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Introduction

Terms of reference

• Subset of children on the autistic spectrum
• Scientific data
• Not anti-vaccine
• Not based upon assumptions
• Not isolated opinion
• Conventional medicine
Clinical history

- normal early development
- developmental regression to autism - MMR
- onset of neurological and gastrointestinal symptoms
- recurrent infections
MMR vaccination in the presence of:

- Strong family history of autoimmune disease
- Intercurrent infection
- Recent or current antibiotic use
- Atopic disease
- Concurrent administration of other vaccines
A temporal association is nothing more than that, in the absence of supportive evidence of causation.
The clinical history
“My child has intestinal problems; I believe they are related to his autism”

Assumption 1.

• “Your child is autistic; they are bound to have bowel problems”
How frequent are bowel-related symptoms in autistic children?

- Unselected series of 385 ASD children compared with 95 developmentally normal controls
  - Significant symptoms in 46% vs 10% (p<0.0001)

  *Melmet R and Schneider C JPN 2000*

- 379 ASD children compared with 44 sibling controls
  - Significant symptoms in 84% vs 31% (p<0.0001)

  *Horvath et al JPN 2000*
Subclinical GI disease and proxy measures of intestinal pathology

- Permeability – increased even in autistic children with no bowel symptoms
  - D’Eufemia et al Acta paediatrica 1996;85:1076-9
- **Not** a diagnostic end-point
- Is there organic intestinal pathology in children with these symptoms?
Clinical features

- Pain
- alternating bowel habit
- soiling
- loss of continence
- fastidious eating habit
- reflux
- night-time waking
Bowel symptoms

• Insidious
• masked by behavioural problems
Do these symptoms reflect underlying pathology?

Were the parents right?
The gut in autistic children

Lancet 1998;351:637-641
American Journal of Gastroenterology 2000;95:2285-2295
The colonic lesion

Class II antigen expression

Normal child  Autistic enterocolitis

Furlano R et al  J. Paediatrics 2001;138:366-372
Autistic enterocolitis
Mucosal immune reaction

Mucosal density of $\gamma\delta$ T cells in autism

[Graph showing mucosal density of $\gamma\delta$ T cells in normal, LNH, autism, Crohn's, and UC conditions]
Autistic enterocolitis
Epithelial pathology

Basement Membrane Thickness in μm

Autistic  LNH/Con.  UC  Crohn’s  Normal
Epithelial proliferation - colon

Autistic enterocolitis

Normal child

Ki-67+ cells as % of total crypt cells

0%
25%
50%
75%

Autistic, LNH/Const, UC, Crohn’s, Normal
The small intestinal lesion

Normal

Autism

CD8+ IEL's (per 100 enterocytes)

Normal controls  Cerebral palsy  Autism  Coeliac disease

p < 0.001
p < 0.00001
p < 0.002
Circulating autoantibody?

IgG

C1q

IgG + C1q

Torrente et al - J Paed Gastroenterol. Nutrition
IgG and C1q in duodenum

IgA and C1q in duodenum
Systemic immunity

infections

• $T_H^2$ skewed
• Raised IgG1
• Low IgG4 &2
• Cutaneous anergy (Merieux multitest)
• Raised $\text{CD3}^+\text{CD8}^+\text{CD28}^-\text{CD45RA}^+$
• $\text{CD4}^+$ and $\text{CD8}^+$ DR4$^+$CD38$^+$
Conclusion: parents were right

Key features

- Developmental regression
- Ileo-colonic LNH - consistent with viral cause
- Entero-colitis – consistent with viral cause
- Immunodeficiency - consistent with viral cause
“After receiving his MMR inoculation at 18 months, Nicholas came down with a very bad case of gastroenteritis. From then on I noticed very distinct changes in his behaviour,” Ang recounts.
Autism & early viral exposures

• In utero & perinatal exposures to measles, mumps, rubella & chickenpox associated with autism

• Concurrent exposure associated with greater risk & more profound autism
  *Deykin & MacMahon*

• Birth cohort effect with measles and rubella epidemics

*Weizman et al*
Assumption 2

- “Coincidence”
- “Symptoms of autism are first noticed in the second year of life when MMR is given”
Onset of behavioural changes in children receiving booster vaccination

Patients 1-11

Age (months) at receipt of MMR/MR

1st dose  
1st regression  
2nd dose  
2nd regression

Onset of behavioural changes in children receiving booster vaccination
Questions

1. Is measles virus present?
   • Viral protein and genetic material

2. Where is it located?
   • Consistent with the pathology

3. How much is there?
   • Anticipate low level infection
2. Can it be sequenced?

3. Can results be confirmed by different technologies?

4. Does the presence of measles virus distinguish autistic children from controls?

5. Can results be confirmed in independent laboratories?
Crohn's disease
Measles virus detection

Serosal granuloma – measles virus N-protein
Immunogold electronmicroscopy
Crohn’s disease

*in cell RT PCR*

- N-gene
- P-gene
- No-primer control
Paramyxovirus gene transcripts in healthy and diseased bowel

- Paramyxovirus gene transcripts detected with MV N-gene and degenerate N-gene primers in 42% with IBD and 17% controls
- High degree of homology with MV
- MV & other paramyxoviridae persists in the gut in a limited or mutated form

