EXPERT REVIEWS

Endophenotypes of obsessive–compulsive disorder: rationale, evidence and future potential

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Samuel R Chamberlain† and Lara Menzies

†Author for correspondence
Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK
Tel.: +44 122 376 7040
Fax: +44 122 333 6968
srchamb@gmail.com

Obsessive–compulsive disorder (OCD) is a heritable and debilitating neuropsychiatric condition. Attempts to delineate genetic contributions have met with limited success, and there is an ongoing search for intermediate trait or vulnerability markers rooted in the neurosciences. Such markers would be valuable for detecting people at risk of developing the condition, clarifying etiological factors and targeting novel treatments. This review begins with brief coverage of the epidemiology of OCD, and presents a hierarchical model of the condition. The advantages of neuropsychological assessment and neuroimaging as objective measures of brain integrity and function are discussed. We describe the concept of endophenotypes and examples of their successful use in medicine and psychiatry. Key areas of focus in the search for OCD endophenotypes are identified, such as measures of inhibitory control and probes of the integrity of orbitofrontal and posterior parietal cortices. Finally, we discuss exciting findings in unaffected first-degree relatives of patients with OCD that have led to the identification of several candidate endophenotypes of the disorder, with important implications for neurobiological understanding and treatment of this and related conditions.

Keywords: endophenotype • functional • human • imaging • inhibition • intermediate • MRI • neuroimaging • obsessive–compulsive disorder • OCD • phenotype • structural

Obsessive–compulsive disorder (OCD) is a prevalent and debilitating neuropsychiatric condition, with the lifetime prevalence estimated to be 2–3% worldwide [1,2]. Diagnostic criteria, according to the Diagnostic and Statistical Manual Fourth Edition (DSM-IV) [3], include the presence of obsessions and/or compulsions that are time consuming and lead to significant functional impairment. Obsessions are recurrent intrusive thoughts that enter into the stream of consciousness and tend to be unpleasant, distressing and difficult to suppress; compulsions are rituals (physical or mental) performed either in response to obsessions or according to rigid rules. Most patients with OCD exhibit multiple types of obsession and compulsion, with analysis of factor structures suggesting the existence of at least four factor dimensions [4–7]. In a recent meta-analysis of the available studies, a four-factor solution was identified: symmetry/ordering, forbidden thoughts, cleaning and hoarding [8]. OCD accounts for a substantial social and economic burden, for example, the economic cost is thought to be over US$8 billion per year in the USA [9]. OCD affects a similar proportion of males and females, tends to initiate in late childhood or early adolescence, and often follows a chronic relapsing–remitting course into adulthood.

Obsessive–compulsive disorder is a moderately heritable condition, although relatively little is known of the etiology [10]. Concordance rates in monozygotic twins with OCD have been historically reported to be 60–90% [11,12]. However, a recent twin study estimated genetic influences over obsessive–compulsive behaviors to be approximately 55% [13], and a thorough review of the OCD twin-study literature indicated a heritability of obsessive–compulsive symptoms of between 45 and 65% in affected children [14]. In adults, a large population-based twin study of 5983 twins and 1304 additional siblings calculated the heritability of obsessive–compulsive behaviors to be 47% [15]. First-degree relatives of patients exhibit an increased risk of also developing clinically significant symptoms compared with the background population [16–19], estimated to be 8.2% compared with 2% in control relatives [18] and reviewed comprehensively by Pauls [20]. Early linkage studies

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suggested a locus on chromosome 9 [21,22]; however, this was not replicated in the largest genome-wide study to date, which instead implicated susceptibility loci on chromosomes 1, 3, 6, 7 and 15 [23]. Interestingly, a very recent study identified a gender-specific suggestive linkage to 11p15 in families with male OCD probands. Candidate genes have also been investigated in OCD (for a review see [24]), for example, the serotonin transporter polymorphism [25] and others, such as brain-derived neurotrophic factor (BDNF) [26] and the glutamate transporter SLC1A1 [27–29]. With the exception of the latter of these, findings have not been consistently replicated and the precise genes conferring vulnerability to the development of OCD and related conditions remain unclear [30]. These replication failures could perhaps be overcome by identifying subclinical, objective biomarkers, for example, from neuroimaging, that are associated with an increased genetic risk for OCD and are more directly regulated by genetic effects than the overt clinical syndrome. This concept is expanded below.

A hierarchical model of OCD

Understanding the pathophysiology of complex neuropsychiatric disorders, such as OCD, is a major challenge for neuroscience today. Unlike disorders with a simple Mendelian genetic basis, for example, autosomal recessive disorders such as cystic fibrosis, we do not have an understanding of the genetic etiology of polygenic disorders, such as OCD, or the importance of interplay between genes and the environment in this etiology.

With the recent advent of genetic technologies, it has become feasible to investigate the role of multiple genes in predisposition to disease. This has led to the formulation of hierarchical models of disease pathology, which aim to understand mechanisms of disease in terms of sequential levels, originating from a basis of the underlying genetic make-up of an individual. To elaborate further, variation in an individual's genotype can modulate protein structure, which can influence protein function and, therefore, the function of cells in which those proteins are expressed. Different cell types act in synergy to produce system-level effects involving many cells within tissues and organs, which then impact upon the exogenous behavior and activity of an organism, and may lead to a clinical diagnosis (or phenotype) (Figure 1). This model can be considered to be hierarchical, with 'knock-on' effects occurring throughout this cascade such that effects at different levels interact and interplay with each other. Environmental factors could also affect disease pathology at all levels of this hierarchy. This theoretical model is likely to be helpful in considering the physiology of an organism in both health and disease, and could facilitate our understanding of pathology in OCD.

Such a model is relatively simple for a Mendelian genetic disorder. For example, in the case of cystic fibrosis, a defect in both copies of a single gene on chromosome 7, which encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein [31], leads to an nonfunctional form of the chloride channel formed by the protein [32]. On a cellular level, this leads to deranged ion transport across cell membranes, and electrolyte and fluid imbalances. At a systems level, the consequence of such effects is to cause a build-up of mucus in many organs, including the lungs and pancreas, which leads to respiratory obstruction, subsequent pulmonary inflammation and frequent respiratory infections, as well as pancreatic insufficiency [33,34]. An understanding of the systems-level effects of cystic fibrosis, and how best to measure these effects, is anticipated to be of use in developing and validating new drugs designed to prevent the onset of chronic lung disease in cystic fibrosis [35]. It is hoped that this will subsume the less effective treatments currently available, which can only alleviate resulting symptoms, for example, physiotherapy to mobilize mucus plugs and antibiotics to treat the secondary lung infections that frequently occur in affected individuals. Thus, visualization of cystic fibrosis in terms of the aforementioned framework (Figure 1) is of potential benefit in improving our understanding and treatment of the disease.

