

## PANDAS (Pediatric Autoimmune Neuropsychiatric Disease Associated with Streptococcus) in Autism? A Case History

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### Abstract

Pediatric Autoimmune Neuropsychiatric Disease Associated with Streptococcus is a recently described neuropsychiatric disorder in children, characterised by the onset of psychiatric symptoms accompanied by abnormal streptococcal serology in prepubertal children. A case of a young boy diagnosed with autism, with symptoms and serology previously described as consistent with this disorder but no apparent history of streptococcal illness, is discussed. In this case, colonic dysbiosis with overgrowth of streptococcal and enterococcal species is identified and the child is treated with probiotics and supplemental nutrients with good clinical response.

**Keywords:** PANDAS, Autism, Colonic Dysbiosis, Streptococcus, Enterococcus, Probiotics.

### Case History

Child A was 5 years old when first seen in consultation, having been diagnosed as having autistic spectrum disorder at age 3 by a tertiary level institution specializing in children's developmental disorders. At the time of first consultation, he had a history of marked behavioural disorders, was frequently aggressive and violent, was delayed in fine motor and gross motor skills, and had initial insomnia.

Child A was the first of two children to unrelated parents. His mother had a history of autoimmune disease, irritable bowel syndrome (IBD), which was better for a gluten-free and casein-free (GFCF) diet, and menses complicated by dysmenorrhoea and PMT. However, pregnancy was uneventful, and his mother continued in full-time employment in a stressful position. Labour was at term, but complicated by failure to progress, resulting in the use of high forceps under an epidural. Child A required initial resuscitation at birth, but responded well with Apgars 6 and 9. As a neonate, he suffered no complications and breast-fed to 8 months of age. His early health was unremarkable, although from 12 months of age he developed recurrent upper respiratory tract infections and had chronic coryza requiring antibiotics on two or three occasions. He had recurrent diarrhoea, which was offensive and pungent. He was an active baby, and was difficult to settle to sleep, apparently needing to be rocked. At age 2, he had operative repair of a left inguinal hernia. He had been

fully immunised, with routine paracetamol administered, had no apparent acute reactions and had no known allergies.

Child A's developmental history was initially normal, with early motor milestones all appropriate, and early eye contact and baby babble. Although there was no clear history of delayed initial language, by age 2 it was apparent that Child A was not as vocal as other children his age, and by age 3 still communicated with single words. He did not point or use gestures but would take his mother by the hand to what he wanted.

By age 2 1/2 years, behavioural abnormalities were becoming apparent. Child A started biting and kicking children and staff members at his preschool, withdrawing himself, not playing with other children and not participating in the centre's activities. His preference was to play in the dirt, and he was continually mouthing and eating dirt. He would often become upset for no apparent reason and would scream and tantrum uncontrollably. He experienced significant difficulty in coping with change, both in his routine and in his physical environment, with great difficulty coping with changing sets of clothing, or changing from one group activity to another in the day care situation. There were ongoing separation problems at preschool, where Child A would scream and kick when dropped off by his parent in the morning. Sleep onset also became increasingly difficult, with up to 1 1/2 hours of crying, screaming, hitting out and biting, with Child A often only settling when he fell asleep with exhaustion. He would often wake during the night and take himself to his parents' bed for the remainder of the night. Whereas previously he had enjoyed a varied diet, he progressively restricted his foods and began craving stir-fry noodles and other carbohydrates. He also developed some abnormal finger movements, with clicking of his fingers close to his eyes.

Understandably concerned, Child A's parents had him assessed by a developmental paediatrician using the Griffiths Mental Development Assessment. He was found to have mild developmental delay in the areas of language (1 1/2 to 2 years age range), personal/social (1 1/2 to 2 years age range), hearing and speech (1 1/2 to 2 years age range) and immaturity in locomotor skills (2 to 2 1/2 years age range) and fine motor development (2 to 2 1/2 years age range). Hearing screening demonstrated

inconsistent hearing responses to low frequency sound (500 Hz). He underwent initial occupational therapy and speech therapy, with little response.

