

## **Status of Serological and Molecular Virological Findings Implicating Measles Virus in the Etiology of Autism**

**Report Prepared for US Congressman, Dave Weldon, Rep. Florida 15th by James Jeffrey Bradstreet, MD, Fellow, AAFP, Founder & Director of Clinical Programs, International Child Development Resource Center (ICDRC), 1688 West Hibiscus Boulevard, Melbourne, Florida 32901 & Adjunct Professor of Neurosciences Department of Psychology Stetson University Celebration, Florida & Visiting Professor of Child Development Department of Pediatrics Southwest College of Naturopathic Medicine Phoenix, Arizona**

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Section 1.

### Introduction and Background of My Involvement in Autism

I am the Director and Founder of the ICDRC in Melbourne, Florida and currently practice as a physician and clinical investigator. Additionally, I serve as an Adjunct Professor of Neurosciences in the Department of Psychology at Stetson University, Florida. I am a Fellow of the American Academy of Family Physicians, an organization with a long history of dedication to public health and preventive medicine. Central to the family practice model of health care is comprehensive inclusion of the whole person and his many organ systems into a unified view of personal health. Part of that process has included the concept of safe vaccinations to prevent potentially devastating infectious diseases. Despite grave concerns, which will be expressed here, regarding the lack of safety of certain vaccine components, I remain dedicated to vaccination - as a concept, while being absolutely convinced more must be accomplished to improve vaccine safety.

My involvement in viral research dates to the mid 70s when I worked as a Robert Wood Johnson Research Fellow at the University of South Florida, College of Medicine. That published work involved investigations into the environmental influences on herpes viral replication (Lancz & Bradstreet 1976). My more specific involvement with the etiology of autism began as a consequence of appreciating that my own son was autistic.

In the processes of seeking answers to my son's specific problems, I became familiar with the works of Warring, Singh, Wakefield, Kawashima, Connolly, and O'Leary. Collectively, these authors built a convincing starting point for my opinion that neurotropic viruses, e.g. measles, must at least be considered as possible etiological agents in the encephalopathic changes observed in autism disordered children. As a result, I set out to contribute additional pieces of data to the growing body of evidence regarding the virology and immunology of autism.

Over the time of the past 6 years I went from knowing only two children with autism, my son and one other, a patient, to now having examined and consulted on over 1000 children with the disorder. In part, this was the result of parents discovering I too had a child with autism and my willingness to listen to their complex stories. My contribution, therefore, comes from the large accumulated data our patients have provided, as well as our efforts to define the biochemistry, immunology and virology of our patients. My perspective remains that of a clinician working on a case by case basis to solve the complex mystery presented by the history, symptoms, physical findings and laboratory data intrinsic to each child.

Further, and as a result of the experience I have acquired of this disorder, I have been made an adjunct Professor of Neurosciences, Department of Psychology, Stetson University, Florida and a visiting Professor of Child Development, Southwest College of Naturopathic Medicine, Phoenix, Arizona. At Stetson University, I teach electrophysiological brain mapping using quantitative EEG methods and low resolution electrotomographic analysis (LORETA), and at SCNM, I teach nutritional interventions for autism spectrum disorders. I am also a coinvestigator on autism research projects at Robert Wood Johnson Medical School, Washington University St Louis - College of Medicine, Arizona State University, and SCNM,

As a result of the tremendous public health consequences of our clinical findings, I have attempted to introduce our data to the public debate regarding autism, MMR and vaccine components as quickly as possible. In doing so, I have presented oral and written testimony to the Institute of Medicine, Deputy Secretary of HHS - Claude Allen, and the US Congress. Various studies are at different degrees of submission for peer review and the status of these findings will be defined as they are presented herein. Additionally, our patients have contributed specimens to Dr Oâ€™Learyâ€™s Unigenetics Laboratory, as well as Dr Singhâ€™s research at Utah State and Michigan State Universities, Dr Connollyâ€™s at Washington University St Louis, along with others. So our population is being defined by numerous medical school researchers and duplicated at several commercial laboratories. The consistency of the findings between these various laboratories is extraordinary.

To the best of my knowledge, I was the first to propose Cerebral Spinal Fluid - PCR investigation in potential MMR cases. These were undertaken by me and my partner Dr Jerry Kartzinel, a board certified pediatrician, in our Florida facility. We combined the PCR - MV studies with immune and autoimmune studies of the CSF and blood of children and developed a classification system for how MV presents as autism.

I do not operate a laboratory or employ scientists. The state of progress in the investigations is therefore dependant upon the various laboratories we utilize, the parentsâ€™ abilities to pay for investigative testing, (generally not covered by state or private insurance) the ability to acquire controls from collaborating institutions, and where applicable, the determinations of institutional review boards.

#### Summary of instructions received

As of July, 2003, I have been asked by Congressman Weldonâ€™s staff to summarize my work, findings and observations regarding the relationship of MMR vaccine to subsequent autistic symptoms. I have further been asked to discuss my experiences, based on the large population of autistics I treat, regarding various subtypes within this disorder. My work is specifically designed to assist individual child medical care planning and to help define potential therapeutic interventions. Nonetheless, of particular interest to public health and legislative policy, are my findings regarding the persistence of MV in the cerebral spinal fluid of a subgroup of children with autism. I will address my rationale for the original investigations, the current state of these observations, and where I am attempting to further these observations.

I will further comment on why the current evidence allows me to diagnosis individual children with MMR-vaccine-induced autistic spectrum disorder. I recognize that autism may present in the absence of vaccination. For my purposes, I am concerned with the biological plausibility of diagnoses rendered, and what supporting data evince those diagnoses. Additionally, I will present the ICDRC data for the RT-PCR/CSF evaluated children we have evaluated to date, and the similarities they generally share with the UK cohort of eight children being evaluated as part of a class action lawsuit in that country. I serve as an expert

witness for the legal firm of Alexander Harris and the UK High Court processing the claims. No child will be identified in anyway which would permit anyone to know their identity. The matters regard the UK cohort, which will be abstracted here, are in the public arena and are entered as evidence in that legal proceeding.

