential for case-control studies. Nevertheless, our systematic review on gut microbiota studies unveiled that 74% of the 69 studies did not report any use of structured diagnostic interview for assessment of diagnosis. In **study I, III,** and **IV**, all study participants were interviewed by experienced psychiatrists using MINI and SCID interview, which strengthens the validity of the study population. Moreover, eight potential healthy controls were excluded from **study III**, due to current psychiatric diagnosis. This illustrates the importance of screening controls for psychiatric diagnosis as well.

Information regarding source of recruitment is important, especially considering potential selection bias. Many studies on executive functions in OCD did not report recruitment methods for patients and/or healthy controls (50). For example, performance of university students on tasks assessing executive functions is probably not representative for the general population (51, 52). In **study I**, participants with OCD with moderate to severe symptoms were recruited from specialized outpatient clinics. Therefore, the results are generalizable and clinically relevant. Moreover, the controls were recruited from the general population and did not differ in educational attainment compared with participants with OCD or BDD.

Reporting participant characteristics is important for comparability between studies. Particularly when multiple confounding factors could be present, such as age-of-onset, educational attainment, and medication status. For microbiome studies especially, matching cases with controls on key confounding factors, such as age and sex, is recommended.

6.5.2 How to study OCD?

Neuropsychological tasks for specific executive functions are standardized in CANTAB. However, it cannot be excluded that the tasks map onto multiple constructs, which means that they may not correspond to specific brain circuits. In fact, most or all of our standard neuropsychological assessments are not sufficiently specific to allow us to identify discrete areas of deficit in particular neuropsychiatric

conditions (49, 202). This notion limits the neurobiological conclusions we can draw based on neuropsychological assessment of executive functions in patients with psychiatric disorders.

Next generation sequencing technology has improved radically the last decade. This creates a dilemma in which older studies using sequencing techniques with lower resolution, such as 16S for microbiota studies, becomes outdated. Furthermore, bioinformatic pipelines are continuously updated and there is a pleiotropy of analysis options that could have a downstream impact on the results (203, 204). To remedy some of the shortcomings in a fast-evolving field of research, sharing raw data in open-source databases is recommended. Moreover, compiling large datasets and use the most updated and adequate bioinformatic tools is critical for future studies on gut microbiome.

6.6 Thoughts on neurobiological research in OCD

Lastly, neurobiological research on OCD and psychiatric disorders in general has had little success in translating the findings into clinically meaningful benefits for the patients. There are several reasons behind this.

First, patients with psychiatric conditions are heterogeneous. For example, major depressive disorder is a disorder that encompasses patients with a mild single episode, to postpartum depression, to severe depression with suicidal ideation. Indeed, patients with severe depression have higher genetic heritability compared with those who suffer from mild depression (205). Clinically we provide different treatments options for these sub-groups of patients depending on the symptoms (206). Building on this premise, focusing on phenotypically distinct cohort of patients could be more fruitful when conducting neurobiological studies. Severe and treatment-resistant patients with OCD is one of them.

Secondly, functional characteristics and adequate effect sizes are two key components for establishing clinical relevance. Gut microbiome studies so far have to a little extent reported interpretable functional analysis of the differences between cases and controls. In **study III**, we reused a grouping of GBM with neuroactive potential previously established by Valles-Colomer et al., to elucidate the functional profiles of gut microbiome in patients with OCD compared with healthy controls. In **study IV**, we used a pre-defined and homogeneous cohort of patients with OCD to study GDRVs with potentially large effect sizes to the phenotypical expression. Thus, the design of study III and IV have prioritized outcomes with clinical relevance.

7 CONCLUSIONS

- Patients with OCD and BDD have comparable executive functions in working memory, cognitive flexibility, and inhibition with healthy controls.

- Evidence for altered gut microbiota in patients with psychiatric disorders including OCD is currently inconclusive.

- Patients with severe and treatment-resistant psychiatric conditions with early age-of-onset is a cohort fit for studying GDRVs.

8 POINTS OF PERSPECTIVE

Patients with obsessive-compulsive disorders (OCD) have debilitating symptoms that impact their daily function. The evidence-based treatments can broadly be divided into CBT, pharmacotherapy, and stimulation-treatment. DBS is a safe and effective treatment for patients with severe and treatment-resistant OCD. Nevertheless, approximately 40% of the patients that receive DBS do not experience significant symptom reduction. Clinically useful predictors of treatment response are currently lacking for this group of patients. GDRVs could potentially moderate treatment response. Therefore, genomic studies of patients with OCD that have received DBS are warranted. However, collecting samples from patients with severe and treatment-resistant OCD requires international collaboration due to the few numbers of patients who have received DBS. Although the scientific value is significant, it has proven harder than expected to acquire samples from this cohort of patients. One major obstacle has been different ethical regulations for genetic studies in each country. There is currently no universal ethical application for genetic studies in the EU.

Psychiatric diagnoses are inherently heterogeneous due to our current diagnostic system because each diagnosis includes a spectrum of severity and phenotypic expression. This complicates neurobiological studies of psychiatric disorders including OCD, as significant findings conducted on a broad spectrum of individuals tend to be of small or moderate effect size and concur with general knowledge rather than shed novel insight on the nature of the disorder. Consequently, the discovery of clinically meaningful findings that lead to new treatment approaches has been challenging. A way forward is to focus on the most disabled patients trans-diagnostically.

Lastly, new research areas tend to gain disproportionally large attention. Gut microbiome is one of the latest. However, new research areas suffer from immature technology and lack of gold-standard procedures, such as bioinformatic analysis and statistics. This hampers the reproducibility of the results, which affects the clinical implication of the results. Publishing the data and code for analysis not only increases the transparency of the study which otherwise is difficult to assess simply by reading the article, but also provides data for future researchers to build upon with improved tools and bioinformatic software. In the long run, sharing data is the foundation for better science.

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10 List of other publications co-authored by the Ph.D. candidate

Menchón JM, Real E, Alonso P, Aparicio MA, Segalas C, Plans G, Luyten L, Brunfaut E, Matthijs L, Raymakers S, Bervoets C, Higueras A, Katati M, Guerrero J, Hurtado M, Prieto M, Stieglitz LH, Löffelholz G, Walther S, Pollo C, Zurowski B, Tronnier V, Kordon A, Gambini O, Ranieri R, Franzini A, Messina G, Radu-Djurfeldt D, Schechtmann G, **Chen LL**, Eitan R, Israel Z, Bergman H, Brelje T, Brionne TC, Conseil A, Gielen F, Schuepbach M, Nuttin B, Gabriëls L. A prospective international multi-center study on safety and efficacy of deep brain stimulation for resistant obsessive-compulsive disorder. Mol Psychiatry. 2021 Apr;26(4):1234-1247. doi: 10.1038/s41380-019-0562-6.

