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1. Introduction

Autism Spectrum Disorders (ASD) are complex neuropsychiatric conditions characterized by stereotypic behaviors and conspicuous deficits in social communication. It has been demonstrated that there is genetic and clinical heterogeneity in these disorders. Inherited and/or de novo genetic factors, as well as environmental and epigenetic components have been shown to be involved in the etiology of ASD. Among the common comorbidities are epilepsy and seizures, gastrointestinal problems, depression, anxiety, hyperactivity (including ADHD), Tourette syndrome, phobias, dysmorphic features, and psychotic disorders [1-5].

The etiological role of genetic factors in ASD has been evaluated by twin studies; the concordance found in monozygotic pairs is higher than the concordance in dizygotic twins (36 to 91% versus less than 32% [6]). Concordance estimates for both types of twins have been reported to be higher if the analyzed phenotype is ASD as opposed to narrow phenotypes [7].

Heritability of ASD has been estimated to be as high as 90%, although a more recent report suggests that it may be about 19-35% in males and 50-63% in females [8]. While some cases are considered sporadic and have been associated with mutations, others are familial and may tend to be associated with relatively common genetic variation [9].

Genes related with synaptic development, signaling, chromatin remodeling, transcription, methylation, neurotropism, and neuroprotection are probably involved in ASD. These genes may include some that code for neurexins, neuroligins, shanks, reelin,
integrins, cadherins, contactins, and neutrophins. Linkage and association with ASD have been described in different regions of autosomes that harbor candidate genes (Table 1, figure 1) [10-17]. In addition, as table 2 shows, the X chromosome has also been implicated in ASD (Table 2, figure 1) [18-20].

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC25A12</td>
<td>Solute carrier family 25 member 12</td>
</tr>
<tr>
<td>OXTR</td>
<td>Oxytocin receptor</td>
</tr>
<tr>
<td>GRIK2</td>
<td>Glutamate receptor ionotropic kainate 2</td>
</tr>
<tr>
<td>RELN</td>
<td>Reelin</td>
</tr>
<tr>
<td>MET</td>
<td>MET protooncogene</td>
</tr>
<tr>
<td>FOXP2</td>
<td>Forkhead box P2</td>
</tr>
<tr>
<td>CADPS2</td>
<td>Calcium dependent activator protein for secretion 2</td>
</tr>
<tr>
<td>EN2</td>
<td>Engrailed 2</td>
</tr>
<tr>
<td>CNTNAP2</td>
<td>Contactin-associated protein-like 2</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>Solute carrier family 25 member 4</td>
</tr>
</tbody>
</table>

Table 1. Genes frequently linked or associated with ASD in autosomes

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLGN4</td>
<td>Neuroligin 4</td>
</tr>
<tr>
<td>NLGN3</td>
<td>Neuroligin 3</td>
</tr>
<tr>
<td>FMR1</td>
<td>Fragile X mental retardation protein</td>
</tr>
<tr>
<td>MECP2</td>
<td>Methyl CpG binding protein 2</td>
</tr>
</tbody>
</table>

Table 2. Genes frequently linked or associated with ASD in the X chromosome

Besides Fragile-X syndrome, other single-gene disorders are sometimes associated with ASD. Rett syndrome (MECP2 gene) is an autism-related condition. Tuberous sclerosis (TSC1 and TSC2 genes) may account for 1 out of 10 cases of ASD. Furthermore, 50% of individuals with Smith-Lemli-Opitz syndrome (DHCR7 gene) and 10% of those with phenylketonuria (PAH gene) may have ASD; in addition, adenylosuccinase deficiency, characterized by psychomotor delay, is often comorbid with epilepsy and autistic features [17, 21-23]. As described earlier, epilepsy is a common comorbidity in patients with ASD, and it has also been associated with region 15q11.1–q13.3 (ubiquitin-protein ligase E3A gene,UBE3A), TSC1 and TSC2 genes, MeCP2, CNTNAP2, SYN1, Fragile X syndrome, 1q21.1, 7q11.23, 16p11.2, 18q12.1, 22q11.2, and 22q13.3 (SHANK 3 gene).
Chromosomal anomalies such as deletions, duplications, inversions, balanced and unbalanced translocations, and aneuploidies have been described in some children with ASD [24]. For example, deletions on region 22q11-q13, which is associated with velo-cardiofacial syndrome, have been identified in about 1% of children with ASD; besides SHANK3, this region harbors two genes that have been associated with ASD, that code for proline dehydrogenase (PRODH) and catechol-o-methyltransferase (COMT) [25]. According to a meta-analysis, copy-number variants and truncating mutations in SHANK genes may be present in almost 1% of patients with ASD (SHANK1 in 0.04%, SHANK2 in 0.17%, and SHANK3 mutations in 0.69%) [26].