## Literature and Evidentiary Review

The material upon which I have relied in order to prepare this report consists of the following:

- a) published medical literature,
- b) research in the process of being published or prepared for submission,
- c) direct examination of and history gathering from numerous children with autism (including a review of past medical records and vaccine cards for all children when available),
- d) the outcomes of specific laboratory tests, e.g. the results of noninvasive and invasive testing,
- e) the individual responses to specific medical interventions and therapies.
- f) the core bundles on the 8 lead UK cases and the summations of the medical records provided by Drs Byers and Walker-Smith. However, my general opinions about causality are not based on an independent review of the 8 case presentations, rather on the essential laboratory and historical evidence provided about the children, the nature of the physical evidence, and my understanding of pathophysiology.

## Epidemiology

I shall refrain from commenting on the epidemiological evidence or any critique of the published epidemiology, except to assert that when defined pathology can be documented in specific children, no amount of epidemiology refutes the importance of that finding. By example, when a child with seizures and autistic regression following vaccination with MMR has measles virus detectable in the CSF, every reasonable clinician should assume a cause and effect relationship, irrespective of the epidemiology.

This, in fact, was expert opinion of the eminent, Dr John Menkes, Professor Emeritus, UCLA, Child Neurology and editor of the standard textbook in the field: Child Neurology. In his opinion report to the UK High Court, he too stated that where MV was detected in the CSF of a child with temporally related regression following MMR vaccination, MV was the etiology of that child's autism "or what we often refer to as autistic encephalopathy.

## Section 2.

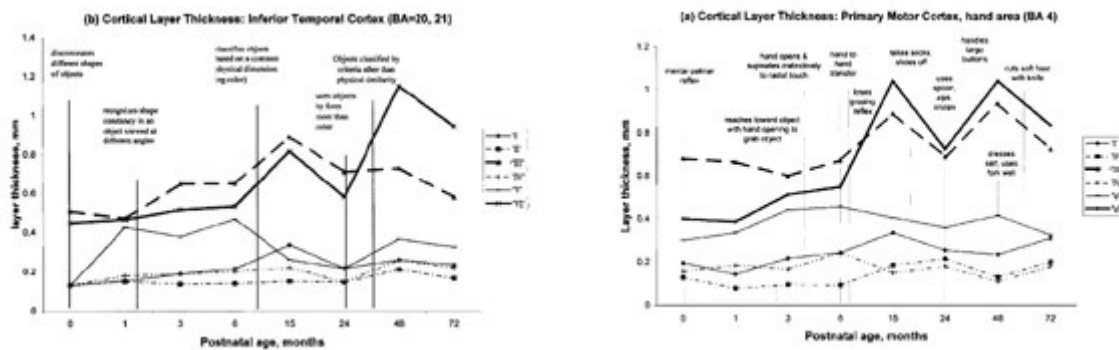
### Event Timing Issues Relating to Subgrouping of Phenotypes

In undertaking the autism biological issues, I think the timing of various events in child development is extremely important. For example, a viral or toxic insult offered to the body at 15 months of life, would obviously have a different impact on neurodevelopment than if it were offered at 15 years of life. Additionally complicating things for child development is the vulnerability of cortical developmental to

disruption. Many neurodevelopmental processes, must occur at specific times in the child's life or they will forever be altered.

The criticality of this timing sequence was most recently carefully documented in the work of Landing, et al (2002), and described in this quote from their work, "From birth to 72 months, without exception, each cortical layer of each of the 41 cytoarchitectonic areas repeatedly thins and thickens in a wave-like fashion. On average, each layer changes its direction of growth 3.5 times over six age periods from birth to 72 months."

These next two graphs from their paper show the importance of timing within the brain's developmental course, and I am using them here to illustrate why even subtle insults to the developing child's brain which coincide with these stages, would have the potential for cataclysmic results.



Of great interest to the autism picture is the timing around the 15 month period " a common time of MMR vaccination. One can readily see that while this is a critical epoch in development, moving 3 months in either direction would create a different type of insult to the developing brain. Hence, phenotypic variation in behavioral and cognitive development would and should be expected.

So, the process of establishing subgroups within the spectrum of autism (ASD) is complicated, not merely by the nature of the environmental hazard " in this case the proposed influence of MMR on brain development, but further by its relationship to the that child's individual cortical developmental processes.

### Section 3.

#### Rationale for Cerebral Spinal Fluid Investigations in Children with Autism following MMR-II. Biological Plausibility.

While our original work in this area has been hotly debated, our medical reasoning for investigating the CSF is quite traditional. Based on the observations of O'Leary and colleagues, who have reported that MV was found to be persistent in the lymphoid follicular tissue of the gut (Uhlmann 2002), and those observations of Kawashima (2000) and his group who could identify MV of vaccine origin in the blood of autistics, when combined with the anecdotal evidence of a historical temporal relationship of ASD symptoms to a live viral, albeit attenuated MV, vaccination, we felt compelled to look for the virus in the CNS.

It is generally believed that the post-measles encephalopathy which occurs as a consequence of wild-type

measles infection is autoimmune in origin (Liebert 1997 & Norrby 1997). We also know MV can be isolated from the CSF of children with acute encephalopathy, but who never manifest a rash or other signs of the disease (Wairagkar 2001). In that study, vaccine records were not available for the children. The detection of MV in the CSF was the basis for the authors concluding MV was the etiological agent for the encephalopathic changes.

Furthering the plausibility and rationale for CSF investigations, Weibel (1998) reported temporal clustering of acute encephalopathy followed by permanent brain injury or death associated with MMR-II (Merck) attenuated measles vaccines. This report is significant because it was generated by the US government agency tasked to follow vaccine reactions.