For OCD, the application of such a model is more complex, since rather than considering single gene effects on the function of proteins, cells and systems, here multiple genes could have many effects at different levels. It is a combination of these genetic factors, together with environmental influences, that ultimately leads to the clinical phenotype that is the presence of the disorder. For example, certain predisposing genes may have measurable effects at cellular or system-levels, but the presence of such implicated genotypes may not necessarily always lead to the clinical phenotype in an individual because this is also affected by the presence of additional genetic variation elsewhere in the genome and environmental stressors.

The notion arises, therefore, that the identification of meaningful measurements at a cellular or systems level, that may represent markers of genetic risk for a disease may be of considerable

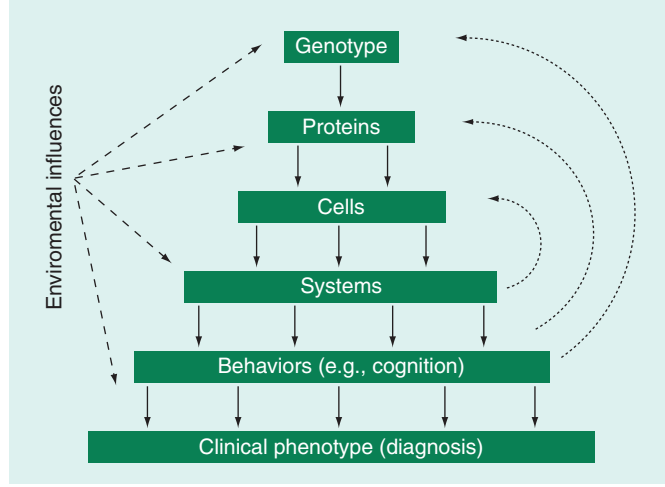

Figure 1. A hierarchical model of levels at which disease pathology may occur. This hypothetical model shows the intermediate levels at which disease pathology may be manifested. Environmental factors may also impact at different levels (dashed arrows). There may also be reciprocal, feedback relationships between levels (dotted arrows). MRI may be a useful tool for investigating the interplay between these levels by mapping brain function at the systems level during modulation by other levels, facilitating our understanding of how each level is involved in ultimately determining a clinical phenotype or diagnosis.

benefit in more precisely defining the full etiology of a disease phenotype, and in developing new targets for treatment strategies. This may be of particular benefit in considering brain-based disorders, such as OCD, due to the complexity of gene expression in the brain and the likelihood that many genes can potentially contribute to disorders affecting brain function [36]. It is now established that OCD is an at least partly heritable disorder with complex polygenetic origins; however, the details of this genetic basis are not yet well understood [30]. Given also that diagnosis of such disorders is currently only categorical and based on (expert) subjective assessment of whether an individual meets a set of clinical criteria, for example the DSM, the identification of more objective measures that contribute to the emergence of a clinical phenotype would be of considerable value in refining diagnosis.

Tools for identifying markers of genetic risk

Precise and objective measurement of brain integrity and function, at a systems level, can be obtained using objective neuropsychological assessment and neuroimaging, for example, MRI. Neuropsychological assessment, using tests such as those in the computerized validated Cambridge Neuropsychological Test Automated Battery (CANTAB), has also been valuable [37–40]. Many of these tests have been adapted in translational models enabling the neural and neurochemical substrates of task performance to be elucidated [37,39]. Furthermore, they can be readily applied consistently across different study sites and are convenient – requiring only a quiet room and standard computer.

Neuroimaging, by contrast, is more costly and available at fewer sites, but on the other hand is likely to be more sensitive to underlying brain changes than measurement of top-level cognition alone. Some of the major advantages of MRI in comparison with other imaging techniques include its minimal invasiveness, lack of radioactivity, the potential for repetitive scanning over time and its widespread availability in hospitals and research centers around the world. Other imaging modalities, such as PET, are limited by some of these aspects. In addition to being objective and sensitive, MRI is a particularly valuable tool for neuropsychiatric research because it is potentially a very rich measure. It can provide a large amount of precise and multidimensional information covering functional and structural changes within gray and white matter over both space and time. MRI measures can also be correlated and associated with behavioral, cognitive or clinical measures to provide further information concerning how system-level variation can affect more distal behavioral and clinical phenotypes.

Neuropsychological and MRI measurements therefore allow one to gain a unique insight into the systems-level function and organization of the brain, and permit investigations to determine if pathology at this level is heritable and how it may impact upon clinical phenotypes. These approaches can provide an informative bridge between genetic-, protein- and behavioral-based effects, and an insight into the relationship between these levels of pathology at vastly different scales in neuropsychiatric disorders.

Endophenotypes

The intervening intermediate phenotypes within the model of disease pathology in Figure 1, lying between genotype and phenotype, may be examples of endophenotypes or markers of increased genetic risk for a disorder. Neuroimaging measures of the structure and function of brain systems are thought to be ideal candidates for such markers of genetic predisposition, due to accumulating evidence supporting their strong heritability [41–43].

Endophenotypes, a term first coined in the 1960s, were defined as "measurable components unseen by the unaided eye on the pathway between disease (phenotype) and distal genotype" [44–46]. This concept has recently attracted considerable scientific interest, particularly in fields such as neuropsychiatry, where disorders may well have an etiology combining polygenic and environmental interactions. This is probably due to frustratingly fruitless attempts to directly correlate (subjectively assessed) clinical characteristics with genetic polymorphisms; it is hoped that endophenotypes will provide a new approach with improved power for gene identification for such disorders [12,47]. The definition of endophenotypes has subsequently evolved; it is generally understood today to be a heritable quantitative trait associated with increased genetic risk for a disorder and, therefore, present in both patients with the disorder in question and their clinically unaffected relatives [48,49].

Importantly, the presence of the endophenotype does not necessarily result in the emergence of the disease phenotype itself. This notion of endophenotypes as heritable 'risk factors' was derived partly from a multifactorial threshold model of complex genetic disorders, which assumes that many factors (genetic and environmental) contribute to a disorder. The effects of each factor are small but do accumulate, and once these effects reach a critical value, the clinical phenotype is precipitated. Endophenotypes have therefore previously been termed 'vulnerability markers' and hypothesized to occur at an increased rate compared with that in the general population, not only in patients with a given disorder but also in their close relatives.