Flygare O, Wallert J, **Chen LL**, Fernández de la Cruz L, Lundström L, Mataix-Cols D, Rück C, Andersson E. Empirically Defining Treatment Response and Remission in Obsessive-Compulsive Disorder Using the Obsessive-Compulsive Inventory-Revised. Behav Ther. 2023 Jan;54(1):43-50. doi: 10.1016/j.beth.2022.06.009. Epub 2022 Jul 15. PMID: 36608976.

Flygare O, **Chen LL**, Fernández de la Cruz L, Rück C, Andersson E, Enander J, Mataix-Cols D. Empirically Defining Treatment Response and Remission in Body Dysmorphic Disorder Using a Short Self-Report Instrument. Behav Ther. 2021 Jul;52(4):821-829. doi: 10.1016/j.beth.2020.10.006. Epub 2020 Oct 24. PMID: 34134823.

Lundström L, Flygare O, Ivanova E, Mataix-Cols D, Enander J, Pascal D, **Chen LL**, Andersson E, Rück C. Effectiveness of Internet-based cognitive-behavioural therapy for obsessive-compulsive disorder (OCD-NET) and body dysmorphic disorder (BDD-NET) in the Swedish public health system using the RE-AIM implementation framework. Internet Interv. 2023 Feb 15;31:100608. doi: 10.1016/j.invent.2023.100608. PMID: 36852382; PMCID: PMC9958485.

Gaengel K, Niaudet C, Hagikura K, Laviña B, Muhl L, Hofmann JJ, Ebarasi L, Nyström S, Rymo S, **Chen LL**, Pang MF, Jin Y, Raschperger E, Roswall P, Schulte D, Benedito R, Larsson J, Hellström M, Fuxe J, Uhlén P, Adams R, Jakobsson L, Majumdar A, Vestweber D, Uv A, Betsholtz C. The sphingosine-1-phosphate receptor S1PR1 restricts sprouting angiogenesis by regulating the interplay between VE-cadherin and VEGFR2. Dev Cell. 2012 Sep 11;23(3):587-99. doi: 10.1016/j.devcel.2012.08.005. Erratum in: Dev Cell. 2012 Dec 11;23(6):1264. Laviña Siemsen, Bàrbara [corrected to Laviña, Bàrbara]. PMID: 22975327.

11 REFERENCES

1. Stein DJ, Costa DLC, Lochner C, Miguel EC, Reddy YCJ, Shavitt RG, et al. Obsessive-compulsive disorder. Nat Rev Dis Primers. 2019;5(1):52.

2. Association AP. Diagnostic and statistical manual of mental disorders (DSM-5®): American Psychiatric Pub; 2013.

3. Postorino V, Kerns CM, Vivanti G, Bradshaw J, Siracusano M, Mazzone L. Anxiety Disorders and Obsessive-Compulsive Disorder in Individuals with Autism Spectrum Disorder. Curr Psychiatry Rep. 2017;19(12):92.

4. Zickgraf HF, Murray HB, Kratz HE, Franklin ME. Characteristics of outpatients diagnosed with the selective/neophobic presentation of avoidant/restrictive food intake disorder. Int J Eat Disord. 2019;52(4):367-77.

5. Ruscio AM, Stein DJ, Chiu WT, Kessler RC. The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. Mol Psychiatry. 2010;15(1):53-63.

6. Sharma E, Sharma LP, Balachander S, Lin B, Manohar H, Khanna P, et al. Comorbidities in Obsessive-Compulsive Disorder Across the Lifespan: A Systematic Review and Meta-Analysis. Front Psychiatry. 2021;12:703701.

7. Frías Á, Palma C, Farriols N, González L. Comorbidity between obsessive-compulsive disorder and body dysmorphic disorder: prevalence, explanatory theories, and clinical characterization. Neuropsychiatr Dis Treat. 2015;11:2233-44.

8. Phillips KA, Kelly MM. Body Dysmorphic Disorder: Clinical Overview and Relationship to Obsessive-Compulsive Disorder. Focus (Am Psychiatr Publ). 2021;19(4):413-9.

9. Phillips KA, Stein DJ, Rauch SL, Hollander E, Fallon BA, Barsky A, et al. Should an obsessive-compulsive spectrum grouping of disorders be included in DSM-V? Depress Anxiety. 2010;27(6):528-55.

10. Jassi A, Krebs G. Body Dysmorphic Disorder. Psychiatr Clin North Am. 2023;46(1):197-209.

11. Fawcett EJ, Power H, Fawcett JM. Women Are at Greater Risk of OCD Than Men: A Meta-Analytic Review of OCD Prevalence Worldwide. J Clin Psychiatry. 2020;81(4).

12. Taylor S. Early versus late onset obsessive-compulsive disorder: evidence for distinct subtypes. Clin Psychol Rev. 2011;31(7):1083-100.

13. Pérez-Vigil A, Fernández de la Cruz L, Brander G, Isomura K, Jangmo A, Feldman I, et al. Association of Obsessive-Compulsive Disorder With Objective Indicators of Educational Attainment: A Nationwide Register-Based Sibling Control Study. JAMA Psychiatry. 2018;75(1):47-55.

14. Pérez-Vigil A, Mittendorfer-Rutz E, Helgesson M, Fernández de la Cruz L, Mataix-Cols D. Labour market marginalisation in obsessive-compulsive disorder: a nationwide register-based sibling control study. Psychol Med. 2019;49(6):1015-24.

15. Weidle B, Jozefiak T, Ivarsson T, Thomsen PH. Quality of life in children with OCD with and without comorbidity. Health Qual Life Outcomes. 2014;12:152.

16. Eisen JL, Mancebo MA, Pinto A, Coles ME, Pagano ME, Stout R, et al. Impact of obsessive-compulsive disorder on quality of life. Compr Psychiatry. 2006;47(4):270-5.

17. Albert U, Maina G, Bogetto F, Chiarle A, Mataix-Cols D. Clinical predictors of health-related quality of life in obsessive-compulsive disorder. Compr Psychiatry. 2010;51(2):193-200.

18. Żerdziński M, Burdzik M, Żmuda R, Witkowska-Berek A, Dę bski P, Flajszok-Macierzyńska N, et al. Sense of happiness and other aspects of quality of life in patients with obsessive-compulsive disorder. Front Psychiatry. 2022;13:1077337.