My approach was to consider CSF testing in the light of immunological assessments combined with any reported temporal relationships between the MMR vaccine and reported ASD symptoms, as well as prior findings of MV by RT-PCR in endoscopic biopsies or blood. My understanding is the UK cohort has not been tested through the methodologies as published by Singh (2003 "in press, 2002, 1998, 1997 & 1993) and Connolly (1999), whereas the US cohort largely has. Therefore, in analysis of the two groups, I will only compare the RT-PCR findings. We did, however use the information provided through Singh and Connolly to contribute to our opinions regarding the justification for CSF diagnostic testing. In retrospect, our logic appears more than reasonable, given the high rate of MV-PCR positives observed (78% of tested CSF are positive for MV).

Methodologies of Specimen Collection and Processing.

Ethical Considerations:

Cerebral Spinal fluid was obtained following informed consent of the parents on 27 children (23 have data on PCR finalized) felt by history, examination and previous testing to be a risk for possible MV in their CSF. Since informed consent had been granted for diagnostic purposes, and this was not a research project, no IRB was required for the procedure or laboratory testing requested (FDA & DHHS: 1980, 1989 & 1991).

Methods

Spinal fluid was obtained after intravenous conscious sedation and local anesthesia, using sterile technique. The fluid was collected in three standard CSF tubes using a commercially available kit from Baxter. Approximately, 4 cc of CSF were obtained. The sample was split between three tubes with one tube going to our local hospital (Health First) for cell count, culture and chemistries: one tube went to Singh at Utah State University for immunological studies, and another tube was sent to Dr O'Leary's Unigenetics Laboratory in Dublin.

For both Dr Singh and Dr O'Leary the specimens were maintained and shipped on dry ice. In the collection of our samples, Dr O'Leary's laboratory always received a minimum of 2 cc of CSF, generally with further preparation by overnight 20C incubation with RNA-Later<sup>®</sup> (Ambion, Austin, TX), and then placed on dry ice. If RNA-Later<sup>®</sup> was not used, the specimens were always on dry ice. The tubes were shipped in their original collection tubes, placed in plastic bags and labeled with the patient's name and date of collection. Expedited shipping was used with 2 day delivery to Ireland, including customs processing, with Monday or Tuesday as the shipping dates so the specimens could arrive midweek for

immediate care at the lab. Dr Singh's studies were sent by next day air.

#### Selection of Laboratories:

In the case of Dr Singh, his was the only significant published laboratory in the autism field which would accept samples from non-university subjects. We had previously cross validated Singh's work using standard split-sample assays with another reference laboratory, and therefore have no reason to believe that his results were anything but reliable. For PCR work, other than Dr Kawashima, only O'Leary's group was publishing and producing in this area.

#### IRB for CSF CONTROLS:

Most recently, Tulane University Medical Center, obtained 3 Control CSF samples under an IRB approved process for study at various centers, including Drs O'Leary's and Singh's laboratories. Children ages 4 to 8 years who had been given standard MMR-II per the CDC guidelines, who also had an indwelling CSF shunt for hydrocephalus were selected as controls following informed consent as set out in the IRB. Up to 10 cc of CSF which would otherwise have been discarded was collected and analyzed, when appropriate, using routine methods. Portions of the specimens were collected in sterile subzero tubes, mixed with RNA-Later® as previously described in our methods section. If studies at Tulane indicted possible infection or bacterial contamination, the specimens were discarded. Only those infection-free samples were forwarded for use as PCR controls. Selection of cases and verification that they did not have autism spectrum disorders was by joint agreement of Dr Jane El-Dahr, Chief of Pediatric Immunology, Allergy and Rheumatology at Tulane and the neurosurgeon working on the shunt. The specimens were collected between November of 2002 and May of 2003. They were continuously kept in the ultracold freezers at the medical school, until shipped on dry ice to ICDRC to be forwarded to Unigenetics.

I have been informed by Dr El-Dahr that the specimens were maintained at dry ice temperatures at Tulane Medical Center. They arrived with abundant dry ice in the shipping container and were immediately transferred to our dry ice freezer. Temperatures at ICDRC were rigorously maintained by assuring dry ice was always present. Prior to shipping to O'Leary's Laboratory and to assure blinding of the controls, 4 other cases were added to the shipping box. We did not provide any known positive controls. These cases were transported in identical tubes (provided by Tulane for matching purposes), with the same color permanent marker and with a similar coding system. No specimens, whether cases or controls, have any names on the tubes, nor is any other documentation provided to the receiving laboratories. The laboratories do not have the control identity code, so they are properly blinded. My Director of Nursing, Esther Kennedy RN, labeled the tubes and prepared them for shipping using the identical protocol as determined in the IRB (including RNA-Later®) and sent them to the Unigenetics laboratory for processing. It was felt by the principle investigator at Tulane, Dr El-Dahr, that this processing step through our clinic to merely add cases to the shipping container was necessary to blind the controls. At our end the specimens from Tulane were never opened, defrosted or managed in any way except to assure they stayed on dry ice throughout shipment to Dublin. A similar process will be used for Dr Singh when controls are forwarded to him.

#### RESULTS OF CONTROL CSF:

Copies of the coding system are maintained at Tulane (by Dr El-Dahr) and at ICDRC (exclusively by myself and Esther Kennedy) for controls, and at ICDRC for cases, again exclusively by myself and Esther Kennedy.

Copies of the control results have been provided to Tulane for verification of the results. Presently, three controls have been accomplished. Of these 3 of 3 are negative. Spinal fluids from lumbar punctures are paucicellular, but always a few cells are present which allows for RNA amplification. This is the expected result, and in complete agreement with the pathophysiological mechanism of MV being put forward as the etiological pathogen in these cohorts of children with ASD.

