THE BASIS OF HOX GENE INVOLVEMENT IN AUTISM

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Abstract: Autism is a childhood neurodevelopmental disorder with marked social deficits, regression or delay of verbal as well as nonverbal communicative skills in addition to a range of stereotypic and restrictive behavior and interests. The evidences from family and twin studies suggest that it is the most heritable neurodevelopmental disorder having involvement of more than fifteen genes that are epistatic, with some contribution of non-genetic factors. During the past decade, tremendous progress has been made in the understanding of the neurobiological basis of autism. Brain stem malformation is suggested to be one of the causative factors of autism and thalidomide embryopathy provides strong evidence in favor of this hypothesis. Neuronal architecture of autistic brain and its similarity with loss of function mutant mice of HoxA1 or HoxB1 provide insight into the participation of these two paralogous genes in the etiology of autism. The genetic basis of autism is reviewed with emphasis on Hox genes as one of the candidate genes for autism in view of a strong correlation of hindbrain abnormality with Hox gene expression.

Key words: Autism, Neurodevelopment, Brain stem, HoxA1, HoxB1

Autism: Diagnosis and its prevalence

Autism is a neurodevelopmental psychiatric disorder in children that is characterized by abnormalities in various domains of social interaction. Leo Kanner first described the disease in 1943 as infantile autism for the social reticence and aloofness in these children [1]. Even though the child looks apparently normal during birth, the manifestation of the disorder becomes quite evident when the child is 2-3 years old and it persists all through his life [2]. The survey conducted by the US Department of Developmental Services has reported a 5.56 fold increase in the prevalence of autism between 1991 and 1997 [3]. This rate is higher than the rate reported for other paediatric disorders like spina bifida, cancer and Down syndrome [4,5]. Even though it is difficult to estimate the prevalence of autism because of such steep rise, a study conducted in the United Kingdom estimated a prevalence rate of 16.8 per 10,000 children [6]. Subsequently, the concern about increasing incidence of autism in different populations spread across different ethnic groups in various parts of the world prompted a population based study in a US metropolitan area that reported a rate of 3.4 per 1000 children for autism. These studies also underscored the long-standing observation that the boys are affected more often than girls and the incidence rate is about four times higher in the former [7]. The most parsimonious explanation that can be ascribed to this escalation in prevalence in the absence of evidence from any major genetic and environmental drifts may include increasing awareness among the people, improved diagnostic tools for the disorder and also due to the broader definition of autism spectrum disorders (ASDs).

DSM IV-TR (Diagnostic and Statistical Manual of Mental Disorders, Edition IV, Text Revision) [8] and ICD-10 (International Classification of Diseases, Tenth Revision) [9] have defined autism based on the triad of impairments which include (1) impairment...
in social interaction, (2) impairment in verbal, nonverbal communication as well as in imaginative play and (3) possession of stereotyped and idiosyncratic behavioral patterns or interests. Autism is associated with a broad spectrum of abnormalities and is generally termed as ASD. Apart from the aforesaid abnormalities, an average of 70% of the cases exhibited mental retardation and approximately 33% were shown to possess epileptic or seizure attack [2]. According to DSM IV, they may also have associated symptoms like hyperactivity, short attention span, impulsivity, aggressiveness, self-injurious behavior and odd responses to sensory stimuli with abnormality in eating or sleeping. The symptoms and features of autism can manifest in different ways ranging from mild to severe. DSM-IV classifies autistic disorder under the umbrella of Pervasive Developmental Disorders (PDD) that include disorders with pervasive as well as severe developmental abnormalities. PDD is categorized into 5 subtypes based on the severity of the heterogeneous behavioral symptoms and age of onset of the disorder. The subtypes of PDD are autistic disorder, Asperger’s syndrome, PDD-not otherwise specified, childhood disintegrative disorder, and Rett’s disorder. Table 1 describes the diagnostic criteria for autistic disorder as per DSM-IV.