De novo chromosomal deletions or duplications have been identified in about 7 to 10% of patients. The 15q11-q13 chromosomal region, that includes a locus for the Prader-Willi/Angelman syndrome, is affected in 1% to 4% of children with ASD. Moreover, the phenotype of a supernumerary chromosome 15 includes autistic features such as developmental delay, mental retardation, neurological signs and behavioral disturbances. A 3.7 megabase deletion (Smith Magenis Syndrome) or duplication (Potocki-Lupski syndrome) on the 17p11.2 region is also associated with ASD [27]. Likewise, deletions on the short arm of chromosome X (NLGN4 gene) and an inversion on 7q (at common fragile sites) have been implicated in ASD [28].

Specific environmental and epigenetic factors have also been associated with ASD. For example, advanced paternal age at conception of the child, especially in non-familial cases, could impact the vulnerability to these disorders with de novo mutations [29, 30]. In addition,
errors in DNA methylation during spermatogenesis may affect the next generations. Recently, it was found in a mouse model that older fathers had a significant decrease in methylation in genomic regions associated with the control of transcription. Indeed, the expression of developmental genes implicated in ASD was dysregulated in the offspring of old fathers [31].

Other non genetic factors that have been associated with ASD include maternal use of certain substances during pregnancy, such as valproic acid, ethanol, thalidomide or misoprostol; low birth weight (or intrauterine growth retardation), congenital rubella and other infections, as well as cerebral palsy [32-38]. Environmental insults may increase the risk to ASD in genetically sensitive individuals, possibly by promoting cellular oxidative stress, and adaptive responses that could include reduced methylation activity, which is the most common epigenetic mechanism. Interestingly, the 15q11-q13 chromosomal region is subject to methylation-dependent genomic imprinting [39]; in addition, GABAergic genes in the same region, GABRB3, GABRA5, and GABRG3, were found to be epigenetically dysregulated in a subset of ASD patients [40].

2. Problem statement

Despite important advances in molecular genetics, the complete spectrum of the genetic component of ASD is still largely unresolved. There are many possible reasons for this partial lack of success. Among them, the complexity of the Central Nervous System, the relatively unique set of risk factors in each patient, which includes the combined effect of genetic (long/short, common, rare, and/or de novo sequences), and the interaction with environmental and epigenetic components. Furthermore, the possibility of genetic loci affecting two or more distinct traits or phenotypes (pleiotropy), and an incomplete penetrance (lack of clinical symptoms in individuals who carry risk alleles) also complicate the genetic studies of ASD.

The core symptoms of ASD, differences in intellectual and language abilities, and comorbidity, among other characteristics help highlight the presence of clinical heterogeneity in ASD. Knowledge about variability of genetic, environmental, and epigenetic factors in these disorders, as well as the presence of clinical heterogeneity, has propelled the search for more homogenous groups of patients through the study of potential endophenotypes. They may contribute to facilitate the identification of genetic risk factors and basic molecular mechanisms involved in ASD.

3. Application area

The concept of phenotype refers to the traits and features that are characteristic of a person. Thus, the phenotype includes body structures, physiological processes, but also conducts that result from the expression of the genotype in a given environment, and a specific epigenetic profile. Each individual has a unique phenotype.
Neuropsychiatric disorders tend to be classified as discrete diagnostic entities. This classification is limited to clinical symptoms and functioning. However, it has been suggested that these phenotypes may be arbitrary and they may encompass a heterogeneous group of subjects [41].

The concept of endophenotype was adopted in Genetics in 1966 for the study of insects, specifically grasshoppers, to indicate an internal phenotype, a behavior or conduct that did not affect the external features of the animal. In 1967 this term was used in the field of human behavior; the goal was to measure situations that were not perceptible without instruments.

In general, endophenotypes are not considered the diagnosis of the disorder per se, or the clinical characteristics observed by the physician when interviewing the patient. They could be defined by internal attributes associated with the disorder or hidden to the naked eye; they could potentially be associated with more basic phenomena and be defined by inherited quantitative traits associated with a genetic risk to a familiar disorder [42, 43].

An endophenotype would ideally have a precise biological meaning, and a more direct relationship with the action of specific genes. Therefore, its study may facilitate the identification of complex behaviors that are associated with genetics in the population or in an important fraction of it. At the end, this is a strategy to try to understand the pathological role of certain genetic variants and to facilitate the identification of the mechanisms involved in disorders. Endophenotypes may be related with neurophysiological activities, neuroanatomy, neuropsychology, cognitive development (such as language and memory), or brain volume [44, 45].