## USE OF PCR DATA IN DETERMINING ETIOLOGY

The present combination of immunological and PCR data is so compelling that even without specific age-matched controls, I consider that it is more than probable that the MMR vaccine is one of the causes of ASD in a number of cases. The rationale for this is straightforward. However, I am aware of two principle objections to the vaccine MV etiology put forward here:

- 1) The MV is present at low levels and is commonly found in the CNS after infection.
- 2) It is a laboratory error secondary to contamination or detecting only "viral residues".

We know vaccine viral persistence (beyond a few days) when it has been described, always occurs in the presence of disease. If we include Kawashima's data with O'Leary's team do have significant negative data on controls which indicates no persistence of vaccine strain in non-cases. I am therefore including Kawashima's data here because it is part of the literature published on the subject:

1. Kawashima found MV of vaccine origin in the blood of children with ASD (Kawashima 2000), but in the CSF in another case of intractable epilepsy, he found wild-type disease despite prior vaccination (Kawashima 1996). 2. Kawashima also found peripheral mononuclear cell, vaccine-strain persistence in children with autoimmune hepatitis years after vaccination (Kawashima 1996). 3. Dr O'Leary's team, in their published and presented works (Martin 2002, Uhlmann 2002 & O'Leary 2000), on the intestinal biopsies of non-autistics, showed they had a very low prevalence of MV (my understanding, is that one case of mixed wild-type and vaccine origin and rare cases of wild-type in the appendix at the time of appendectomy) in controls, whereas there was a high percentage of MV of vaccine origin in the autism cases (Sheils 2002).

I believe the observations regarding intestinal findings and CSF findings must be viewed jointly. It seems unfounded to presume one strain (vaccine) resides in the gut, and another strain (wild type) resides in the CSF/brain in all cases, even though that may be biologically and remotely possible.

Sonoda and Nakayama (2001), in their benchmark work reported the detection of MV (of wild-type) in the CSF and blood of children with encephalitis, acute measles, or SSPE, even when viral isolation techniques (culture) were negative. Thus, the wild-strain virus can persist in a noninfectious or non-culturing state in symptomatic individuals manifesting serious diseases or acute measles, and is best detected by RT-PCR.

The specifics of the allelic discrimination findings are the expertise of others, but what has been presented to me thus far, is compelling from the clinician's perspective. Given the insensitivity of other methods as reported by Sonada and Nakayama, RT-PCR techniques represent the most appropriate diagnostic intervention when MV persistence is suspected in the brain. It should then be followed by allelic discrimination when possible, if there is concern about the source of the pathogen, as is the case here. Further, Dr O'Leary's methodologies and laboratory detection methods have been replicated and

validated by several independent laboratories. This was a requirement of the UK class-action lawsuit.

As I attempt to form my diagnostic impression of the children, I must include the possibility the vaccine failed to provide adequate protection from wild-type MV disease. But few diagnostic options will remain when the children have no measles by history, apart from the vaccination exposures. So, when the laboratory reports PCR-MV detection on CSF samples of symptomatic and vaccinated individuals, I am left with these options, if one is to believe the vaccine is not the primary strain present: 1. Following vaccination the child developed suboptimal levels of immune response to protect from wild disease, but modified the course of the wild infection so that it was never apparent clinically (occult infection). 2. This occult infection is also a persistent occult infection (detectable years after the onset of symptoms), which is symptomatically manifesting as the symptoms we refer to as autism. 3. The occult infection was acquired shortly after the vaccination, because the histories are consistent with ASD symptomology within months in the majority of cases. 4. Theoretically, however, both wild and vaccine strains could be persisting.

If these were the findings which explain our cases, it would be extraordinary. An occasional case of subacute sclerosing panencephalitis (SSPE) has been documented in the medical literature, for children who have previously been vaccinated (Miki 2002 & Jin 2002). These studies and other related studies which identify strains with specificity in SSPE, all find wild-type MV irrespective of prior vaccination programs (Santibanez 1999, Yamaguchi 1997, Katayama 1995 & Godec 1990). None of these report autistic symptoms. A single case report of delayed onset post-vaccination measles inclusion body encephalitis (for which allelic discrimination was also utilized) found vaccine strain in the brain of a Canadian boy 8 months after vaccination (Bitnun 1991).

I reviewed all the reported complications of the wild-type measles outbreaks which have occurred worldwide since 1991 (as can be found on the CDC web site or in the MMWR). As is well established, wild-type measles does cause symptomatic encephalitis or encephalopathy (although infrequently), but there are no reports of autism from wild-type measles. Therefore, it is logical to assume the MV present in the CSF are not of the wild-type. Even if they were, the vaccine-induced alteration of immune response to that wild-type virus would be the likely precipitating event leading to the unexpected course. Thus, causality (in this scenario) is still secondary to the vaccine, because absent the vaccine, the child's measles infection would have been typical of the familiar wild-type measles diseases.

Regarding the contamination argument, it is the burden of Unigenics Ltd experts to speak to that point as it relates to their end, but I can assure Dr Weldon that no contamination could possibly enter into any of the CSF samples at ICDRC. Extreme caution was taken to exercise sterile technique. I do know that the contamination issues have been raised by the defendants in the UK legal matter and it has been effectively dismissed by the data offered by the laboratory. Further we: 1. do not have a laboratory, 2. do not culture or keep viruses onsite, 3. label specimen containers prior to use, 4. do not open containers in the clinic, 5. use spinal kits which are sterile and prepackage by the manufacturer, 6. use fresh bottles of RNA-Later<sup>®</sup>, 7. never pipette out of the bottle. 8. store the specimens in our facility on dry ice.

Where we have gathered intestinal biopsy material on our own patients (in collaboration with gastroenterologists in Florida), either I or my nurse were personally present and immediately put the sample on RNA-later<sup>®</sup> and then dry ice within 24 hours, or when not present, we instructed via written procedures with verbal confirmation the exact same methods to the hospital surgical staff.

