Endophenotypes in Autism Spectrum Disorders

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Introduction

The term "autism spectrum disorder" (ASD) designates a diagnostic category encompassing all those patients who, despite a surprising degree of interindividual variability in clinical symptomatology, share two essential features: (a) deficits in social interaction and communication and (b) behavioral abnormalities, in the form of either repetitive behaviors ("stereotypies"), difficulties in adapting to change ("insistence on sameness"), or restricted interests. Genetics provides strong contributions to the pathophysiology of ASD (Persico and Bourgeron 2006; Persico 2012). Twin studies performed two decades ago converged upon heritability estimates greater than 90 %, with monozygotic twins displaying concordance rates of 73–95 % compared to 0–10 % in dizygotic twins (Steffenburg et al. 1989; Bailey et al. 1995). Furthermore, first-degree relatives show mild traits qualitatively similar to those found in their autistic family members, justifying the definition of an "autism spectrum" or "extended phenotype" (Piven et al. 1997). However, these genetic underpinnings are neither simple nor consistent: "Syndromic autism" is part of a known genetic or chromosomal syndrome in approximately 10 % of cases; another 7–10 % of patients display "monogenic autism," stemming from de novo mutations or copy number variants (CNVs) in the form of pathogenic microdeletions or microduplications; and the remaining majority of cases suffers from "polygenic autism," originating from multiple-hit gene-gene and gene-environment interactions typical of nonlinear complex

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genetics (Persico 2012). Indeed, a very recent twin study has reported heritability estimates down to 37 % as compared to 55 % variance explained by shared environmental factors (Hallmayer et al. 2011). During the same two decades, the incidence of ASD has dramatically risen from 2 to 5/10,000 to approximately 1–2/1,000 children for strict autism (Fombonne 2009) and 6 – 10/1,000 for the broader ASD category (Baron-Cohen et al. 2009). Broader diagnostic criteria and increased awareness in the medical community have likely contributed to this trend (Rutter 2005; King and Bearman 2009), but a real increase in incidence is also likely (Grether et al. 2009; Hertz-Picciotto and Delwiche 2009). Indeed, ASD may be drifting from a low incidence, primarily monogenic disorder, to a high incidence, primarily polygenic disorder with complex genetic and environmental components.

Only a thorough understanding of the pathophysiology of a disease can pave the path to effective treatments, to real prevention, and to reliable prognostic predictions. In this regard, the heterogeneous and multifactorial mechanisms underlying complex disorders always pose a remarkable challenge. It is precisely within this context that significant contributions can come from the study of endophenotypes, literally "phenotypes not visible from the outside." These quantitative, measurable, familial, and heritable traits associated with the complex disease of interest can in fact serve multiple purposes, starting from the identification of subgroups of patients apparently similar at the clinical level yet different in their pathophysiological underpinnings.

Endophenotypes and Biomarkers: Similarities and Differences

Before examining the nature and potential significance of the best-established endophenotypes in ASD, we shall now define some terms whose correct understanding and appropriate use is a useful prerequisite. The term "genotype" refers to the DNA sequence encoding for a given "phenotype," which is an observable characteristic of a given organism. Phenotypes are the result of an interaction between genotype, environment, and chance. Monogenic Mendelian disorders display tight genotype-phenotype correlations, leaving relatively little space for environmental influences and stochastic events. Complex polygenic disorders do not follow genotype-phenotype correlations as strictly, because the interaction between multiple gene variants, environmental influences, and stochastic events generates broad interindividual phenotypic variability. This is especially true for behavioral disorders caused by abnormal neurodevelopment, because despite being hard wired under genetic programming, the central nervous system (CNS) is indeed structured to undergo environmentally driven adaptive plasticity.

Almost 40 years ago, Gottesman and Shields (1973) used the term "endophenotypes" in the context of genetic theories of schizophrenia, referring to internal phenotypes detectable using a "biochemical test or microscopic examination." They adapted this term from previous work by John and Lewis (1966), who used it in an entirely different context, namely, insect biology and evolution. In their paper, John and Lewis (1966) state that the geographic distribution of

Table 1 Similarities and differences between endophenotypes and biomarkers

Definition criteria	Endophenotype	Biomarker
1. Associated with the disease in the general population	+	+
2. Segregates with the disease in the family	+	+
3. Heritable (i.e., it has a genetic basis)	+	0/+
4. Familial (i.e., intermediate phenotype in first-degree relatives)	+	0/(+)
5. Trait dependent	+	0/(+)

grasshoppers depends on characteristics found not so much in their "exophenotypes," but instead in "the endophenotype, not the obvious and external but the microscopic and internal."