Ward B et al AAP 2000
Measles virus protein
Methods

- Immunohistochemistry
  - Polyclonal
  - Monoclonal (WHO: NP-B, CL-120, F-Ost, M263)

- Immunogold electronmicroscopy

- Flow cytometry

- Serology
Results

**Immunohistochemistry**

- Cases negative for
  - RAd68⁻
  - Adenovirus
  - HSV I & II
  - Rubella
  - Mumps
  - HIV
  - P. Carini
  - No 1⁰ antibody
  - Pre-immune RAd68⁺ serum & murine IgG fraction
MV⁺CD45⁺  CD3⁺ lamina propria  CD3⁺ epithelium

MV⁺CD45⁺

CD3⁺ lamina propria

CD3⁺ epithelium

a

b

c
d

e

f
Measles virus gene
Sequence comparison for positive samples from 3 autistic children

MCS
Schwarz
1
2
3

8393  8401  8411  8421  8431
AAGTTGCC  AATGGAGACA  TGCTCCAGC  AGGCCGTGAA  GGGTAAATC

8441  8451  8561  8471  8481
CAAGCAGTCT  GCGAGAATCC  CGAGTGGGCA  CCATTGAAGG  ATAACAGGAT

8491  8501  8511  8521  8531
TCCTTCATAC  GGGGTCTTGT  CTGTTGATCT  GAGTCTGACA  GTTGAGCTTA

8541  8551  8561  8571  8581
AAATCAAAT  TGCTGGGGA  TTCGGGCCAT  TGATCACACA  CGGTTCAAGG

8591  8601  8611  8621  8631
ATGGACCAAT  ACAAAATCAA  CCACAACAAT  GTGTATTGCC  TGACTATCCC

MCS
Schwarz
1
2
3

8641  8651  8661  8671
GCCAATGAAG  AACCTAGCCT  TAGGTGTAAT  CAACAC

Measles virus
H-gene
Patients and Controls

Positive control material

- Measles virus infected Vero cells
- Measles infected brain samples (SSPE)

Negative control material

- Myocardium, SiHa, BC-1, BC-3, cervix, HeLa, normal brain
Solution phase RT-PCR

Quantitation

localisation

Southern blot

Sequencing

TaqMan real time RT PCR

in cell RT PCR
Optimisations:

A) cDNA amplicons derived from F, H and N measles virus genes using control measles RNA

B) Different oligonucleotide probe concentrations for in situ hybridisation after RT in cell PCR (measles transfected vero cells)
Results
Southern Blot analysis of PCR positive cases and controls

DIG oligo 3’ end labelled & DIG luminescent detection kit
Sequence of F-gene PCR product

**Forward strand:**

```
GAGANNNNGNACCTTTTGGGNNANTAGGGTCCA
AATTTGTTTCTCAGCNCTCTCCTGTATTCTGCAATCTCT
ACCCTNNTGAGTTATGAGAGAGTTATATTGGGCA
TTAATTTTATGACTAATGATTGATGGCTGGAACGAGTC
```

**Reverse strand:**

```
GGGAGGAGGAGTNGANGGNTTTATGGTGAATAA
AATANTGNCANNNAACTCTCTCCTCAATAACTGCGAC
GAGGGTAGNAAGATTTGNNAGTTAGGAGACACTA
GAGAACATTTTGGACAATTAGAGATGCACTTAATGCAATGACCCAAC
```

Questions & Answers

1. Is measles virus present?
   - Viral protein and genetic material ✔

2. Where is it located?
   - Consistent with the pathology ✔

3. How much is there?
   - Anticipate low level infection ✔
Questions and Answers

2. Can it be sequenced? ✓

3. Can results be confirmed by different technologies? ✓

4. Does presence of measles virus distinguish autistic children from controls? ✓

5. Can results be confirmed in independent laboratories? ✓
Assumption 3: “this work has not been scientifically reviewed”

- British Society of Gastroenterology *Gut* 1998a
- U.S.- Canadian Academy of Pathology March. *Lab. Invest.* 2000a
- American Gastroenterological Assn. May 2001
The “gut-brain axis”
Environmental insult in genetically susceptible child

Gut infection & damage

Impaired metabolism and increased absorption of gut-derived chemicals

Direct influences on brain growth & behaviour

Summary
Conclusions

- Immune-mediated inflammatory bowel disease – consistent with viral cause
- Presence of measles virus
- Perception of health care professionals is of a substantial increase in numbers of affected children
Key to understanding the problem I

Changing patterns of exposure to childhood infections

Implications for chronic disease
Measles mortality
England & Wales
<15 y.o.a
Factors influencing the manifestation and outcome of measles infection

- Age of exposure
- Dose/intensity of exposure
- Material circumstances
- Social factors
- Interaction with other infections
Viral interference

- The interaction of measles with another virus may be a risk factor for IBD


- Precedents for: measles and herpes zoster
  - hepatitis B & C
  - hepatitis B & Delta
  - HIV and herpes viruses
  - HIV and hepatitis C
  - MMR vaccine
Immunopathological consequences of:

- High zone tolerance
- Low zone tolerance
- Consequences of loss of tolerance