As you know, I was present, during Congressional testimony conducted into vaccine-related autism concerns on April 25, 2001, when Dr Gershon put forward the viral residue notion. This does not seem credible on the surface, since viral protein can be detected (Wakefield, during the same hearings, 2001), which demands active viral encoding of proteins and gene expression, not inactive "dead" residues. Unigenetics Laboratory maintains standard testing measures to assure accuracy and prevent undetected contamination of samples.

#### Section 4. Biological Evidence of Causality in the ICDRC, MMR-Linked Cohort:

The data will show that there is more than sufficient biological evidence to establish the role of MMR in causality for at least some subgroups of ASD children. Further, the abundance of published research by a diverse group of investigators from numerous medical institutions, offers evidence that this is not a narrowly held opinion.

To define the cohort better, we have extensive records on all children, and I have direct access to all records including those of my partner, Dr Kartzinel. The children were selected because either I (25/27 cases) or Dr Kartzinel (2/27 cases) felt there was a high index of suspicion regarding MV in the onset or etiology of symptoms, (with the exception of #27, a female with cerebral palsy, but also with an atypical pattern of brain autoimmunity for CP [more typical of ASD]). The persistence of bowel problems and the insistence of the parents to exclude MV from the differential diagnosis were used as indications for the procedure in that case. She also seemed to improve modestly with elimination of gluten and casein). All have a history of either ongoing or past protracted bowel complaints. Most parents noted abrupt changes "declines in their children following the MMR and most often we had detected anti-Brain antibodies; either through Dr Anne Connolly at Washington University " St Louis (Connolly 1999), or with Dr Singh or Specialty Laboratory prior to investigation by lumbar puncture. It is of note that #27 had high levels of anti-MBP at Specialty Lab. Additionally, she had antibodies to brain endovascular as found by Dr Connolly at WUSTL, and this is typically significant for the ASD group as well (ibid). This added to the need to clarify this child's pathology.

Occasionally, the parents and records note the decline post MMR was subtle for the first few months then rapidly accelerated, as long as 6 to 9 months post vaccination. This is temporally consistent with the previously cited case of delayed reactions to MMR in the Canadian child (Bitnun 1999), although that child had MIBE, not ASD.

All children were diagnosed with ASD or as in the one child CP/ASD-like condition, by either a developmental pediatrician, child psychiatrist or child neurologist and had an established diagnosis prior to referral to our center. In all cases, I concurred with the previous diagnosis. In the seven US children who underwent endoscopy, LNH was present in all. Of these 5 of 6 had MV detected through Unigenetics Ltd, and one child's specimens were lost in transit. In the other children, a history of protracted abnormal bowel habits was consistent with autistic enterocolitis, but we were unable to obtain endoscopy for a variety of reasons. Further, 3 of the 4 children with positive bowel biopsies for MV were also positive for MV in the CSF. Table 1 documents the distribution of the data.

Findings from Dr Singh's Laboratory: I am including Singh's data in tabular form here (Table 1) because, (a) we do have it, (b) the data was part of our initial investigations into the role of MV in the CSF, and (c) Singh has published his methodologies in peer-reviewed medical journals as noted previously. My

opinion in this matter is not, however, dependant upon Dr Singh's findings, although they provide additional insights into the workings of the immune system for this cohort.

We are not implying this overall cohort represent likely statistics for the population of autistics at large. No one has the means to invasively sample that population at this time. Once our data is published, such a study may be determined to be ethical and then funded. Section 4. Biological Evidence of Causality in the ICDRC, MMR-Linked Cohort:

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Table 1. Shows the results of my initial CSF investigations thus far.(Click link above to view)

Table 1: All children received MMR-II vaccine between the ages of 12 and 18 months. All children have/had significant intestinal symptoms as manifested by all or part of the following: chronic constipation, diarrhea, undigested food in feces, bloating, apparent pain, and/or persistent pathogenic organisms. All children have the diagnosis of either Autism or PDD-NOS (DSM-IV) as established by an outside observer other than ourselves with the exception of #27 female who has cerebral palsy with autistic features. Blanks represent studies the parents elected not to have performed for financial reasons, with the exception of the bowel biopsies where a lack of access to pediatric gastroenterologists willing to perform endoscopies in the presence of autism, severely limited our access to this aspect of diagnostic testing. (We can identify the children from our original spreadsheet, but Federal HIPPA regulations prevent any disclosures which would allow anyone to identify the child).

Section 5. Analysis of the Data

Clinically, this is a work in progress; however the findings of the completed work can be summarized as:

- 1) The cohort for CSF - PCR with finalized data (N=23) contains 18 boys and 5 girls with ages ranging from 2 to 14 years.
- 2) 18/23 (78%) CSF are positive for MV- F gene by PCR.
- 3) Of the 18 positives 4/5 girls and 14 of 18 are boys.
- 4) 10/23 (43%) CSF are immunologically reactive to brain autoantibodies.
- 5) 3/23 (13%) CSF have detectible MV antibodies (IgG)

6) 6/23 (26%) CSF have the unique antibody to H Protein of MV present.

7) Whole blood detection of MV " F gene by PCR was positive in 10 of 22 (45%), but in 4 cases less than 1 gene per ng of RNA was detected.

8) In the sera, 14/17 (82%) react to brain autoantibodies (nearly identical to the PCR data), and 10/12 (83%) have the unique H protein antibody published by Singh (2003 & 2002).

9) In cases where bowel biopsies could be obtained, 5 of 6 (83%) were positive for MV, which is consistent with the data previously reported (Uhlmann 2002 & Martin 2002).

10) There is also a high degree of correlation between positive bowel biopsies and positive CSF in this small cohort (80% of those with positive biopsies were further positive for MV in CSF).

11) For the Controls: 3 of 3 are reported as negative.