19. Fernández de la Cruz L, Isomura K, Lichtenstein P, Rück C, Mataix-Cols D. Morbidity and mortality in obsessive-compulsive disorder: A narrative review. Neurosci Biobehav Rev. 2022;136:104602.

20. Isomura K, Sidorchuk A, Brander G, Jernberg T, Rück A, Song H, et al. Risk of specific cardiovascular diseases in obsessive-compulsive disorder. J Psychiatr Res. 2021;135:189-96.

21. Isomura K, Brander G, Chang Z, Kuja-Halkola R, Rück C, Hellner C, et al. Metabolic and Cardiovascular Complications in Obsessive-Compulsive Disorder: A Total Population, Sibling Comparison Study With Long-Term Follow-up. Biol Psychiatry. 2018;84(5):324-31.

22. Dell'Osso B, Benatti B, Grancini B, Vismara M, De Carlo V, Cirnigliaro G, et al. Investigating duration of illness and duration of untreated illness in obsessive compulsive disorder reveals patients remain at length pharmacologically untreated. Int J Psychiatry Clin Pract. 2019;23(4):311-3.

23. Skoog G, Skoog I. A 40-year follow-up of patients with obsessive-compulsive disorder [see commetns]. Arch Gen Psychiatry. 1999;56(2):121-7.

24. Samuels J, Bienvenu OJ, Krasnow J, Wang Y, Grados MA, Cullen B, et al. An investigation of doubt in obsessive-compulsive disorder. Compr Psychiatry. 2017;75:117-24.

25. Bhikram T, Abi-Jaoude E, Sandor P. OCD: obsessive-compulsive ... disgust? The role of disgust in obsessive-compulsive disorder. J Psychiatry Neurosci. 2017;42(5):300-6.

26. Belloch A, Fornés G, Carrasco A, López-Solá C, Alonso P, Menchón JM. Incompleteness and not just right experiences in the explanation of Obsessive-Compulsive Disorder. Psychiatry Res. 2016;236:1-8.

27. Ahmari SE, Spellman T, Douglass NL, Kheirbek MA, Simpson HB, Deisseroth K, et al. Repeated cortico-striatal stimulation generates persistent OCD-like behavior. Science. 2013;340(6137):1234-9.

28. Fitzgerald KD, Welsh RC, Stern ER, Angstadt M, Hanna GL, Abelson JL, et al. Developmental alterations of frontal-striatal-thalamic connectivity in obsessive-compulsive disorder. J Am Acad Child Adolesc Psychiatry. 2011;50(9):938-48.e3.

29. Poli A, Pozza A, Orrù G, Conversano C, Ciacchini R, Pugi D, et al. Neurobiological outcomes of cognitive behavioral therapy for obsessive-compulsive disorder: A systematic review. Front Psychiatry. 2022;13:1063116.

30. Park HR, Kim IH, Kang H, McCairn KW, Lee DS, Kim BN, et al. Electrophysiological and imaging evidence of sustained inhibition in limbic and frontal networks following deep brain stimulation for treatment refractory obsessive compulsive disorder. PLoS One. 2019;14(7):e0219578.

31. Le Jeune F, Vérin M, N'Diaye K, Drapier D, Leray E, Du Montcel ST, et al. Decrease of prefrontal metabolism after subthalamic stimulation in obsessive-compulsive disorder: a positron emission tomography study. Biol Psychiatry. 2010;68(11):1016-22.

32. Baldermann JC, Schüller T, Kohl S, Voon V, Li N, Hollunder B, et al. Connectomic Deep Brain Stimulation for Obsessive-Compulsive Disorder. Biological Psychiatry. 2021;90(10):678-88.

33. Goodman WK, Storch EA, Sheth SA. Harmonizing the Neurobiology and Treatment of Obsessive-Compulsive Disorder. Am J Psychiatry. 2021;178(1):17-29.

34. Rudebeck PH, Rich EL. Orbitofrontal cortex. Curr Biol. 2018;28(18):R1083-r8.

35. Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, et al. Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. Am J Psychiatry. 2011;168(7):718-26.

36. Rolls ET. The cingulate cortex and limbic systems for emotion, action, and memory. Brain Struct Funct. 2019;224(9):3001-18.

37. Meek BP, Fotros A, Abo Aoun M, Modirrousta M. Improvements in error-monitoring and symptoms following low-frequency rTMS of dorsal anterior cingulate cortex in obsessive compulsive disorder; a randomized, sham-controlled study. Brain Cogn. 2021;154:105809.

38. Lebow MA, Chen A. Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Mol Psychiatry. 2016;21(4):450-63.

39. Uddin LQ, Nomi JS, Hébert-Seropian B, Ghaziri J, Boucher O. Structure and Function of the Human Insula. J Clin Neurophysiol. 2017;34(4):300-6.

40. Viol K, Aas B, Kastinger A, Kronbichler M, Schöller HJ, Reiter EM, et al. Erroneously Disgusted: fMRI Study Supports Disgust-Related Neural Reuse in Obsessive-Compulsive Disorder (OCD). Front Behav Neurosci. 2019;13:81.

41. Burbaud P, Clair AH, Langbour N, Fernandez-Vidal S, Goillandeau M, Michelet T, et al. Neuronal activity correlated with checking behaviour in the subthalamic nucleus of patients with obsessive-compulsive disorder. Brain. 2013;136(Pt 1):304-17.

42. Jones DT, Graff-Radford J. Executive Dysfunction and the Prefrontal Cortex. Continuum (Minneap Minn). 2021;27(6):1586-601.

43. Diamond A. Executive functions. Annu Rev Psychol. 2013;64:135-68.

44. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD. The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: a latent variable analysis. Cogn Psychol. 2000;41(1):49-100.

45. Menzies L, Chamberlain SR, Laird AR, Thelen SM, Sahakian BJ, Bullmore ET. Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. Neurosci Biobehav Rev. 2008;32(3):525-49.

46. Beucke JC, Sepulcre J, Buhlmann U, Kathmann N, Moody T, Feusner JD. Degree connectivity in body dysmorphic disorder and relationships with obsessive and compulsive symptoms. Eur Neuropsychopharmacol. 2016;26(10):1657-66.

47. Bettcher BM, Mungas D, Patel N, Elofson J, Dutt S, Wynn M, et al. Neuroanatomical substrates of executive functions: Beyond prefrontal structures. Neuropsychologia. 2016;85:100-9.

