Criteria for Autism Spectrum Disorder: A Redefinition

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ABSTRACT

Diagnostic criteria for Autism Spectrum Disorder (ASD) have been based on the clinical presentation in the past. However, there is a need for redefining these criteria with the use of neuroimaging and genetic data. This study aimed to provide an updated understanding of ASD criteria by incorporating recent advances in neuroimaging and genetics.

INTRODUCTION

ASD is a neurodevelopmental disorder characterized by deficits in social communication and repetitive behavior. The current diagnostic criteria are based on clinical symptoms, but recent advances in neuroimaging and genetics suggest the need for a redefinition.

MATERIALS AND METHODS

Bacterial strains and plasmids. For initial cloning and maintenance of the DNA fragments pUC18 vector (Pharmacia), together with E. coli strain XL-1 blue and for protein production, expression vector pET23a (+) (Novagen) and bacterial strain BL21 (DE3) pLysS were used. Bacterial transformation and DNA manipulations were performed as described by Sambrook et al. [15].

Keywords: Heat-labile enterotoxin, Heat-Stable enterotoxin, E. coli.

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**Polymerase Chain Reaction.** PCR was performed using two sets of primers designed for the amplification of LT-B gene from the standard strain H10407 with the following sequences: 5’CCGAATTCCGGGATGAATTATGAATAAAG3’ and 5’AGCTGAGAGTTTCCATCTGATTGCGC3’ containing EcoRI and PstI sites respectively. Ventā DNA polymerase (New England Biolabs) was used for amplification and the PCR conditions were as follows: 94°C 1 min, 60°C 45s, 72°C 30s. This program was performed for 25 cycles. DNA sequencing was performed by the dideoxy chain termination technique of Sanger et al. [16].

**Construction of synthetic ST gene.** The gene encoding ST was assembled synthetically using two sets of overlapping oligomers 5’CGGGATCCTGC TGTAAGATTATACTGATTG CGGC3’ and 5’CGGG ATCCACATCCTGTACAGGCAGGATTACA3’ dNTPs and Taq DNA polymerase. The size of the fragment was assessed by SDS-PAGE and the sequence was verified by DNA sequencing.

**Construction, cloning and expression of the fused gene.** The LT-B gene was cloned in pUC18 digested with SmalI (pUC-LTB); the resulting construct was digested with BamHI and filled in with Klenow. The blunt ended ST gene was cloned in this construct (pUC-LTB-ST) and transformed into E. coli strain XL-1 blue. For expression, the fused genes were digested with EcoRI and HindIII and cloned into pET23a (+) digested with the same enzymes.

E. coli BL21 (DE3) pLysS transformed with pET-LTB/ST was cultured in LB supplemented with ampicillin (100 μg/ml) at 37°C with good aeration to early exponential phase (OD 600 approximately 0.2-0.5). IPTG was added to a final concentration of 0.5 mM and the culture was allowed to continue growing for 3-4 h. Bacteria were harvested by centrifugation and LTB/ST was released using sonication or 8 M urea [17].

**Detection of the expressed fusion peptide.** The reversed Passive Latex Hemagglutination kit and Colist EIA kit (Denka Sieken co. Ltd.) were used according to manufacturer’s instruction to detect LTB and ST respectively. SDS-PAGE, Western blot and dot blot immunoassay using antibodies provided in the kits were also used for analysis of the expressed protein [14].

**Toxicity test.** The suckling mouse assay for ST was performed essentially as described by Giannella [18]. Newborn suckling mice (1 to 4 days old) were randomly divided into the groups of three. Each mouse was inoculated intra-gastrically with 1, 100, 1000 ng of the LTB/ST fusion polypeptide stained with 2% Evans blue. At 3h postinoculation, the mice were killed, the abdomens were opened, and the entire intestine from each mouse was removed and the ratio of the gut weight to that of the remaining carcass (G/C) was calculated. The mean G/C ratio was then calculated for each group. The G/C ratios of >0.09 are considered positive. A wild ETEC isolate producing ST was used as positive and PBS as negative control. One ng of the toxins produces positive reaction in infant mice [12].

**RESULTS AND DISCUSSION**

The B subunit of heat-labile enterotoxin of E. coli is part of a contiguous operon encoding a single polycistronic mRNA for synthesis of both A-and B-subunit precursor polypeptides that contain N-terminal hydrophobic signal sequences [12]. In the present study, the full coding region of LTB including the signal sequence, which could direct the expressed protein to the bacterial preplasmicspace was amplified. On the other hand, the human ST is synthesized as a 72 amino acids (AA) residue precursor, which is proteolytically processed to produce a 19 AA peptide constituting the mature toxin [19]. In this study, the constructed gene coded for a truncated form of the toxin with only 13 AA and it was cloned after LTB with a segment coding for 7 AA separating the two genes.

Previously, It had been shown that the alteration of the carboxyl terminus of the LTB affects its receptor-binding properties, therefore ST had been fused to the 5’ end of the LTB or CTB [21]. However, Clements [12] showed that the fusion of the gene for mature ST to the carboxyl terminus of LTB would be functional if a 7 AA linker containing two prolines separates the two moieties. But, in the absence of the linker, no ST antigen could be detected. Therefore, in the present study, we used 7 AA linker containing 2 prolines to separate the two
could correspond to the pentameric form of LTB/ST (Fig. 2 and 3).

The monomeric form was not detected in either SDS-PAGE or Western blot, which could be due to the low expression or the lack of recognition by the antibody raised against the pentameric form as suggested by Sewani et al. [20]. Therefore, urea was used for extraction since it had been shown [17] that urea could facilitate the release and help antigen-antibody recognition of the LT from clinical isolates. The immunodot blot assay was performed on the supernatants obtained after urea extraction and by sonication. As it is shown in Fig. 4, the urea-extracted protein produced much stronger signals.

Sandkvist et al. [21] had shown that two constructs, in which 12 and 17 AA had been added to the carboxyl-end of the LTB, the fused proteins were not transported to the preplasmic space and the expression level was low. In the present study, the carboxyl terminus of LTB had been altered by the addition of 24 AA (ST plus the flanking AA), which might have resulted in the low expression of the fused protein or its anchorage to the cell membrane resulting in its low recovery when sonication had been used. However, it must be noted that Aitken et al. [14] reported no such difficulties with the fused protein obtained from the fusion of LTB with a gene
coding for a precursor of human ST. The fused protein was tested in the suckling mouse assay for toxicity and the amount of up to 1 mg of the fused protein failed to elicit any reaction. Whether this non-toxic fusion protein can give rise to protective antibody needs to be studied.

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REFERENCES