Research Communication

Uncovering neural tube defects in humans through candidate gene expression patterns in mouse embryos

Nor Linda Abdullah, Mustakiza Muslimin, Hoo Wan Mun, Fahimah Noor Ngah, Sheikh Mohd Norhafiz Abdul Aziz, Nor Syazwani Yip, Noraishah Mydin Abdul-Aziz*.

Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

ABSTRACT

Neural tube defects (NTDs) are the leading cause of disability in humans arising from the malformation of the central nervous system. The genes responsible and their involvement in causing neural tube defects in humans are poorly understood. Gene expression analysis in a whole organism enables the identification of the possible role of the gene being studied. If the gene is expressed in a particular tissue at a certain period of development, this spatiotemporal pattern of the gene of interest signals the possibility that the gene serves a function of being switched on in those tissues at that particular time. In this report, we have identified possible gene candidates in the mouse which may be required for the development of the neural tube, the precursor to the brain and the spinal cord. Development of the brain occurs by closure of the anterior neuropore (forms the cranial neural tube) while the spinal cord forms due to resolution of the posterior neuropore (forms the caudal neural tube). The genes *Tiam1* and *T-cadherin* were found to be likely candidate genes for the development of the spinal cord and may serve as potential human NTDs genes.

Keywords neural tube development, Eph receptor tyrosine kinase, ephrin ligand, neurulation, gene expression

*Corresponding author: Dr.Noraishah Mydin Abdul Aziz, Department of Parasitology, Faculty of Medicine, University of Malaya, 50360 Kuala Lumpur, Malaysia.
E-mail: noisha@um.edu.my

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Introduction

*Eph receptor A7* and its cognate ligand *ephrin-A5* were the first among the Eph family of receptor tyrosine kinases to have been identified as genes responsible for the development of the presumptive mouse brain (Holmberg et al. 2000). Failure in the development of the presumptive brain and spinal cord results in neural tube defects (NTDs). NTDs occur in high frequencies; approximately one in every 1000 live births in humans (Copp et al. 2003). As neural tube development takes place in the human embryo approximately 28 days after fertilization, NTDs usually develop before most women would even know that they are pregnant. The mouse model is used extensively in NTD studies to represent most mammals as discoveries from mice are comparable to humans (Wyszynski 2006). Majority of human NTDs are low-penetrance nonsyndromic NTDs which are multifactorial in nature and are likely to be represented in the mouse as null-mutant heterozygotes or mutants with partial gene function or hypomorphs (Harris & Juriloff 2007). Therefore, if any of the known 190 mouse NTD genes have a role in human NTDs, those genes are likely to be involved in a regulatory manner influencing partial-function (Harris & Juriloff 2007; Copp & Greene 2010).

However, this does not abrogate nor diminish the importance of fundamental gene expression studies in the mouse which serves to pinpoint potentially important human NTD genes such as the grainyhead-like 3 gene (*Grhl3*). *Grhl3* is expressed in the neuroepithelium during neurulation (Gustavsson et al 2007). It produces thoracolumbar spina bifida when genetically ablated in the mouse and is implicated with the *Ptk7* gene in causing open spina bifida in humans (Ting et al. 2003; Stiefel et al. 2003).

Many mutant mice develop NTDs due to folate-sensitivity (Martinez-Barbera et al. 2002; Harris & Juriloff 2007). However, the *Grhl3* mutant mouse as well as the *EphA7* mutant is folate-resistant (Ting et al. 2003; Holmberg et al. 2000). The Eph receptors and ephrin ligands have been implicated in neurulation, in particular, adhesion and fusion of the neural folds, in the presumptive brain as well as the spinal cord (Holmberg et al. 2000; Abdul-Aziz et al. 2009). Two Rho GTPase-associated molecules which have been particularly implicated in Eph and ephrin signalling are the neuronal guanine nucleotide exchange factor (*Ngef*), better
known as Ephexin and the Rac-1 specific guanine nucleotide exchange factor, called T-cell lymphoma invasion and metastasis 1 (Tiam1) (Tolias et al. 2005; Knoll & Drescher 2004; Tanaka et al. 2004; Winning et al. 2002; Shamah et al. 2001). Eph-interacting exchange protein or ephexin for short is one of the novel family members of Dbl oncoproteins of the guanine nucleotide exchange factors, GEFs (Shamah et al. 2001). Ephexin is found on chromosome 1 in mouse and located on 2q 37 of human chromosome (Rodrigues et al. 2000). Ephexin has previously been reported to be expressed in the rat brain and rat spinal cord during embryonic day (E) 15 till E17 (Shamah et al. 2001). Rac-1 specific guanine nucleotide exchange factor Tiam1 is known to act downstream of Ephs and ephrins interaction in an in vitro culture system (Tolias et al. 2005; Tanaka et al. 2004). Tiam1 has been previously reported to be expressed in the epithelium and is involved in apical-basal polarity (Mertens et al. 2006).

