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Endophenotypes in the genetic research of ADHD over the last decade: have they lived up to their expectations?

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“...research into candidate endophenotypes for attention-deficit/hyperactivity disorder lags somewhat behind, compared with similar studies in other psychiatric disorders...”

The endophenotype concept was introduced over 35 years ago in psychiatric research [1]. It was observed that, in complex disorders, multiple phenotypes could arise from the same genotype, as well as that multiple genotypes could give rise to the same phenotype. Thus, the direct link between genotype and phenotype is often only weak. Endophenotypes are proposed to intermediate between genotype and phenotype, and can be defined as ‘heritable, quantitative traits that index an individual’s liability to develop or manifest a given disease’ [2]. As such, it follows that the endophenotype is heritable (and familial), in which the same genes partly influence the endophenotype and phenotype. Furthermore, the endophenotype is associated with the disorder (i.e., present in affected individuals), but it also observable in nonaffected first-degree relatives of an affected individual, since first-degree relatives are likely to carry some of the susceptibility genes of the disorder [3]. Fundamental to the endophenotypic concept are the assumptions that the endophenotype is influenced by fewer genes than the phenotype and/or is less removed from relevant gene action than the phenotype, hence providing greater power for genetic analyses. Endophenotypes are not readily observable, unlike phenotypes, but require some sort of measurement. Candidate endophenotypes may be anatomical, neurochemical, neurophysiological or cognitive in nature.

The main objectives for including endophenotypes into genetic studies of psychiatric disorders are to unravel the

modes of action of known risk genes and to discover new risk genes for the disorder. Additional advantages of studying endophenotypes instead of/or in addition to phenotypes are that endophenotypes can often be more objectively (and therefore reliably) measured than phenotypes and provide more insight into the neurobiological underpinnings of the disorder (and are, thus, more useful for creating animal models) than phenotypes. Endophenotypes are more often quantifiable instead of dichotomous phenotypes based on the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV diagnostic categories (hence providing more statistical power).

Given these advantages, it is surprising that research into candidate endophenotypes for attention-deficit/hyperactivity disorder (ADHD) lags somewhat behind, compared with similar studies in other psychiatric disorders, such as schizophrenia, (manic) depression and alcoholism. Perhaps this may be related to the disappointing findings of the first couple of ADHD endophenotype studies. These studies focused on cognitive functioning and examined several basic characteristics of an endophenotype, namely whether both affected children and their nonaffected family members performed worse than controls on cognitive measures. One of the first studies that included (nonaffected) family members of ADHD patients was conducted by Murphy and Barkley [4]. They observed that parents of children with ADHD demonstrated no impairments on

laboratory measures of sustained attention, memory, cognitive flexibility, encoding and impulsivity, despite showing impairment in social and psychological functioning. Similar normal cognitive functioning was found in a large group of parents of ADHD children on tasks measuring set shifting, sustained attention and visual information processing [5]. These findings did not support the viability of cognitive functions as candidate endophenotypes for ADHD. However, given that symptoms of ADHD are predominantly present in childhood and often (strongly) improve during adolescence and adulthood, it may be more opportune to study endophenotypes in child siblings. One of the first studies that included nonaffected (nonreferred) siblings of children with ADHD was conducted by Seidman and colleagues [6]. Disappointingly, their findings also did not provide much confidence in finding cognitive ADHD endophenotypes. They assessed executive, attention and memory functions in the children, and observed that siblings without ADHD were similar to controls on virtually all measures. They concluded that 'neuropsychological deficits are unlikely to constitute an endophenotype to ADHD'. So far, not so good.

"...support was found for temporal processing and/or response variability as candidate endophenotypes for ADHD..."

Perhaps the wrong cognitive functions were studied in search for candidate ADHD endophenotypes? In one of the most influential papers on candidate cognitive endophenotypes of ADHD, it was proposed that ADHD research should focus on three prime endophenotypic candidates: 'a specific abnormality in reward-related circuitry that leads to shortened delay gradients, deficits in temporal processing that result in high intrasubject intertrial variability, and deficits in working memory' [3]. None of these functions were studied before as candidate endophenotypes. It was only until recently (2007), that abnormalities in reward-related circuitry were studied endophenotypically in a large sample of dizygotic twin pairs discordant for ADHD [7]. Motivation and delay aversion, two reward-related functions, were examined. Unexpectedly, both the group of affected children and the nonaffected siblings performed normally on the tasks, suggesting motivation and delay aversion were less suitable as endophenotypic candidates compared with the range of other functions examined in this study, such as executive functions, processing speed and response variability. More evidence for ADHD endophenotypic qualities has been gathered for temporal processing. In several studies, support was found for temporal processing and/or response variability as candidate endophenotypes for ADHD using widely different tasks [7-12]. Similarly, support was found for verbal and visuospatial working memory as candidate endophenotypes for ADHD [7,8,13]. It, therefore, appears that two of the three suggested prime candidate cognitive endophenotypes [3] are supported by research results, yet more research is needed to confirm/depute abnormalities in reward-related circuitry as ADHD endophenotypes.

