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Lack of Association between Measles Virus Vaccine and Autism with Enteropathy: A Case-Control Study*

A Critique of the study designed and published to discredit the work of Dr. Andrew Wakefield, and deny autistic children their day in court.

By Marcella Piper-Terry, M.S.

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A Critical Analysis of Hornig, Briese, et. al's Case-Control Study Regarding the Association between Measles Virus Vaccine and Autism with Enteropathy.

By Marcella Piper-Terry, M.S.

This is the list of authors and their affiliations:

Mady Hornig[1*], Thomas Briese[1], Timothy Buie[2], Margaret L. Bauman[3], Gregory Lauwers[4], Ulrike Siemetzki[1], Kimberly Hummel[5], Paul A. Rota[5], William J. Bellini[5], John J. O'Leary[6], Orla Sheils[6], Errol Alden[7], Larry Pickering[8], W. Ian Lipkin[1*]

1 Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York, United States of America, **2** Division of Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital, Boston, Massachusetts, United States of America, **3** Department of Neurology, Harvard Medical School and Departments of Neurology and Pediatrics and Learning and Developmental Disabilities Evaluation and Rehabilitation Services (LADDERS), Massachusetts General Hospital, Boston, Massachusetts, United States of America, **4** Department of Pathology of Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts, United States of America, **5** Measles, Mumps, Rubella, and Herpesvirus Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **6** Department of Histopathology, Trinity College Dublin, Dublin, Ireland, **7** American Academy of Pediatrics, Elk Grove Village, Illinois, United States of America, **8** National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

Pretty impressive.

Many people may think we could automatically assume this study was conducted and reported accurately, at least. Most people would probably never even THINK of questioning a study done by this group, what with the varied and stellar associations listed.

ABSTRACT

Background

The presence of measles virus (MV) RNA in bowel tissue from children with autism spectrum disorders (ASD) and gastrointestinal (GI) disturbances was reported in 1998. Subsequent investigations found no associations between MV exposure and ASD but did not test for the presence of MV RNA in bowel or focus on children with ASD and GI disturbances. Failure to replicate the original study design may contribute to continued public concern with respect to the safety of the measles, mumps, and rubella (MMR) vaccine.

Methodology/Principal Findings

The objective of this case-control study was to determine whether children with GI disturbances and autism are more likely than children with GI disturbances alone to have MV RNA and/or inflammation in bowel tissues and if autism and/or GI episode onset relate temporally to receipt of MMR. The sample was an age-matched group of US children undergoing clinically-indicated ileocolonoscopy. Ileal and cecal tissues from 25 children with autism and GI disturbances and 13 children with GI disturbances alone (controls) were evaluated by real-time reverse transcription (RT)-PCR for presence of MV RNA in three laboratories blinded to diagnosis, including one wherein the original findings suggesting a link between MV and ASD were reported. The temporal order of onset of GI episodes and autism relative to timing of MMR administration was examined. We found no differences between case and control groups in the presence of MV RNA in ileum and cecum. Results were consistent across the three laboratory sites. GI symptom and autism onset were unrelated to MMR timing. Eighty-eight percent of ASD cases had behavioral regression.

MV RNA – Measles Virus RNA (genetic material from the Measles Virus)

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MV RNA – Measles Virus RNA (genetic material from the Measles Virus)

<u>Comment</u>: The above paragraph contains a lot of information. For those who are not accustomed to reading research studies, it can be intimidating and hard to understand.

It is often easier to understand things if we break them down.

Methodology/Principal Findings

Let's start with the first sentence, which states the objective, or reason, for the study. This is where the research hypothesis comes from.

The objective of this case-control study was to determine whether children with GI disturbances and autism are more likely than children with GI disturbances alone to have MV RNA and/or inflammation in bowel tissues and if autism and/or GI episode onset relate temporally to receipt of MMR.

What this says is that they are looking to answer 2 questions:

1. **Are children with autism (ASD) & gastrointestinal disorders (GI) more likely to have evidence of MV RNA (genetic material from the measles virus) &/or inflammation of the bowel tissues than children with GI disorders who do not have ASD?**
2. **Is the timing of autism diagnosis and onset of GI disorders related to when a child receives the MMR vaccine?**

Methodology/Principal Findings

On to the 2nd sentence:

The sample was an age-matched group of US children undergoing clinically-indicated ileocolonoscopy.

"Age-matched" means the children who were studied are assumed to have been of comparable ages with no difference between children who were in one study group and children who were in another study group.

"clinically-indicated ileocolonoscopy" means that all children in the study, regardless of whether or not they had previously received an autism diagnosis, were suffering from gastrointestinal disorders that were severe enough to warrant them undergoing an ileocolonoscopy. To perform this medical procedure without the patient having met the criteria for clinical necessity would be unethical.

Methodology/Principal Findings

2nd sentence, continued…

The sample was an age-matched group of US children undergoing clinically-indicated ileocolonoscopy.

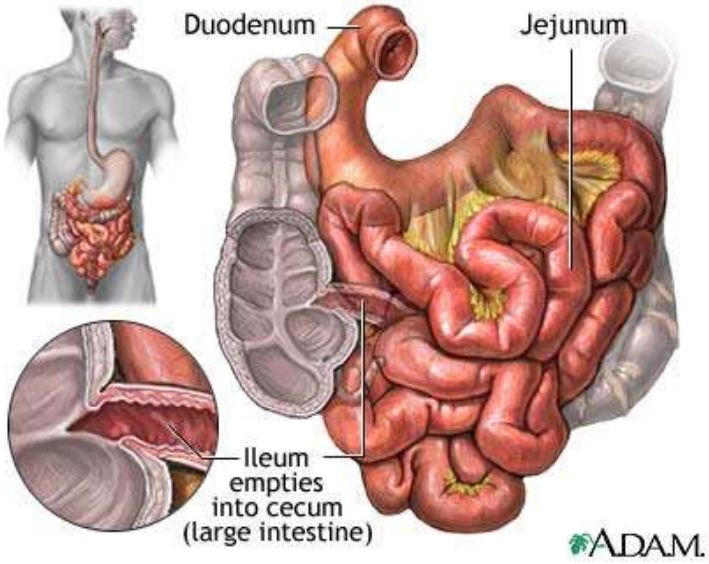
Comment: The fact that all children in this study were suffering from gastrointestinal disorders severe enough to warrant ileocolonoscopy provides us with the first issue of concern regarding the outcome of the study.

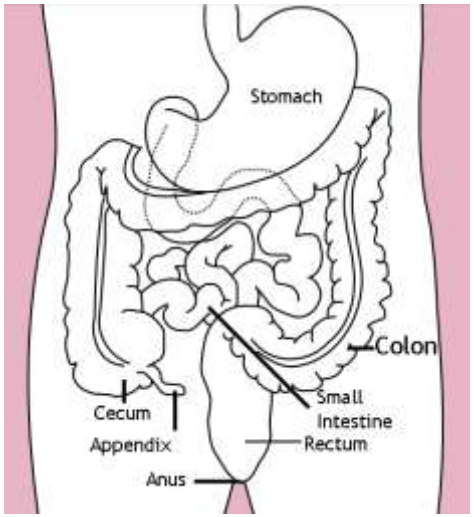
Because there are no true "controls" (i.e., children who did not have gastrointestinal disorders, we cannot make generalizations about the outcome of this study to "normal" children, or to the population at large. If study results could be generalized at all (applied beyond those children studied) it would only be appropriate to do so to children who were similar to those studied…children with ASD and GI, and children with GI without ASD. The limitations that result from lack of a true "control" group are not the fault of the researchers (since it would have been unethical to perform ileocolonoscopy on healthy children), but it is a limitation that should be noted, and which impacts the reliability of the results.