Family studies and genetic basis of autism

Earlier, it was believed that “frozen motherhood” might be responsible for the developmental abnormalities observed in autistic children. Some reports have even highlighted the measles, mumps, rubella (MMR) vaccination and inflammatory bowel diseases as possible causes of autism, but more recently these arguments are fairly ruled out [10-13]. But there are reports speculating that the mercury based preservatives used in the vaccines may pose the risk for autism [14-16]. Autistic phenotype is frequently associated with some genetic disorders like fragile-X syndrome, tuberous sclerosis, phenylketonuria and neurofibromatosis [17-21]. But this accounts only to a small proportion (~5-14%) of the total autistic population. Apart from these, several environmental factors are also considered to be the causative determinants for the occurrence of ASDs. For example, in utero exposure of the embryo to rubella infection during first trimester or teratogens like ethanol, valproic acid, thalidomide and misoprostol were found to increase the risk of developing this disorder [22-28]. These studies have provided some insights into possible pathological basis and the putative period during fetal development when such assaults could lead to autism.

Convincing evidences from the epidemiological and family studies including twins and siblings substantiate that autism is a genetically inheritable disorder with the participation of some unknown non-genetic factors [29,30]. The recurrence rate of autism in the sibling of an affected child is around 2-6% and the ratio decreases to less than 1% in the case of more distant cousins [31]. The rate further decreases in general population where it is only 0.04-0.1% [32-34]. Thus the chance of having autism in the families of autistic patients is 40-100 times higher than its occurrence in the general population [2]. The information that has been gathered from twin studies showed that the concordance rate for autism in monozygotic (MZ) twins is 70-90%, where as the rate decreases to 0-25% in the case of dizygotic (DZ) twins [35-38]. In spite of sharing 100% of their genes in the case of MZ twins, the concordant rate of 70-90% indicates the involvement of non-genetic elements apart from a strong genetic predisposition [39]. Among the complex genetic disorders, autism has a relatively high heritability of approximately 90% [37]. The observed decrease in the rate of incidence of the disease in the family as the degree of genetic relationship increases through generations suggests the involvement of multiple interacting genes as risk factors of autism [40,41]. It may be such that the interplay of multiple genes increases the susceptibility to autism rather than directly causing the disorder provided favorable non-genetic factors also co-exist [7]. Studies based on genome-wide mapping, cytogenetic analysis and candidate gene approach support that autism is an oligogenic disorder of epistatic nature.

In order to identify the specific chromosomal regions that may serve as likelihood regions for susceptibility to autism, many investigators have carried out genome-wide screening strategy [40,42-47]. First genome-wide screening for autism was published in 1998 by International Molecular Genetic Study of Autism Consortium using samples of DNA from 99 multiplex autism families and has suggested the participation of six chromosomal regions such as 4p, 7q, 10p, 16p, 19p and 22p, based on a multipoint logarithm of the odds score (MLS) more than or equal to 1. Subsequent studies calculated an odds ratio of 3-3.6 to be highly significant for genetic linkage and showed a region on 7q with a MLS of 3.55 [42,48].
The contributions of different regions of chromosomes 1, 2, 4, 5, 6, 7, 13, 15, 16, 17, 19 and the X chromosomes have been suggested through this approach. Even though the data compiled from all genome scan studies provide nominal suggestions of the possible involvement of 17 autosomes and X-chromosomes in the expression of autism phenotype, independent studies could support only a few of these loci as candidate regions. Taking into consideration the broad phenotype and the suggestive evidences of participation of different chromosomal loci, autism is now defined as a complex genetic disorder with multiple atiology.