#### CONCLUSIONS REGARDING THE TABLE FINDINGS:

As we have approached our patients, we use the data gathered from the CSF, blood and bowel, to form a clinical diagnosis. This process is based on the following conclusions:

1) Viral persistence in the presence of a high degree of clinical suspicion is a likely and common (78%) observation. This strongly supports the suspicions of the parents, the role of MV from MMR in the etiology, and the clinical diagnoses we presumed as a basis for our CSF investigations.

2) From a CSF immunological perspective, there is more heterogeneity than from a viral PCR perspective, implying PCR data is a more sensitive tool than the selected immunological studies in the CSF. It could also mean the immune systems of children respond to MV persistence in various and/or inadequate ways. Additionally, we likely need to expand the testing of immunological studies in the CSF to screen for other markers of inflammation and autoimmunity.

3) Typical IgG MV antibodies are uncommon findings in CSF, despite viral persistence. Two of the three which were positive had the highest ratio of MV to Total RNA.

4) When autoantibodies to brain in CSF are examined " nearly half - 43% are reactive.

5) One child without detectable CSF MV, but with bowel and blood MV had autoantibodies to MBP in the CSF and IgG antibodies to MV and anti- H protein of MV in the CSF. The results were repeated and equal on two different spinal tap procedures. This is highly suspicious for previous CNS presence of the virus, but with ongoing autoimmunity to the brain.

6) Serum positive reactions to anti-MBP and H-protein antibodies are highly correlated with the persistence of MV in the CSF, although there are a few exceptions in each category.

7) Blood appears to be an inadequate predictor of MV in other places, as several were negative in blood, but positive in either CSF or Bowel. The reverse is also true as several children with negative CSF or Bowel by

PCR were positive in the blood.

8) The consistency between our cohort and the previously reported MV persistence in the GI tract implies a high correlation between the US and the UK cohorts. When added to the high correlation between MV in the gut (83% in our documented gut group) and MV in the CSF (80% in the same subgroup), the pattern of this observation is consistent with the virus gaining access to preferred replicating sites.

9) Based on our data, a positive gut biopsy for MV combined with a high index of clinical suspicion, is a good proxy-marker for direct CNS involvement of the MV when one lacks specific information about the CSF. A larger cohort of cases would be ideal and combining our data with the data from the lead cases could give us a larger pool to analyze.

Comparing the ICDRC - CSF Cohort to the UK "Lead Cases Cohort.

This is an interesting way of expanding the database to provide a larger view of this disorder. I have read the case summaries and presentations of the 8 children and find them generally similar, phenotypically, to our cohort.

At this time, 7 of the 8 cases (4 selected by each side of this matter) have had CSF-PCR finalized. I understand one additional case selected by the defendants will not be accomplished.

In looking at the specifics of the UK cohort, all have lower ratios of MV F-gene to RNA total, than the findings in the US cohort. There are differences in the vaccine schedules in the US, and we provide significantly more complex vaccine programs than the UK " with the addition of hepatitis B at birth and thereafter, regardless of the mother's hepatitis profile. The UK provides targeted Hepatitis B vaccination. We also provided compulsory hemophilus influenza B (HIB) vaccines several years prior to the UK program (<http://www.immunisation.nhs.uk/vaccines.html>). This is, at least, a possible explanation of the difference in the data. Despite this observation, however, approximately 43% (3 of 7) are positive for the MV- F gene. All of the UK cohort have MV in the gut and 5 of 6 have MV in the blood. Based on the US cohort I might have predicted a higher percentage of positive CSF since we saw an 80% correlation between gut and CSF.

After review of the case summaries of Drs Byers and Walker-Smith, I have observed similar bowel and immunological findings exist between both the US and UK cohorts. This is in addition to the commonality of the MV " PCR findings. Thus, the concept of coexistent immune dysregulation and MV persistence in either the gut or brain, has been replicated in two separate and distinct populations. The cohesive features of both groups are: 1.Occurrence of ASD symptomology following MMR (regardless of other vaccines co-administered). 2.Persistence of MV in bowel and/or brain " CSF. 3.Immunological dysregulation tending towards immune deficiency (as a group). 4.Histories of gluten and casein intolerance. 5.LNH findings at the time of endoscopy " in that portion of our cohort which has undergone endoscopy.

## IMPLICATIONS OF THE OBSERVED SIMILARITIES OF THE UK AND US COHORTS

The observed similarities of these two separate groups of autistic children are striking. One cannot help but be impressed that essentially identical pathology exists on both sides of the Atlantic. This, despite the obvious fact, that our children were endoscoped by a completely distinct group of pediatric gastroenterologists with no ties to the Royal Free group. Further, the spinal taps were performed by two

different groups, my own in Florida, another in the Detroit area, where the UK children had CSF samples taken. I have never had any communication with the doctors in Detroit who performed the spinal taps. Taken together, the distinctions among medical providers investigating the children's health issues, contributes further evidence of the objectivity of the findings.

As a clinician, I take laboratory data as fact, unless evidence provides me a reason to doubt the findings. In this way, genetic data is generally accepted in the medical community as precise. We rely on it routinely in our evaluations. Until such time as proof to the contrary is presented, dismissing molecular pathology data would be completely cavalier behavior for any clinician. I, therefore, rely on the data provided by Dr O'Leary's team as fact.

Working from that basis forward, we then see what can only be described as a new clinical entity. It is no wonder then that so many physicians have run into difficulties when faced with the MMR issue in autism. I will not go into a review of medical history, but we are all taught the amazing difficulties new ideas and new diagnoses face prior to being fully accepted by the medical community. MMR in autism is absolutely typical of what one would expect from the profession, when faced with both the newness of the data and the implications thereof.

From my perspective, this new entity is a disorder characterized by nearly simultaneous disturbances of gastrointestinal and cerebral function. The pathogen can be isolated from both sites in the majority in the US group and three-sevenths in the UK group. The virus has been demonstrated to be active through protein expression and immune activation (Wakefield 2001). Failure to isolate the virus from the brain or CSF in no way precludes us from assuming the vaccine virus is the cause of symptoms; either by past infection as is commonly understood, or ongoing autoimmunity as discussed, or still further through the opioid mechanism described by many authors and recently reviewed by Wakefield (2002).