48. Gruner P, Pittenger C. Cognitive inflexibility in Obsessive-Compulsive Disorder. Neuroscience. 2017;345:243-55.

49. Henderson VW. Chapter 17: cognitive assessment in neurology. Handb Clin Neurol. 2010;95:235-56.

50. Abramovitch A, Mittelman A, Tankersley AP, Abramowitz JS, Schweiger A. Neuropsychological investigations in obsessive-compulsive disorder: A systematic review of methodological challenges. Psychiatry Res. 2015;228(1):112-20.

51. Jefferies-Sewell K, Chamberlain SR, Fineberg NA, Laws KR. Cognitive dysfunction in body dysmorphic disorder: new implications for nosological systems and neurobiological models. CNS Spectr. 2017;22(1):51-60.

52. Greenberg JL, Weingarden H, Reuman L, Abrams D, Mothi SS, Wilhelm S. Set shifting and visuospatial organization deficits in body dysmorphic disorder. Psychiatry Res. 2018;260:182-6.

53. Dunai J, Labuschagne I, Castle DJ, Kyrios M, Rossell SL. Executive function in body dysmorphic disorder. Psychol Med. 2010;40(9):1541-8.

54. Hanes KR. Neuropsychological performance in body dysmorphic disorder. J Int Neuropsychol Soc. 1998;4(2):167-71.

55. Labuschagne I, Rossell SL, Dunai J, Castle DJ, Kyrios M. A comparison of executive function in Body Dysmorphic Disorder (BDD) and Obsessive-Compulsive Disorder (OCD). Journal of Obsessive-Compulsive and Related Disorders. 2013;2(3):257-62.

56. Browne HA, Gair SL, Scharf JM, Grice DE. Genetics of obsessive-compulsive disorder and related disorders. Psychiatr Clin North Am. 2014;37(3):319-35.

57. Mataix-Cols D, Boman M, Monzani B, Rück C, Serlachius E, Långström N, et al. Population-based, multigenerational family clustering study of obsessive-compulsive disorder. JAMA Psychiatry. 2013;70(7):709-17.

58. Arumugham SS, Cherian AV, Baruah U, Viswanath B, Narayanaswamy JC, Math SB, et al. Comparison of clinical characteristics of familial and sporadic obsessive-compulsive disorder. Compr Psychiatry. 2014;55(7):1520-5.

59. Nestadt G, Samuels J, Riddle M, Bienvenu OJ, 3rd, Liang KY, LaBuda M, et al. A family study of obsessive-compulsive disorder. Arch Gen Psychiatry. 2000;57(4):358-63.

60. Pauls DL, Alsobrook JP, 2nd, Goodman W, Rasmussen S, Leckman JF. A family study of obsessive-compulsive disorder. Am J Psychiatry. 1995;152(1):76-84.

61. Taylor S. Etiology of obsessions and compulsions: a meta-analysis and narrative review of twin studies. Clin Psychol Rev. 2011;31(8):1361-72.

62. Hudziak JJ, Van Beijsterveldt CE, Althoff RR, Stanger C, Rettew DC, Nelson EC, et al. Genetic and environmental contributions to the Child Behavior Checklist Obsessive-Compulsive Scale: a cross-cultural twin study. Arch Gen Psychiatry. 2004;61(6):608-16.

63. Sidore C, Busonero F, Maschio A, Porcu E, Naitza S, Zoledziewska M, et al. Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. Nat Genet. 2015;47(11):1272-81.

64. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.

65. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53.

66. Meier SM, Agerbo E, Maier R, Pedersen CB, Lang M, Grove J, et al. High loading of polygenic risk in cases with chronic schizophrenia. Mol Psychiatry. 2016;21(7):969-74.

67. Zhu Q, Ge D, Maia Jessica M, Zhu M, Petrovski S, Dickson Samuel P, et al. A Genome-wide Comparison of the Functional Properties of Rare and Common Genetic Variants in Humans. The American Journal of Human Genetics. 2011;88(4):458-68.

68. Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. Nat Rev Genet. 2016;17(1):9-18.

69. Strom NI, Yu D, Gerring ZF, Halvorsen MW, Abdellaoui A, Rodriguez-Fontenla C, et al. Genome-wide association study identifies new locus associated with OCD. medRxiv. 2021:2021.10.13.21261078.

70. Nagel M, Jansen PR, Stringer S, Watanabe K, de Leeuw CA, Bryois J, et al. Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. Nature Genetics. 2018;50(7):920-7.

71. Pardiñas AF, Holmans P, Pocklington AJ, Escott-Price V, Ripke S, Carrera N, et al. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. Nat Genet. 2018;50(3):381-9.

72. Ripke S, Neale BM, Corvin A, Walters JTR, Farh K-H, Holmans PA, et al. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421-7.

73. Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. Cell. 2019;179(7):1469-82.e11.

74. Brakoulias V, Starcevic V, Belloch A, Brown C, Ferrao YA, Fontenelle LF, et al. Comorbidity, age of onset and suicidality in obsessive-compulsive disorder (OCD): An international collaboration. Compr Psychiatry. 2017;76:79-86.

75. Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J, Duncan L, et al. Analysis of shared heritability in common disorders of the brain. Science. 2018;360(6395).

76. Mahjani B, Klei L, Mattheisen M, Halvorsen MW, Reichenberg A, Roeder K, et al. The Genetic Architecture of Obsessive-Compulsive Disorder: Contribution of Liability to OCD From Alleles Across the Frequency Spectrum. Am J Psychiatry. 2022;179(3):216-25.

77. Cappi C, Brentani H, Lima L, Sanders SJ, Zai G, Diniz BJ, et al. Whole-exome sequencing in obsessive-compulsive disorder identifies rare mutations in immunological and neurodevelopmental pathways. Transl Psychiatry. 2016;6(3):e764.

78. Halvorsen M, Samuels J, Wang Y, Greenberg BD, Fyer AJ, McCracken JT, et al. Exome sequencing in obsessive-compulsive disorder reveals a burden of rare damaging coding variants. Nat Neurosci. 2021;24(8):1071-6.

79. Cappi C, Oliphant ME, Péter Z, Zai G, Conceição do Rosário M, Sullivan CAW, et al. De Novo Damaging DNA Coding Mutations Are Associated With Obsessive-Compulsive Disorder and Overlap With Tourette's Disorder and Autism. Biol Psychiatry. 2020;87(12):1035-44.

80. McGrath LM, Yu D, Marshall C, Davis LK, Thiruvahindrapuram B, Li B, et al. Copy number variation in obsessive-compulsive disorder and tourette syndrome: a cross-disorder study. J Am Acad Child Adolesc Psychiatry. 2014;53(8):910-9.