Studies relating to the toxicological basis of autism

The studies using teratogens revealed that autism could be linked to teratogen exposure during early gestation period and indicated early brain stem injury as one of the root causes for autism. A previous study indicated that specific developmental stages could be identified during which the specific morphological embryopathy occurred as a result of the toxic insult by thalidomide and reported that thumb was affected at an early period of gestation i.e., 20 days of gestation, the ears from 20-33 days, and limb formation from 25-35 days [49]. Studies on a thalidomide-exposed population with developmental anomalies like stunted arms and legs, abnormal or missing ears and thumbs as well as disorders of eye and facial muscles presented an interesting correlation with the structural abnormalities often found in the autistic individuals [27,50]. Studies on thalidomide exposed population showed that 15 out of 86 cases were noted with ear malformations and of which four cases (5% of the total) were diagnosed with psychiatric problems and subsequently detected it as ASD [27,49]. Another study on the prevalence of autism in Nova Scotia, provided evidences for ear anomalies in autistic patients as compared to their unaffected siblings or people with mental retardation [51,52]. Thus it can be argued that these autistic individuals could have had the toxic hit between the 20-24 days of gestation [27,53,54]. At the same time when thalidomide embryopathy was published, Christianson et al [55] reported case studies relating to the incidence of autism with another teratogen, valproic acid (VPA). VPA is an antiseizure drug that was known to cause developmental and neurological problems in offsprings [56]. These subjects also exhibited the limb and craniofacial anomalies as observed in thalidomide victims. Afterwards in a clinical study conducted in 57 children with fetal anticonvulsant syndrome, 46 children were exposed to anticonvulsant drugs (VPA alone or in combination with other medications) in utero and among them 5 of them were diagnosed with ASDs [57].

Neuronal basis of CNS malformation in autism

This critical time period during embryogenesis (i.e., 20-24 days) marks the closure of neural tube and differentiation of the central nervous system (CNS) into rhombomeres [28,58,59]. At this stage, the early CNS development takes place with the generation of only a few of the first formed neurons. Most of these early neurons are motor neurons of the cranial nerve that are innervated into the muscles of the eyes, face, tongue, throat as well as to jaw and the cell bodies of these neurons are positioned in the brain stem [60]. Since motor neuron generation and ear development occurs simultaneously, it can be envisaged that thalidomide exposure can also cause abnormal development of craniofacial innervations in victims with autism [54,58]. Such abnormalities are also quite often reported for idiopathic cases of autism and it has been noted that autistic subjects possessed eye or facial anomalies or both.

These studies, clearly suggest brain stem pathology in the manifestation of autism in thalidomide victims. Brain stem is the region of the brain, which connects spinal cord with the rest of the brain. Any injury to the brain stem may interfere with proper brain development and maintenance of synaptic plasticity in different brain regions that are involved in higher level functioning such as speech, which is impaired in autistic cases. Rodier and his group went on to investigate whether the suggested malformation of the brain stem in thalidomide subjects could be detected in the brain structure of an autistic patient or not [28]. They compared the histology of the autistic brain with that of the controls and found that there exists reduction in the number of neurons in facial nucleus, absence of superior olive and shortening of the brain stem. They had also noted that some of the neurons of hypoglossal nucleus failed to differentiate [28]. A schematic representation of the anatomical differences of normal and autistic brain is shown in figure 1. Detailed structural analysis of the autistic brain showed that the fibers that usually outline superior olive and facial nucleus in normal cases were found to pass through the regions of these
nuclei, where they are supposedly positioned and thus the area of these nuclei has never been occupied. This information reveals that these neuronal structures are not at all developed during CNS differentiation in autistic brains rather than being lost at a later developmental stage. Apart from these studies, it has also been shown that the prenatal treatment of valproate in rats showed reduction in the number of purkinje cells in the cerebellum, which is also one of the brain anomalies observed in autism [59]. All these data led to further advancement in this field by administering VPA in rats to use it as an animal model that could reproduce the early brain injury and behavioral abnormalities attributed to autistic phenotype [61,62].

**Involvement of Hox genes in neurodevelopment**

The neuropathology of autism observed in human beings and animals had striking similarity with a knockout mouse, which is engineered to delete HoxA1 gene. The results from those studies on knockout mice showed that the function of this protein bears a prominent role in hind brain development [63,64]. HoxA1 is expressed in hindbrain only during the early developmental period when the closure of neural tube occurs [65]. The period of expression of this gene is very much linked with the time of incidence of teratogenic embryopathy observed in thalidomide exposed autistic subjects. On comparison, the histological abnormalities observed in human autistic brain and the brain of HoxA1 knockout mouse overlapped in many a respect in the absence of posterior part of facial nucleus and superior olive with maldevelopment of external ears plus shortening of brain stem at the fifth rhombomere. Based on these supporting evidences it is postulated that HoxA1 gene is one of the probable candidate genes that can be studied to understand the pathology of autism.