He was further assessed at a tertiary developmental clinic at age 3 years 4 months, using the Childhood Autism Rating Scale (CARS). It was apparent that Child A displayed impairment in three crucial areas of development. Firstly, his verbal communication consisted of very little spontaneous expressive language and was marked by unintelligible jargon and frequent repetition of words and sentences. Secondly, Child A experienced significant difficulty relating to both his peers and unfamiliar adults on a social level, preferring to play alone and often withdrawing into his own space. His emotional responses could also be quite inappropriate, and he had difficulty understanding the needs or feelings of others. Thirdly, Child A's play had a definite repetitive quality and tended to lack imagination and spontaneity. On that occasion, he met the criteria for a diagnosis of mild-moderate autism, CARS score 35. He was not apparently tested for inborn errors of metabolism at that time.

Child A was also informally assessed by a speech therapist, confirming marked difficulties with both expressive and receptive language. She confirmed difficulties in imaginative play, decreased range of movement and control of his tongue, with difficulties in pronunciation of some (t, d, n, l, s), poor speech intelligibility, poor initiation of conversation, poor sequencing of speech, inability to follow simple 2 stage commands, lack of understanding of abstract questions and inappropriate behaviour with marked impulsivity. Child A was commenced on an intensive intervention program, including speech pathology, occupational therapy, therapy from a Special Education Teacher and behavioural management therapy by a psychology student. He also commenced a GFCF diet. He made good progress, particularly in relation to his language and communication skills. His response and adaptation to change had also improved. However, he continued to have difficulties with socialisation and easy aggression and was quite unpredictable in his physical outbursts, in which he would scratch, bite and strike out at people. If he hurt himself in any way, he would attack the person next to him. His mother described him as being bossy, needing to be in control, and, if not in control, would get upset. He had also developed new obsessions with dinosaurs, Spiderman, and Australian Football League (AFL), which he would continually talk about to other people. Physically, his nasal congestion had cleared and stool odour and consistency had improved, although he continued to be a chronic mouth breather.

Child A was re-assessed after 13 months, at age 4 years 5 months, using the Weschler Preschool and Primary Scale of Intelligence 3<sup>rd</sup> Edition (Australian Adaptation), Vineland Adaptive Behaviour Scales, CARS, and Developmental Behaviour Checklist. He refused to respond to one out of eight core sub-tests

during assessment, (coding sub-test) so it was not possible to establish a Full Scale IQ score on that occasion. However, the test profile revealed a relatively even scatter of sub-test scores across both scales and placed Child A's verbal and non-verbal reasoning skills within the mild disability to borderline level of ability: verbal IQ 74 (range 69-82, 95% confidence interval), performance IQ 79 (range 73-87, 95% confidence interval). Child A's personal independence, as assessed on the Vineland Adaptive Behaviour Scales, fell within the mild disability range with delay in most skills, but particular difficulties within the area of socialization: communication domain 65±6, age equivalent 2 years 1 month; daily living skills domain 60±7, age equivalent 2 years 2 months; socialization domain 48±7, age equivalent 2 years 3 months; motor skills domain 5 years 11 months; adaptive behaviour composite 55±5. Child A's behaviour as re-assessed on the CARS, continued to be consistent with a diagnosis of autism, total score 31, although it was noted that he had made some progress in his ability and desire to communicate.

Child A was investigated by a paediatrician with postgraduate training and interest in nutritional medicine. He was found to have a normal Full Blood Count (except for anisocytosis and poikilocytosis, abnormally shaped rbc, on film), Liver Function Testing (exclude Gilbert's syndrome, with resultant difficulty in glucuronidation of toxins and metabolites), Gliadin Abs, endomyseal Ab and tissue transglutaminase Ab, (markers for celiac disease) and levels of Immunoglobulins E, A and G, and T cell lymphocyte subsets (immune system markers); vitamin B12, red blood cell folate, caeruloplasmin, serum copper, zinc, mercury and lead levels. A comprehensive parasitology test (GSDL USA) was performed assessing aerobic gastrointestinal flora, which demonstrated high levels of Alpha haemolytic streptococcus, gamma haemolytic streptococcus, *Citrobacter freundii* complex, and *Candida albicans*, with absent *Lactobacillus* species (Table 1). According to his mother, he was treated with two courses of Diflucan, with no apparent benefit, some probiotics (Flora Care, Metagenics: *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* NCFM, *Bifidobacteria lactis*) and some generic homoeopathics for neurotransmitters (Neuro 1, Neuro 2, Metagenics).