81. Gazzellone MJ, Zarrei M, Burton CL, Walker S, Uddin M, Shaheen SM, et al. Uncovering obsessive-compulsive disorder risk genes in a pediatric cohort by high-resolution analysis of copy number variation. J Neurodev Disord. 2016;8:36.

82. Grünblatt E, Oneda B, Ekici AB, Ball J, Geissler J, Uebe S, et al. High resolution chromosomal microarray analysis in paediatric obsessive-compulsive disorder. BMC Med Genomics. 2017;10(1):68.

83. Mahjani B, Bey K, Boberg J, Burton C. Genetics of obsessive-compulsive disorder. Psychol Med. 2021;51(13):2247-59.

84. Borre YE, Moloney RD, Clarke G, Dinan TG, Cryan JF. The impact of microbiota on brain and behavior: mechanisms & therapeutic potential. Advances in experimental medicine and biology. 2014;817:373-403.

85. Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell. 2016;164(3):337-40.

86. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489(7415):220-30.

87. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. Nat Rev Neurosci. 2011;12(8):453-66.

88. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nature reviews Neuroscience. 2012;13(10):701-12.

89. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Molecular psychiatry. 2014;19(2):146-8.

90. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;141(2):599-609, .e1-3.

91. Kelly JR, Borre Y, C OB, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. Journal of psychiatric research. 2016;82:109-18.

92. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99(4):1877-2013.

93. Dinan TG, Cryan JF. Brain-Gut-Microbiota Axis and Mental Health. Psychosom Med. 2017;79(8):920-6.

94. McGuinness AJ, Davis JA, Dawson SL, Loughman A, Collier F, O'Hely M, et al. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. Mol Psychiatry. 2022;27(4):1920-35.

95. Nikolova VL, Smith MRB, Hall LJ, Cleare AJ, Stone JM, Young AH. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. JAMA Psychiatry. 2021;78(12):1343-54.

96. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 2019;4(4):623-32.

97. Samochowiec J, Misiak B. Gut microbiota and microbiome in schizophrenia. Curr Opin Psychiatry. 2021;34(5):503-7.

98. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504(7480):446-50.

99. Sun J, Wang F, Hong G, Pang M, Xu H, Li H, et al. Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. Neurosci Lett. 2016;618:159-66.

100. Kratsman N, Getselter D, Elliott E. Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model. Neuropharmacology. 2016;102:136-45.

101. Yamawaki Y, Fuchikami M, Morinobu S, Segawa M, Matsumoto T, Yamawaki S. Antidepressant-like effect of sodium butyrate (HDAC inhibitor) and its molecular mechanism of action in the rat hippocampus. The World Journal of Biological Psychiatry. 2012;13(6):458-67.

102. Rees JC. Obsessive-compulsive disorder and gut microbiota dysregulation. Medical hypotheses. 2014;82(2):163-6.

103. Turna J, Grosman Kaplan K, Anglin R, Van Ameringen M. "WHAT'S BUGGING THE GUT IN OCD?" A REVIEW OF THE GUT MICROBIOME IN OBSESSIVE-COMPULSIVE DISORDER. Depression and anxiety. 2016;33(3):171-8.

104. Kantak PA, Bobrow DN, Nyby JG. Obsessive-compulsive-like behaviors in house mice are attenuated by a probiotic (Lactobacillus rhamnosus GG). Behavioural pharmacology. 2014;25(1):71-9.

105. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature. 2019;574(7776):117-21.

106. Brander G, Rydell M, Kuja-Halkola R, Fernandez de la Cruz L, Lichtenstein P, Serlachius E, et al. Association of Perinatal Risk Factors With Obsessive-Compulsive Disorder: A Population-Based Birth Cohort, Sibling Control Study. JAMA psychiatry. 2016;73(11):1135-44.

107. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(26):11971-5.

108. Goedert JJ, Hua X, Yu G, Shi J. Diversity and composition of the adult fecal microbiome associated with history of cesarean birth or appendectomy: Analysis of the American Gut Project. EBioMedicine. 2014;1(2-3):167-72.

109. Kauer J, Pelchat ML, Rozin P, Zickgraf HF. Adult picky eating. Phenomenology, taste sensitivity, and psychological correlates. Appetite. 2015;90:219-28.

110. Mataix-Cols D, Frans E, Pérez-Vigil A, Kuja-Halkola R, Gromark C, Isomura K, et al. A total-population multigenerational family clustering study of autoimmune diseases in obsessive-compulsive disorder and Tourette's/chronic tic disorders. Mol Psychiatry. 2018;23(7):1652-8.

111. Baj J, Sitarz E, Forma A, Wróblewska K, Karakuła-Juchnowicz H. Alterations in the Nervous System and Gut Microbiota after β-Hemolytic Streptococcus Group A Infection-Characteristics and Diagnostic Criteria of PANDAS Recognition. Int J Mol Sci. 2020;21(4).

112. Murphy TK, Sajid MW, Goodman WK. Immunology of obsessive-compulsive disorder. The Psychiatric clinics of North America. 2006;29(2):445-69.

113. Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity. 2012;37(4):601-10.

114. Sommer F, Backhed F. The gut microbiota--masters of host development and physiology. Nature reviews Microbiology. 2013;11(4):227-38.

115. Quagliariello A, Del Chierico F, Russo A, Reddel S, Conte G, Lopetuso LR, et al. Gut Microbiota Profiling and Gut-Brain Crosstalk in Children Affected by Pediatric Acute-Onset Neuropsychiatric Syndrome and Pediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococcal Infections. Front Microbiol. 2018;9:675.

116. Turna J, Grosman Kaplan K, Anglin R, Patterson B, Soreni N, Bercik P, et al. The gut microbiome and inflammation in obsessive-compulsive disorder patients compared to age- and sex-matched controls: a pilot study. Acta Psychiatr Scand. 2020.

117. Domènech L, Willis J, Alemany-Navarro M, Morell M, Real E, Escaramís G, et al. Changes in the stool and oropharyngeal microbiome in obsessive-compulsive disorder. Sci Rep. 2022;12(1):1448.

118. Nezgovorova V, Reid J, Fineberg NA, Hollander E. Optimizing first line treatments for adults with OCD. Compr Psychiatry. 2022;115:152305.

119. Foa EB, Yadin E, Lichner TK. Exposure and response (ritual) prevention for obsessive-compulsive disorder: Therapist guide, 2nd ed. Exposure and response (ritual) prevention for obsessive-compulsive disorder: Therapist guide, 2nd ed. New York, NY, US: Oxford University Press; 2012. p. x, 182-x, .