By definition, an "endophenotype" must satisfy a set of five criteria summarized in Table 1. Briefly, it must be (a) *associated with the disease of interest in the general population*, meaning it must be significantly more frequent or elevated among patients compared to population controls, especially if the latter have been selected for unaffected status; (b) *associated with the disease of interest within the family*, where endophenotype and illness must co-segregate; (c) *heritable*, indicating that it must have a genetic basis; (d) *familial*, meaning that it should have highest frequency or intensity among patients, intermediate frequency or intensity among their unaffected family members, and lowest frequency or intensity among population controls, especially if selected for unaffected status; and (e) *trait dependent* and not state dependent, meaning it must act as a "risk tag," consistently detected in a liable individual regardless of whether he/she is in a state of acute illness or in remission (Gottesman and Gould 2003).

Endophenotypes can contribute to solve important questions on etiological models of complex disorders. The rationale underlying the use of endophenotypes in psychiatry stems from the multifactorial and polygenic origin of psychiatric disorders in the vast majority of patients (Gottesman and Gould 2003). Simple, quantitative, and heritable phenotypes associated with the disease should in principle be linked more easily to the underlying pathophysiological mechanisms and to a smaller set of specific genes, compared to abnormal human behaviors. In addition, endophenotypes can also be used to guide the refinement of diagnostic categories, to develop valid animal models, and to search for new pharmacological treatments in targeted subgroups of patients. Synonyms highlighting specific aspects of the overall definition of an "endophenotype" include the terms "intermediate phenotype," "subclinical trait," and "vulnerability marker."

"Biological markers" of disease or "biomarkers" must instead be distinguished from "endophenotypes," as all endophenotypes are also biomarkers but not vice versa (Table 1). A biomarker can be defined as a biological variable associated with the disease of interest and measurable directly in a given patient or in his/her biomaterials using sensitive and reliable quantitative procedures. Biomarkers are not necessarily heritable: They merely tag for the presence/absence of the disease, with no implication on the existence of an underlying genetic mechanism. Secondly, the lesser the biomarkers act as trait-dependent and familial "intermediate

phenotypes," the more discriminatory power they possess (i.e., the better they separate out affected and unaffected individuals in the general population). In principle, a reliable set of autism biomarkers could foster earlier and more reliable diagnoses; predict developmental trajectories and treatment response; identify individuals at high risk, eventually leading to the establishment of preventive health-care strategies; contribute to dissect ASD into more discrete clinical entities; and perhaps even reveal unknown causes of autism or pathophysiological processes underlying the disease, at least in some cases. The revolutionary employment in clinical settings of plasma lipoproteins and cholesterol levels as high-risk biomarkers for cardiovascular disease is an obvious example of the dramatic medical progress brought about by the use of biomarkers in disease prevention. To this date, many autism biomarkers have been proposed (Ecker et al. 2010; Wang et al. 2011; Veenstra-VanderWeele and Blakely 2012), but scientific, ethical, clinical, and practical issues still pose a major challenge to their use in clinical practice (Walsh et al. 2011). The biological complexity and heterogeneity of ASD, coupled with the low sensitivity and specificity of any single biomarker, prompt the development of age- and sex-specific diagnostic panels, each including several parameters likely belonging to different domains (biochemical, brain imaging, dysmorphological, electrophysiological, genetic, immunological, etc.). Within this framework, *several endophenotypes will probably be incorporated into these diagnostic panels and be used as biomarkers, while not all biomarkers qualify for endophenotype status as many lack heritability, familiality, and trait dependence* (Table 1).

A detailed description of autism biomarkers is beyond the scope of this chapter, which shall now focus on the best-characterized endophenotypes in autism spectrum disorder.

Endophenotypes in Autism Spectrum Disorder

Many studies have attempted to identify endophenotypes associated with autism and to assess their heritability, familial aggregation, and trait dependence. Autism endophenotypes can be grouped into at least seven categories: biochemical, morphological, hormonal, immunological, neurophysiological/neuroanatomical, neuropsychological, and behavioral. A summary of autism endophenotypes in each category and an index of the evidence supporting their endophenotype definition based on the five criteria stated above are presented in Table 2. Each category will now be analyzed in detail.

Biochemical Endophenotypes

Serotonin (5-HT) blood levels represent the best-characterized biochemical endophenotype in autism research. Elevated 5-HT blood levels have been consistently recorded in approximately 30–50 % of individuals with ASD

Table 2 Autism endophenotypes and strength of the evidence supporting their definition (+++ conclusive, ++ probable, + possible)