When studying complex polygenic disorders, it must be appreciated that epigenetic and pleiotropic mechanisms may also be at work. Epigenetic processes affect the phenotype without changing the underlying DNA sequence and can include imprinting, X chromosome inactivation, DNA methylation and chromatin remodeling. The role of epigenetics has been considered particularly in schizophrenia to date [50] but may also be important in disorders such as OCD. Pleiotropy refers to the ability of a single gene to have multiple, differential effects in different cell types over time, meaning that a single genetic variation can have wide-reaching and variable effects across an organism and lead to multiple phenotypic changes. In particular, transcription factor mutations can lead to a variety of different effects across different cell types through their complex roles in gene regulation and determining gene expression. It has, therefore, been proposed that pleiotropic transcription factor mutations could account for heterogeneous symptoms of neuropsychiatric disorders, such as schizophrenia. For example, the basic helix–loop–helix transcription factor, neurogenin 1, has been implicated in increasing risk

for schizophrenia by impacting on several aspects of the phenotype, including total cerebral gray matter volume, temporal gray matter volume and cognitive deficits [51].

Endophenotypes may be important in studying complex, polygenic disorders, since they represent a deconstruction of the behavioral phenotype into biologically simpler measures that lie hypothetically closer in the chain of causality to the genes underlying the disease. For example, an endophenotype for a complex brain disorder, such as OCD, could be quantative and symptom derived, neurophysiological, biochemical, endocrinological, neuroanatomical or cognitive in nature, and so may exist at a level more proximal to our genes than complex behaviors. We discuss the identification of such candidate endophenotypes for OCD in neurocognitive and neuroanatomical domains later in the text. The identification of heritable quantitative traits could be used to facilitate the identification of susceptibility genes, for example using a linkage or quantitative trait loci (QTL) approach. This would be predicted to render these genetic techniques with increased statistical power compared with the use of more distal and complex behavioral phenotypes. While the validity of increased genetic tractability for endophenotypes over behavioral and clinical exophenotypes has been disputed by some [52], the validation of objective, reliable quantitative traits would at least improve diagnostic precision, reducing the inherent heterogeneity that may subtend a clinical diagnosis and therefore should, be advantageous in the genetic deconstruction of a disorder.

Utility of endophenotypes

The endophenotype strategy has been demonstrated in non-neuropsychiatric disorders. For example, genes predisposing to cardiac clinical syndromes, such as syncope, ventricular arrhythmia and sudden cardiac death, have been identified using quantitative variation in the electrocardiographic QT interval as an endophenotype [53–55]. An elongated QT interval was found to occur in both affected individuals and their asymptomatic close relatives. Using this quantitative trait, measured by ECG, linkage and association studies identified a susceptibility locus for cardiac arrhythmias at chromosome 11p15.5. Initially, the actual gene involved in long QT (LQT) syndrome at this locus was thought to be the Harvey-ras 1 gene; however, subsequent work then suggested that the LQT locus was more centromeric than previously thought [56] and the *LQT1* gene was later identified to be *KVLQT1*, a gene that codes for a voltage-gated potassium channel [57]. The use of an endophenotype measured using ECG was instrumental in initiating searches for the *LQT1* gene in this region of chromosome 11. Additionally, genes predisposing to asthma have been identified using serum levels of IgE as an intermediate phenotype; the heritable quantitative trait of serum IgE has been linked to chromosome 13 and both variation in IgE levels and occurrence of severe clinical asthma are associated with variation in a gene known as *PHF11* on this chromosome [58–60].

It has been postulated that endophenotypes may have a particular utility in understanding neuropsychiatric disorders. The identification of a well-defined etiology for complex brain disorders and an understanding of their genetic basis has met with only limited success to date, probably for several reasons. First, the inherent imprecision of categorical psychiatric diagnoses that rely on broad and relatively subjective criteria, for example, as set out in the DSM-IV, is a limiting factor, meaning that many individuals labeled with the same diagnosis are likely to be suffering from one of a heterogeneous group of disorders. Essentially, psychiatric diagnoses are currently based on subjective application of somewhat arbitrary criteria. It is conceivable and considered to be very likely that these broad clinical phenotypes subtend a great deal of heterogeneity, that is several, or even many, different genetic factors can impact differently upon different intervening levels within the hierarchical model of disease pathology, yet still be undistinguishable at the level of the clinical neuropsychiatric phenotype. Understanding of these intervening levels and the interplay between them may, therefore, aid diagnostic classification of such disorders on an objective, genetic basis. Second, the immense complexity of the brain compared with other organs in terms of the variety of cell types, their wide range of biochemical and molecular profiles (approximately 16,000 genes out of our total genome of 30,000 are expressed in the brain) [36], their connections to numerous other cells and the potential for these factors to be modified by an individual's prior experiences via plasticity mechanisms perhaps suggests that disorders affecting the brain might well present a substantial challenge for research [48]. It may be logically inferred that the endophenotype strategy, which identifies more tractable measures of brain function upon which the genetic effects may be more easily understood, might be more telling than the use of behavioral observations that has predominated to date.

One problem relating to the utility of endophenotypes is that in order to maximize their value, endophenotypes should be more highly heritable than the clinical disorder (phenotype). A recent study considering endophenotypes of schizophrenia estimates the heritability of 12 potential endophenotypes to be between 24 and 55%, yet estimates of the heritability of schizophrenia have been reported as high as 80% [61], suggesting that this may not always be the case. However, as discussed below, in the search for genes predisposing to alcoholism, it has been found that some electrophysiological endophenotypes obtained from EEG are more highly heritable than the alcohol-dependence diagnosis itself, supporting use of the endophenotype strategy [62]. There is also encouraging evidence that neuroimaging measures are also highly heritable [41–43]. Since little is known currently concerning the heritability of endophenotypes of OCD, it will be important to assess this before immediately assuming that use of endophenotypes in genetic association studies will be more enlightening than those using the clinical phenotype.