Child A was re-assessed at age 5 years 6 months by an occupational therapist at the same tertiary institution at which he had been diagnosed with autism, using the Peabody Development Motor Scales. In the Fine Motor Component, he scored 7 in the subtests for grasping and visual motor integration, (standard score 8-12 being average), placing him below average. Gross Motor assessment (clinical observation- Ayres) demonstrated difficulties in gross motor planning and bilateral co-ordination, low muscle tone and below-average sensory-motor skills. It was noted that he tried hard to maintain attention and concentration when attempting tasks.

Table 1: Faecal Microbial analysis 1

Bacteriology	Result	Sensitivity
Beneficial bacteria		
Lactobacillus sp	No growth	
Escherichia coli	4+	
Bifidobacterium	2+	
Additional bacteria		
Alpha-haemolytic Streptococcus	4+	
Gamma-haemolytic Streptococcus	2+	
Bacillus sp	3+	
Citrobacter freundii complex	4+	Ceftriaxone, Ciprofloxacin, Trimethoprim/Sulfa
Mycology		
Candida albicans	2+	Fluconazole, Itraconazole, Ketoconazole

Note: Lab Testing by Great Smokies Diagnostic Laboratories, USA

As there had been only partial response to nutritional therapies to that time, he was then tested for urinary pyrroles (said to be a biochemical marker for behavioural abnormalities requiring high dose vitamin B6), which were mildly elevated. He was then commenced on the 'Pfeiffer' nutritional protocol, with specific supplements, including high dose zinc and vitamin B6. Unfortunately, he reacted adversely to this protocol, becoming more angry, with continual biting.

Once those particular supplements were ceased, he gradually settled, although would still be unpredictably aggressive. His obsessions, need for control and lashing out when hurt continued.

At the first consultation with the author, he was re-investigated, the only serological abnormality detected being raised streptococcal titres, with ASOT 252 (0-300) and antiDNase B 400 (0-300), markers of recent infection with beta-haemolytic streptococcus, in particular GABHS (group A beta-haemolytic streptococcus). However, Child A had no apparent history of beta-streptococcal illness (eg tonsillitis, scarlet fever, impetigo, cellulitis), nor of any other streptococcal illness (eg otitis media or pneumonia, both commonly due to *Strep pneumoniae*) at any time. He also had no history of staphylococcal disease, which can cause some similar illnesses (impetigo, cellulitis).

As gastrointestinal symptoms were prominent in his history, a repeat faecal microbiological stool analysis was performed, analysing aerobic and anaerobic flora (Bioscreen, Melbourne), which demonstrated significant colonic dysbiosis similar to that previously demonstrated. In particular, there was a significant over growth of E coli, enterococcus and streptococcus in the aerobic flora, Prevotella and clostridia in the anaerobic flora, together with absent lactobacillus species.

Notably, there was no candidial species grown, perhaps as a result of treatment with Diflucan (Table 2).

Table 2: Faecal Microbial analysis 2

Bacteria	CFU/GM	Normal range	% distribution	Normal range
Aerobes				
Total aerobes	1.7 +08	1.0 +07 – 1.0 +08		
Escherichia coli	1.7 +08	7.0+6 – 9.0+7	96.2%	70-90%
Enterococcus spp	2.4 +06	<5.0 +5	1.4%	< 5%
Streptococcus – total	4.2 +06	<3.0 +05	2.4%	<5%
Streptococcus non-haemolytic	4.2 +06	<3.0 +05	2.4%	<5%
Yeasts				
	0	<1.0 +04		
Anaerobes				
Bacteroides fragilis spp	1.9 +10	9.0 +7 – 9.5 +11	30.7%	90-95%
Prevotella spp	2.0 +10	<5.0 +08	32.0%	<10%
Clostridium spp	2.3 +10	<5.0 +08	37.3%	1-10%