Despite not being put forward as one of the three most likely ADHD endophenotypic candidates [3], inhibition (or interference control) has been given the most attention from researchers aiming to detect cognitive ADHD endophenotypes [7,13-18]. Consistently, inhibition or interference control fulfilled the investigated basic characteristics of an endophenotype, such as being impaired in affected and nonaffected family members, and correlating between siblings. In addition, the brain activation during inhibition was similarly altered in ADHD-affected children and their nonaffected siblings. Thus, as with deficits in temporal processing and working memory, impaired inhibition or interference control can be considered a prime cognitive endophenotype for ADHD.

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Compared with the growing knowledge on cognitive endophenotypes for ADHD, relatively little is known about neurophysiological and neuroimaging measures as candidate endophenotypes for ADHD. Undoubtedly, this is related to the higher time and financial costs of these measures compared with the quickly and cheaply administered cognitive measures. Nevertheless, these measures are most likely worth the investment of extra time and money. For example, in a recent report on EEG measures in ADHD-affected sibling pairs [19], it was shown that EEG measures correlated moderately between siblings during baseline conditions and significantly during cognitive activation. Furthermore, specific event-related potential components related to action monitoring and initial error processing were also found to be putative endophenotypes [20]. Neuroanatomical measures have also proved to be sensitive to familial ADHD effects. It was shown that nonaffected children had similar reductions in prefrontal and occipital gray and white matter as their affected siblings and also showed a trend towards having overall smaller brain volumes as did their affected siblings [21]. Furthermore, orbitofrontal volume loss in ADHD was determined by genetic effects in a sample of monozygotic twin pairs concordant for ADHD [22], suggesting structural MRI (sMRI) measures have also proved a promising area for endophenotypic research. A recent study indicated that unaffected siblings of individuals with ADHD show deficits similar to affected probands in prefrontal areas for unexpected events and in cerebellum for events at unexpected times [23]. Thus, although limited in number, the studies that have employed sMRI, functional MRI (fMRI) and neurophysiological measures provide support for the viability of such measures in the search for ADHD endophenotypes.

However, although informative, these initial endophenotypic studies may best be viewed as forming the basis from which more comprehensive studies must follow. These more comprehensive studies should incorporate information from all levels (i.e., genotype, endophenotype and phenotype) and aim at the

two main objectives for including endophenotypes into genetic studies of psychiatric disorders: unraveling the modes of action of known risk genes and discovering new risk genes for the disorder. Thus far, a multitude of studies has concentrated on the first aim. For example, the relationship between ADHD risk genes, such as the dopamine receptor 4 and 5 genes (*DRD4* and *DRD5*, respectively) the dopamine transporter gene (*DAT1*), the monoamine-oxidase type A gene (*MAOA*), the catechol-*O*-methyltransferase gene (*COMT*), the dopamine β -hydroxylase gene (*DBH*) and candidate endophenotypic measures (predominantly cognitive measures) have been studied. It is not my intention to systematically review all of these studies, but it is safe to say that results are, at best, inconsistent. Findings can often not be replicated or even reverse genetic effects on cognitive functions are found [24]. Small sample sizes, differences in sample ascertainment (different ADHD subtypes, clinically referred or not and including controls or not), differences in used cognitive paradigms, differences in ADHD measurement methods (interview or questionnaires), differences in participant characteristics (e.g., age, sex and comorbidity) and differences in the studied polymorphisms may all hamper comparability between findings, limiting the possibility to unravel the modes of action of known risk genes.

What about the second objective for including endophenotypes into genetic studies of psychiatric disorders, namely to discover new risk genes for ADHD? In order to do this, linkage analyses are necessary, which require substantial sample sizes to obtain adequate power. Thus far, only one study has been published using cognitive candidate ADHD endophenotypes in a linkage design [25]. However, results were promising. Two significant genome-wide linkage signals were found on 2q21.1 and on 13q12.11, and ten additional suggestive linkage signals were found. The logarithm of the odds ratio scores of the two genome-wide significant linkage signals were among the highest ever reported in ADHD genetic research, supporting the use of endophenotypes to detect new risk loci. Similar promising findings using endophenotypes in linkage have been reported by the Collaborative Study on the Genetics of Alcoholism [26].