Methodology/Principal Findings

3rd sentence:

Ileal and cecal tissues from 25 children with autism and GI disturbances and 13 children with GI disturbances alone (controls) were evaluated by real-time reverse transcription (RT)-PCR for presence of MV RNA in three laboratories blinded to diagnosis, including one wherein the original findings suggesting a link between MV and ASD were reported.





The drawing on the left shows the Ileum and the right shows the location of the Cecum.

Comment: There's a lot of real estate in the GI tract that was not investigated in this study. Again, results from this particular study can only be applied to what was actually studied.

Methodology/Principal Findings

4th sentence:

The temporal order of onset of GI episodes and autism relative to timing of MMR administration was examined.

Comment: "temporal order of onset" means this was *ordinal data*. They ranked the participants with regard to the amount of time (in months) between when they were given the MMR and when their gastrointestinal problems began. This is different from using *interval data* (number of months) and looking at the difference between the two groups. If the researchers had used *interval data*, they would have been able to use *parametric analysis.* With *ordinal data,* they were limited to use of *non-parametric* tests.

The difference between non-parametric and parametric analysis is important because parametric tests are more reliable statistical measures and convey increased validity of results. Non-parametric tests are weaker and less reliable. Therefore, it is preferable to use parametric analysis whenever possible.

Methodology/Principal Findings

5th sentence:

We found no differences between case and control groups in the presence of MV RNA in ileum and cecum.

Comment: If the researchers had truly been looking for differences, they would have used the more powerful statistical analysis.

There was no valid reason for not using interval data and parametric tests. Interval data is numbers. Interval data includes things like the number of days, weeks, or months between the administration of the MMR vaccine and onset of gastrointestinal dysfunction and/or onset of symptoms of autism.

They HAD numbers. They CHOSE to convert the interval data to ordinal data.

Since it is preferable to use interval data whenever possible, one must question the rationale behind the conversion of the raw data. One possible reason for converting interval data to ordinal data would be to weaken the analysis and decrease the probability that the results would be statistically significant.

Methodology/Principal Findings

5th sentence, continued…

We found no differences between case and control groups in the presence of MV RNA in ileum and cecum.

A second reason for using ordinal, rather than interval data would be because of the unequal sample sizes. This study looked at two groups of children. The autism/GI group had 25 kids and the GI ALONE group had 13 kids. The statistical power of the test could have been improved by adding another 12 kids to the GI ALONE group, which would have provided equal numbers of subjects in each of the two study groups. If they had done this, they could have used interval data and performed a parametric analysis.

Apparently the researchers were unable to find an additional 12 children who did not have autism along with their gastrointestinal disorders.

I think it says something when children with autism are easier to find than children without autism.

Methodology/Principal Findings

5th sentence, continued…

We found no differences between case and control groups in the presence of MV RNA in ileum and cecum.

In research, "no differences" does not mean there was no difference. It means there may have been a difference but the degree of difference was not great enough for the results to obtain statistical significance. Statistical significance is important because it decreases the probability that findings of the study are related to chance. Statistical significance has to do with the validity of the results.

The most important factor that influences the probability of obtaining statistical significance is sample size. The smaller the sample size, the harder it is to obtain statistical significance. For results to be applicable beyond the research setting, sample size should be 100 or more participants. While it is quite common for research to be conducted with small sample sizes, it is a basic tenet of research that any results obtained from studies with small sample sizes should be replicated with larger samples before making any assumptions about whether or not the findings may be true for the population at large.

Methodology/Principal Findings

5th sentence, continued…

We found no differences between case and control groups in the presence of MV RNA in ileum and cecum.

Comment: Note that this sentence does not say they found No MV RNA.

What the above sentence says is they found "no differences" between case and control groups. They actually found MV RNA in BOTH groups of children…

Among the 38 children in this study, ALL of whom were suffering from gastrointestinal dysfunction severe enough to warrant undergoing ileocolonoscopy, measles virus RNA was found in the gastrointestinal tracts in BOTH GROUPS.

Methodology/Principal Findings

6th sentence:

Results were consistent across the three laboratory sites.

Comment: If the methodology is flawed, the results of the study are not valid. What is in question is the analysis of the data by the researchers, not the analysis of the tissue samples by the laboratories.

This child is "posturing." He is putting pressure on his belly because he is in pain.

The fact that laboratory analysis of the tissue samples obtained from children with severe gastrointestinal dysfunction "were consistent across the three laboratory sites" is relevant only so far as it provides support for the hypothesis that the presence of measles virus RNA in the GI tract is not only associated with autism, but also with gastrointestinal dysfunction in children who have not received an autism diagnosis.

Methodology/Principal Findings

7th sentence:

GI symptom and autism onset were unrelated to MMR timing.

Comment: This statement is misleading at best.

The following statement would have been more appropriate:

"In this study, which included a total of 38 subjects, all of whom suffered from clinically significant gastrointestinal dysfunction, the presence of Measles Virus RNA was found in children with autism and GI dysfunction, and in children who had not received an autism diagnosis but who suffered from GI dysfunction. It appears there may be an association between MMR vaccine and gastrointestinal dysfunction in children, regardless of whether or not they have been diagnosed with autism. However, the results of this study must be viewed with caution, due to several factors that impact the reliability and validity of obtained results. Factors that must be considered when interpreting the results of this study include the very small sample sizes, inequality between study groups, lack of a true "control" group, and the use of ordinal data and non-parametric tests of statistical significance. While no significant differences were found between children with autism and GI dysfunction and children with GI dysfunction alone, further research is necessary to determine the relationship between MMR and GI dysfunction, and larger samples should be analyzed to determine if the results of this study may be generalized to the population of children who routinely receive the MMR vaccine."

Methodology/Principal Findings

8th sentence:

Eighty-eight percent of autism cases had behavioral regression.

Comment: Children who regress after the MMR vaccine DO display behaviors that are different than what they displayed prior to the vaccine. They often stop making eye-contact, stop using words they had learned previously, and display other characteristics that earn them the autism diagnosis. They also often have chronic diarrhea, pain in their guts, and other gastrointestinal problems.

The next two slides present photos of a child who developed gastrointestinal dysfunction and regressive autism within hours of receiving the MMR vaccine at 15 months of age.

Methodology/Principal Findings

8th sentence:

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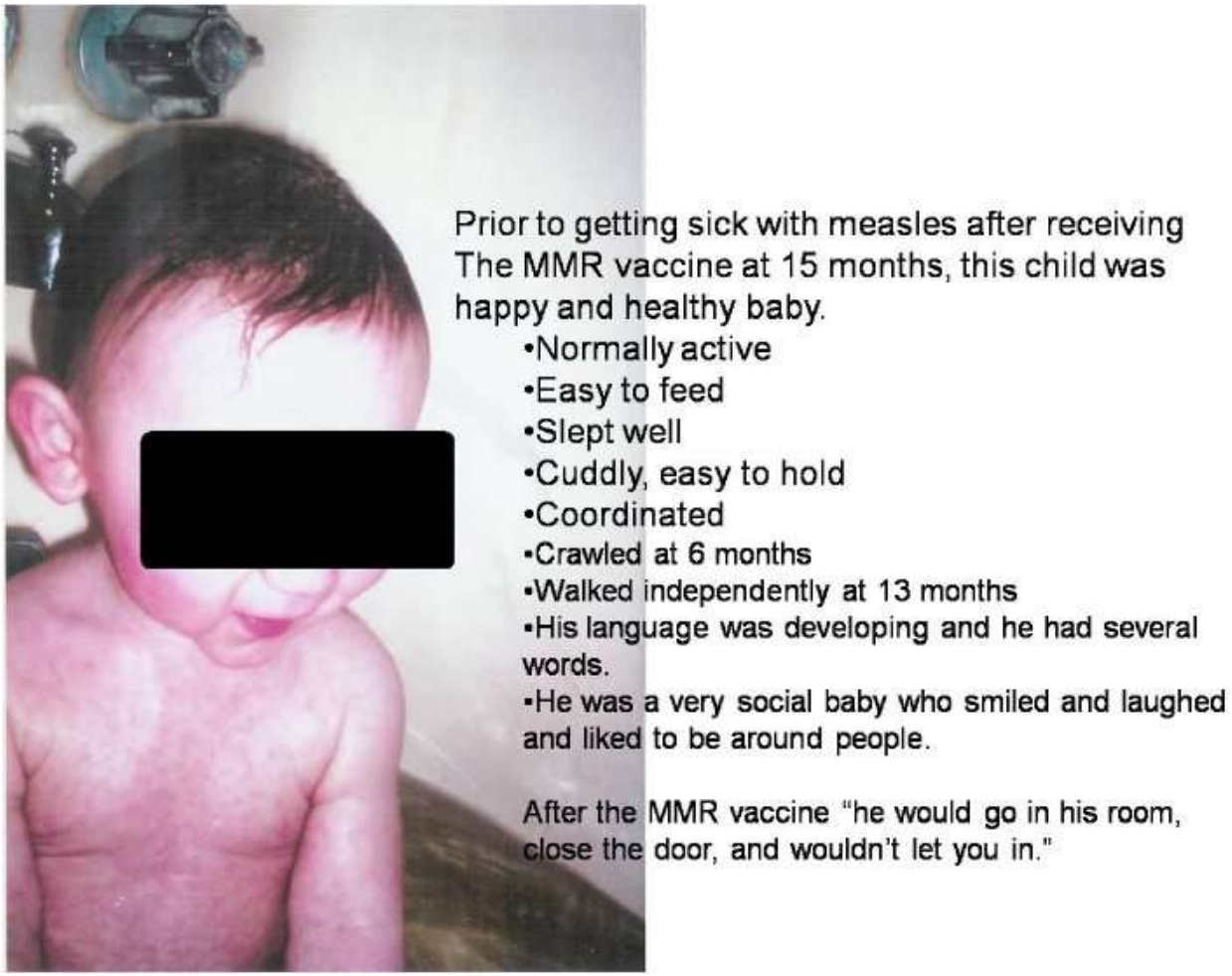

Methodology/Principal Findings

8th sentence:

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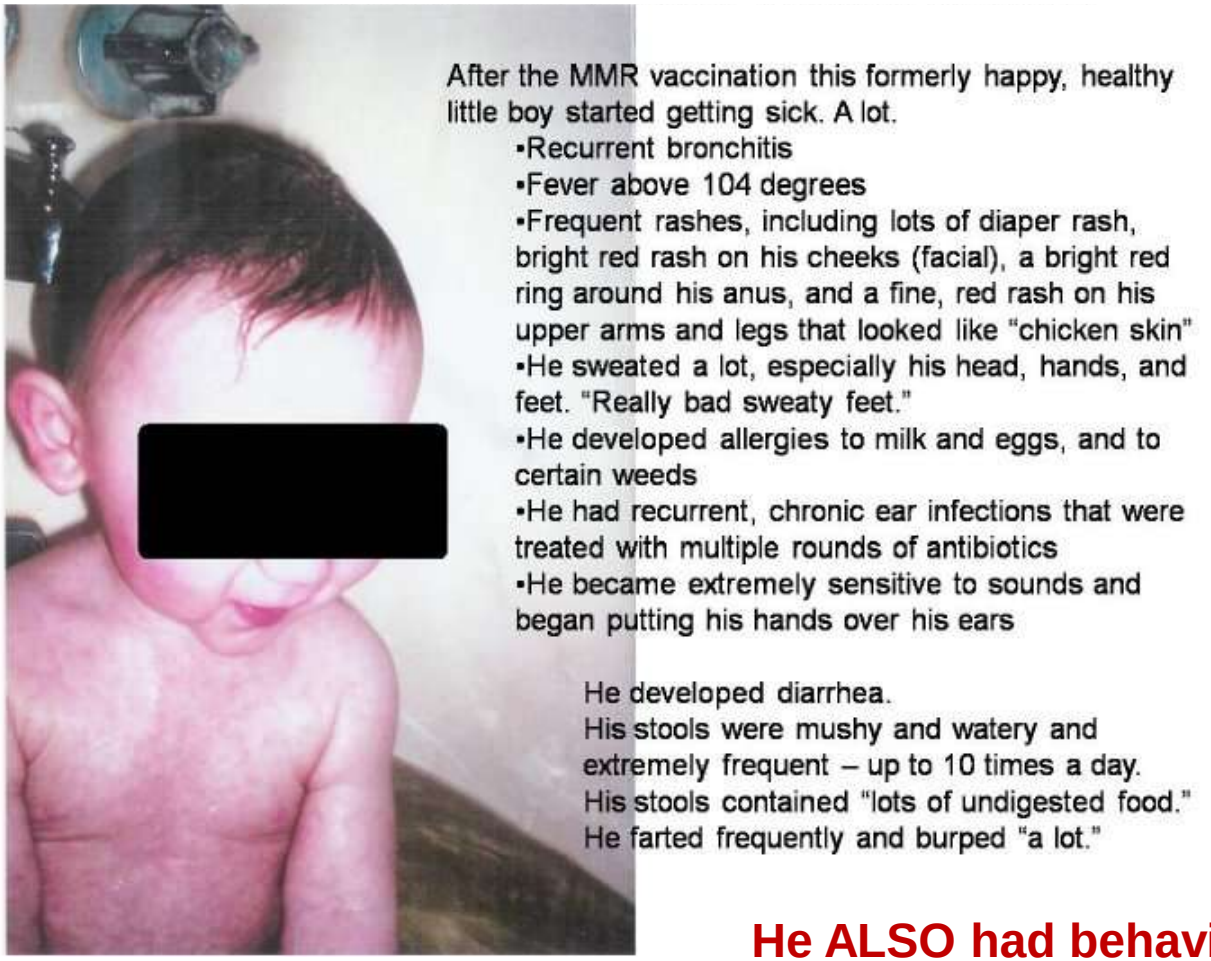

He ALSO had behavioral regression.

Conclusions/Significance

First sentence:

This study provides strong evidence against association of autism with persistent MV RNA in the GI tract or MMR exposure.

Comment: NO. The statistical analyses applied in this study are not powerful enough to provide "strong evidence" for or against anything. Add the unequal study groups and small group sizes, and the only conclusions that should appropriately be applied are those that relate to the subjects themselves.

This is the same argument that is made by the medical community when they tell parents of children with autism that we cannot draw any conclusions about autism and MMR based on observations of our own children. It's a case study. You cannot generalize from a case study. Period.

Conclusions/Significance

Second sentence:

Autism with GI disturbances is associated with elevated rates of regression in language or other skills and may represent an endophenotype distinct from other ASD.

Comment: Yes, and No. Autism with GI disturbances IS associated with elevated rates of regression. That's why it's called "regressive autism" – and it IS different from autism that is present from birth.

The term "endophenotype" implies a more purely genetic cause. According to the endophenotype hypothesis, children with autism and gastrointestinal disorders who regress "behaviorally" would have done so regardless of whether or when they receive the MMR vaccine because they are genetically programmed to do so. While it may be true (and I believe is likely true) that some children are genetically predisposed to be more severely damaged by the MMR vaccine than others, to say that this study provides STRONG EVIDENCE for a purely genetic cause is fantasy.