Note: counts exponential eg 1.7+07 = 1.7x10<sup>n</sup>, where n=7; Lab testing by Bioscreen, Melbourne

This profile would likely result in changed colonic pH secondary to raised Enterococcus and Streptococcus species, as they are obligate D-lactate producers on fermentation of glucose (van der Wiel-orstanje & Winkler, 1975). This can result in modification of faecal microbial metabolism particularly the Bacteroides and Bifidobacterium spp, resulting in a decreased production of volatile fatty acids, (Edwards, Duerden, & Read, 1985) which are necessary for colonocyte cell nutrition, thus resulting in increased epithelial barrier dysfunction increasing passive intestinal permeability to small and large molecules. Overgrowth of Enterococcus and Streptococcus spp may also affect the distribution of the anaerobic flora, production of primary bile acids and influence the bile acid composition in both bile and the intestine, (Salvioli, Salati, & Bondi, 1982) thus conceivably affecting absorption of fats and excretion of toxins. The overgrowth of Prevotella spp supports this premise, as Prevotella are saccharolytic, bile-sensitive, haem-dependant anaerobes.

Intestinal permeability testing (ARL Pathology, Melbourne) demonstrated reduced mannitol (5.9%, RR 9.5-25), indicative of malabsorption, and a raised lactulose:mannitol ratio (.04, RR <.04), indicative of increased pore size between intestinal cells, commonly known as 'leaky gut', further evidence for the effect of the altered microbial flora. Several markers have been used to test for increased intestinal permeability: polyethylene glycol (PEG), Cr-EDTA, and lactulose to mannitol ratio. Lactulose:mannitol is widely accepted

and most frequently taken for testing small intestinal permeability. In IBD, lactulose:mannitol is predictive of disease activity and relapse. (Vogelsang, 2008)

Hair mineral analysis (Trace Minerals Inc USA) was performed as a marker of overall mineral status. This form of testing is routinely used in forensic medicine. The results were markedly abnormal, with overall nutrient deficiency, imbalances indicating stress responses, and significant mineral toxicities (Table 3).

Table 3: Hair mineral analysis

Mineral	Lab Ref Range	High/Low
<b>Nutritional elements</b>		
Calcium	25 22-97	Low/normal
Magnesium	2.3 2-11	Low/normal
Sodium	83 4-36	High
Potassium	88 2-24	High
Copper	1.6 .9-3.9	
Zinc	6 10-21	Low
Phosphorus	9 11-20	Low
Iron	1.3 .5-1.6	
Manganese	.045 .014-.130	
Chromium	.12 .02-.04	High
Selenium	.07 .03-.18	
Boron	.06 .02-.91	
Cobalt	.002 .001-.003	
Molybdenum	.005 .003-.008	
Sulphur	3743 3545-5336	
<b>Toxic elements</b>		
Arsenic	.031 <.20	High
Uranium	.0008 <.0170	
Beryllium	.0001 <.0010	
Mercury	.04 <.18	
Cadmium	.004 <.14	
Lead	.40 <.30	High
Aluminium	1.6 <1.8	

Note: Lab testing by Trace Elements Inc, USA

Physical examination demonstrated tongue protrusion, chronic mouth breathing with abnormal palatal development, frontal and maxillary sinus congestion, marked ligamentous laxity and retained primitive fear reflexes. A provisional diagnosis of Pediatric Autoimmune Neuropsychiatric Disease Associated with Streptococcus (PANDAS) secondary to intestinal streptococcal dysbiosis and chronic sinusitis, with increased intestinal permeability, malabsorption and activation of the gut associated lymphoid tissue, was made. This situation was exacerbated by clostridial toxins and mineral toxicity.