Hox genes are a group of transcription factors known as homeobox genes that play a pivotal role in the morphogenesis during embryo development in animals. The function of these proteins is also implicated in the development of brain stem and establishment of body axis during embryogenesis [66,67]. Hox genes contain a 180 nucleotide sequence which codes for a highly conserved 60 amino acids homeodomain. These proteins represent the largest group of DNA binding proteins and function as transcription factors by binding to specific DNA sequences through helix turn helix motifs present in the homeodomain [68]. These genes, initially identified in Drosophila within the homeotic gene complex, are highly conserved in animal kingdom. The number of Hox genes increased in vertebrate lineage during evolution as it evolved from invertebrates [69]. In mammals, there exist four clusters of Hox genes, termed A, B, C & D on different chromosomes with amplified copies generated by tandem duplication in each cluster and the location of HoxA, HoxB, HoxC and HoxD are on chromosomal regions 7p15.3, 17q21.3, 12q13.3 and 2q31 respectively. The genes with same rank in different complexes are evolutionarily related and are called paralogues. A total of 39 Hox genes occupy these four clusters in humans and are arranged in 13 paralogous groups [70]. An important characteristic of Hox genes is that the rostrocaudal and temporal expression of the genes in the body is determined by the order of arrangement of these genes on the chromosome [71,72]. The pattern of activation of Hox genes follows colinearity rule in such a way that the one located in the 3’ region is expressed first and that too in the anterior part of the embryo and subsequently the genes located 5’ to the former gets transcribed in the more caudal regions [73,74]. The first four paralogue members (Hox A, B, C & D, 1-4) are expressed within the hindbrain during embryonic development [75-77].

**Role of Hoxa1 and Hoxb1 in the pathology of autism**

In humans, the paralogous genes HoxA1 and HoxB1 are located on chromosomes 7p and 17q respectively [78,79]. Studies on loss of function mutants reveal that HoxA1 is involved in the maintenance as well as generation of hindbrain segments and HoxB1 has a role in conferring specific identity to rhombomere r4 cells [80-84]. Thus the importance of these genes in the hindbrain development are well recognised through these experiments. Based on the information gathered from these reports, a study was conducted in Caucasian population with 57 probands, 166 relatives of these probands and 119 unrelated adults as controls to probe into the possibility of association of the allelic variants of the two genes with autism [85]. They identified a substitution variant A218G at the exon 1 of HoxA1 gene and a 9 base pair insertion variant (insertion allele) after the base 88 in the HoxB1 gene coding for an additional histidine-serine-alanine. In case of HoxA1, the substitution allele
Table 1: Diagnostic Criteria for Autistic Disorder

A. A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3).
1. Qualitative impairment in social interaction, as manifested by at least two of the following:
   a. Marked impairment in the use of multiple nonverbal behaviours such as eye-to-eye gaze, facial expression, body postures, and gestures, to regulate social interaction.
   b. Failure to develop peer relationships appropriate to developmental level.
   c. A lack of spontaneous seeking to share enjoyment, interests or achievements with other people eg: by a lack of showing, bringing or pointing out objects of interest.
   d. Lack of social or emotional reciprocity.
2. Qualitative impairments in communication as manifested by at least one of the following:
   a. Delay in, or total lack of, the development of spoken language not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime.
   b. In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others.
   c. Lack of varied, spontaneous, make-believe play or social imitative play appropriate to developmental level.
3. Restricted, repetitive and stereotyped patterns of behaviour, interests and activities, as manifested by at least one of the following:
   a. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus.
   b. Apparently inflexible adherence to specific nonfunctional routines or rituals.
   c. Stereotyped and repetitive motor mannerisms eg: hand or finger flapping or twisting, or complex whole-body movements.
   d. Persistent preoccupation with parts of objects.

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:
1. Social interaction
2. Language as used in social communication
3. Symbolic or imaginative play.