120. Andersson E, Enander J, Andrén P, Hedman E, Ljótsson B, Hursti T, et al. Internet-based cognitive behaviour therapy for obsessive-compulsive disorder: a randomized controlled trial. Psychol Med. 2012;42(10):2193-203.

121. Craske MG, Treanor M, Conway CC, Zbozinek T, Vervliet B. Maximizing exposure therapy: an inhibitory learning approach. Behav Res Ther. 2014;58:10-23.

122. Öst LG, Riise EN, Wergeland GJ, Hansen B, Kvale G. Cognitive behavioral and pharmacological treatments of OCD in children: A systematic review and meta-analysis. J Anxiety Disord. 2016;43:58-69.

123. Melin K, Skarphedinsson G, Thomsen PH, Weidle B, Torp NC, Valderhaug R, et al. Treatment Gains Are Sustainable in Pediatric Obsessive-Compulsive Disorder: Three-Year Follow-Up From the NordLOTS. J Am Acad Child Adolesc Psychiatry. 2020;59(2):244-53.

124. Hansen B, Kvale G, Hagen K, Havnen A, Öst LG. The Bergen 4-day treatment for OCD: four years follow-up of concentrated ERP in a clinical mental health setting. Cogn Behav Ther. 2019;48(2):89-105.

125. Havnen A, Hansen B, Öst LG, Kvale G. Concentrated ERP Delivered in a Group Setting: A Replication Study. Behav Cogn Psychother. 2017;45(5):530-6.

126. Lundström L, Flygare O, Andersson E, Enander J, Bottai M, Ivanov VZ, et al. Effect of Internet-Based vs Face-to-Face Cognitive Behavioral Therapy for Adults With Obsessive-Compulsive Disorder: A Randomized Clinical Trial. JAMA Netw Open. 2022;5(3):e221967.

127. Aspvall K, Andersson E, Melin K, Norlin L, Eriksson V, Vigerland S, et al. Effect of an Internet-Delivered Stepped-Care Program vs In-Person Cognitive Behavioral Therapy on Obsessive-Compulsive Disorder Symptoms in Children and Adolescents: A Randomized Clinical Trial. Jama. 2021;325(18):1863-73.

128. Soomro GM, Altman D, Rajagopal S, Oakley-Browne M. Selective serotonin re-uptake inhibitors (SSRIs) versus placebo for obsessive compulsive disorder (OCD). Cochrane Database Syst Rev. 2008;2008(1):Cd001765.

129. Bloch MH, McGuire J, Landeros-Weisenberger A, Leckman JF, Pittenger C. Meta-analysis of the dose-response relationship of SSRI in obsessive-compulsive disorder. Mol Psychiatry. 2010;15(8):850-5.

130. Bloch MH, Landeros-Weisenberger A, Kelmendi B, Coric V, Bracken MB, Leckman JF. A systematic review: antipsychotic augmentation with treatment refractory obsessive-compulsive disorder. Mol Psychiatry. 2006;11(7):622-32.

131. Thorén P, Asberg M, Cronholm B, Jörnestedt L, Träskman L. Clomipramine treatment of obsessive-compulsive disorder. I. A controlled clinical trial. Arch Gen Psychiatry. 1980;37(11):1281-5.

132. Skapinakis P, Caldwell DM, Hollingworth W, Bryden P, Fineberg NA, Salkovskis P, et al. Pharmacological and psychotherapeutic interventions for management of obsessive-compulsive disorder in adults: a systematic review and network meta-analysis. Lancet Psychiatry. 2016;3(8):730-9.

133. Insel TR, Murphy DL, Cohen RM, Alterman I, Kilts C, Linnoila M. Obsessive-compulsive disorder. A double-blind trial of clomipramine and clorgyline. Arch Gen Psychiatry. 1983;40(6):605-12.

134. Isomura K, Nordsletten AE, Rück C, Ljung R, Ivarsson T, Larsson H, et al. Pharmacoepidemiology of obsessive-compulsive disorder: A Swedish nationwide cohort study. Eur Neuropsychopharmacol. 2016;26(4):693-704.

135. Mancebo MC, Eisen JL, Pinto A, Greenberg BD, Dyck IR, Rasmussen SA. The brown longitudinal obsessive compulsive study: treatments received and patient impressions of improvement. J Clin Psychiatry. 2006;67(11):1713-20.

136. Flygare O, Andersson E, Ringberg H, Hellstadius AC, Edbacken J, Enander J, et al. Adapted cognitive behavior therapy for obsessive-compulsive disorder with co-occurring autism spectrum disorder: A clinical effectiveness study. Autism. 2020;24(1):190-9.

137. Eisen JL, Sibrava NJ, Boisseau CL, Mancebo MC, Stout RL, Pinto A, et al. Five-year course of obsessive-compulsive disorder: predictors of remission and relapse. J Clin Psychiatry. 2013;74(3):233-9.

138. Mataix-Cols D, Fernández de la Cruz L, Nordsletten AE, Lenhard F, Isomura K, Simpson HB. Towards an international expert consensus for defining treatment response, remission, recovery and relapse in obsessive-compulsive disorder. World Psychiatry. 2016;15(1):80-1.

139. Denys D. Pharmacotherapy of obsessive-compulsive disorder and obsessive-compulsive spectrum disorders. Psychiatr Clin North Am. 2006;29(2):553-84, xi.

140. Dell'Osso B, Benatti B, Buoli M, Altamura AC, Marazziti D, Hollander E, et al. The influence of age at onset and duration of illness on long-term outcome in patients with obsessive-compulsive disorder: a report from the International College of Obsessive Compulsive Spectrum Disorders (ICOCS). Eur Neuropsychopharmacol. 2013;23(8):865-71.

141. Hirschtritt ME, Bloch MH, Mathews CA. Obsessive-Compulsive Disorder: Advances in Diagnosis and Treatment. Jama. 2017;317(13):1358-67.

142. Pampaloni I, Marriott S, Pessina E, Fisher C, Govender A, Mohamed H, et al. The global assessment of OCD. Compr Psychiatry. 2022;118:152342.

143. Lundström L, Flygare O, Ivanova E, Mataix-Cols D, Enander J, Pascal D, et al. Effectiveness of Internet-based cognitive-behavioural therapy for obsessive-compulsive disorder (OCD-NET) and body dysmorphic disorder (BDD-NET) in the Swedish public health system using the RE-AIM implementation framework. Internet Interv. 2023;31:100608.

144. Olatunji BO, Davis ML, Powers MB, Smits JA. Cognitive-behavioral therapy for obsessive-compulsive disorder: a meta-analysis of treatment outcome and moderators. J Psychiatr Res. 2013;47(1):33-41.