Miscellaneous Information…

- **Funding:** This work was supported by CDC grant U50 CCU522351 to AAP and by National Institutes of Health awards AI57158 (Northeast Biodefense Center-Lipkin), HL083850, and NS47537. Role of Study Sponsors: Members of the funding organization (AAP) and its sponsor (CDC) participated along with experts in virology and neurovirology, autism pathogenesis, and vaccine design and safety; representatives of the autism advocacy community; and study collaborators in an Oversight Committee that reviewed and agreed to all aspects of study
- **Competing interests:** Authors JOL and OS were compensated for expert witness statements concerning MMR vaccine and autism on behalf of claimants in litigation in the United Kingdom.

Comments:

Northeast Biodefense Center is funded by HHS "in excess of $9 million per year." The Center supports "investigator-directed research with an emphasis on the development and testing of vaccine, therapeutic and diagnostic concepts."

http://www.nystar.state.ny.us.pr/03/press26-03.htm

Competing interests… this speaks for itself.

Miscellaneous Information…

- **Editor:** Mark R. Cookson, National Institutes of Health, United States of America
- **Received:** June 29, 2008; **Accepted:** August 8, 2008; **Published:** September 4, 2008
-

Comments: This article was published with lightening speed, and unlike the majority of journal articles, it is available on the internet with full access. That's unusual. I guess the people who funded (paid for) the research wanted anyone who was concerned about MMR and Autism to be able to read this for themselves so they could see there was nothing to worry about.

The editor is from NIH. This might be a good time to review the CBS interview of Dr. Bernadine Healy, former head of NIH, to recall what she had to say about the interest of NIH in identifying susceptible groups of children, and pursuing a MMR/Autism connection.

Here is the link to the CBS interview with Dr. Healy: http://www.cbsnews.com/video/watch/?id=4088138n

From the INTRODUCTION…

Beginning in 1998, Wakefield and colleagues reported intestinal abnormalities, including reactive lymphoid hyperplasia in ileum, in children with autism and other developmental disturbances [1]–[8]. These findings, combined with parent-reported associations of timing of onset of behavioral abnormalities with MMR administration, led to the hypothesis that MMR contributed to autism pathogenesis [1]. Subsequent studies from this group reported MV RNA in bowel biopsies and peripheral blood mononuclear cells (PBMC) from children with ASD [9]–[12].

*Over 20 epidemiologic studies reported no temporal relationship between MMR and ASD [13]–[33], and three studies found no MV RNA in PBMC of ASD children [34]–[36]; however, no published studies from other research groups have addressed whether MV RNA is present in bowel of ASD children with GI disturbances. Here we report **independent**, blinded analysis of **ileal and cecal tissues** from children with ASD and GI disturbances and children with GI disturbances but **no neurological deficits** for the presence of MV RNA in three laboratories, including the one where the original reports of an association between ASD and MV were obtained.*

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Comments: I question the appropriateness of use of the word "independent" since the research facility that conducted the study receives in excess of $9 million dollars a year from HHS and their primary focus includes vaccine development.

Ileal and cecal tissues (as an earlier slide shows) constitute only a very small portion of the bowel and an even smaller portion of the gastrointestinal tract. (This is just a reminder.)

From the INTRODUCTION…

*… Here we report **independent**, blinded analysis of **ileal and cecal tissues** from children with ASD and GI disturbances and children with GI disturbances but **no neurological deficits** for the presence of MV RNA …*

Comments: The abstract states that "controls" were 13 children with gastrointestinal disturbances and no *diagnosis of autism*. In the introduction, we are told the 13 children with gastrointestinal disturbances severe enough to warrant ileocolonoscopy actually had "*NO NEUROLOGICAL DEFICITS.*"

I find this difficult to believe, since somewhere between 80 & 95% of the neurotransmitters responsible for memory, attention, and learning are produced in the gastrointestinal tract. I have never seen ANYONE, child or adult who had significant GI disturbance and had NO NEUROLOGICAL DEFICITS. I think it is more likely that the 13 children in the "control group" differed in degree regarding neurological performance and therefore had not received a formal diagnosis of ASD or other neuro-developmental disorder. This could well be due to the age of participants and the measures used to assess their neuropsychological development.

From the RESULTS…

Forty-seven children were recruited. Six recruits did not complete the study: 3 potential cases dropped out prior to colonoscopy; 1 potential case and 2 potential controls completed colonoscopy but had incomplete clinical assessments. No differences were found in age, sex, or case-control status between study completers and non-completers. An additional 2 potential cases were excluded for failure to meet diagnostic inclusion criteria (below cutoffs for autistic disorder [AUT] on ADI-R); and 1 case was excluded because no bowel biopsy material was available. The final study population consisted of 25 cases (AUT/GI group) and 13 controls (GI control group) presenting consecutively for ileocolonoscopy who received at ***least one dose of MMR*** *and completed all study procedures.*

Comment: **The lack of control in dosage of MMR further calls into question any results of the study and contributes to the inability to generalize results of this study beyond the subjects themselves. Non-parametric tests, as noted previously, allow for the use of ordinal data, but by using them, researchers sacrifice power of the test. Additionally, validity of results when employing non-parametric tests is dependent on assumption of equality between subjects on basically all factors, including age, race, socioeconomic status, AND in this case, dosage of MMR vaccine.**

From the RESULTS…

The clinical indications for endoscopic/colonoscopic procedures commonly noted in both AUT/GI and GI groups included recurrent abdominal pain (RAP), gastroesophageal reflux, vomiting, and food allergies. Although the more subjective factor of RAP was frequently present in both cases (36%) and controls (38%), it was rarely the sole rationale for GI examination in either group (1 of 25 cases, or 4%; 2 of 13 controls, or 15%; P = 0.27).

<u>Comment:</u> Notice that in this explanation, the presence of recurrent abdominal pain (RAP) alone is swept aside as if it is insignificant because it was "rarely the sole rationale for GI examination in either group." Specifically, there was only one child in the autism/GI group and only two in the GI alone group ("controls").

Also notice that in the AUT/GI group one child constitutes 4% of the group, and in the "control" group 2 children comprises 15% of the group.

From the RESULTS…

The clinical indications for endoscopic/colonoscopic procedures commonly noted in both AUT/GI and GI groups included recurrent abdominal pain (RAP), gastroesophageal reflux, vomiting, and food allergies. Although the more subjective factor of RAP was frequently present in both cases (36%) and controls (38%), it was rarely the sole rationale for GI examination in either group (1 of 25 cases, or 4%; 2 of 13 controls, or 15%; P = 0.27).

Comment: **Notice the "P = 0.27" - This tells you whether or not a particular finding is statistically significant. What we are usually looking for is P<.05. As noted before in this presentation, it is REALLY hard to get significant results with small group sizes… virtually impossible when you're talking about the differences between 1 child and 2 children.**

From the RESULTS…

Median *age at receipt of first MMR was similar for cases [15.3* ***(1.7)*** *months] and controls [16.0* ***(4.9)*** *months]. The majority of study subjects were in the 3–5 year age stratum and below the age recommended for second MMR (4–6 years [37]); expectedly, 80% of cases and 69% of controls received only one MMR prior to the study (P = 0.36). Consistent with the older age of girls in the study, there was a trend toward a higher proportion of girls than boys receiving a second MMR (P = 0.13). None of the children received MV-containing vaccines other than MMR.*

Comment: What I want you to see here is the numbers; specifically the median age at receipt of first MMR. The median age for the two groups (15.3 & 16.0, respectively) is similar. To understand the meaning of this statement, one has to understand the meaning of the term "median."