C. The disturbance is not better accounted for by Rett’s Disorder or Childhood Disintegrative Disorder.

(A218G) changed the primary structure of the protein by altering the 2nd histidine into arginine (H73R) in a string of 10 histidines of the domain critical for interacting with other proteins. This is a highly conserved region of the protein among rat, human and mouse, varying only in the number of histidines in the string. Its function may depend strongly on the integrity of the protein and the change H73R may only partially reduce the function of the protein because of the physicochemical properties of the two basic amino acids. The study by Ingram et al.[85] reported a significant deviation from Hardy-Weinberg equilibrium resulting from an excess of AG genotype of HoxA1 in autism cases. Their study also showed a significant deviation from the Mendelian expectation in gene transmission among the affected offsprings. However, they could not detect any association of HoxB1 insertion allele polymorphism with autistic cases. Another independent study conducted by Johnson et al. [86] was in agreement with Ingram’s observations on HoxA1 polymorphism. Two more single nucleotide base substitutions of HoxB1 like A315T and G456A were identified by Li et al. [87]. They found that these are in tight linkage disequilibrium with the 9 base pair insertion. They genotyped HoxA1 and HoxB1 variants in 110 multiplex families and concluded that it is unlikely to have an association between autism and polymorphisms in HoxA1 and HoxB1. Subsequently four independent studies also failed to replicate the initial findings and could not detect any linkage or association of these polymorphisms with autism [88-91]. Interestingly, a recent report of a case control and family based association analysis performed with 127 autistic patients and 174 ethnically matched controls suggested an association of A allele of HoxA1 with autism [92].

As autism is a multifactorial disorder, the susceptibility factor shared by this gene polymorphism can only be partial. When viewed from the background that the Hox gene variations are also observed in control populations, a direct involvement of the Hox genes in the development of autism is equivocal. It could be the result of complexities and epistatic nature of susceptible genes, which in conjunction with an adverse environmental influence might trigger the development of the spectrum of behavioral phenotypes observed in autism. Even though the correlation of brain stem abnormalities and Hox gene expression with the narrow window of teratogen induced insult during embryogenesis is suggestive of a possible participation of Hox genes in autism, it warrants more studies in different ethnic populations to confirm the hypothesis.

Conclusion:

Autism is a complex disorder that sprouts within the initial three years of life and causes a life long
disability in the affected person. It markedly impairs one’s ability to interact and communicate and often shows abnormal repetitive as well as idiosyncratic behavior. The prevalence of the disease is ever increasing with a male to female ratio of 4:1. Family and twin studies enlighten the fact that it is more of a genetic disorder, but the involvement of non-genetic factors cannot also be ruled out. These studies also furnished the information that autism is not due to a single gene, but the multiple genes that are epistatic in nature are involved in its pathology.

The promising results from teratological and neuroanatomical studies recommended the brain stem injury as one of the causes of autism. The brain stem injury around 20-24 days of gestation marks the early stages of CNS development and that coincides with the expression of Hox genes. But studies on genetic variations of HoxA1 and HoxB1 with autism have come up with conflicting results and more research on this aspect among different populations are needed to validate this hypothesis. Manifestation of autism is apparent as a spectrum of abnormalities that represent an array of neurobiological heterogeneity which in turn may reflect the complexity in genetic make up. Autism is diagnosed solely on the basis of behavioral anomalies, the association of Hox genes based on biological basis of autism may provide more meaningful approach for linkage studies. Even though most of the association studies could not replicate the original findings by Ingram’s group [85], but the possible role of HoxA1 and HoxB1 in the pathology of autism cannot be ruled out. An integrated approach by psychiatrists, psychologists, neurologists, neuroscientists, geneticists, special educators, speech therapists and occupational therapists is needed to explain the pathology of autism, which may lead to suitable tools for better management of the affected individuals. Considering the worldwide prevalence of 15-20 autistic children in 10,000 individuals, extracts a figure of 1.5 to 2 million autistic population in India. But the research contribution from India towards the understanding of autism is very limited with a few reports on epidemiological and clinical studies. Our team and a research group from All India Institute of Medical Sciences, New Delhi have already initiated investigations and the main focus of research is related to genetic and immunological basis of autism respectively.

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