145. Jakubovski E, Diniz JB, Valerio C, Fossaluza V, Belotto-Silva C, Gorenstein C, et al. Clinical predictors of long-term outcome in obsessive-compulsive disorder. Depress Anxiety. 2013;30(8):763-72.

146. Knopp J, Knowles S, Bee P, Lovell K, Bower P. A systematic review of predictors and moderators of response to psychological therapies in OCD: do we have enough empirical evidence to target treatment? Clin Psychol Rev. 2013;33(8):1067-81.

147. Cervin M, Mataix-Cols D. Empirical severity benchmarks for obsessive-compulsive disorder across the lifespan. World Psychiatry. 2022;21(2):315-6.

148. Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, et al. The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. Arch Gen Psychiatry. 1989;46(11):1006-11.

149. Stern RS, Marks IM, Mawson D, Luscombe DK. Clomipramine and exposure for compulsive rituals: II. Plasma levels, side effects and outcome. Br J Psychiatry. 1980;136:161-6.

150. Nuttin B, Cosyns P, Demeulemeester H, Gybels J, Meyerson B. Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. Lancet. 1999;354(9189):1526.

151. Rück C, Karlsson A, Steele JD, Edman G, Meyerson BA, Ericson K, et al. Capsulotomy for obsessive-compulsive disorder: long-term follow-up of 25 patients. Arch Gen Psychiatry. 2008;65(8):914-21.

152. Greenberg BD, Gabriels LA, Malone DA, Jr., Rezai AR, Friehs GM, Okun MS, et al. Deep brain stimulation of the ventral internal capsule/ventral striatum for obsessive-compulsive disorder: worldwide experience. Molecular psychiatry. 2010;15(1):64-79.

153. Denys D, Mantione M, Figee M, van den Munckhof P, Koerselman F, Westenberg H, et al. Deep brain stimulation of the nucleus accumbens for treatment-refractory obsessive-compulsive disorder. Archives of general psychiatry. 2010;67(10):1061-8.

154. Mallet L, Polosan M, Jaafari N, Baup N, Welter ML, Fontaine D, et al. Subthalamic nucleus stimulation in severe obsessive-compulsive disorder. N Engl J Med. 2008;359(20):2121-34.

155. Alonso P, Cuadras D, Gabriëls L, Denys D, Goodman W, Greenberg BD, et al. Deep Brain Stimulation for Obsessive-Compulsive Disorder: A Meta-Analysis of Treatment Outcome and Predictors of Response. PLoS One. 2015;10(7):e0133591.

156. Menchón JM, Real E, Alonso P, Aparicio MA, Segalas C, Plans G, et al. A prospective international multi-center study on safety and efficacy of deep brain stimulation for resistant obsessive-compulsive disorder. Mol Psychiatry. 2021;26(4):1234-47.

157. Mar-Barrutia L, Real E, Segalás C, Bertolín S, Menchón JM, Alonso P. Deep brain stimulation for obsessive-compulsive disorder: A systematic review of worldwide experience after 20 years. World J Psychiatry. 2021;11(9):659-80.

158. Saleh C, Fontaine D. Deep brain stimulation for psychiatric diseases: what are the risks? Curr Psychiatry Rep. 2015;17(5):33.

159. Nguyen TT, Hathaway H, Kosciolek T, Knight R, Jeste DV. Gut microbiome in serious mental illnesses: A systematic review and critical evaluation. Schizophr Res. 2019.

160. Schalla MA, Stengel A. Gastrointestinal alterations in anorexia nervosa - A systematic review. Eur Eat Disord Rev. 2019;27(5):447-61.

161. Cheung SG, Goldenthal AR, Uhlemann AC, Mann JJ, Miller JM, Sublette ME. Systematic Review of Gut Microbiota and Major Depression. Front Psychiatry. 2019;10:34.

162. Furlani B, Kouter K, Rozman D, Videtič Paska A. Sequencing of Nucleic Acids: from the First Human Genome to Next Generation Sequencing in {COVID}-19 Pandemic. Acta Chim Slov. 2021;68(2):268-78.

163. Enander J, Andersson E, Mataix-Cols D, Lichtenstein L, Alström K, Andersson G, et al. Therapist guided internet based cognitive behavioural therapy for body dysmorphic disorder: single blind randomised controlled trial. Bmj. 2016;352:i241.

164. Enander J, Ivanov VZ, Andersson E, Mataix-Cols D, Ljótsson B, Rück C. Therapist-guided, Internet-based cognitive-behavioural therapy for body dysmorphic disorder (BDD-NET): a feasibility study. BMJ Open. 2014;4(9):e005923.

165. Naesström M, Hariz M, Strömsten L, Bodlund O, Blomstedt P. Deep Brain Stimulation in the Bed Nucleus of Stria Terminalis in Obsessive-Compulsive Disorder-1-Year Follow-up. World Neurosurg. 2021;149:e794-e802.

166. Pasolli E, Asnicar F, Manara S, Zolfo M, Karcher N, Armanini F, et al. Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. Cell. 2019;176(3):649-62.e20.

167. Nielsen HB, Almeida M, Juncker AS, Rasmussen S, Li J, Sunagawa S, et al. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nat Biotechnol. 2014;32(8):822-8.

168. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics. 2019;36(6):1925-7.

169. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011;43(5):491-8.

170. Fromer M, Purcell SM. Using XHMM Software to Detect Copy Number Variation in Whole-Exome Sequencing Data. Curr Protoc Hum Genet. 2014;81:7.23.1-7..1.

171. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-91.

172. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581(7809):434-43.

173. Wells G, Shea B, O'Connell J. The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of Nonrandomised Studies in Meta-analyses. Ottawa Health Research Institute Web site. 2014;7.

174. Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PLoS One. 2013;8(7):e67019.

175. Hofmann B. Incidental findings of uncertain significance: To know or not to know--that is not the question. BMC Med Ethics. 2016;17:13.

176. de Kovel CGF, Syrbe S, Brilstra EH, Verbeek N, Kerr B, Dubbs H, et al. Neurodevelopmental Disorders Caused by De Novo Variants in KCNB1 Genotypes and Phenotypes. JAMA Neurol. 2017;74(10):1228-36.

177. Abramovitch A, Abramowitz JS, Mittelman A. The neuropsychology of adult obsessive-compulsive disorder: a meta-analysis. Clin Psychol Rev. 2013;33(8):1163-71.

