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Genetic and non-genetic animal models for autism spectrum disorders (ASD)

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ABSTRACT

Autism spectrum disorder (ASD) is associated, in addition to complex genetic factors, with a variety of prenatal, perinatal and postnatal etiologies. We discuss the known animal models, mostly in mice and rats, of ASD that helps us to understand the etiology, pathogenesis and treatment of human ASD. We describe only models where behavioral testing has shown autistic like behaviors. Some genetic models mimic known human syndromes like fragile X where ASD is part of the clinical picture, and others are without defined human syndromes. Among the environmentally induced ASD models in rodents, the most common model is the one induced by valproic acid (VPA) either prenatally or early postnatally. VPA induces autism-like behaviors following single exposure during different phases of brain development, implying that the mechanism of action is via a general biological mechanism like epigenetic changes. Maternal infection and inflammation are also associated with ASD in man and animal models.

1. Introduction

Human ASD is a heterogeneous group of neurobehavioral problem with different recognized genetic and environmental origins. Due to the complexity of these pathologies and the lack of a definite known diagnostic marker, ASD is defined by phenotypic behavioral traits. The DSM 5 defines ASD as a neurobehavioral disorder manifested by persistent deficits in social and communication interaction, deficits in developing, understanding and maintaining relationships, as well as abnormal and fixed interests and repetitive behavior, with various degrees of severity [1,2]. Symptoms must be present at early childhood and interfere with daily function. The etiology is largely unknown, and seems to be the result of genetic and environmental interaction [3].

In the last years environmental exposures, especially during pregnancy, are increasingly being recognized as potential risk factors for ASD, and the possibility that the prenatal environment affects fetal programming is a promising direction for research. In addition, in many children with ASD a variety of gene mutations and of changes in gene expression, most of them related to the brain development and function were found. In addition, children with various well defined clinical entities (i.e. Fragile X, tuberous sclerosis, Rett syndrome) exhibit ASD like behavior.

In addition to complex genetic susceptibility, as evidenced from twin studies, epigenetic changes have also been proposed [2]. Other mechanisms are: immune dysregulation that include abnormal levels of cytokines and growth factors, fetal and maternal antibodies to brain tissue, microglial activation, and others [4]. Additional proposed mechanisms are increased oxidative stress, mitochondrial dysfunction, abnormalities in brain serotonin, abnormal white matter connectivity and altered synapses resulting from genetic changes such as changes in ERK pathway [4].

A variety of morphological and functional changes have been demonstrated in the brain of children or adults with ASD. However, their presence is inconsistent and is generally not related to the severity of the symptoms. Hence, in spite of the existence of various imaging and neurobehavioral tools for the diagnosis of ASD, its diagnosis relies on clinical behavioral grounds.

Experimental animal models are of importance for the understanding of the etiology and pathogenesis of any human disease, including ASD. However, these models are appropriate mainly when the same diagnostic markers demonstrating resemblance to the human situation are used. This is relatively easy whenever there are distinct markers for a disease. For example, in animal models of diabetes, one has to show similar metabolic derangements. However, it is more difficult to prove that an experimental animal is the suitable model for ASD in man, since it is based on behavioral

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changes and the behavior of a mouse or a rat is not the same as the behavior of a child or adult. Indeed, a variety of neurobehavioral tests have been used to demonstrate in the animal models a behavioral pattern that mimics the behavior of ASD children. Another approach is to create genetic models that are similar to the human genetic changes observed sometimes in children with ASD. In these models, neurobehavioral tests often demonstrate an autistic like behavior as well as distinct changes in the brain that resemble those sometimes found in children with ASD.

Despite the heterogeneity of the clinical symptoms, animal studies allow coherent investigations of the circuits, cells, and pathophysiological processes affected in autism [5].

Basically, there are two types of animal models for ASD: environmentally induced, by exposure of the pregnant animals to certain chemicals or infection/inflammation and those induced by genetic manipulations. The first are relevant mainly if it is shown that the same chemicals are also relevant for human; for example VPA. VPA was found to significantly increase the rate of ASD among offspring of treated mothers [6,7] and was thereafter shown to induce autism-like behavior in rats and mice [8]. Genetic animal models of ASD are also important especially when they mimic the ASD symptoms that are present in distinct human genetic diseases such as fragile X and tuberous sclerosis. Mouse models should therefore be based on a known genetic cause of a disease, reflect key aspects of the human symptoms and, if possible, respond to treatments that are effective in the human disease [9]. However, "new" genetic changes in animals that induce ASD like symptoms may also be of importance since they may "push" to seek similar changes in man.