Any biomarker should contribute to identify the presence of a given disorder, but it may be influenced by the health status of the person or by the effects of environmental factors. In order to be considered as an endophenotype, the biomarker is expected to be heritable, to cosegregate with the disorder, and as such, to be influenced by the genetic component of the disorder [46]. Endophenotypes have sometimes been referred to as intermediate phenotypes, but some authors do not agree on the indistinctive use of these two terms. An intermediate phenotype could be considered as a subclinical picture of psychopathology [47].

Different endophenotypes may be associated with ASD. Ideally, each one should:

a. Cosegregate with the disorder.
b. Be involved in the etiology rather than the effects of the disorder.
c. Be, at least partially, heritable.
d. Be stable even if the symptoms of the disorder are not manifested.
e. Be found in unaffected relatives of patients more frequently than in the general population.
f. Be associated with alleles that contribute to quantitatively distinguish patients and unaffected relatives from people with no ASD.
g. Vary in a continuous way in the general population.
h. Be objective and confirmed by different levels of analysis.
i. Be identified for genetically related disorders [48-51].

Thus far, the identification of endophenotypes has been successful in several fields of Medicine. Endophenotypes were useful in the identification of genes for QT syndrome, idiopathic hemochromatosis, juvenile myoclonic epilepsy, and familial adenomatous intestinal polyposis. Biochemical assays, physiological measures and challenges contribute as primary evidence of pathology in the detection of increased risk to diabetes, hypercholesterolemia, obesity, hypertension, and osteoporosis [48, 52, 53]. Furthermore, different endophenotypes have been proposed for neuropsychiatric disorders.

4. Research course

First degree relatives of patients are key elements in the study of endophenotypes. They are considered at-risk individuals. Even so, only a relatively small percentage of siblings of an individual with ASD will develop such disorders, but a larger proportion is expected to exhibit phenotypes that may fulfill the requirements of endophenotypes.

It has been shown that siblings of patients with ASD as well as other first degree relatives sometimes exhibit social, communication, and/or learning deficits, and ASD are more frequent in them than in the general population. Several possible endophenotypes for ASD have been proposed so far and are related with language and communication and/or with cognition, anatomy, or neurophysiology.

5. Methods

Medline database was searched for articles related with autism and autism spectrum disorder endophenotypes (query terms: autism spectrum disorders and endophenotypes, neuroanatomy, neurophysiology, endocrinology, cognition, social communication, and language. OMIM compendium was searched for disease and gene names. We will discuss potential endophenotypes based on studies in which one or more of their characteristics were fulfilled.

6. Status

Potential endophenotypes related with language and social communication

A continuous distribution of social deficits has been described in the general population, being more frequent in boys than in girls and more prevalent in brothers and fathers than in sisters and mothers of patients with ASD [54]. The estimated frequency of such disorders in siblings ranges from 2.6% to 61.7% [55-59].

It has been suggested that siblings later diagnosed with ASD may be distinguished from other siblings and low-risk infants by unusual eye contact, visual tracking, disengagement of visual
attention, orienting to name, imitation, and delayed social expressive and receptive language [60].

Moreover, there are reports of poor social and communicative performance in parents without a diagnosis of ASD and siblings of children with such disorders [61]. Families with multiple affected individuals were compared with single-incidence autism families, and Down syndrome families as controls. In most cases with multiple affected relatives, both parents had autism-related characteristics. This was not observed in single-incidence families [62].

Furthermore, children with ASD and their parents have been shown to have an impaired performance in rapid automatized naming. Siblings of patients had intermediate performances when compared with controls [62, 63].

 Poor verbal imitation and reduced response to vocal approaches have also been described in children with ASD or children who later developed ASD [64, 65]. In a study, 100% of the 12-month-old infants with no risk for ASD responded on the first or second name call, but 14% of at risk infants responded later. At two years of age developmental problems were identified in many of the children who failed to respond [66].

Impairment in joint attention abilities at age 20 months was also associated with later social and language symptoms [67]. At that same age, children with ASD failed to orient towards biological motion [68]. In addition, affected children who were three to four years old performed worse not only in social orienting and joint attention, but also in attention to the distress of someone else when compared with children without ASD [69].

There are genes that have been associated with language and social impairment in children with ASD and their siblings. This may be illustrated by a familial mutation of the MET gene. The mutation consists of a deletion of 4 exons that cause a frameshift and a premature stop codon. On the homologous chromosome 7 the patient carried an allele that was associated with a decrease in MET expression.