In summary, endophenotypes could have a particularly valuable role to play in neurology and psychiatry research where much is still unknown concerning the pathway between genes and behavior, although much work is still required in order to fully validate them. Endophenotypes may reduce the need to use subjective clinical and behavioral phenotypes, which probably encompass a considerable degree of heterogeneity. They may be of benefit in allowing the identification of predisposing genes for disorders

by facilitating studies using gene association, linkage and QTL mapping, clarifying disease classification and diagnosis, and enabling the development of more tractable animal models for diseases where the phenotype is strongly 'human' in quality and so difficult to study in animals. Endophenotypes of a brain-based nature, for example, measurements from neuroimaging may be especially useful in identifying genes involved in neuropsychiatric, disorders. As proof of concept for a role for brain-based measures in gene identification, this gene mapping technique has already been used to relate measures of cortical and noncortical volumes in mice to chromosome regions where genetic variation affects this brain-based measure [63]. Identification of such complex trait loci can then be used to pinpoint genes within these chromosomal regions that may affect this phenotype. This is of benefit since it can lead to the formation of an *a priori* hypothesis for the investigation of single nucleotide polymorphisms (SNPs) in these genes that may be responsible for phenotypic variation.

Examples of endophenotypes in neuropsychiatry to date

Since Gottesman and Shields invoked the endophenotype concept to consider the genetic basis of schizophrenia in 1973 [46], the concept has lain largely dormant in neuropsychiatry until relatively recently. Now, 30 years later, with the advent of neuroimaging techniques, batteries of objective cognitive tests and advancing genetic technologies, there has been a resurgence of interest in identifying intermediate phenotypes and using these to improve our understanding of the genetic basis of neural disorders [49,64].

Two striking examples of the potential promise of endophenotypes in neuropsychiatry so far are considered below. First, a sensory gating abnormality known as a deficit in prepulse inhibition has been found in both schizophrenia patients and their unaffected first-degree relatives [65]. Prepulse inhibition is measured by EEG, using the P50 suppression test. Here, two auditory stimuli are presented to an individual 500 ms apart, and a positive event-related response to each is measured by EEG. In normal individuals, the neuronal response to the second stimulus is of lower amplitude than the first. The extent of this suppression is reduced in patients with schizophrenia and their relatives, suggesting that variation in this trait is heritable. Association studies using this quantitative trait identified a susceptibility locus on chromosome 15, in the α-7-subunit gene (*CHRNA7*) of the nicotinic cholinergic receptor [66]. A specific functional SNP was then identified in the promoter region of this gene that was associated with the deficit in prepulse inhibition. A preliminary study of a molecularly based treatment for schizophrenia using an α-7-agonist, DMXB-A, has now been performed, which, even in a small patient sample, led to a significant improvement in P50 inhibition together with improvements in cognition on a testing battery [67].

A second success story of endophenotypes leading to gene identification comes from the Collaborative study on the Genetics of Alcoholism (COGA) [62]. Here, the initial identification of electrophysiological endophenotypes (from EEG and event-related potentials [ERPs]) helped to identify two genes associated with alcohol dependence, *GABRA2* [68] and *CHRM2* [69], and the use of endophenotypes was advantageous compared with clinical diagnoses in terms of the strength and localization of the linkage signal.

Neurobiology of OCD

The neuropsychology and neurobiology of OCD have been reviewed in-depth elsewhere [70–78]. In this review, we focus selectively on research within our group that identified neurobiological abnormalities in patients with OCD and then subsequently in unaffected first-degree relatives of patients.

Evidence from cognitive studies

The last decade has seen a preponderance of studies that have sought to address whether or not patients with OCD exhibit cognitive deficits across a variety of domains (for in-depth reviews see [10,75,79]). The findings are somewhat heterogeneous, which probably reflects variability in task sensitivity, failure to control for depressive mood, comorbidities and medication history. Domains of interest have included action monitoring and memory (since the repetitive symptoms manifested in OCD could theoretically stem from deficits in action monitoring or memory); motor inhibitory control (since repetitive symptoms could reflect top-down failures in inhibition); flexibility (since compulsions are often performed according to rigid rules); and decision-making/reversal of responses (since these functions are thought to be dependent on orbitofrontal integrity, see later discussion of neural dysfunction).

Few studies have investigated cognition in OCD patients who were free from axis-I comorbidities. Comorbidities represent an important potential confounder in cognitive studies, since, for example, depression has been linked to cognitive deficits in itself. The following section focuses on studies conducted in patients with archetypal washing/checking OCD symptoms who had no axis-I comorbidities according to self-report regarding lifetime history of diagnoses and screening with the Mini International Neuropsychiatric Inventory (MINI) [80].

In one study, we measured motor inhibition and cognitive flexibility (set-shifting) in 20 OCD patients, 17 patients with trichotillomania (an impulse control disorder characterized by repetitive hairpulling) and 20 healthy controls [81]. We focus on this study here, since it formed the basis of follow-up endophenotype work. Inhibition was measured with the stop-signal task, which is dependent on the integrity of the right inferior frontal gyrus and noradrenergic neurotransmission [39,82–85]. On this task, volunteers observe a series of directional arrows on-screen, and make speeded motor responses (left or right button) depending on the direction of each arrow. On a subset of trials, the computer generates a stop-signal (i.e., auditory tone) some variable time after presentation of the go stimulus, and volunteers attempt to suppress their response. By varying the time between the go and stop stimuli, this task uses a highly sensitive algorithm to estimate the time taken for the brain to stop responses (referred to as the 'stop-signal reaction time'). Cognitive flexibility was measured with the set-shift task from the CANTAB, which is dependent on dorsolateral circuitry.

This task decomposes different components of flexibility, including the ability to shift attention away from a previously relevant stimulus dimension on to a dimension that was never previously relevant (extradimensional [ED] set-shift). OCD patients showed impaired stop-signal reaction times and ED set-shifting, whereas patients with trichotillomania showed deficits only in terms of stop-signal reaction times. Thus, OCD appeared to be characterized by inhibitory dyscontrol spanning not only the motor but also attentional domain, suggestive of dysregulation of both inferio–frontal and more dorso–lateral circuitry.

Evidence from neuroimaging

Neuroimaging studies of OCD were first performed in the late 1980s. These functional metabolic studies, using PET to predominantly investigate glucose utilization across the brain either at rest or following symptom provocation, suggested that people with OCD had increased metabolic activity in bilateral orbitofrontal cortex (OFC) and in striatal regions, such as the head of the caudate [86–93]. It is worth noting that the PET OFC findings can be considered to be relatively robust, whereas findings relating to striatal metabolism have been more variable [78,94].

Findings from PET led to the development of an orbitofronto–striatal circuit hypothesis that built upon a framework from animal studies of the existence of relatively segregated fronto–striatal circuits [95,96] between different frontal cortical regions and particular parts of the basal ganglia (Figure 2). It was the affective, orbitofrontal–striatal circuit that was purported to be relevant to OCD [10,97].