Biochemical	
Elevated serotonin blood levels	+++
Oligopeptiduria	++
Abnormal plasma levels of free fatty acids	+
Morphological	
Macrocephaly/macrosomy	+++
Minor physical anomalies	++
Endocrine	
Low melatonin plasma levels	+++
Low oxytocin plasma levels	++
Immunological	
Increased production of pro-inflammatory cytokines and IL-10	+++
Increased number of CD8+ "naïve" T lymphocytes	+++
Decreased number of CD4+ and CD8+ differentiated T lymphocytes	+++
Neurophysiological/Neuroanatomical	
Abnormal activation of the prefrontal cortex during a nonsocial visual attention task	+++
Hypoactivation of the fusiform gyrus bilaterally, left dorsolateral prefrontal cortex, right inferior temporal gyrus in response to the vision of human movement	+++
Abnormal mirror neuron circuitry	+
Reduced activation of the prefrontal cortex during a visual imagery task	+
Reduced responsiveness of the auditory cortex during auditory evoked potentials	+
Neuropsychological	
Abnormal visual scanning of human faces assessed by eye tracking	+++
Deficits in some executive functions (spatial working memory and strategic planning)	+++
Behavioral	
Absence or delay in verbal language development	++
I.Q.	+
"Savant" skills	+

(Anderson et al. 1990). Piven et al. (1991) measured 5-HT in platelet-rich plasma recording basal levels in 10 unaffected controls, higher levels among 23 autistic individuals from simplex families (i.e., with only one affected child), and highest levels in five autistic individuals belonging to multiplex families (i.e., families with two or more autistic children). Family members of autistic patients display levels intermediate between the excess of their autistic sibling and the basal levels recorded in the general population. The hyperserotonemia found in autistic individuals is due to excessive accumulation of 5-HT in platelets, whereas free 5-HT plasma levels are not different between patients and controls (Cook et al. 1988; Anderson et al. 1990; Piven et al. 1991). Serotonin uptake in platelets is mediated by the same 5-HT transporter expressed in neurons (Lesch et al. 1993). The density of 5-HT transporters on the platelet membrane (Vmax) is increased in autism, while the affinity (Kd) of the 5-HT transporter for serotonin is unchanged

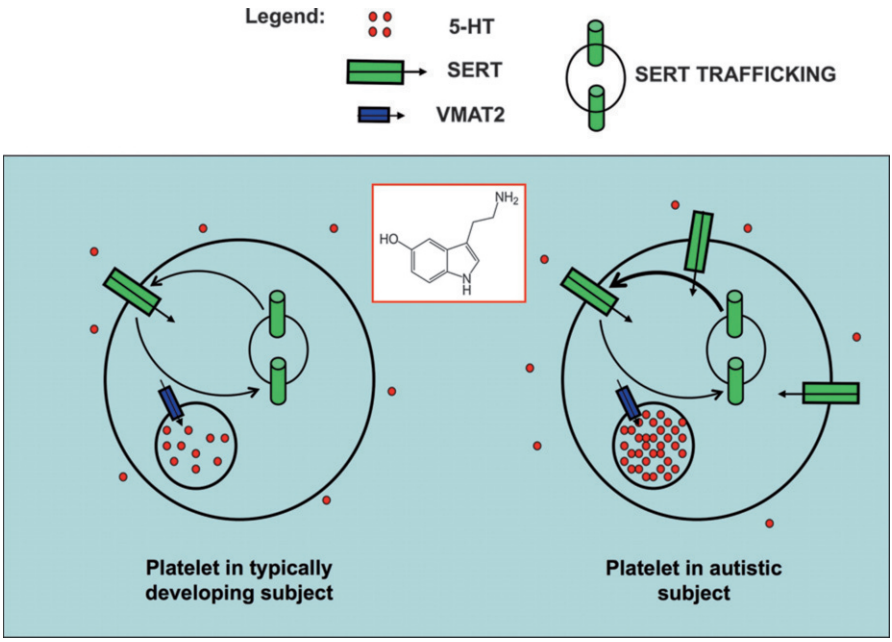

Fig. 1 Biochemical endophenotypes: elevated serotonin (5-HT) blood levels in autistic patients are due to excessive 5-HT uptake into platelets, mediated by the same 5-HT transporter expressed in the brain

(Katsui et al. 1986; Marazziti et al. 2000) (Fig. 1). In autistic children, 5-HT blood levels are especially elevated before puberty, whereas after puberty, difference between autistics and controls becomes less pronounced (McBride et al. 1998).

Abnormal concentrations of urinary solutes have also been described in subgroups of autistic individuals ranging from 10 % to 60 % depending on their ethnic origin (Reichelt et al. 1997; Yap et al. 2010). These solutes have been initially designated as "oligopeptides" (Reichelt et al. 1997), and the overall phenomenon of "oligopeptiduria" has recently been shown to be associated with autism and familial (Sacco et al. 2010). However, the exact chemical nature of urinary solutes is still controversial: For example, the existence of casein-derived urinary oligopeptides with opioid activity has not been confirmed (Hunter et al. 2003; Dettmer et al. 2007; Cass et al. 2008). Furthermore, urinary solutes likely represent a chemically heterogeneous set of molecules at least partly originating from gut bacteria and their derivatives produced through hepatic metabolism, such as *p*-cresol and *p*-cresylsulphate, respectively (Yap et al. 2010; Altieri et al. 2011).