ASD in human is manifested by a set of behavioral changes that appear at early childhood. The most prominent are impaired social interaction, impaired language and motor behavior, including stereotyped and repetitive motor movements and limited interest in the surroundings. These behaviors can also be observed and monitored in various animal models by using specific tests that were developed for the measurement of such behavioral modifications. Although it is valid to argue that animal behaviors are different from those in men, the fact that they can be specifically demonstrated in the relevant models points to their similarity to the human behavior. As most genetic and non-genetic models of ASD are in mice and rats, the more common and reliable behavioral tests were developed in these animals. In spite of some limitations they are generally considered to represent adequately human behaviors. In the tables we name, wherever relevant, the different behavioral tests used by the investigators to demonstrate autistic like behaviors in their models. It should be remembered, however, that not all tests used by the different investigators are unanimously accepted as representing ASD like behaviors. Hence, this might be a weakness of some of the studies.

The purpose of the present review is to summarize the data on more common experimental animal models of ASD either as a result of prenatal exposure (i.e. VPA, inflammation) or those following genetic manipulations. Most models are in mice and rats. The current review includes only investigations in which animals were evaluated by behavioral studies and the changes mimic ASD like behavior. The impacts of the genetic background or environmental insults on the phenotypic traits, anatomical, biochemical, physiological markers and treatment modalities are described whenever applicable. The tests used, the neurobehavioral changes and mode of treatment in each study are described in the tables of the genetic models, as well as the specific gene defects. In the tables that describe the VPA induced models we also added the time of exposure and dose of VPA.

1.1. Genetic animal models of autism: implication for human studies (Tables 1–3)

Several human syndromes derived from a single gene mutation increase the risk for ASD. The more common aberrations are Fragile X syndrome, a mutation in *FMR1* [10], Rett syndrome, a mutation in *MECP2* [11], tuberous sclerosis, mutations in *TSC1* or *TSC2* [12] and Timothy syndrome, a mutation in *CACNA1C* [13]. Copy-number variants that lead to inherited maternal 15q11–13 duplication resulting in Prader-Willi syndrome [14] and other duplications like the *NPHP1* gene [15] are also associated with autistic traits. The development in strategies for the identification of genetic variants also led to the description of new syndromic forms of ASD and enabled the association between phenotype and genetic traits .

The identification of such genetic variations with the development of new strategies for genetic engineering facilitated the development of genetic animal models of ASD. Mice are the predominant animal model for ASD owing to their genetic tractability and their ability to demonstrate analogs of behavioral deficits associated with ASD [5]. Other animal models include rats [16], zebrafish [17], song birds [18] and the newly introduced macaque monkeys [19]. Until now, only few of the current animal models were proven valid for evaluation of a known human aberration by establishment of a measurable marker and an offer of a treatment option.

2. Animal models for genes of a syndromic disorder predisposing to autism (Table 1)

The animal models representing known human syndromes derived from a gene mutation exemplify the difficulties in establishing a valid genetic trait.

2.0.1. Fragile X (FMR1)—Table 1

Children with Fragile X syndrome which is the most frequent inherited cause of mental retardation have increased rates of ASD. The Fragile Mental Retardation 1 locus (*FMR1*) resides in the X chromosome and expansion of triplet repeats in the untranslated region of the *FMR1* gene prevents synthesis of the *FMR1* gene product FMRP. FMRP is an RNA-binding protein that modulates mRNA trafficking, dendritic maturation and synaptic plasticity. Rodents, mostly mice knock out for the *FMR1* gene, were shown to present autistic traits. Different traits depend on the background strain. Some of the studies also found structural, biochemical and physiological abnormalities including abnormal dendritic spine morphology [20], elevated phosphorylation of translational control molecules and exaggerated protein synthesis in the hippocampus [21]. Additionally, enhanced metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) recordings were described [21,22], implying reduction in the efficacy of neuronal synapses following a stimulus. Treatment modalities by genetic engineering and medications are meant for prevention of exaggerated protein synthesis [21] rescue of synaptic tonic inhibitory currents [23] activation of translation to overcome the lack in Fmrp [24] and genetic engineering for correction of the synaptic mGluR signaling [22]. However, environmental stimulation was successful as well [20].

2.0.2. Rett syndrome and MECP2 *mutations (Table 1)*

Rett syndrome, an X linked disease that affects girls, is characterized by neurodevelopmental delay, ASD and seizures. It is caused by mutations in the gene encoding for the methyl-CpG binding protein 2 (*MECP2*) that binds to methylated-CpG dinucleotides and influences gene expression. *MECP2* is expressed widely, but is