The median is the mid-point of the range of scores. For the following group of scores: 1, 3, 5, 7, 9, 11, 13, 15, 17…the median is 9 because it's the score in the middle; there are four scores below and four scores above. For the following group of scores: 1, 5, 7, 8, 9, 17, 29, 53, 99… the median is also 9, because it's the score in the middle. As this illustration shows, the median doesn't tell us much about the group, or the differences between two groups.

From the RESULTS…

Median *age at receipt of first MMR was similar for cases [15.3* ***(1.7)*** *months] and controls [16.0* ***(4.9)*** *months].*

<u>Comment:</u> In the two groups of scores [1, 3, 5, 7, 9, 11, 13, 15, 17] and [1, 5, 7, 8, 9, 17, 29, 53, 99], the second group has a lot more variability between scores.

The number in parentheses (above) is a measure of variability. Notice that the "cases" (AUT/GI) group [15.3(1.7) months] has less variability than the "control" (GI) group [16.0 (4.9) months]. In this instance, the measure of variability gives us more information about the subjects in each of the two groups, while considering the median alone is not very informative. Looking at the variability in each group, we can determine that in the AUT/GI group, the children were much more likely to be vaccinated at or close to 15.3 months, since the variability between scores was small (1.7 months). In the "control" group, the median age of first MMR vaccine was 16 months, but the amount of variability (4.9 months) was much larger, meaning that *<u>more of those children were not vaccinated according to the recommended childhood schedule.</u>*

Since children in the "control" group did not develop autism, a reasonable hypothesis may be that there is a protective benefit from delaying administration of the MMR vaccine. Of course, we cannot make that kind of assumption from this study alone, due to previously mentioned problems related to small sample size and the use of non-parametric measures.

From the RESULTS…

Clearance of MV depends on development of adaptive immunity. As cell-associated MV RNA may be present transiently after receiving MMR [38]–[39], timing of vaccination relative to biopsy was potentially important. Parental reports of timing of MMR receipt 6 months or more prior to biopsy were in accord with pediatric provider immunization charts for the final study population with the exception of one control boy whose immunization record revealed receipt of a second MMR 3.5 months prior to biopsy. This subject was retained in final analyses after determining results to be the same both with and without inclusion of his data. The median MMR-biopsy interval was similar for cases [40.8 (26.7) months] and controls [39.8 (21.1) months], and was not influenced by sex (Table 1). Older age at biopsy was associated with a longer MMR-biopsy interval, independent of case status (Spearman rank correlation, Rho = 0.65, p<0.0001).

Comment: The only comment I have about this is that during the first two years of a child's life, things happen incredibly quickly. While the difference in a few months may not seem huge when we are talking about adults, in infants, the developing immune system strengthens not only with each passing month, but with each passing week, day, and hour. It may be that the children in this study whose parents did not follow the recommended vaccine schedule, and who were vaccinated when they were older, did NOT develop autism because their adaptive immunity was stronger at the time of vaccination.

SUBJECT CHARACTERISTIC		AUT/GI CASES	GI CONTROLS
SEX	Male	23 (92)	9 (69)
n (%)	*3–5 years*	*15*	*7*
	6–7 years	*6*	*1*
	8–10 years	*2*	*1*
	Female	2 (8)	4 (31)
	3–5 years	*0*	*1*
	6–7 years	*0*	*1*
	8–10 years	*2*	*2*
	All subjects	25 (100)	13 (100)
ETHNICITY	Caucasian	18 (72)	12 (92)
n (%)	Asian	4 (16)	0 (0)
	Hispanic	2 (8)	0 (0)
	African-American	1 (4)	1 (8)
AGE STRATUM	3–5 years	15 (60)	8 (61)
n (%)	6–7 years	6 (24)	2 (15)
	8–10 years	4 (16)	3 (23)
AGE AT BIOPSY	All subjects	5.5 (3.0)[a]	5.1 (3.0)
in years, median (IQR)			
AGE AT FIRST MMR	All subjects	15.3 (1.7)[b]	16.0 (4.9)
in months, median (IQR) [RANGE]		[12.2–22.8]	[5.6–20.5]
TIME FROM LAST MMR TO BIOPSY	All subjects	40.8 (26.7)[c]	39.8 (21.1)
in months, median (IQR) [RANGE]		[23.7–97.9]	[3.5–64.5]
TOTAL NUMBER OF MMR VACCINES	All subjects	20[d]	31
% receiving 2 doses			
TOTAL NUMBER OF ALL VACCINES	All subjects	17 (4)*	20 (1)
median (IQR) [RANGE]		[13–21]	[15–22]

[a]Mann-Whitney U, one-tailed, $P = 0.67$.
[b]Mann-Whitney U, one-tailed, $P = 0.15$.
[c]Mann-Whitney U, one-tailed, $P = 0.50$.
[d]X^2, Fisher's exact test, one-tailed, $P = 0.36$.
*Mann-Whitney U, one-tailed, $P = 0.04$.
doi:10.1371/journal.pone.0003140.t001

Table 1. Subject characteristics. Remember that what's in parentheses is a measure of variability. Also remember that with non-parametric tests, we must assume that groups are virtually the same with regard to characteristics. When working with small groups, the bigger the variability, the less likely you are to achieve significance in results.

Mann-Whitney U
Is a non-parametric test.

Neuropsychiatric Status

AUT diagnoses were confirmed for all cases. ***Absence of AUT, other ASD, or other developmental disturbances was <u>confirmed</u> for controls.***

<u>Comment:</u> The authors state that absence of developmental disturbances was confirmed for controls. Yet, 69% of controls were between 3-5 years of age (see Table 1).

The problem with the above statement is that there is VERY POOR reliability and validity in ANY *standardized* measures used to assess neurodevelopmental disorders in children under 6 years of age.

It is IMPOSSIBLE to say that the ABSENCE of other ASD (such as Asperger's Syndrome or Non-Verbal Learning Disability), or other developmental disturbances (such as ADHD and Language Based Learning Disabilities) was CONFIRMED in children between 3-5 years of age.

In order to make any type of assumption about this, the researchers will need to follow the subjects throughout AT LEAST their elementary school years, and possibly beyond, since a great many children on the ASD continuum are not officially diagnosed until their cognitive, social, and behavioral challenges become more salient in the classroom environment.

Timing of Event, Table 2.

Median AUT onset age was 13.5 **(7.0)** months (*Table 2*). Cases had a high rate of CPEA-defined behavioral regression (loss of language and/or other skills following acquisition), 88%, compared to published rates of 20–40% for the general ASD population [27], [40].

Timing of event	AUT/GI cases (n = 25)	GI controls (n = 13)
First MMR vaccine	15.3 (1.7)[a]	16.0 (4.9)
age in months, median (IQR)		
First episode of GI disturbance	12.0 (17.5)[b]	2.0 (19.5)
age in months, median (IQR)		
MMR before GI onset *[n (%)]*	12 (48)[c]	3 (23)
MMR after GI onset *[n (%)]*	13 (52)	10 (77)
Autism onset	13.5 (7.0)	Not applicable
age in months, median (IQR)		
MMR before autism onset *[n(%)]*	13 (52)	Not applicable
MMR after autism onset *[n (%)]*	12 (48)	Not applicable
GI onset before autism *[n (%)]*	16 (64)	Not applicable
GI onset after autism *[n (%)]*	9 (36)	Not applicable
MMR before GI onset *[n (%)]*	5 (20)	Not applicable
<AND>		
GI onset before autism *[n (%)]*		

Key: *MMR*, Measles-Mumps-Rubella vaccine.
[a]Mann-Whitney U, one-tailed, $p = 0.15$.
[b]Mann-Whitney U, one-tailed, $p = 0.29$.
[c]X^2, Fisher's exact test, one-tailed, $p = 0.13$.
doi:10.1371/journal.pone.0003140.t002

Comment: 48% of the Autism/GI group Developed GI problems AFTER the MMR. 23% of "controls" developed GI problems AFTER the MMR. If you look very closely You will see that the analysis of the difference between groups was performed using Chi-square, and the obtained value was P=0.13. This is not significant, because it does not reach the level of P<.05. BUT… it WAS PRETTY DARN CLOSE, even with VERY SMALL groups AND groups of UNEQUAL size: AUT/GI = 12; Controls = 3.