Morphological Endophenotypes

Macrocephaly can be defined, together with hyperserotonemia, as the best-established endophenotype in autism research (Sacco et al. 2007). Approximately 20 % of all

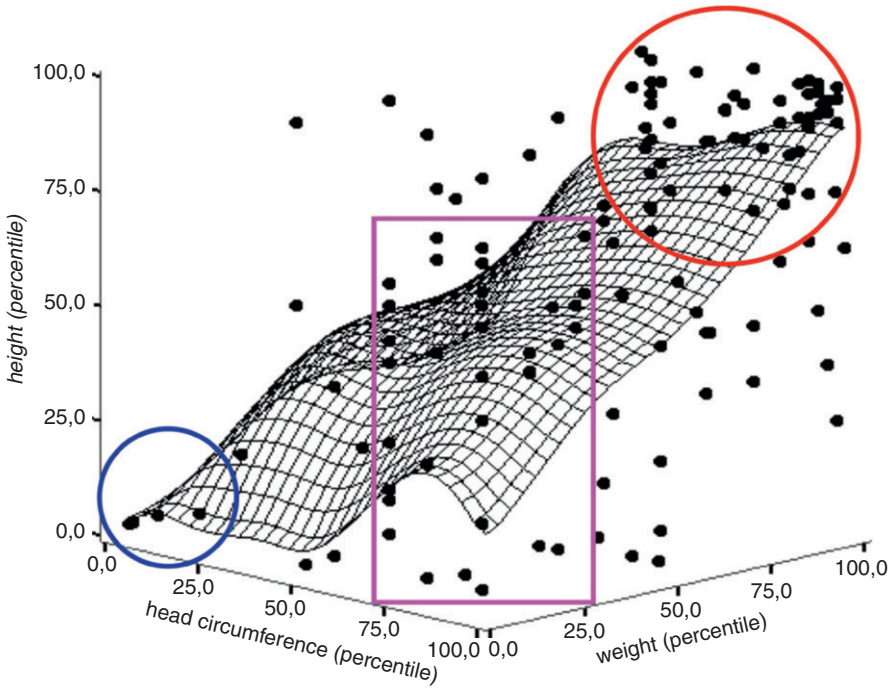

Fig. 2 Morphological endophenotypes: correlations among head circumference, height, and weight distinguish autistic individuals with macrosomy (*red*) from those with relative macrocephaly (*purple*) and with microsomy (*blue*) (From Sacco et al. 2007, modified)

autistic children consistently display head circumference measures above and beyond the 97th percentile, regardless of race and ethnicity. Head growth in these children follows a peculiar nonlinear developmental trajectory: (1) It is well within normal limits during prenatal life and at birth. (2) It starts accelerating during the first year of postnatal life, peaking sometime between 6 months and 4 years of age. (3) It then decelerates so that at puberty, typical head size is nonsignificantly larger among autistic individuals compared to controls (Courchesne et al. 2007). This "triphasic" development in head size (normal-accelerated-decelerated) is paralleled by a similarly disharmonious brain growth, especially evident in the overgrowth of the frontal and temporal lobes, shown in postmortem studies and using neuroimaging techniques in vivo (Courchesne et al. 2007). Macrocephaly is highly familial, and 45 % of macrocephalic autistic patients have at least one macrocephalic parent (Miles et al. 2000; Sacco et al. 2010): In simple terms, this indicates that some genes contributing to macrocephaly also increase the risk of giving birth to an autistic child. Finally, in most autistic children, macrocephaly is part of a broader macrosomic phenotype, characterized by an excess also in height and weight (Fig. 2): This macrosomic endophenotype is interestingly associated with a positive history for allergies or autoimmune disorders both in the patient and in his/her first-degree relatives,

as well as with obstetric complications during pregnancy (Sacco et al. 2007). These surprising findings strongly point toward macrocephaly as part of a systemic stimulation of growth processes pathophysiologically linked with abnormal functioning at the level of the CNS and immune system. The mTOR pathway represents the most likely candidate both linked with autism and able to mediate at the intracellular level either genetically based or immune-produced overstimulation of brain and systemic growth (Ma and Blenis 2009).

The presence of minor physical anomalies represents another morphological endophenotype of great interest. In addition to macrocephaly, also an abnormal cephalic index and palate morphology were identified as the most frequent minor physical anomalies in 24 ASD individuals (Tripi et al. 2008). "Dense surface-modeling" techniques applied to contrast facial morphology in 72 ASD children, 128 first-degree relatives, and 254 controls detected a significant and atypical facial asymmetry in ASD children, especially dominant on the right supraorbital and anterior periorbital regions (Hammond et al. 2008). Mothers of ASD children interestingly display a vertical asymmetry, especially evident in the orbital regions. Since brain and skull are known to develop in parallel, with bone assuming the underlying brain shape, these fronto-orbital asymmetries may well represent markers of abnormal frontal lobe development, in accordance with neuroimaging studies (Courchesne et al. 2007). Alternatively, genetic factors could influence facial morphology and brain development at the same time (Hammond et al. 2008).