A sibling who was also a carrier of the deletion had language and social impairment [70]. MECP2 gene has been associated with difficulty in recognizing emotional expressions and less time spent looking at facial features [71]; it has also been described as a regulator of the expression of genes involved in social behavior [72]. In animal models, social deficits are observed when the activity of specific genes is lacking, such as FMR1 and Mu-opiod receptor gene (OPRM1) [73].

Cognitive, anatomical and neurophysiological candidate endophenotypes

Atypical patterns of brain activation, for example, in the fusiform gyrus and the amygdala have been identified in individuals with ASD during face processing [74, 75]. In adults, parents and siblings of individuals with ASD were less able to discriminate subtle differences between faces than control individuals, but performed better than adults with ASD. Relatives were significantly worse at identifying expressions of fear and disgust than controls and did not show the typical sensitivity to direct eye gaze direction as opposed to averted eye-gaze, a characteristic observed in adults with ASD [76]. In a prospective study, 6 to 10 month-old
infants were asked to view faces with eye gaze directed toward or away from the infant. Characteristic components of event-related potentials were associated with ASD diagnosed later, at 36 months [77]. In one study, functional magnetic resonance imaging and eye tracking in unaffected siblings of individuals with ASD were performed. There were important differences in gaze fixation and brain activity in response to images of human faces when unaffected siblings were compared with typically developing controls. Siblings performed in a similar way than the ASD patients. When amygdala volume was compared, individuals with ASD and their siblings had similar volumes, which were significantly reduced when compared with volumes in the control group [78]. Small differences were independently found in the left amygdala when subjects with ASD or siblings were compared with controls [79]. Moreover, boys with ASD and unaffected brothers of individuals with ASD showed deficits in a visual attention task. Atypical fronto-cerebellar activation was identified in patients and unaffected brothers when they were compared with controls [80, 81].

Differences in gray matter in the cerebellum, parietal lobe, and left occipital lobe have been described between patients with ASD and controls. In some cases, differences between siblings without ASD and controls have also been reported [79, 82]. Furthermore, deficits in frontal, temporal, and occipital lobes have been identified in discordant monozygotic twins. Eight out of nine of the unaffected twins showed a social or language delay [83]. The functional magnetic resonance imaging response to happy facial expression was different between unaffected siblings and healthy controls without a family history of ASD. Here there were no statistically differences in response between unaffected siblings and patients with ASD [84].

The CNTNAP-2 gene (at 7q35 region) is mutated in individuals with intellectual disability, seizures, autistic features, and language impairment. Connectivity in the frontal lobe has been associated with CNTNAP-2 genetic variants [85].

White matter structure was analyzed in a voxel-based diffusion tensor imaging study. Children with ASD and their unaffected siblings had significantly reduced white matter fractional anisotropy values in the frontal parietal and temporal lobes when compared with controls. There were no significant differences in white matter structure between the ASD and sibling groups [86].

In other studies, children with ASD and their parents showed eye movement abnormalities that suggested a poorer spatial accuracy with respect to controls. The authors proposed that the spatial working memory may be affected in parents [87].

It has been suggested that ASD is characterized by a tendency towards local rather than global information processing. This has been called weak central coherence, which has also been reported in fathers of boys with ASD, as indicated by piecemeal processing in four tests [88]. In addition, some parents preferred nonsocial activities and ability in detail-focused processing, as is expected in subjects with ASD. A questionnaire completed by parents discriminated between ASD individuals and controls, but did not differentiate siblings [89]. In addition, atypical activation of temporal and frontal regions during the Embedded Figures Task was found in subjects with ASD and in unaffected siblings. The reduced activation on the ASD
group when compared with controls was correlated with the severity of reciprocal social interaction deficits [90].

Regarding body measurements, although there is a high degree of variability in head circumference of children with ASD, the rate of macrocephaly is increased in these patients, if compared with the general population. Mean standardized head circumference and frequency of macrocephaly were similar in patients with ASD and their parents. Increased head circumference was associated with ASD severity and with a late onset of language [91]. Macrocephaly appearing in the first year of life has been described in subgroups of patients with ASD, mental retardation, and/or language delay who carry mutations on the Phosphatase and tensin homolog (PTEN) gene. Numerous relatives of individuals with a PTEN mutation also had macrocephaly and mental retardation [92-94]. Mutations in the hepatocyte cell adhesion molecule gene (HEPACAM) have also been associated with macrocephaly and ASD [95].