On the basis of this metabolically derived hypothesis, there have been many structural neuroimaging studies of OCD. Initially, many of these studies were region-of-interest (ROI) based, and on the basis of the *a priori* OFC–striatal hypothesis, a small number of regions for assessment of volume in case–control studies of OCD were selected. There are several potential limitations of this study design. First, the restriction to only a few brain regions may mean that other critical brain regions are overlooked. Second, the lack of definitively agreed anatomical boundaries for specific brain regions means that there are differences between the anatomical landmarks used to define brain regions, making it difficult to authoritatively compare across studies. Third, the manual and technically laborious nature of the ROI technique means that only small samples are typically considered feasible for analysis. Finally, even when authors assess the volume of a large number of ROIs, efforts are not always made to correct for the multiple comparisons entailed.

Despite these potential limitations of the ROI technique, it is striking that a number of studies have reported a reduction in volume of the OFC in OCD [98–102]. The results concerning striatal regions are again less consistent [94], but there are several findings of structural abnormalities in the striatum in patients with OCD compared with healthy controls [103–106].

A recently available and powerful imaging analysis technique is that of computational morphometry. This automated analysis method permits an unbiased, objective analysis of structural differences across the whole brain, without the need for a potentially restrictive *a priori* hypothesis, and for which appropriate corrections for multiple comparisons can be applied. There are four studies reporting voxel-based univariate analysis of brain structure differences in OCD [107–110].

The findings from these whole-brain studies of OCD give some support for the OFC–striatal model; there is evidence of reduced gray matter in the OFC in OCD [107,109,110]. Pujol *et al.* also reported bilaterally increased striatal gray matter in patients compared with controls [109]. However, it is of interest that all four studies found evidence of gray matter abnormalities in OCD patients in a distributed set of regions, by no means exclusively limited to the OFC–striatal circuit. For example, both Kim *et al.* [108] and Valente *et al.* [107] report parietal abnormalities in patients – a region that had not particularly been focused upon in OCD prior to these studies; while van den Heuvel *et al.* found decreased gray matter in the left dorsolateral prefrontal cortex, left inferior frontal cortex and bilateral medial prefrontal cortex, as well as the left OFC [110]. The aforementioned studies not only

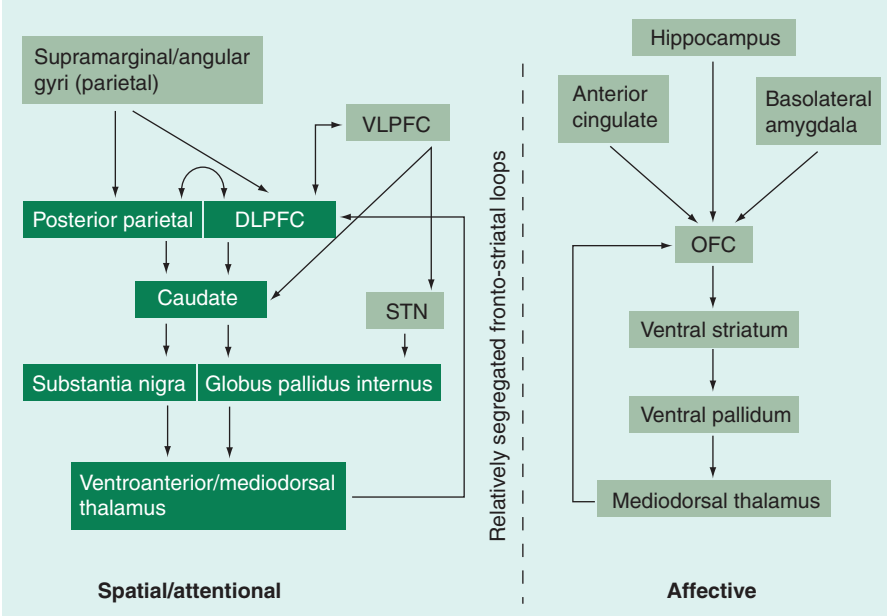

Figure 2. Fronto–striatal loop circuitry salient to obsessive compulsive disorder.
Traditionally, obsessive compulsive disorder was thought to be characterized by changes in the affective loop (right) involving the orbitofrontal cortex. More recently, other circuitry has also been implicated in the pathophysiology, notably (left) the spatial loop involving the dorsolateral and posterior parietal cortices.
DLPFC: Dorsolateral prefrontal cortex; OFC: Orbitofrontal cortex; STN: Subthalamic nucleus; VLPFC: Ventrolateral prefrontal cortex.
Reproduced with permission from Elsevier Limited [115].

explored overall structural abnormalities in the OCD patients as a whole, but also the relationship between neuroimaging abnormalities and the expression of different symptom dimensions. For example, in the cardinal study of van den Heuvel *et al.*, symmetry/ordering was associated with lower global gray and white matter volumes; contamination/washing with lower gray matter volumes in the bilateral caudate; harm/checking with lower bilateral temporal lobe gray matter; and symmetry/ordering with lower right motor cortex, left insula and left parietal cortex gray matter volume [110]. These findings highlight the heterogenous nature of OCD and raise the possibility of distinct neural abnormalities relating to different symptom dimensions.

While some of these findings could represent confounds relating to small sample sizes, use of medications or comorbidity with other psychiatric illness apart from OCD in patients, for example, major depressive disorder, they are at least suggestive of the notion that OCD results from abnormalities in large-scale distributed neural systems. This proposal of systems-level dysfunction in OCD is also supported by functional MRI studies that, using a variety of symptom provocation and cognitive (e.g., working memory, response inhibition and attentional) paradigms, have again suggested large-scale neurocognitive abnormalities in OCD. For example, in addition to the OFC–striatal dysfunction suggested by a reversal learning study [111], studies of planning and response inhibition [112,113] have also implicated another of the fronto–striatal circuits, namely the dorsolateral prefrontal loop that comprises the dorsolateral prefrontal cortex, posterior parietal cortex, thalamus, caudate and other basal ganglia regions. Functional MRI case–control studies of OCD were recently subjected to a meta-analysis using a technique known as activation likelihoood estimation and this again showed dysfunction across distributed brain regions [114,115].