This *STRONGLY SUGGESTS* that IF THE SAMPLE SIZE HAD BEEN LARGER, RESULTS WOULD HAVE BEEN SIGNIFICANT.

Timing of Event, Table 2, continued

Timing of event	AUT/GI cases (n = 25)	GI controls (n = 13)
First MMR vaccine	15.3 (1.7)[a]	16.0 (4.9)
age in months, median (IQR)		
First episode of GI disturbance	12.0 (17.5)[b]	2.0 (19.5)
age in months, median (IQR)		
MMR before GI onset *[n (%)]*	12 (48)[c]	3 (23)
MMR after GI onset *[n (%)]*	13 (52)	10 (77)
Autism onset	13.5 (7.0)	Not applicable
age in months, median (IQR)		
MMR before autism onset *[n(%)]*	13 (52)	Not applicable
MMR after autism onset *[n (%)]*	12 (48)	Not applicable
GI onset before autism *[n (%)]*	16 (64)	Not applicable
GI onset after autism *[n (%)]*	9 (36)	Not applicable
MMR before GI onset *[n (%)]*	5 (20)	Not applicable
<AND>		
GI onset before autism *[n (%)]*		

Key: *MMR*, Measles-Mumps-Rubella vaccine.
[a]Mann-Whitney U, one-tailed, $p = 0.15$.
[b]Mann-Whitney U, one-tailed, $p = 0.29$.
[c]X^2, Fisher's exact test, one-tailed, $p = 0.13$.
doi:10.1371/journal.pone.0003140.t002

Comment: Researchers reported No Difference in age of 1st MMR vaccine between the two groups.
As Table 2 shows, results were P=0.15.

Again, this is VERY CLOSE to achieving significance, and given the small sample sizes and unequal number of subjects, *STRONGLY SUGGESTS* that if the samples had been larger, EVEN using the non-parametric measure, there would have been a statistically-significant difference.
In other words, the entire outcome of this study would very likely have been flipped, IF THE SAMPLE SIZES HAD BEEN LARGER and IF THERE HAD BEEN MORE EQUAL DISTRIBUTION OF SUBJECTS.

It really is too bad the researchers couldn't find another 12 children who did not have autism.

Real-time RT-PCR assays

Prior to examination of study samples, performance of the four different primer sets (two for H gene, two for F gene) was evaluated for the 12 cloned target regions using synthetic RNA standards. A lower limit of detection of 50 RNA molecules per reaction was confirmed for each primer set in all laboratories.

All laboratories correctly identified all positive controls using pre-established criteria for positivity (positive results in at least two of three wells with at least one of the primer pairs for F and one of the primer pairs for H). All laboratories correctly identified all negative controls.

Concordance across laboratories was achieved in the initial round of real-time RT-PCR assays for all positive and negative results with the exception of a single study sample, an ileal biopsy from a control. An additional three samples, one ileal sample (from a control) and two cecal samples (one case, one control) yielded signal in at least one assay in one laboratory but did not meet criteria for positivity. All four samples were retested as below to resolve discrepancies.

As detailed above, only one sample met the pre-established definition of discordance; in this instance, an ileal sample from a control was positive with all four MV primer pairs in a single laboratory. Neither of the other two laboratories reported positive wells with any primer/probe combinations for this sample. The amplification product from this reaction was sequenced and determined to contain the engineered restriction site, confirming that it represented the synthetic transcript control. This sample was classified as negative. Aliquots of the three other samples that had yielded signal in one assay in a single laboratory were shipped to all three laboratory sites for retesting under new IDs. Two negative and one positive control were included to ensure blinding and monitor assay performance. Repeat testing of these three discordant samples with the F or H gene sequence primer/probe set responsible for the initial single positive finding failed to reproduce positive results in any of the three laboratories on the second round. In all three instances, results were negative on second round testing, including the one laboratory initially reporting positive results for a single primer pair.

I am not a geneticist and have never learned anything about PCR assays. I am not prepared to comment on this. I am not arguing that the labs were obtained incorrectly; I assume they were. However, even the best raw data is meaningless when study design and statistical analyses are not carefully controlled and applied correctly.

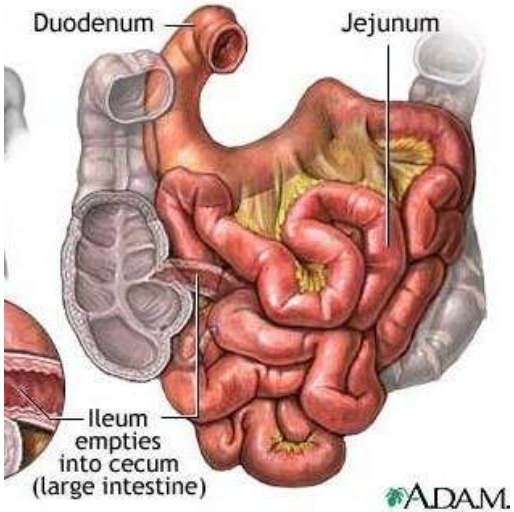
MV RNA in bowel biopsies

Analyses in all three laboratories found two ileal biopsy samples with MV F gene and H gene RNA: one from a boy in the AUT/GI group, the other from a boy in the control group. Real-time RT-PCR indicated a range of 2–7 molecules per PCR reaction, corresponding to approximately 50–500 MV RNA molecules per 100 ng of total RNA extract (Table 3). Sequence analysis confirmed that products of these samples were authentic. MV RNA was not detected in cecum of these subjects, or in ileum or cecum of any other subject. The presence of MV sequences was not associated with an AUT diagnosis (cases, 4%, controls, 8%).

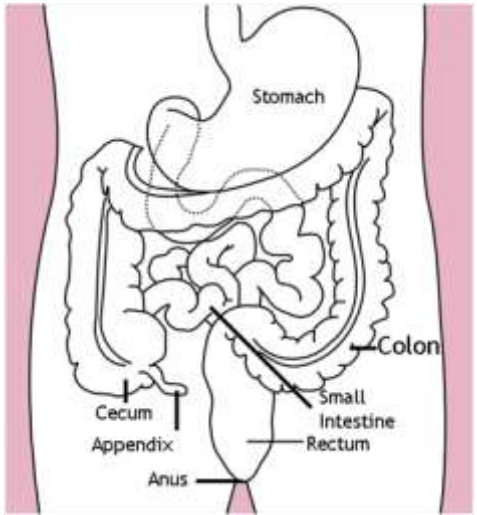
Comment: This is where the authors get their conclusion that there was no difference between children with autism/GI and GI ALONE with regard to the presence of MV RNA. MV RNA was found in ONE subject in EACH GROUP, so there was NO DIFFERENCE between groups.

There are two likely reasons as to why they only found MV RNA in two children:

(1) Group size was too small; and (2) They only looked in the ileum and cecum.