Hormonal Endophenotypes

Potential roles for oxytocin (OT) in autism were initially hypothesized based on its involvement in the establishment of social bonds in prairie voles, as compared to their less affiliative counterpart, the mountain voles (Young et al. 1998). Plasma levels of OT were then found to be significantly lower among ASD children compared to matched controls, especially in a subgroup of autistic individuals (Modahl et al. 1998). Physiologically oxytocin plays a major role in the establishment of affiliative bonds also in higher species, including humans (Feldman 2012). Unfortunately to this date, no study reports to our knowledge about plasma OT levels in family members, so this parameter should be viewed as a candidate endophenotype still awaiting for a definitive confirmation. Nonetheless, polymorphisms of the OXTR gene, encoding for the OT receptor, are associated not only with autism but also with pair bonding, social behavior, emotional affect, and autism spectrum traits continuously distributed in the general population (Lucht et al. 2009; Walum et al. 2012). Furthermore, OT blood levels are negatively correlated with 5-HT blood levels in autistic children (Hammock et al. 2012): Given the intermediate 5-HT blood levels recorded in first-degree relatives (see above), a mirroring trend is to be reasonably expected with OT blood levels. The intranasal administration of OT determines in autistic individuals a significant reduction in repetitive behaviors, increased social interactions, and improved social skills and emotion recognition (Hollander et al. 2007). Strikingly, similar increases

in OT salivary levels are recorded in 5-month-old infants and in their fathers when *only the latter* are administered intranasal OT prior to face-to-face engagement, opening a new perspective on the treatment of ASD and other disorders using the parent, rather than the child, as a pharmacological target and as therapeutic mediator (Weisman et al. 2012).

Melatonin (MT) has been known for a long time to play a crucial role in circadian and seasonal rhythms, in the modulation of immune responses and in neuronal plasticity. MT synthesis in the pineal gland is inhibited by light and stimulated by darkness. MT is formed from 5-HT, which is transformed into *N*-acetylserotonin and then into MT, the latter step through the action of the enzyme acetylserotonin methyltransferase (ASMT). Plasma levels of MT are abnormally low in many autistic children, due to a deficit in ASMT activity recorded in the platelets both of ASD patients and of their parents (Melke et al. 2008). In the same study, two polymorphisms located in the promoter region of the ASMT gene were also significantly associated with autism and the "in vivo" assessment of the circadian synthesis of MT unveiled the absence of physiological nocturnal increases in MT plasma levels in the autistic child and in his/her mother, both carrying the ASMT polymorphisms associated with autism (Melke et al. 2008). Blunted MT plasma levels and altered circadian rhythmicity in MT synthesis and release are also supported by other studies and thus appear to represent a reliable and valid endophenotype in ASD (Rossignol and Frye 2011; Tordjman et al. 2012). Its possible link with a disrupted sleep-wake cycle is especially interesting, as this occurs frequently in many autistic children especially during their early infancy and they seemingly benefit from MT as a sleep-promoting agent (Rossignol and Frye 2011; Cortesi et al. 2012).

Immunological Endophenotypes

It is well known that the major histocompatibility complex (MHC), many cytokines, and the immune cells responsible for their secretion can influence neurodevelopment both pre- and postnatally, whereas in the adult, an activation of the immune system can profoundly affect cognitive and affective functioning. Multiple abnormalities have been recorded in the immune system of many autistic individuals (Ashwood et al. 2006), including elevated plasma levels of IL-1 and IFN-γ accompanied by elevated levels of TNF-α in the cerebrospinal fluid, enhanced production of the anti-inflammatory cytokine IL-10, and an abnormal post-thymic maturation of T lymphocytes, with increased counts of "naïve" and decreased counts of differentiated T lymphocytes (i.e., CD4+ and CD8+ T cells). Importantly, the same abnormalities, albeit generally less prominent, have also been found in the unaffected first-degree relatives of ASD patients (Saresella et al. 2009), thus fully qualifying as an autism endophenotype. Conceivably, non-autistic first-degree relatives could carry more efficient anti-inflammatory mechanisms, able to contain and to compensate for a liability to neuroinflammation seemingly shared by all family members. Collectively these results support the existence of

a dysfunctional immune response in a consistent subgroup of ASD patients, possibly distinguishable even at the clinical level (see the "ICS" patient cluster in Sacco et al. (2012)). Finally, approximately 7 % of mothers and 21 % of autistic children carry autoantibodies directed against a variety of brain antigens, especially localized in GABAergic neurons (Croen et al. 2008; Rossi et al. 2011). Preliminary results limited to ASD children positive to 45 kD and 62 kD autoantibodies indicate that they do not belong to the "ICS" patient cluster, clinically enriched in "immune, circadian, and sensory abnormalities" (Sacco et al. 2012), but rather to the "S" cluster enriched in motor stereotypes, at least in reference to the 62 kD autoantibody (Persico et al. manuscript in preparation). This result is consistent with studies previously reporting stereotypic behavior, cognition, and language to be more severely affected in children harboring these cerebellum-directed antibodies (Goines et al. 2011; Wills et al. 2011).