In response to these findings, newer imaging studies of OCD have employed a multivariate, or multivoxel, analysis approach, designed to capture brain dysfunction at an optimized level for detecting system-level changes. For example, Soriano-Mas *et al.* used such a technique to study structural changes in gray matter across the whole brain, and found evidence of gray matter decreases in a system of regions, including the medial prefrontal, posterior cingulate, insula cortices and cerebellum, and gray matter increases within bilateral ventral striatum, posterior thalamus, cerebellum and medial midbrain [116]. Remarkably, this study was also able to use imaging data on an individual subject level to predict whether that subject was a patient with OCD or a healthy control. Additionally, a recent study using PET to investigate brain differences in OCD tested both a univariate and multivariate approach, and concluded that a multivariate approach was more suitable for characterizing brain dysfunction in OCD [117]. Finally we have recently employed a multivariate analysis technique, known as partial least squares [118,119], to identify abnormalities in brain structure at a systems level in both patients with OCD and their healthy, unaffected relatives [120]. Since this research describes an endophenotype of OCD, it will be discussed further in the next section.

Endophenotypes of OCD

The first direct step towards identification of an endophenotype is to investigate whether cognitive and neural abnormalities detected in patients with the given condition also occur in people at increased risk of developing the condition irrespective of symptom manifestation. Endophenotypes should by definition exist in unaffected relatives of patients, even in the absence of clinical phenotype (top-level symptoms) [48,121,122]. Given the potential of neurocognitive and neuroimaging indices as endophenotypes of OCD that may facilitate identification of genes predisposing to the disorder, we have begun investigating whether such endophenotypes exist. We have employed a paired relative-proband design involving patients with OCD, their unaffected and healthy first-degree relatives (preferably same-sex sibling of similar age), and a third group of unrelated healthy control volunteers with no known family history of the disorder. The aim of this design is to permit the assessment of familial and perhaps genetic factors contributing to OCD, as outlined in Figure 3.

Cognitive

Two behavioral neurocognitive studies have been conducted to

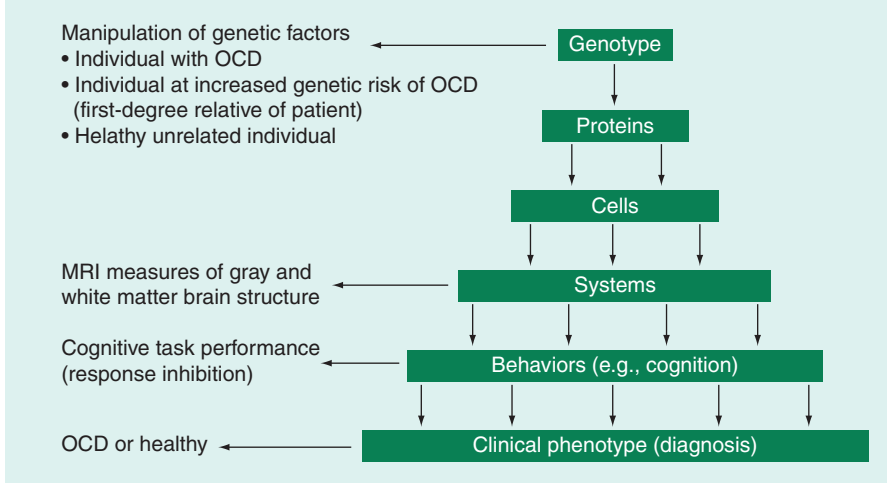

Figure 3. Investigating the effects of genetic factors on brain systems and cognition using an endophenotype design. The rationale for using an endophenotype design to investigate whether individuals with 'at-risk genotypes' for OCD (affected patients and their unaffected first-degree relatives) show commonalities at the brain systems level, in contrast to healthy volunteers. Such systems-level measures may therefore represent markers of increased genetic risk for OCD (endophenotypes), but not necessarily always result in the clinical phenotype. Identification of endophenotypes may aid diagnostic classification of OCD and facilitate studies to identify underlying risk genes for the disorder.
OCD: Obsessive compulsive disorder.

date that sought to address whether cognitive deficits in OCD also exist in unaffected relatives versus control subjects with no family history of the condition. The first [123] followed-up the aforementioned study [81], which identified response inhibition and cognitive flexibility deficits in patients with OCD compared with healthy controls. Patients with archetypal OCD (without comorbidities) were recruited, and each patient participated with a first-degree relative (by preference a similarly aged same-sex sibling) who had never experienced clinical OCD and was free from current axis-I disorders. Matched healthy controls were enrolled, who did not have a known family history of OCD. The sample size was 20 participants per group. Both OCD patients and their unaffected relatives showed impaired response inhibition and ED set-shifting versus controls. Indeed, relatives did not differ significantly from patients in the magnitude of impairment. These data suggest that impaired motor inhibitory control and attentional flexibility exist in the absence of clinically significant symptoms and medication confounds in people at familial risk of the condition.

The other available behavioral study was conducted in unaffected first-degree relatives of patients with OCD, unaffected first-degree relatives of patients with autism and healthy controls (n = ~50 per group) [124]. The battery included executive planning (Tower of London task, akin to the Tower of Hanoi), verbal fluency, design fluency and trail-making tests. Relatives of patients with OCD and relatives of patients with autism exhibited executive planning impairment on the Tower of London task versus the controls.

Neuroimaging

Having observed the impairment of response inhibition on the stop-signal task in both patients and their relatives compared with healthy volunteers described previously, we used a larger sample (n = 31 per group) to confirm that patients and relatives showed a deficit in response inhibition compared with healthy controls, and employed MRI to assess whether there were brain structural patterns related to these inhibitory impairments [120]. Using a multivoxel analysis approach (partial least squares) we combined data from the stop-signal task with neuroimaging data to identify large-scale structural brain systems in which variation in gray matter density was associated with performance variation on the stop-signal task. Poorer ability on the motor inhibition task (longer stop-signal reaction time) was associated with increased gray matter in middle and posterior cingulate, parietal and occipital cortices, and striatum, and with decreased gray matter in bilateral orbitofrontal, inferior frontal and temporal cortices (Figure 4). The strongest endophenotypic findings, which is between-group differences in gray matter, were located in orbitofrontal/inferior frontal cortex (significantly reduced gray matter in both patients and relatives compared with healthy controls) and within parieto-occipital cortex (significantly increased gray matter in patients and relatives, compared with controls). Interestingly, using two different analyses (within-pair correlation, between each patient and their relative, and a novel permutation test) we identified significant familial effects on the MRI measures but not the cognitive marker of SSRT. This aids the validation of brain-based measures as markers of risk for OCD and suggests that brain-system indices are more tightly regulated by familial (perhaps genetic) effects than cognitive measures of behavior and the clinical phenotype, providing optimism regarding the