178. Snyder HR, Kaiser RH, Warren SL, Heller W. Obsessive-compulsive disorder is associated with broad impairments in executive function: A meta-analysis. Clin Psychol Sci. 2015;3(2):301-30.

179. Mesholam-Gately RI, Giuliano AJ, Goff KP, Faraone SV, Seidman LJ. Neurocognition in first-episode schizophrenia: a meta-analytic review. Neuropsychology. 2009;23(3):315-36.

180. Mann-Wrobel MC, Carreno JT, Dickinson D. Meta-analysis of neuropsychological functioning in euthymic bipolar disorder: an update and investigation of moderator variables. Bipolar Disord. 2011;13(4):334-42.

181. Snyder HR. Major depressive disorder is associated with broad impairments on neuropsychological measures of executive function: a meta-analysis and review. Psychol Bull. 2013;139(1):81-132.

182. Vaghi MM, Vértes PE, Kitzbichler MG, Apergis-Schoute AM, van der Flier FE, Fineberg NA, et al. Specific Frontostriatal Circuits for Impaired Cognitive Flexibility and Goal-Directed Planning in Obsessive-Compulsive Disorder: Evidence From Resting-State Functional Connectivity. Biol Psychiatry. 2017;81(8):708-17.

183. Hampshire A, Zadel A, Sandrone S, Soreq E, Fineberg N, Bullmore ET, et al. Inhibition-Related Cortical Hypoconnectivity as a Candidate Vulnerability Marker for Obsessive-Compulsive Disorder. Biol Psychiatry Cogn Neurosci Neuroimaging. 2020;5(2):222-30.

184. Turna J, Grosman Kaplan K, Anglin R, Patterson B, Soreni N, Bercik P, et al. The gut microbiome and inflammation in obsessive-compulsive disorder patients compared to age- and sex-matched controls: a pilot study. Acta Psychiatr Scand. 2020;142(4):337-47.

185. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, et al. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am J Clin Nutr. 2018;107(6):965-83.

186. Yap CX, Henders AK, Alvares GA, Wood DLA, Krause L, Tyson GW, et al. Autism-related dietary preferences mediate autism-gut microbiome associations. Cell. 2021;184(24):5916-31.e17.

187. Ou Y, Belzer C, Smidt H, de Weerth C. Development of the gut microbiota in healthy children in the first ten years of life: associations with internalizing and externalizing behavior. Gut Microbes. 2022;14(1):2038853.

188. Askeland RB, Hannigan LJ, Ask H, Ayorech Z, Tesli M, Corfield E, et al. Early manifestations of genetic risk for neurodevelopmental disorders. Journal of Child Psychology and Psychiatry. 2022;63(7):810-9.

189. Rosario-Campos MC, Leckman JF, Mercadante MT, Shavitt RG, Prado HS, Sada P, et al. Adults with early-onset obsessive-compulsive disorder. Am J Psychiatry. 2001;158(11):1899-903.

190. Stewart SE, Geller DA, Jenike M, Pauls D, Shaw D, Mullin B, et al. Long-term outcome of pediatric obsessive-compulsive disorder: a meta-analysis and qualitative review of the literature. Acta Psychiatr Scand. 2004;110(1):4-13.

191. Geller DA, Homayoun S, Johnson G. Developmental Considerations in Obsessive Compulsive Disorder: Comparing Pediatric and Adult-Onset Cases. Front Psychiatry. 2021;12:678538.

192. Janowitz D, Grabe HJ, Ruhrmann S, Ettelt S, Buhtz F, Hochrein A, et al. Early onset of obsessive-compulsive disorder and associated comorbidity. Depress Anxiety. 2009;26(11):1012-7.

193. Mahjani B, De Rubeis S, Gustavsson Mahjani C, Mulhern M, Xu X, Klei L, et al. Prevalence and phenotypic impact of rare potentially damaging variants in autism spectrum disorder. Mol Autism. 2021;12(1):65.

194. Havdahl A, Niarchou M, Starnawska A, Uddin M, van der Merwe C, Warrier V. Genetic contributions to autism spectrum disorder. Psychol Med. 2021;51(13):2260-73.

195. Kvarnung M, Nordgren A. Intellectual Disability & Rare Disorders: A Diagnostic Challenge. Adv Exp Med Biol. 2017;1031:39-54.

196. Fu JM, Satterstrom FK, Peng M, Brand H, Collins RL, Dong S, et al. Rare coding variation provides insight into the genetic architecture and phenotypic context of autism. Nat Genet. 2022;54(9):1320-31.

197. Grassi G, Cecchelli C, Mazzocato G, Vignozzi L. Early onset obsessive-compulsive disorder: the biological and clinical phenotype. CNS Spectr. 2021:1-7.

198. Bar C, Kuchenbuch M, Barcia G, Schneider A, Jennesson M, Le Guyader G, et al. Developmental and epilepsy spectrum of KCNB1 encephalopathy with long-term outcome. Epilepsia. 2020;61(11):2461-73.

199. Bar C, Breuillard D, Kuchenbuch M, Jennesson M, Le Guyader G, Isnard H, et al. Adaptive behavior and psychiatric comorbidities in KCNB1 encephalopathy. Epilepsy Behav. 2022;126:108471.

200. Lappalainen T, MacArthur DG. From variant to function in human disease genetics. Science. 2021;373(6562):1464-8.

201. Ruan H, Wang Y, Li Z, Tong G, Wang Z. A Systematic Review of Treatment Outcome Predictors in Deep Brain Stimulation for Refractory Obsessive-Compulsive Disorder. Brain Sci. 2022;12(7).

202. Kashyap H, Abramovitch A. Neuropsychological Research in Obsessive-Compulsive Disorder: Current Status and Future Directions. Front Psychiatry. 2021;12:721601.

203. Gao B, Chi L, Zhu Y, Shi X, Tu P, Li B, et al. An Introduction to Next Generation Sequencing Bioinformatic Analysis in Gut Microbiome Studies. Biomolecules. 2021;11(4).

204. Nearing JT, Douglas GM, Hayes MG, MacDonald J, Desai DK, Allward N, et al. Microbiome differential abundance methods produce different results across 38 datasets. Nat Commun. 2022;13(1):342.

205. Clements CC, Karlsson R, Lu Y, Juréus A, Rück C, Andersson E, et al. Genome-wide association study of patients with a severe major depressive episode treated with electroconvulsive therapy. Mol Psychiatry. 2021;26(6):2429-39.

206. Meltzer-Brody S, Colquhoun H, Riesenberg R, Epperson CN, Deligiannidis KM, Rubinow DR, et al. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet. 2018;392(10152):1058-70.