Neurophysiological and Neuroanatomical Endophenotypes

The impressive technological advances in electrophysiological and brain imaging techniques occurred during the last two decades have greatly contributed to the identification of novel neurophysiological and neuroanatomical endophenotypes in ASD. In general, morphological studies simply address the size of cerebral structures, whereas functional neurophysiological or brain imaging studies typically apply precise cognitive tasks to highlight the underlying activation of specific brain regions and, more recently, of distributed neural networks. The two best-defined endophenotypes are summarized in Table 2 and briefly described here:

(a) During a nonsocial visual attention task, autistic individuals display an abnormally delayed and long-lasting activation of the prefrontal cortex, with inadequate functional connectivity between distant brain regions, whereas unaffected brothers display an atypical enhanced activation of the prefrontal cortex in the presence of intact functional connectivity (Belmonte et al. 2010). This enhanced activation of prefrontal cortical regions strongly points toward the existence of compensatory mechanisms possibly mediated by broader recruitment and alternative routes for information processing in non-autistic family members.

(b) Assessing cerebral responses to movement using fMRI, ASD children and their unaffected siblings, but not typically developing children, display an hypoactivation of a neural network encompassing the left ventrolateral prefrontal cortex, the right amygdala, the right posterosuperior temporal sulcus (pSTS), the ventromedial prefrontal cortex, and the form gyrus bilaterally (Kaiser et al. 2010). However, the pSTS displays a "state-dependent" hypoactivation, directly correlated with the severity of social deficits, whereas a "trait-dependent" hypoactivation shared by ASD patients and first-degree relatives affects the fusiform gyrus bilaterally, the left dorsolateral prefrontal cortex, and the right inferior temporal gyrus (Kaiser et al. 2010). Hence, the latter identifies a familial endophenotype presumably based upon vulnerability genes shared by affected and unaffected individuals within the family.

Only among unaffected siblings, an activation is encountered in the right pSTS and in the ventromedial prefrontal cortex, again suggesting the existence of compensatory mechanisms able to efficiently counteract the increased liability to develop an ASD shared by autistic and non-autistic family members (Kaiser et al. 2010).

A wealth of electrophysiological and brain imaging studies has documented over the years abnormalities in brain morphology and information processing among autistic individuals; unfortunately, the lack of data on first-degree relatives hampers at this time their inclusion among those parameters satisfying the five criteria set for an appropriate definition of an "endophenotype." For example, the recent discovery of mirror neurons, encoding motor acts in accordance with their final goal regardless of whether the action is performed by the individual himself or it is observed as another individual performs the same action, spurred interest into their potential role in ASD. Subsequent studies have demonstrated an atypical activation of motor neurons localized in the operculum of the inferior frontal gyrus, during the imitation and observation of human actions and emotional expressions (Dapretto et al. 2006). Autistic individuals seemingly lack an understanding of the mental state of the agent ("theory of mind"), necessary to empathically recognize the goal of the action, although they do recognize the motor act itself. It is still somewhat debated whether this deficit is primary or whether it stems from an insufficient feeding of sensory information to the mirror system, especially in the social and affective realms. Most importantly for our purposes, it is still unknown whether this deficit is also at least partly shared with non-autistic family members and thus represents a true endophenotype. Similarly, multidimensional MRI-based approaches are seemingly able to distinguish autistics from controls and from patients with ADHD with up to 90 % sensitivity and 80 % specificity, but whether these neocortical and subcortical differences are genetically based and familial or rather the consequence of a long-standing pathological process remains to be established as first-degree relatives were not assessed in these studies (Ecker et al. 2010, 2012). The same limitation holds true for many other neurophysiological and neuroanatomical parameters found to be abnormal in autism, such as the reduced amplitude and longer latency of the N1c wave recorded using auditive-evoked potentials, reflecting an hypoactivation of the auditive system in autism (Bruneau et al. 2003), and the hyperactivation of prefrontal regions and of the inferior parietal cortex both involved in selective attention and likely underlying the excessive attention to object details typical of many autistic children (Gomot et al. 2008).