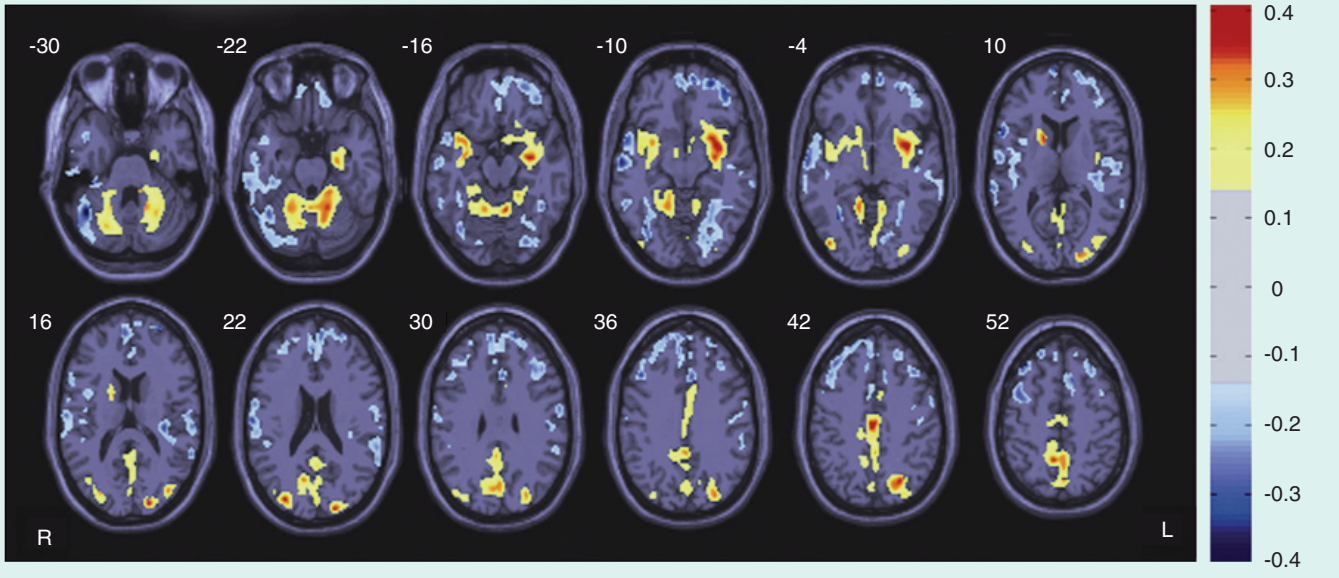

Figure 4. Brain maps of regions where brain gray matter density correlated with inhibitory control (stop-signal reaction times). Red/yellow: areas in which increased gray matter density was associated with prolonged SSRT (impaired response inhibition); blue regions: areas where decreased gray matter density is associated with prolonged SSRT. Color bar indicates strength of correlation between SSRT and gray matter density for each voxel; R and L markers indicate side of the brain, numbers denote the *z*-dimension of each slice in MNI space.
Reproduced with permission from [120].

accuracy of our hierarchical model of disease pathology (Figure 1) and the familiality of this endophenotype.

We have recently also found evidence for endophenotypes of OCD in white matter brain tissue, using diffusion tensor imaging (DTI), again using the same three-group design with patients, relatives and control volunteers (n = 30 per group) [125]. DTI measures the diffusion of water molecules, the extent and direction of which is dependent upon the medium in which it occurs. For example, diffusion can be isotropic where there are no cellular structures to restrict diffusion, for example, in cerebrospinal fluid (CSF), or the structures restricting diffusion occur in roughly the same amount in all directions, such as, in gray matter. By contrast, when tissue structure is highly directional, such as within white matter tracts, diffusion is found to be anisotropic. Fractional anisotropy (FA) can be calculated from DTI data and describes the extent of anisotropy (or differential diffusion of water in a particular orientation) within a given voxel. We identified a region of right parietal matter where FA was significantly reduced in both patients and their relatives compared with healthy controls, and a region of right medial frontal white matter where FA was significantly increased in both patients and relatives compared with controls. It is of interest that these findings of white matter differences are located close to regions previously found to be structurally and functionally abnormal in OCD; for example, there are a considerable number of previous findings of parietal, cingulate and medial frontal abnormalities in OCD (for a review see [115]). These findings provide preliminary evidence that familial and perhaps genetic risk for OCD is mediated by a large-scale dysconnectivity, for example involving fronto-parietal regions.

On the basis that there are encouraging indications of endophenotypes of OCD relating to brain structure, it is interesting to consider whether intermediate phenotypes might also be evident at a functional neuroimaging level. These might be expected to show functional deficits in brain circuits thought to underlie OCD, such as involving orbitofrontal and parietal cortices. On the basis of what is known of the neuropsychology of OCD, functional probes of cognitive flexibility and inhibitory control processes are paramount in the search for functional imaging endophenotypes of the disorder [10,81,123]. Such functional abnormalities, likely to be more closely related to genetic effects than a distal clinical phenotype, if indeed evident in patients and their relatives, could be considered to predispose individuals to the onset of the disorder. We have used fMRI to assess whether patients and their relatives show deficits in such circuits during performance of a cognitive flexibility paradigm [126]. For the purpose of the imaging study, all participants were pretrained on the task in order to reduce the likelihood of performance differences that could be conceived as potential confounding factors when analyzing the imaging data. In a sample of 14 patients, 12 relatives and 13 healthy controls, we identified strikingly reduced activation in the orbitofrontal and posterior parietal cortex in both patients and relatives compared with controls, during the reversal learning element of the task, which is when subjects had to learn that their previously correct answer was now incorrect and reverse their responses accordingly [127]. This study provides further evidence that brain-based markers of large-scale dysfunction, in particular involving orbitofrontal and parietal regions may be of considerable value in identifying the genetic underpinnings of OCD.