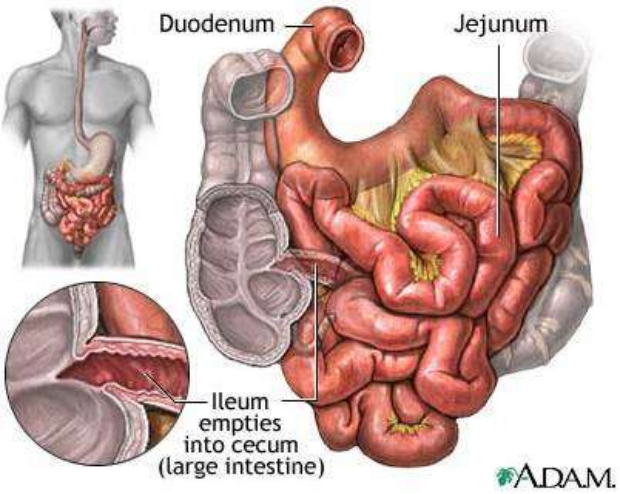
The ileum and cecum are only a small fraction of the area that comprises the GI tract.



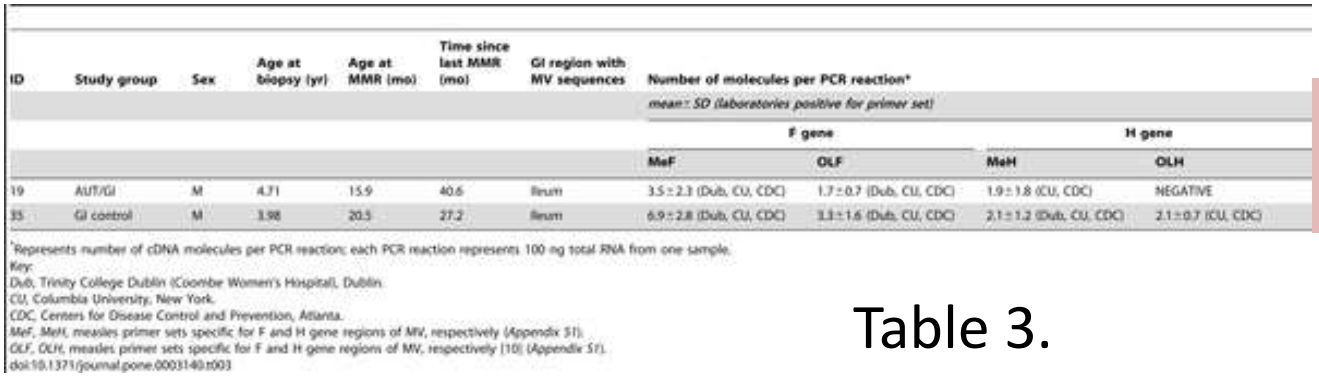
MV RNA in bowel biopsies

Both subjects with positive samples had reactive lymphoid follicles (RLF). In the AUT/GI subject, RLF were present in both small and large intestine; the control had RLF restricted to colon. Endoscopy revealed inflammation in both subjects: the case had nonspecific gastritis; the control had acute distal esophagitis. ***Other cases and controls had RLF and/or inflammation in their upper and lower GI tracts, but MV sequences were not detected in their GI samples.***

Comment: "Other cases and controls had RLF and/or inflammation in their upper and lower GI tracts, but MV sequences were not detected in their GI samples." (*Because they only looked at the ileum and cecum!*)



At left is the GI tract showing relative size and position of the ileum and cecum. Even though children with Autism/GI had evidence of inflammation (RLF) in both small and large Intestine, the researchers concluded there was no link between MMR and autism because they didn't find MV RNA in the ileum and cecum. Why would they stop there?



ID	Study group	Sex	Age at biopsy (yr)	Age at MMR (mo)	Time since last MMR (mo)	GI region with MV sequences	Number of molecules per PCR reaction* mean± SD (laboratories positive for primer set)			
							F gene		H gene	
							MeF	OLF	MeH	OLH
19	AUT/GI	M	4.71	15.9	40.6	Ileum	3.5±2.3 (Dub, CU, CDC)	1.7±0.7 (Dub, CU, CDC)	1.9±1.8 (CU, CDC)	NEGATIVE
35	GI control	M	3.98	20.5	27.2	Ileum	6.9±2.8 (Dub, CU, CDC)	3.3±1.6 (Dub, CU, CDC)	2.1±1.2 (Dub, CU, CDC)	2.1±0.7 (CU, CDC)

*Represents number of cDNA molecules per PCR reaction; each PCR reaction represents 100 ng total RNA from one sample.
Key:
Dub, Trinity College Dublin (Coombe Women's Hospital), Dublin.
CU, Columbia University, New York.
CDC, Centers for Disease Control and Prevention, Atlanta.
MeF, MeH, measles primer sets specific for F and H gene regions of MV, respectively (Appendix S1).
OLF, OLH, measles primer sets specific for F and H gene regions of MV, respectively [10] (Appendix S1).
doi:10.1371/journal.pone.0003140.t003

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Table 3.

ID	Study Group	Sex	Age at Biopsy (yr)	Age at MMR (mo)	GI region w/ MV sequences	Number of molecules per PCR reaction* Mean +/- SD (laboratories positive for primer set)			
						F gene		H gene	
						MeF	OLF	MeH	OLH
19	AUT/ GI	M	4.71	15.9	Ileum	3.5 +/- 2.3 (Dub, CU, CDC)	1.7 +/- 0.7 (Dub, CU, CDC)	1.9 +/- 1.8 (Dub, CU, CDC)	Negative
35	GI control	M	3.98	20.5	Ileum	6.9 +/- 2.8 (Dub, CU, CDC)	3.3 +/- 1.6 (Dub, CU, CDC)	2.1 +/- 1.2 (Dub, CU, CDC)	2.1 +/- 0.7 (Dub, CU, CDC)

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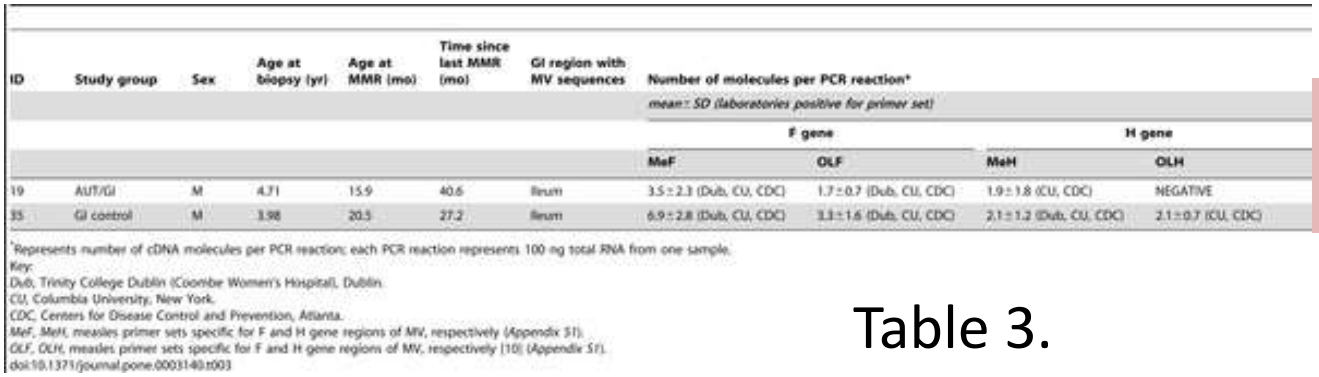
MeF, MeH, measles primer sets specific for F and H gene regions of MV, respectively (Appendix 51).
OLF, OLH, measles primer sets specific for F and H gene regions of MV, respectively [10] (Appendix 51).

