# Expression of autism susceptibility genes in the earliest stages of human cerebral cortex development

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## 1) Introduction

Autism spectrum disorder (ASD) is a developmental disorder; symptoms include: problems with communication, a failure to empathise with the emotion of others and a variety of repetitive stereotypical behaviours.
Previous research into expression levels of ASD candidate genes using microarray technology identified some genes which showed a gradient expression across the rostral-caudal axis of the developing cortex (Ip *et al.*, 2010). This lead to the hypothesis that these ASD candidate genes have a previously unexpected role in the early stages of cerebral cortex development.

**Aims:**

1. Select a subset of ASD candidate genes to analyse
2. Identify whether this subset of ASD candidate genes are expressed during early fetal cerebral cortex development
3. Investigate whether there is qualitative evidence for a difference in expression levels of the subset of genes between the front (rostral) and rear (caudal) cerebral cortex.

## 2) Methods

A subset of 20 ASD candidate genes were selected from the genotator online database which overlapped "Autism" and "Autistic Disorder" categories. PubMed was used to retrieve information regarding the developmental expression of these genes in model organisms and their pathways.
The list of 20 genes was narrowed down to 4 genes based upon the high expression levels of these genes in the developing mouse brain (data from Allen Brain Atlas (See Figure 1)):

- **BDNF** (Brain Derived Neurotrophic Factor)
- **GABRB3** (Gamma-aminobutyric acid receptor subunit beta-3)
- **NRXN1** (neurexin 1) – both alpha and beta isoforms
- **SHANK3** (SH3 and multiple ankyrin repeat domains 3).

Using the online Primer-BLAST program, gene specific primer sequences (incorporating all RNA isoforms) were designed, together with specific primers to the α and β NRXN1 isoforms (Supplementary information). The forward and reverse primers were approximately 20 bases long and spanned two exons.

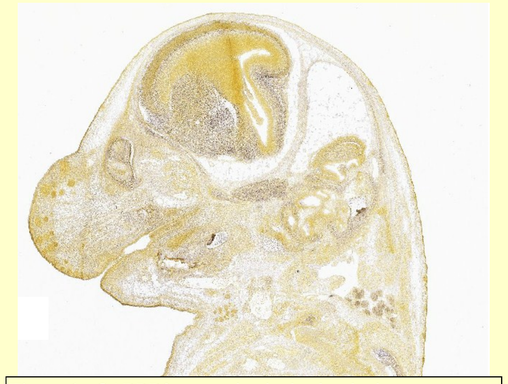


**Figure 1** - GABRB3 expression in the developing mouse brain at stage E15.5 (sagittal view). Image taken from the Allen Brain Atlas. Purple staining is indicative of GABRB3 expression.

Using mRNA prepared from human rostral or caudal fetal cortex, first strand cDNA was synthesised using reverse transcriptase and Polymerase chain reaction (PCR) which was optimised for each primer pair. An amplicon is generated by the PCR reaction if the specific gene sequence is present in cDNA. The products of the PCR are subjected to gel electrophoresis and are then stained with Gel Red and visualised under UV light.

This procedure allowed the visual analysis of the expression of the selected genes from different caudal and rostral samples aged 8 (post conception weeks) PCW or 11PCW.
An average qualitative expression based upon the intensity of the amplicon was made for the 5 genes at 8 PCW and 11PCW from both caudal and rostral samples (Table 1).

**Table 1**

| GENE | 8PCW | | 11 PCW | |
|---|---|---|---|---|
| BDNF | R_N1519 | ++ | R_N1403 | |
| | C_N1519 | +++++ | C_N1403 | |
| | | | R_N1415 | + |
| | | | C_N1415 | + |
| | | | R_N1505 | |
| | | | C_N1505 | + |
| GABRB3 | R_N1519 | +++++ | R_N1403 | ++ |
| | C_N1519 | ++++ | C_N1403 | |
| | | | R_N1415 | ++++ |
| | | | C_N1415 | + |
| | | | R_N1505 | + |
| | | | C_N1505 | ++ |
| NRXN1α | R_N1519 | +++++ | R_N1403 | ++ |
| | C_N1519 | ++++ | C_N1403 | + |
| | | | R_N1415 | +++++ |
| | | | C_N1415 | ++ |
| | | | R_N1505 | |
| | | | C_N1505 | ++++ |
| NRXN1β | R_N1519 | + | R_N1403 | + |
| | C_N1519 | | C_N1403 | |
| | | | R_N1415 | + |
| | | | C_N1415 | |
| | | | R_N1505 | |
| | | | C_N1505 | + |
| SHANK3 | R_N1519 | ++ | R_N1403 | + |
| | C_N1519 | ++ | C_N1403 | + |
| | | | R_N1415 | ++ |
| | | | C_N1415 | + |
| | | | R_N1505 | + |
| | | | C_N1505 | + |