Limitations: specificity and utility of OCD findings

It is important to question the specificity of the cognitive and imaging abnormalities detected in patients with OCD and their symptomatically unaffected relatives reported in the above studies from several points of view. First, nothing is known of the relationship between these candidate endophenotypes and predisposition towards different OCD symptom dimensions [4,128]. The above studies focused on patients with archetypal washing/checking symptoms without hoarding, and assumed OCD to be a somewhat unitary entity. As described earlier, there is evidence that distinct symptom dimensions are associated with different neural abnormalities [110]. Second, it is not yet known whether these markers are specific for OCD itself or rather would also exist in the context of other axis-I disorders. ADHD is highly heritable and stop-signal impairment has been posited as a candidate endophenotype for this condition [129]. With regard to studies by our group [120,125,127,130], recruits undertook clinical assessment using the MINI tool, which screened for several axis-I disorders but not ADHD. However, none of the OCD patients or relatives in these studies reported a diagnosis of ADHD when asked about lifetime incidence of psychiatric diagnoses. Furthermore, no lifetime diagnoses of ADHD were identified in OCD patients' notes. These studies did not measure subclinical Attention-deficit hyperactivity disorder (ADHD) symptomatology. Elsewhere it has been found that up to 30% of children/adolescents with OCD also exhibit ADHD and vice versa, with family study suggestive of overlapping etiology between these two disorders [131,132]. We hypothesize that cognitive and imaging abnormalities in patients with OCD and their relatives may also exist to some extent in patients with ADHD and their relatives, reflecting this overlapping presumed etiology. It will be important for future research to overcome these limitations in current knowledge regarding the specificity of candidate OCD endophenotypes.

It is also interesting to consider which endophenotypes might be of most use in aiding the search to elicit the genetic factors that increase the risk of developing OCD. We have presented evidence for the existence of cognitive, structural (in both gray and white matter) and functional endophenotypes of OCD. The ultimate tests of such endophenotypes will be to confirm their heritability and to assess whether they can be used successfully to identify specific genetic loci in which variation contributes to genetic risk of developing OCD. Further work is required in order to provide an answer to this. However, of interest, in accordance with our model of disease pathology (Figure 1) suggesting that structural brain measures are likely to be more directly regulated by genotype than cognitive measures, we found that brain structure measures were more directly related to familiality than cognitive markers, suggesting that this model does have some validity. Of course, the utility of endophenotypes of different domains is also related to the cost of obtaining such measures and their availability;

the limitations of neuroimaging compared with neuropsychological testing in terms of cost and accessibility have already been alluded to earlier.

Expert commentary

As can be seen, the concept of endophenotypes – despite being proposed some 50 years ago – has received scant application in the context of psychiatry. It has taken time for technology to catch up with the concept via the development of objective and validated brain-based markers. Preliminary inroads have been made into the identification and validation of objective endophenotypes for OCD using measures of orbitofrontal circuit function. It will be important for subsequent studies to replicate these findings and expand upon them. For example, dorsolateral prefrontal dysfunction has been identified during executive planning in OCD patients [112] and it will be important to examine wheather similar deficits exist in unaffected relatives; and indeed the relationship between dysfunction in the OFC and dorsolateral prefrontal cortical circuits. That cognitive and brain (structural and functional) abnormalities exist in unaffected first-degree relatives of patients with OCD has profound implications. These findings suggest that some of the cognitive and brain changes previously associated with OCD were not attributable to the symptoms themselves or directly affiliated with them. Rather, they represent markers existing in those at increased genetic and/or familial risk, which may actually predispose to the illness. Cognitive deficits in clinically unaffected relatives, as well as patients, could interfere with everyday function and quality of life, and thus should be examined as novel treatment targets, for example, with cognitive enhancers and cognitive therapies. These findings could also account for discrepancies in the previous OCD cognitive and brain literature: not all studies have screened control groups for a family history of the illness. Relatively little is known regarding the interaction between clinical phenotypes, intermediate phenotypes (endophenotypes) and environmental factors, such as exposure to life stressors or learning of behaviors (such as rituals) between family members. Population-based twin studies have supported the existence of a broad OCD phenotype whose expression is influenced by both genes and environmental factors (36 and 64%, respectively) [133,134]. Limitations in a current understanding of the genetic and environmental factors and their interactions could be addressed in future by studying the evolution of ritualistic behaviors over time the and by conducting further twin studies. We need to address not only those factors predisposing to OCD, but also those mechanisms that trigger the phenotype.

Five-year view

Studies should use cognitive and MRI measures in conjunction with whole-genome scans and QTL mapping and investigate whether such measures can assist in the identification of people at risk of OCD in order to pre-empt symptoms and, ideally, obviate them with novel treatments (psychological and/or pharmacological). It will be important to identify not only similarities, but also differences in neural and cognitive function between patients and their relatives, since this would give insights into why some individuals at genetic/familial risk develop symptoms while others do not. Another key area will be to explore overlapping and distinct endophenotypes between axis-I disorders, since OCD may constitute part of a spectrum of conditions characterized by impaired inhibitory control and common risk factors [135–137]. We speculate that OCD may share (to some extent) overlapping endophenotypes with conditions such as trichotillomania and ADHD, which link with common genetic and environmental mediators.

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Key issues

- Obsessive–compulsive disorder (OCD) is a neuropsychiatric condition characterized by recurrent intrusive distressing thoughts and/or repetitive physical or mental rituals. It is debilitating and prevalent.
- Psychiatric conditions such as OCD can be conceptualized hierarchically, from underlying genetic–environmental factors, to neural circuitry and cognitive function, to the top level expression of symptoms.
- Endophenotypes represent objective markers manifested at some intermediate point along this hierarchy between disease and the underlying genetic–environmental diatheses.
- OCD is associated with abnormal function of neural circuitry involving the orbitofrontal cortex, posterior parietal cortex and basal ganglia.
- Patients with OCD and their unaffected relatives showed impaired inhibitory control on objectively measured tests. These deficits were related to brain gray matter structural abnormalities.
- The orbitofrontal cortex was underactivated in OCD patients and relatives during a cognitive probe in functional MRI flexibility paradigm.
- Preliminary evidence of white matter endophenotypes of OCD further suggests that this disorder is mediated by large-scale dysconnectivity, for example between frontal and parietal cortical areas.
- Further work is required to replicate and extend these findings, validate these measures fully as endophenotypes and use them to augment genetic studies of OCD.
- It is hoped that endophenotyping strategies will lead to early detection of those at risk and the optimization of disease classification and treatment algorithms.

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Affiliations

- Samuel R Chamberlain
Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK
and
Brain Mapping Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK
and
Queen Elizabeth II Hospital, Welwyn Garden City, UK
and
Behavioural and Clinical Neurosciences Institute, University of Cambridge, UK
Tel.: +44 122 376 7040
Fax: +44 122 333 6968
srchamb@gmail.com
- Lara Menzies
Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK
and
Brain Mapping Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK
and
Queen Elizabeth II Hospital, Welwyn Garden City, UK