"...the future of ADHD genetic studies looks promising, since endophenotype studies are increasingly comprehensively designed and are employing larger sample sizes than before."

So, have endophenotypes in the genetic research of ADHD lived up to their expectations? Yes and no. Yes, because more and more is known about the mechanisms of action of ADHD risk genes by linking them to candidate ADHD endophenotypes. For example, well-designed studies, such as those conducted by Durston and colleagues [27], provide promising new leads into the modes of action of ADHD risk genes on brain activation patterns, which may, ultimately, translate into individualized treatments targeting genotype/fMRI activation profiles. No, because, as yet, no consistent pattern of findings on the modes of action of known risk genes has emerged from the current literature.

However, compared with endophenotypic research in other fields (i.e., schizophrenia, [manic] depression and alcoholism), endophenotypic research into ADHD is still in its primary phase. For example, candidate ADHD endophenotypes that emerge from literature comparing affected children and their nonaffected family members to controls may not always be true candidate endophenotypes. A comprehensive review on this issue has been published recently [28]. The authors state that a true candidate endophenotype mediates between genes and disorder. However, it may also be possible that the 'candidate' endophenotype fulfills several criteria of an endophenotype but, nevertheless, is not an endophenotype in the strict sense. For example, the 'candidate' endophenotype may actually be a consequence of the disease, may be an epiphenomenon (i.e., is related to the same gene[s] as the disorder but does not mediate between genes and disorder), may be related to entirely different genes than the disorder, or may arise from nongenetic factors. In the case that the trait is actually an epiphenomenon or related to environmental factors, it is possible that both ADHD patients and their nonaffected siblings will differ from controls [28], causing a false conclusion to be drawn on the usefulness of the trait as ADHD endophenotype. Thus, future studies should try to determine whether a trait meets all relevant characteristics, as has been done comprehensively by Waldman and colleagues [29,30]. Nevertheless, the future of ADHD genetic studies looks promising, since endophenotype studies are increasingly comprehensively designed and are employing larger sample sizes than before.

"...candidate ADHD endophenotypes that emerge from literature comparing affected children and their nonaffected family members to controls may not always be true candidate endophenotypes."

Several recommendations can be made for future ADHD endophenotypic studies. First, substantially larger sample sizes are necessary to prevent spurious associations between risk genes and candidate endophenotypes. Second, ADHD involves widespread cognitive disturbances, such as those of attention, executive functions, state regulation and motivation, motor control and temporal information processing [31]. These various functional domains can be associated with abnormal anatomic findings in brain regions implicated in these functions. Comprehensive endophenotype batteries for ADHD must, therefore, assay all of these relevant variables, preferably in combination with sMRI, fMRI and/or neurophysiological measures. Third, it would greatly benefit comparability of research findings when the same tasks are used across studies; that is, the current use of multiple variations of tests for the same cognitive domains prevents thorough generalization of the research findings. Composing a freely accessible computer-driven battery with a broad range of tasks that have been examined for their validity, reliability and, preferably, for their heritability, would make it possible to combine different samples across research sites to a larger sample. This would greatly enhance comparability and power of genetic research results. Fourth, samples should preferably be studied longitudinally. The

phenotypic presentation of ADHD and, possibly, also the genetic effects on candidate cognitive endophenotypic and phenotypic measures are strongly influenced by age [24,32]. These developmental effects can only be reliably studied in a longitudinal design. Fifth, comorbid disorders should be assessed in relation to genetic and endophenotypic variables of ADHD. Only in this way, light may be shed on the shared and unique developmental pathways leading up to ADHD with/without comorbidities. Moreover, hypothetically, what appears as a candidate ADHD endophenotype, may actually be an endophenotype of a comorbid disorder. For example, a recent study showed that increased response variability, considered a core cognitive feature of ADHD, was more strongly related to autism spectrum disorders than to ADHD [33]. Of course, this one study does not imply that increased response

variability is not a viable ADHD endophenotype; however, such results do warrant the importance of studying ADHD in a broader phenotypic context. Taking into account these recommendations will hopefully improve consistency among research results and, as such, increase our understanding into the genetic basis of ADHD.

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