Computational Detection of Homologous Recombination Hotspots in X-Chromosome Autism-Associated Genes

Introduction

Recently, changepoint analyses by Environmental Protection Agency (EPA) scientists (McDonald & Paul 2010) identified changes as young as autism disorder data from US and Denmark. We have carried out further analyses and found evidence of changepoints in Figure 1 by US, summarised in Table 1. In the countries studied, the only universal environmental cause correlated with the change point was the identification of the human DNA residues.

The safety of human DNA residues has been debated for 50 years (Sheng et al. 2009). Potential dangers of the residues include autoimmune reactions to the non-host human DNA or improper integration of DNA fragments into the host genome or host mitochondrial genome during base repair by homologous recombination.

This study focuses on improved integration of the residual DNA as a possible contributor to autism, particularly in genetically susceptible infants. It is known from gene therapy studies that injected naked DNA can be transported to the brain (Deng et al. 2001); that improperly integrated therapeutic DNA has caused cancer in young children (Hacini-Bel-Abna et al. 2008); and that shorter DNA fragments have a higher probability of entering the nucleus (Luchardt et al. 2002). To investigate whether improperly integrated DNA can contribute to autism, we are undertaking the following: (1) measure the amount and length distribution of residual human DNA in vaccines; (2) predict sites of DNA insertion via homologous recombination (HR) and measure insertion rates; (3) model how brain cell function might be affected, either via loss of the ability to make proper connections or via selective growth of cells with improperly integrated DNA at the expense of healthy cells; (4) conduct epidemiology studies comparing autism rates in children injected with vaccines containing human DNA residues.

Fig. 1: Changepoint analysis for US/DOE and CA/DDS Autistic Disorder

DNA levels and lengths

Vials of Meruvax II (rubella, Meneve&Co. Inc.) and Havrix (hepatitis A, Glaxo Smith Kline Biologicals) were heat-maintained by placement in a 50°C water bath for 2 hours. Meruvax contents were reconstituted in Tris-EDTA (TE) solution then loaded into 4% agarose gel. After electrophoresis, gels were stained with SYBR Green dye (Invitrogen). Human DNA was quantified by labeling double stranded DNA (dsDNA) with picogreen (Invitrogen) and single-stranded (ssDNA) with oligone (Invitrogen), then reading with a spectrophotometer.

Fig. 2: Levels and residual size (SYBR gold) of human dsDNA (picogreen assay) and ssDNA (oligogreen assay) in Havrix (Hepa) and Meruvax II (Rubella)

Analysis in injected mice showed significant expression of the human DNA fragments in the brain, with a higher expression of ssDNA than dsDNA. In the brain, ssDNA was found to be more than 100 times more abundant than dsDNA.

Recombination Hotspots

Chromosomal coordinates of hotspots (Myers et al. 2004) were overlaid with coordinates (transcription starts and ends) of autism-associated genes downloaded from the ACMap database. This procedure finds 8-kb hotspot regions in the genome. More localized searches for hotspot motifs were done using BLAST. Gene coordinates are from build 36. BLAST is focused on X-chromosome genes due to the >1.5 male/female SSD.

Fig. 4: X-chromosome autosome-associated genes with recombination hotspots.

Discussion

Changepoint analysis of autism disorder demonstrates a temporal correlation with events associated with human DNA residues in vaccines. The levels of residual DNA are well over FDA-recommended limits. To reduce the risks of integrating residual DNA, recombinant vaccines were made to fragment the DNA. Unfortunately, in vitro studies in model organisms have shown that the majority of these vaccines have a higher chances of evading the immune response. Cell culture experiments are in progress to determine the rate and sites at which these residual DNA fragments integrate into the genome.

Our data show that the majority of these vaccines have a higher chance of integrating the DNA, which these DNA residuals might integrate into the genome and predict that disruption of exons and non-coding regions due to neurogenesis. Neurin binding to neurexin is critical for synapse maturation and function in the brain. Across the entire X-chromosome, the majority of recombination hotspots are located outside of the transcribed regions of genes (Myers et al. 2004). In contrast, we find that there are 119 gene hotspots that contain hotspots within the transcribed regions. Among all 228 published autism-associated genes, 119 genes have a combined total of 536 hotspot regions within transcribed regions. Moreover, we find almost perfect matches to the most common recombination hotspot motif (Myers et al. 2008) inside exons of two X-chromosome neurexin genes. Mouse models have demonstrated that loss of binding of NLGNX to neurexin leads to deficits in social interactions and communication that is similar to autism spectrum disorder (Janhainen et al. 2008).

Summary

1. Meruvax-ll contains >140ng/mL ssDNA and >30ng/mL dsDNA. The FDA-recommended amounts are <10ng/mL.
2. There are 515 autism-associated genes in the X-chromosome with recombination hotspots inside the transcribed regions.
3. NLGNX (exons 2) and NRXN1 (exons 2,3) contain near matches to the most common recombination hotspot motif in humans. Structural modeling showed that exon 2 is involved in the binding to neurexin (NRXN1), which is important for synaptic formation.

References


Table 1: Events related to vaccines with human DNA residuals

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Events Related to Vaccine with Human DNA Residuals</th>
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<tr>
<td>HAVRIX</td>
<td>Vaccine contains &gt;140ng/mL ssDNA and &gt;30ng/mL dsDNA.</td>
</tr>
<tr>
<td>Meruvax II</td>
<td>Vaccine contains &gt;140ng/mL ssDNA and &gt;30ng/mL dsDNA.</td>
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On day 1(-1), U937 cells were permeabilized with 0.2% saponin and then treated with DAPI to inhibit cell proliferation and to label endogenous cellular DNA blue. On day 2, 0.2ug Cy3-labeled red COT I DNA fragments were added to the culture. Nuclear COT I DNA accumulation (red) is evident after 24 hours and persist out to 72 hours.

Fig. 3: Human DNA accumulation in nucleus of human U937 cells

Table: Prevalence (per 10) for various vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
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<tbody>
<tr>
<td>HAVRIX</td>
<td>276.00</td>
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<tr>
<td>Meruvax II</td>
<td>35.74</td>
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</tbody>
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Fig. 5: 3-dimensional models for NLGNX 4 and NRXN1 (neurexin) demonstrate that exo2 of NLGNX is involved in binding. (Fabricry et al. 2007)

Conflict of Interest: None