Neuropsychological Endophenotypes

Visual scanning of human faces may represent the most studied neuropsychological parameter thanks to the development of "eye tracking" technologies. Autistic individuals have been consistently shown to spend significantly more time scanning the mouth and neck regions compared to the eyes, which represent by far the region most targeted by typically developing controls (Spezio et al. 2007). Visual face

processing was also assessed among parents of autistic children, who display similar abnormalities when aloof and socially isolated, while parents with well-developed social skills employ face-scanning strategies superimposable to those applied by controls (Adolphs et al. 2008).

Studies assessing executive functions have unveiled deficits in spatial working memory and in strategic planning. These deficits were recorded not only in autistic patients but also among their unaffected first-degree relatives (Delorme et al. 2007). Interestingly, these cognitive endophenotypes appear to be shared between autism and obsessive-compulsive disorder (Delorme et al. 2007).

Behavioral Endophenotypes

As previously discussed, using the term "endophenotype" to designate observable behavioral parameters is theoretically inappropriate. It is especially inappropriate when referring to signs or symptoms listed among the diagnostic criteria of the disorder, such as the presence of stereotypic behaviors in the case of ASD. Nonetheless, several genetic studies published in international journals have used the term "endophenotype" in reference to heritable cognitive parameters such as I.Q., non verbal communication, language development, and social adaptation. Liu et al. (2008) used as endophenotypic variables the ADI-R total scores in social interaction, stereotypic behaviors and restricted patterns of interests, age at first words, age at first sentence, and presence/absence of verbal language, as well as I.Q. Several studies, such as those by Bradford et al. (2001), Spence et al. (2006), and Alarcón et al. (2008), used verbal language development as an endophenotypic variable. The latter study identified the autism gene CNTNAP2 (contactin-associated protein-like 2), a member of the neurexin gene family highly expressed in the frontotemporal subcortical circuits involved in executive functions, such as the attentional system which interestingly also represents a necessary precursor for language development. The identification of different loci in studies employing similar phenotypic measures pertaining to language development probably reflects the large number of genes involved in this function and the similarly high degree of genetic heterogeneity underlying verbal communication deficits in ASD.

"Savant" skills, hyperdeveloped cognitive abilities present in a small subset of ASD individuals, represent another interesting behavioral phenotype. Wallace et al. (2009) assessed in great detail the neuropsychology and brain morphometry of a single autistic subject with extraordinary skills in mathematics and art. In addition to I.Q., mathematical skills, memory, and visuospatial functions, three additional domains relevant to the development of savant skills were assessed: weak central coherence, implicit learning, and speed of information processing. The neurocognitive profile of this autistic patient is characterized by exceptional memory, mathematical skills, and visuospatial functions, as well as knowledge of calendar structure and weak central coherence. This translates into an extraordinary memory for details and a relative inability to recall essential data in adaptive contexts, normal implicit learning, and insufficient visual exploratory skills.

Brain imaging analysis revealed significantly reduced neocortical thickness in regions involved in social cognition, as well as in the medial and superior prefrontal cortex, whereas the superior parietal lobule, involved in visuospatial and mathematical functions, was significantly thicker.

These behavioral "endophenotypes" clearly carry a significant heuristic potential, and some have provided sizable contributions to our understanding of the processes underlying autism, especially in the genetics field. However, they cannot be typically related to an intermediate phenotype measurable among first-degree relatives. At least at this stage, rather than being defined "endophenotypes," they should be viewed as clinical features.

Conclusions

ASD represents a genetically complex and heterogeneous disorder, characterized by constellations of signs and symptoms displaying variable developmental trajectories and response to treatment in different patients. The study of endophenotypes is bound to acquire increasing relevance in the clinical management of autistic patients in coming years: Their intermediate position between genotype and behavior offers a distinct advantage to investigators striving toward a classification of autistic patients based upon the underlying pathophysiology. Despite the large number of genetic and environmental factors involved in autism pathogenesis, these factors must converge upon a relatively limited number of intracellular biochemical pathways and neurodevelopmental mechanisms which, through endophenotypes and biomarkers, can be conceivably identified and corrected administering specifically targeted pharmacological agents. A pathophysiologically based and genetically driven psychopharmacology is still in its infancy. However, clinical trials with IGF1 in girls with Rett syndrome carrying MeCP2 mutations and with rapamycin in macrocephalic/macrosomic autistic individuals carrying mutations in the PTEN gene clearly demonstrate that its development has begun (Tropea et al. 2009; Crino 2011). Working from the opposite end, also the identification of patient clusters based not only on clinical features but also on patient- and family-history variables, as well as several biochemical and morphological endophenotypes described in this chapter, spurs hope into clinical applications of these clustering strategies and into their possible contribution to genetic and neurophysiological studies (Sacco et al. 2010, 2012).