## 3) Results

The Gel Electrophoresis photographs (Figures 2 & 3) shows a qualitative method of measuring the gene expression within the fetal DNA samples. This is an example of the gel electrophoresis data produced for 8PCW and 11PCW samples. R denotes a rostral sample and C denotes a caudal sample. The numbers at the top of the photographs refer to the well number in the gel. 100bp DNA ladder was used in all gel electrophoresis procedures. Each gel ran a positive and negative control - the negative control (water replaced the cDNA) checked for contaminants in the reagents, the positive control (10PCW cDNA and PRKCB primers) tested for a successful PCR reaction.

Figure 2
GABRB3 - 8PCW samples

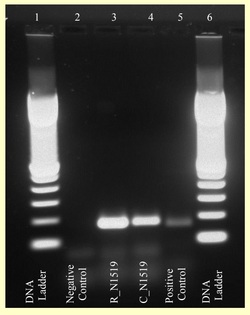
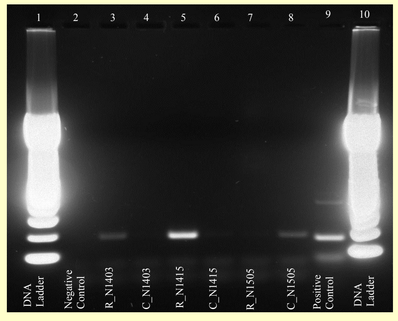



Figure 3
GABRB3—11PCW samples




Gel Electrophoresis of gene specific amplicons was performed , Table 1 contains a summary of the results. + denotes an intensity value between 1 and 5. Trends in the expression patterns of the genes are evaluated in the Discussion section.

## 4) Discussion

The expression of all four genes (and the two isoforms of NRXN1) are clearly detectable in both 8PCW and 11PCW samples. BDNF, GABRB3, NRXN1α and SHANK3 expression levels suggest that there is increased expression at 8PCW compared to 11PCW and that there is a higher level of expression at 11PCW in rostral samples compared to caudal samples.

The data suggests there is a higher expression of NRXN1α compared to NRXN1β which is present at a much lower detectable level. There is a possibility that the PCR reaction using NRXN1β primers was less efficient than the reaction using NRXN1α primers.

In the data that I collected it is apparent that the N1505 sample does not follow the same trends as the other 11PCW samples. The level of rostral expression for all genes using the N1505 sample is very low. This suggests that R_N1505 is an outlier. This could indicate that the cDNA was degraded which would be detected using a spectrophotometer.

## 5) Conclusion & Further implications for research

Four genes from a subset of ASD candidate genes were selected which have been detected in early fetal cerebral cortex development of mouse and other model organisms. The expression of these genes is clearly detectable in 8pcw and 11pcw cDNA samples. Qualitative evidence of differential expression patterns between the rostral and caudal samples using BDNF and GABRB3 primers were obtained which supports the evidence of the microarray data.

Further research is needed, whereby quantitative expression data is collected by conducting real time PCR using rostral and caudal fetal samples which is compared to the expression levels of a house-keeping gene e.g. GAPDH (which is always expressed at a consistent level). Real time PCR allows the comparison of the initial starting concentration of cDNA to the concentration of the amplified DNA. The real time PCR results will also confirm the expression data collected by PCR and Gel Electrophoresis in these analyses. The further research should consider the inclusion of more experimental samples for both 8PCW and 11PCW than have been used in this research. The use of more samples will either support or oppose the evidence for the trends that are described in this work.

## 6) Acknowledgements

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Websites:
Genotator online database (http://genotator.hms.harvard.edu/), PubMed (www.ncbi.nlm.nih.gov/pubmed), Primer-BLAST program (www.ncbi.nlm.nih.gov/tools/primer-blast/), Allen Brain Atlas (http://www.brain-map.org/).

References:
Ip, B.K., Wappler, I., Peters, H., Lindsay, S., Clowry, G.J. and Bayatti, N. (2010) 'Investigating gradients of gene expression involved in early human cortical development', *J Anat*, 217(4), pp. 300-11.