<u>Comment:</u> Remember back a few slides back, when I hypothesized that perhaps the GI controls only developed GI disorders and not GI disorders with Autism because their parents did not follow "the schedule" and they received the MMR later, therefore giving their immune systems more time to develop?
A look at the Age at MMR column would seem to support this hypothesis.
That is, it would, if we could make generalizations from this study.



ID	Study group	Sex	Age at biopsy (yr)	Age at MMR (mo)	Time since last MMR (mo)	GI region with MV sequences	Number of molecules per PCR reaction* mean± SD (laboratories positive for primer set)			
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doi:10.1371/journal.pone.0003140.t003

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Table 3.

ID	Study Group	Sex	Age at Biopsy (yr)	Age at MMR (mo)	GI region w/ MV sequences	Number of molecules per PCR reaction* Mean +/- SD (laboratories positive for primer set)			
						F gene		H gene	
						MeF	OLF	MeH	OLH
19	AUT/ GI	M	4.71	15.9	Ileum	3.5 +/- 2.3 (Dub, CU, CDC)	1.7 +/- 0.7 (Dub, CU, CDC)	1.9 +/- 1.8 (Dub, CU, CDC)	Negative
35	GI control	M	3.98	20.5	Ileum	6.9 +/- 2.8 (Dub, CU, CDC)	3.3 +/- 1.6 (Dub, CU, CDC)	2.1 +/- 1.2 (Dub, CU, CDC)	2.1 +/- 0.7 (Dub, CU, CDC)

*Represents number of cDNA molecules per PCR reaction; each PCR reaction represents 100 ng total RNA from one sample.
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MeF, MeH, measles primer sets specific for F and H gene regions of MV, respectively (Appendix 51).
OLF, OLH, measles primer sets specific for F and H gene regions of MV, respectively [10] (Appendix 51).

Comment: The authors reported that the absence of Autism, other ASDs, and other developmental disorders was *"confirmed"* in the GI Controls. As noted previously in this critique, the absence of ADHD, Asperger's Syndrome, Non-Verbal LD, or Language-Based Learning Disabilities cannot be determined prior to at least six years of age, due to lack of reliability and validity in standardized psychometric measures for children younger than six years. Yet, the age of the GI control subject in Table 3 was 3.98 years. It is *impossible* to *confirm* either the absence *or* the presence of many neurodevelopmental disorders at this age. It is simply too soon to tell.

Timing of MMR, GI episodes and Autism

If MMR is causally related to either GI disturbances or AUT it should precede their onset. Similarly, if GI disturbances contribute to AUT they should precede onset of AUT. We approached temporal relationships in the following manner: subjects with MMR administration and GI onset in the same month were considered to have MMR administration before the onset of GI episodes; subjects with GI episode and AUT onset within the same month were considered to have GI onset before AUT onset; and subjects with MMR and AUT onset within the same month were considered to have MMR onset before the onset of AUT.

Comment: MMR, Autism onset, and GI episode were only considered related if they occurred "in the same month." I wonder if that means that if a child received the MMR on February 28th and regressed into autism on March 2, it would be assumed there was no association between MMR and autism because the two events did not happen in the same month.

You wouldn't THINK the researchers would do this, but there are a lot of things about this study you wouldn't THINK such stellar researchers would do.

It might be interesting to check this out further…

Timing of MMR, GI episodes and Autism

There were no significant differences in the proportion of cases and controls with MMR before onset of GI episodes: 12 of 25 cases (48%) received MMR before GI episodes began as compared with 3 of 13 controls (23%; P = 0.13; Table 2).

Comment: "There were no significant differences…" The key word here is "significant." The difference in onset of GI episodes between 12 children in the autism group and 3 children in the GI control group was not significant.

This should come as no shock. As has been stated previously, the most influential factor impacting the ability to obtain statistical significance is sample size. Unequal numbers of subjects between samples is another factor that adds to the difficulty of obtaining statistical significance, so we should not be surprised that when comparing differences between one group of 12 children and one group of 3 children, "no significant differences" were found. However, the obtained result was close to being statistically significant (P=0.13), once again giving STRONG SUGGESTION that IF the sample size had been larger and IF the number of subjects in each group had been equal, we would be looking at completely different results from this analysis.

Timing of MMR, GI episodes & Autism

To determine whether our data supported the hypothesis that GI pathology contributes to ASD pathogenesis, we examined the temporal relationship between MMR immunization, first GI episode, and AUT onset. If the putative relationship of MMR to GI pathology and AUT is valid, MMR must precede GI dysfunction and AUT, and GI dysfunction must precede AUT. If GI dysfunction contributes to AUT independent of MMR, it is necessary only that GI dysfunction precede development of AUT. X^2 analyses indicated no role for MMR in either the pathogenesis of AUT or GI dysfunction (Table 4). ***Only 5 of 25 subjects (20%) had received MMR before the onset of GI complaints and had also had onset of GI episodes before the onset of AUT (P = 0.03).***

Comment: **Did they *really* say that "ONLY 5 of 25 subjects (20%)" of this VERY SMALL sample had received MMR before the onset of GI complaints and had ALSO had onset of GI episodes BEFORE the onset of Autism?**

"ONLY" 20% !!!

Timing of MMR, GI episodes & Autism

Only 5 of 25 subjects (20%) had received MMR before the onset of GI complaints and had also had onset of GI episodes before the onset of AUT (P = 0.03).

"ONLY" 20% !!!

A ratio of "ONLY 5 of 25" regressing into autism and GI dysfunction after MMR translates to 1 in 5 children!

The authors of this study don't think that's a big deal??

Is THIS why they are so unconcerned about the fact that Autism currently affects 1 of every 90 to 100 American children?

Table 4.

ORDER OF EVENTS	CASES (n = 25)	
	GI before ASD	GI after ASD
	n (%)	*n (%)*
MMR before GI	5 (31)[†]	7 (78)
MMR after GI	11 (69)	2 (22)

[†] X^2, Fisher's exact test, one-tailed, $p = 0.03$.

doi:10.1371/journal.pone.0003140.t004

Comment: **In their interpretation and discussion of these findings (previous slides) the authors conclude there is no relationship between the MMR vaccine and GI dysfunction with regressive autism. As evidence to back up their claim, they note that a higher percentage of children who regressed into autism after receiving the MMR actually had GI dysfunction BEFORE they were vaccinated. (see table above)** ***This fact seems like a VERY STRONG ARGUMENT for NOT Vaccinating children with GI Dysfunction with the MMR.***

Looking at this table, what I interpret from the data is that THERE IS A RELATIONSHIP between MMR, GI dysfunction, and regressive autism, and we need MORE RESEARCH into the exact nature of this relationship to determine how these factors interact to produce susceptible groups of children who are more vulnerable to vaccine damage.

THIS IS UNACCEPTABLE!!!!

When researchers at one of the most prestigious facilities in the United States cannot find enough children without autism to study, so that their results will not be invalidated by small size, unequal numbers between study groups, and statistical measures that lack power, something is desperately wrong.

When those same researchers, whose facility receives more than

9 MILLION DOLLARS each year of taxpayer money have the GALL to state that their results indicate no problem because

"ONLY 5 of 25"

children in their VERY SMALL study developed gastrointestinal dysfunction and regressive autism after receiving the MMR vaccine, *SOMETHING IS DESPERATELY WRONG!*

After spending many hours critically reviewing this study and putting together this presentation, the only conclusion I come up with is

WE ARE BEING LIED TO and OUR CHILDREN ARE BEING SACRIFICED.

PLEASE FORWARD THIS TO EVERYONE YOU KNOW. This HAS to STOP.