The two main limitations of endophenotyping in autism at this time are (a) the lack of "cross-talk" between endophenotyping modalities and (b) many ASD biomarkers to this date that have been assessed only in patients and controls, leaving unexplored their familiality and heritability. To this date, extremely few studies address the degree of correlation between different endophenotypes (see Hammock et al. (2012)), and the number of studies characterizing patients using more than one endophenotype is extremely limited (see Sacco et al. (2010)). It will be very important that in coming years, first-degree relatives be included in case–control studies as a way to investigate

the familial underpinnings of putative endophenotypic variables. Furthermore, longitudinal studies exploring the possible association between the most reliable endophenotypes and developmental trajectories or treatment response will be critical in translating knowledge into patient management.

Key Terms

Biomarker. A biomarker is a compound used as a sign of a particular biological condition. It has to be associated with the state of interest and it should be quantifiable directly on a given patient or more often on his/her biological specimens, using sensitive and reliable quantitative procedures.

Endophenotype. Endophenotypes are heritable quantitative traits distributed continuously among patients and first-degree relatives. They can either have a role in the etiological processes leading to the disease or at least characterize a relatively homogeneous subgroup of patients.

Familiality. The endophenotype found in affected family members is also present in non-affected family members at higher frequency/amounts compared to the general population.

Hyperserotonemia. Abnormally elevated serotonin (5-HT) blood levels. In autistic children, it is due to an excessive accumulation of 5-HT in platelets.

Macrocephaly. Head circumference greater than the 97th percentile expected by sex and age.

Trait dependence. Presence of a given parameter in a disease-liable individual, regardless of whether the illness is active or in remission.

Key Facts of Endophenotypes in Psychiatry

- "Genotype" refers to the DNA sequence encoding for a given "phenotype."
- "Exophenotypes" are observable characteristics of a given organism.
- "Endophenotypes" are internal phenotypes detectable using a biochemical test, brain imaging technique, or microscopic examination.
- Endophenotypes can be especially useful in psychiatry, because they stem from a relatively small set of genes, and are generally easier to link to an underlying pathophysiological mechanism as compared to abnormal human behavior. They also characterized subgroups of patients presumably sharing common pathophysiological underpinnings.
- Endophenotypes must be associated with the disease of interest in the general population, meaning they must be significantly more frequent or elevated among patients compared to population controls.
- Endophenotypes must be associated with the disease of interest within the family, where endophenotype and illness must co-segregate.
- Endophenotypes must be heritable, meaning they must have a genetic basis.

- Endophenotypes must be familial, meaning that they must have highest frequency/amounts among patients, intermediate frequency/amounts among unaffected family members, and lowest frequency/amounts among population controls.
- Endophenotypes must be trait dependent, meaning they must be detected in disease-liable individuals regardless of whether the illness is active or in remission.

Summary Points

- This chapter defines the concept of "endophenotype," offering a comprehensive overview of the best-established endophenotypes in autism spectrum disorder.
- An "endophenotype" in an internal phenotype, detectable using a biochemical test, brain imaging technique, or microscopic examination.
- Endophenotypes must be associated with the disease in the general population and within families, genetically based, familial, trait dependent, and not state dependent.
- Endophenotypes should be distinguished from biomarkers, compounds associated with the disease of interest and measurable either on a given patient or on his/her biological specimens, using sensitive and reliable quantitative procedures. Biomarkers are associated with the disease in the general population and within families, but they are not necessarily genetically based nor familial, and they are often state dependent.
- Endophenotypes in autism can be grouped into at least seven categories: biochemical, morphological, hormonal, immunological, neurophysiological/neuroanatomical, neuropsychological, and behavioral.
- Biochemical endophenotypes include excessive serotonin blood levels and abnormally elevated urinary solute concentrations.
- Morphological endophenotypes encompass macrocephaly and macrosomy, as well as the presence of minor physical anomalies.
- Hormonal endophenotypes include reduced melatonin and oxytocin blood levels.
- Various immunological parameters are abnormal in a consistent subgroup of ASD patients and in their first-degree relatives (elevated levels of several pro-and anti-inflammatory cytokines, abnormalities in T lymphocytes and NK cells, etc.).
- At least two neurophysiological and neuroanatomical endophenotypes have been described in ASD, but their number will likely increase as future studies enroll also first-degree relatives in addition to cases and controls.
- Neuropsychological endophenotypes include gaze abnormalities recorded by eye tracking and deficits in executive functions, such as spatial working memory and strategic planning.

- Behavioral endophenotypes include several variables like stereotypic behaviors, verbal language, I.Q., and savant skills, among others. Although their definition of "endophenotype" is inappropriate, these overt clinical characteristics have been successfully employed especially in genetic studies.
- Future studies will have to measure several endophenotypes and assess their within-subject correlation. Biomarker studies will greatly benefit from assessing endophenotype status by recruiting first-degree relatives in addition to cases and controls.

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