Other researchers have documented impairment in cortical sensory processing in ASD cases. For example, it has been suggested that early auditory responses as well as transient memory are impaired in some children with ASD; auditory stimuli produce messages that are sent from the brainstem to the thalamus and into the auditory cortex. Event related potentials were found to be delayed in affected children, and indicated a deficiency to achieve typical maturational development of the auditory system in individuals with ASD [96, 97]. In addition, children who were at risk for ASD showed atypical neural lateralization to speech in their first year of life [98].

Impairment in cortical sensory processing has been found in patients with Rett syndrome. In addition, auditory and visual evoked potentials in mice that were heterozygous for a loss of function variant of the MeCP2 gene demonstrated impairments in sensory processing. For example, increased N1 amplitude, latency of auditory P2 component, and suppression of beta-band activity were identified. There was a decreased suppression of induced gamma-band activity [99]. Gamma-band activity differences have been identified between parents of children with ASD and control adults [100].

Another area of potential endophenotypes is related with movement. There have been reports that suggest that specific motor developmental clues may be identified early in infants who will develop ASD. These signs tend to be persistent, and may be associated with abnormalities at the cerebellar, vestibular, cortical or fronto-striatal level [101]. Moreover, infants who were 18-month old and who had an older sibling with ASD were at higher risk for a delay in postural change and stability, language production and comprehension if compared with infants with no risk for ASD [56]. An association of motor anomalies and specific genes has been proposed. MeCP2, SHANK3, 22q13.3, Neurexin 1 (NRXN1) deletion, 15q11.1-q13.3, and KIAA0442 gene (known as Autism Susceptibility Candidate 2 or AUTS2) have been associated with hypotonia, stereotypes and/or motor delay; RNA-binding protein FOX1 (RBFOX1) has been associated with motor asymmetry [102].
7. Results

According to the studies so far discussed, different techniques have proven to be useful in the identification of endophenotypes or potentially promising biomarkers. Social deficits, as well as cognitive, anatomical, and/or neurophysiological findings are encouraging and in some cases are associated with candidate genes. Endophenotypes may help detect children who are at higher risk for ASD. This could promote better and earlier diagnosis and treatment, as well as the establishment of prevention strategies. It is also probable that new drug targets can be identified and that personalized treatments can be available.

The precise genetic, environmental, and epigenetic factors that increase susceptibility to ASD may vary in several ways from one affected child to another. For this reason, it is very important to continue the search for means that allow us to form less heterogenous groups of patients. Endophenotypes, at least in some cases, could contribute to achieve this goal. They could help recognize risk factors that participate or affect common pathways or processes, such as chromatin remodeling and transcription that may affect neurodevelopment.

In some instances, de novo mutations associated with ASD may be highly penetrant, and in absence of a family history of ASD, endophenotypes may not be observed in relatives, but could still contribute to an earlier identification of symptoms in the carrier of the mutation; the next generations could also benefit from early assessments and prevention strategies. On the other hand, there will be families in which relatives will probably share enough risk factors with the patient, as to exhibit one or more endophenotypes or ASD.

8. Future research

Further studies in patients and relatives, preferably of first and second degree, will help determine if biomarkers fulfill the criteria of heritability and cosegregation.

Knowledge about the neuroetiology of ASD will be enriched by research in areas such as neuroanatomy, physiology, and social behavior. Multiple investigations are underway, and risk factors for ASD will continue to be identified, possibly in more homogenous groups of patients.

Future research should ideally involve longitudinal research in large samples that include twin pairs, nuclear families and/or extended pedigrees. These conditions may facilitate the identification or confirmation of endophenotypes, in which the criteria of heritability and cosegregation are fulfilled. Whenever possible, it will be useful to obtain detailed information about clinical and demographic aspects, not only regarding the individual with ASD, but about first and second degree relatives. While the grouping of individuals in a dichotomous way, as affected or unaffected will continue to be evaluated, and offers advantages, this approach can be complemented with the evaluation of endophenotypes [46].
9. Conclusion

Several social, cognitive, anatomical, and neurophysiological biomarkers are under investigation and may be associated with candidate genes. Given that certain deficits have been identified in first degree relatives, mainly in siblings and parents of individuals with ASD, it is highly probable that at least some of them are indeed true endophenotypes. Longitudinal studies with large samples of cases and controls, but also of nuclear and extended families are needed in order to determine if all the requirement for endophenotypes are fulfilled. Once this phenotypes are confirmed, they may help detect infants and first degree relatives with an increased risk for ASD. This represents an excellent opportunity to initiate preventive measures and/or an early treatment when necessary. An early identification of risk may facilitate a better prognosis.

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