While it will take further efforts to completely characterize this new disorder, the pathogenic role of MV of vaccine origin in the role of a subtype of autism should not be denied. It would be parallel to denying the role of hepatitis virus in liver disease, or HIV in AIDS. In both of these other examples, no epidemiology was required to establish causality, because the pathogen could be isolated in the disease state. In hepatitis, AIDS and MMR-Autism, we see the persistence of a pathogen for long periods of time before overt symptoms. We also see the persistence of the pathogen in its preferred organs or cell types in other infectious disorders, just as we do in the MMR-Autism cohorts. To deny the role of MV as the agent of cause in this condition, therefore would require departure from commonly accepted medical principles and traditions. I have absolutely no basis to conclude anything but a cause and effect relationship. In our small group of controls we see no evidence of MV persistence post-vaccination, just as we expected.

Section 6. Using the findings to improve patient outcomes in the US cohort.

In the process of defining this condition, it is my intent to assist the children in their recovery. The new findings provide both comfort in knowing the cause, but simultaneously, concern secondary to near total absence of specific antiviral means to deal with this new entity. Based on the documented persistence of the MV in many of these children and the autoimmune findings in most, along with the poor digestion and chronic diarrhea and/or constipation, we have attempted to address these serious issues in the following ways:

1. General supportive nutrition, with special attention to protein intake, minerals (especially zinc and selenium), cysteine/sulfur, and antioxidants. The rationale is based on an abundance of literature which supports the importance of these substances in immunocompetence and detoxification. (Turchan 2003, Grimm 2001, Cousins 2000, Droge 2000, Sprietsma 1999, & Lichtenstein BS 1995).

2. Digestive assistance with supplemental digestive enzymes. We in particular prefer plant derived enzymes with a spectrum of pH functional ranges broad enough to encompass all expected ranges in the gut. We also use peptidase containing enzymes to reduce the load of potential opioid peptides. Our anecdotal impression from hundreds of parents is that this approach is often reasonably successful in improving digestion and adverse behaviors. The work of respected researchers has led to a greater understanding of the significant gastrointestinal pathology recognized to exist in children with attention deficit and autism spectrum disorders. There is emerging evidence of the potential role and application of protease- and peptidase-containing enzymes in addressing overall protein digestion as well as opioid peptide problems commonly seen in autism. Clinicians have reported that many gastrointestinal conditions seen in children with ASD have responded favorably to the use of broad-spectrum microbial and plant-derived enzyme supplementation including a full range of protease-, peptidase-, and DPP-IV-containing enzymes – the opioid digestive enzymes. (Brudnak 2002, 2001, Knivsberg 2002, Bradstreet 2001 & Zorn 1996)

3. Targeted immune support with a variety of different agents. For example, we use inositol hexaphosphate and inositol in equal ratios (IP-6) to boost NK cell activity in those NK cell deficient children based on published work on the role of this product. (Grases 2002, 2001, Shamsuddin 1999).

4. Immunovirâ,ç (inosine pranobex) is an immunomodulator of the potentiator type (Milano 1991 & Tsang 1987) which has demonstrated an enhancing effect on the function and number of various cells of the immune system, particularly T lymphocytes. Immunovirâ,ç has the ability to enhance the functions of various cells within the immune system and it seems likely that these effects are responsible for its clinical efficacy. Therapy with an agent capable of enhancing certain aspects of the immune response is a logical approach to the treatment of disorders associated with an underlying cellular immune defect. SSPE, MV persistence in the brain, is one of its approved indications in many countries (Anlar 1997). It is an adjunctive in SSPE and unlikely to be curative in its own right. However, the safety and tolerance make it a reasonable option, and we have several children whom we have seen significant improvement with the medication including child #1 and 2 from the table (the highest viral ratios we have seen in the CSF).

5. Targeted antivirals are possible considerations, but based on the SSPE literature, few options exist (Solomon 2002). Ribavirin was one of the first antiviral drugs ever discovered, and has been recently reviewed by Snell (2001). It is approved in the United States in an aerosol form for the treatment of a severe lung infection (RSV) in infants, and in an oral tablet for Hepatitis. It is being studied in combination with reverse transcriptase inhibitors, as an anti-HIV treatment. More recently, it has shown activity against hepatitis A, C and B. Ribavirin is available in numerous countries in an oral, tablet form. It has been used off-label in SSPE with variable results as noted in the previously cited papers. We attempted to use it in our CSF positive children – whom also had seizures or severe encephalopathy, but with mixed results. MV positive autistic children were far more sensitive to the hemolytic anemia effects of the drug than hepatitis C infected children (Chang 2002). On the favorable side, 2 of 6 children did get significant reduction in diarrhea without complications on low dose Ribavirin. This was reported back to the manufacturer’s chief medical officer, and we further consulted several drug companies regarding potential anti-MV drugs in the pipeline. None appear imminent.

6. Interferons. We have tried several different oral interferon products off-label and none has been impressively positive, while alpha interferon products were often harmful, causing fevers and temporary set-backs in the overall functioning of the children adversely affected. All children regained lost ground after cessation, but in one case that took almost 6 months. This negative outcome likely speaks volumes to the range of immune dysfunction and could aid future therapeutic development (Solomon 2002- IBID).