We are also interested in the EphB receptors as potential gene candidates expressed during the development of the presumptive brain and spinal cord. EphB3 has previously been reported to be expressed in the embryonic brain specifically the cerebrum (Kamitori et al. 2005). EphB4 is expressed in venous endothelial cells (Wang et al. 1998). They remain expressed at lower levels in adult tissues and are up-regulated in pathological conditions such as cancer.

Another candidate which we are interested in is the glycosylphosphatidyl inositol (GPI)-anchored T-cadherin molecule. T-cadherin is of particular interest as GPI-anchored molecules have been shown to be required for neural tube closure (O'Shea & Kaufman 1980, Abdul-Aziz et al. 2009). T-cadherin has only been previously reported to be localized in the trunk of the chick embryo (Ranscht & Bronner-Fraser 1991).

To assess the roles of genes during the development of the neural tube, whole mount in situ hybridization was performed on mouse embryos. The technique of whole mount in situ hybridization allows for spatiotemporal expression analysis of cloned genes (Neidhardt et al. 2000; Wilkinson 1998). It is considered a powerful technique that enables gene expression and localization patterns to be analyzed on a whole tissue and thus gives valuable hints as to the possible role of a gene during embryogenesis (Neidhardt et al. 2000; Wilkinson 1998). Using a background of RNA transcription process, sense strand and antisense strand were generated. Antisense probe reads the DNA template of the gene while sense strand contains the reverse strand read-out that is unable to be read when undergoing expression (Neidhardt et al. 2000).

**Methods**

**Embryology**
Non-mutant random-bred CD1 mice (Charles River, UK) were paired overnight and females were checked for copulation plugs the following morning, designated embryonic day (E) 0.5. Embryos were explanted at E8.5 and E9.5 (Cockcroft 1990).

**Gene detection**
Whole-mount in situ hybridization was performed using digoxigenin-labelled cRNA Tiam1, T-cadherin, Ephexin, EphB3 and EphB4 probes (Copp et al. 1999; Abdul-Aziz et al. 2009). This allows observation of the gene without sectioning of the embryo.

**Results**

*Tiam1, T-cadherin, Ephexin, EphB3 and EphB4 are expressed in the cranial and spinal neural tube during neurulation. (Figure 1)*

![Figure 1](image_url)

**Figure 1** Tiam1, T-cadherin, Ephexin, EphB3 and EphB4 are expressed in the cranial and spinal neural tube during neurulation. Red arrows show gene expression. A, Tiam1 is expressed ubiquitously in the embryo with a significantly higher expression in the posterior neuropore as shown by red arrow at late E9.5. B, T-cadherin is expressed strongly in the posterior neuropore at late E9.5. C, Ephexin is expressed in the forebrain, branchial arches, periphery of presumptive eye and otic vesicle as well as forelimb at early E10.5. D, EphB4 is expressed throughout the embryo, most significantly in the trunk and cranial regions with distinct expression in the presumptive eye and rhombomere at late E9.5. E, EphB3 is expressed in the posterior neuropore, forebrain and forelimb bud at early E10.5. F shows no expression in the E9.5 embryo which is the sense (negative control) probe for Tiam1.
**Discussion**

Gene expression analysis is a powerful technique to screen for potential genes which may be important for the successful development of the presumptive brain and spinal cord. Whole mount *in situ* hybridization provides a means to visualize the spatiotemporal patterns of these genes during this important developmental period. However, most gene expressions dissipate after their functions have been achieved, making this method impractical for human embryos due to its scarcity. Ironically, this very fact makes it crucial as a tool to study the mechanism of the development of the pre-cursor of the brain and spinal cord, the neural tube, as most genes are not expressed when and where it is not required. It is this fact that makes the mouse model an indispensable tool to study neural tube closure in humans.

In this study, gene expression in embryos were studied between E8.5 – E10.5 which is the developmental period for neural tube closure. *Tiam1* and *T-cadherin* are strong candidates that may play a role during neural tube closure as they are expressed clearly in the spinal neural tube at E9.5, a period which is crucial for closure of the neural tube as they are expressed throughout the embryo which is stronger in the trunk and cranial regions. *EphB4* is expressed throughout the embryo, most significantly in the trunk and cranial regions with distinct expression in the presumptive eye and rhombomere at late E9.5 (Fig.1D). *EphB3* is expressed in the posterior neuropore, forebrain and forelimb bud at early E10.5 (Fig.1E). Fig.1F shows no expression in the E9.5 embryo which is the sense (negative control) probe for *Tiam1*.

**Conclusion**

*Tiam1* and *T-cadherin* may be required for spinal neural tube closure. The roles of *Tiam1* and *T-cadherin* have yet to be uncovered in humans NTDs.

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