7. Intravenous Immunoglobulin (IVIG): Although this process is

incapable of clearing the virus from its intracellular residence, it does seem to ameliorate the adverse immunological (Gupta 1999 and 2000), particularly the autoimmune, issues, in the children (Bradstreet 1999). It has also been observed in our population to improve weight gain and decrease the symptoms of LNH. We have directly observed significant improvement in gut inflammation, such as noted in child #2. And consistent with the observations of others regarding related symptoms to ASD, we have observed cessation of seizures (Villani 2002 & Mikati 2002), and decreases in obsessive behaviors in some cases (Sewdo 2002, Weir 2000, Perlmutter 1999, & Singer 1999). We have tracked the children over time and seen anti-MBP return to normal with IVIG therapy. This improvement further seems to correlate with favorable behavioral outcomes and supports the observations of Dr Singh, as well as the potential role of MV induced autoimmune reactions in the etiological changes leading to autism. However IVIG is extremely expensive and most families cannot afford the treatments unless they have insurance approval. Section 7. Future plans for our efforts.

It is abundantly clear that our data need to be published, and we are preparing a report for that purpose. While I have " in the past - informed both our Deputy Secretary of HHS and provided the US Congress (2001 & 2002) with oral and written testimony, no remedial efforts or vaccine recalls have been initiated. We assume this is because: 1) the data was not published with controls 2) the risk of measles epidemics seems greater to public health officials than the risk of autism " although our present controlled data should convince them otherwise, and 3) the negative consequences disclosure of the facts will have on public confidence in vaccine programs in general.

We need to address these concerns and help public health officials develop safer vaccine policies. I believe the findings described in the US and UK cohorts, when combined with the observations of many others, help light the way to safe vaccine policy. For over three decades, MV vaccines using either live natural-route inoculation, or killed nasal administration (including more recent development of subviral noninfectious units) have been tested, but remain unavailable to the public (Roth 2003, Liashenko 1999, Etchart 1997, de Haan 1995, Muller CP 1995, Cernescu 1984, Khaletskiaia 1969, Bellanti 1969).

Further, we need a multicenter investigation with biotech cooperation to find specific interventions for post-MMR related neurodevelopmental and gastroenterological disorders. Of greatest concern are those children with persistence of MV in the CSF. This is going to be extraordinarily challenging. The virus seems to exist in low copy numbers and is likely reproducing slowly (as vaccine strain viruses are designed to do). Perhaps of greater concern is the way the virus induces both local and system immunological changes through the continued \_expression of antigenic proteins. We are continuing to gather CNS/CSF controls for MV and immunological comparison in conjunction with Tulane University. We will encourage additional medical schools to pursue the CSF issues in a broader way, while making efforts at reproducing our findings with Dr Lipkin at Columbia.

We are presently working with Dr Connolly at WUSTL, to define the immunological issues to a broader array of brain proteins in an effort to reproduce the findings of Dr Singh and uncover the specific pathogenic autoimmune markers. We will continue to perform lumbar punctures for CSF studies. We have an approved IRB with Wake Forrest to do immune chip array CSF marker characteristics. We are in the response to questions phase of NIH funding for this study. We have collated the data for ammonia levels in the gut population and they are significantly elevated. This data was presented at the Autism 1 conference by Prof Adams our collaborator at ASU.

We are pursuing the cysteine deficiency issues and the best replacement methods. It would be ideal to define the mechanism of cysteine/glutathione depletion in these children.

Within a few months we will have a pediatric gastroenterology department at ICDRC Florida Hospital campus in Orlando, and we intend to create a center of autism treatment excellence.

## Section 8. Summary and Opinions

My remarks here relate only to those children we are discussing as the ICRDC (US) cohort and as it relates to the UK (lead cases) cohort. The US cohort lends clear support to the broader findings, while validating the observations of Wakefield and colleagues.

Piece by piece over the last 10 years the necessary components required to unravel this disorder have come together. Once Dr O'Leary's team published their landmark findings regarding MV persistence in the GI tract of child with autism, I felt we had the justification to look for the virus in the most logical place one could expect, if it were persisting in encephalopathic children. The entire clinical picture for our cohort presented the justification we required to look at the children's spinal fluid.

Evaluating their CSF has led to what I believe is the most stunning discovery of my career: measles virus of vaccine origin (based on the gut findings) as at least one etiological agent of ASD. The quality of Dr O'Leary's team and laboratory made this discovery possible. But it was the careful documentation of the gastrointestinal disorders by the team at the Royal Free which provided the momentum necessary to take the next steps. Drs Singh and Connolly, provide further evidence that the brain "either through direct invasion, disruption of endothelium, or via immune/autoimmune mechanisms" is a critical target organ within the scheme of autism spectrum disorders. Tulane University, through the efforts of Dr Jane El-Dahr, has contributed the controls.

As a clinician, it is my duty to formulate a diagnosis with the facts I have. In this case, those facts are impressively consistent with only one condition, MV persistence with the associated neurological, clinical and immunological changes we know as ASD.

We have two cohorts, in different countries, with differing vaccine schedules, with shared similar immune and dietary disorders, which notably, share exposure to the attenuated strain of MV in the MMR vaccine. That strain has been identified in their symptomatic gastrointestinal pathology (UK cohort), and we await the findings on the CSF allelic discrimination, but regardless of that outcome, MV is detected in the CSF of cases and not in 3 of 3 controls. Thus the vaccine has either altered the course of wild-type infection, while not defending against it, or vaccine-strain has set up a persistent and symptomatic presence in the brain of children with encephalopathy and autistic features. That final portion of the mystery (allelic discrimination) should be resolved within the next few weeks to months.

The individual variations within the groups most likely results from an individual's own genetic predisposing factors, further influenced by the timing of the vaccine during the course of neurodevelopment, and further combining with individual environmental stressors.

What we see in our cohort is different from what is known about wild-type disease, although it seems more closely related to acute postinfectious measles encephalitis (APME). It lacks the obvious findings of SSPE or

MIBE. An excellent review of the current state of Medicine's understanding of CNS-MV infections was recently published by Schneider-Schaulies and colleagues (2003). These next two graphs from their paper show the importance of timing within the brain's developmental course, and I am using them here to illustrate why even subtle insults to the developing child's brain which coincide with these stages, would have the potential for cataclysmic results.

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