Child A was commenced on a new protocol, incorporating the use of the homeopathics specific to his symptom picture and physical findings, and high dose nutritional supplements. Homeopathy is a form of therapy, widely used in Europe, and recognized by the World Health Organization. It is a highly individualised therapy, in which the symptoms of the patient are matched to the symptoms that a particular substance would invoke if given in toxic doses. The first

homeopathic remedy Child A was given was the remedy Stramonium, on the basis of his pattern of uncontrollable aggression with scratching, biting, and striking out at others. In addition he was commenced on a broad-spectrum multi-vitamin/mineral formulation in an organic whole food base, a high potency formulation with magnesium, B6, zinc and other cofactors, a buffered vitamin C and bio flavinoid formulation, a bovine colostrum formulation with other nutrients directed to the gut associated lymphoid tissue, a broad-spectrum probiotic, ('Tridophilus', Biomedica: lactobacillus, bifidobacteria) and Omega-3 essential fatty acids. His diet was modified to include organic, whole foods, with an emphasis on 'primitive' traditional and fermented foods, wheat and cow's dairy free.

Child A was not seen again until three months later. He had progressed well, had stopped biting, and was coping well at school, with good concentration and expanding interests. His bowels had normalised, although his mother had noticed diarrhoea over the vacation when they had been less careful about supplements and diet. He had also started tantruming, which similarly settled once regular supplementation was re-instated. Specific probiotics for his particular gut bacterial profile were commenced, ('Synbac' Nutrisearch: Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium longum, Saccharomyces boulardii): and digestive enzymes were added to his protocol. A brief review two months later, found that he no longer had problems with anger or biting, his bowels were normal, sleep onset was normal, and he was concentrating well in school. His vocabulary and articulation were gradually improving. He continued with his supplements and homeopathics.

These improvements had continued when reviewed another four months later. At that time, his mother stated that his behaviour had improved overall 'ten times', his vocabulary was more fluid and extensive, and his interests had expanded including playing team sport. He no longer had problems with aggression, but would be anxious, especially if faced with a new task. He continued to suffer chronic mouth breathing, was diagnosed with chronic sinusitis and commenced on a herbal antimicrobial formulation to treat this in addition to his previous supplements.

Child A was reviewed nine months later, then aged 7 years and in mainstream school, in an age-appropriate class with an aide 15 hours/week He was coping well, 'average' in reading and spelling, although 'struggling' with mathematics, in which he was about one year behind in level of function. His behaviour, according to his mother, was 'fantastic': he was co-operative, conversed well, was rarely frustrated and had no tantrums. He was still sensitive to being hurt, and was having ongoing speech therapy for oromotor dyspraxia with tongue thrusting. Although he no longer was mouth breathing during the day, he was still mouth breathing during sleep, and had a class one malocclusion with

protrusion of his front teeth, so he was fitted with a pre-orthodontic silicon splint. He continued to demonstrate some visuospatial difficulties in that he would put his shorts on backwards and shoes on the wrong feet. He also demonstrated some continued sensory difficulties in his tendency to grab and squeeze his friends. He continued with his obsessions with dinosaurs, AFL and Spiderman. Child A also had continuing initial insomnia. CARS was re-assessed on the basis of parental history and observation: at that time, he scored 21 and no longer met the criteria for autism. He was continued on his modified diet and supplements.

When last reviewed, another 2 years later, after the summer school holidays, Child A had deteriorated somewhat. He had been eating wholemeal wheat bread for some time, but remained cow's dairy free and had not been on regular supplementation. If upset, he would get angry, kick, yell, scream, slam doors, throw things, and chase and strike his younger sister. He was sensitive to loud sounds, and was easily annoyed and irritated, and was obsessing about dinosaurs, AFL and spiderman, as he had when younger. However, he was generally doing well socially and at school and was regularly swimming and playing tennis and Oztag. CARS was performed based on observation and parental history: at that time he scored 22.5, the predominant difficulty being with emotional responses. Baseline serology and hair mineral analysis were ordered, and he was recommenced on a wheat-free diet, homeopathics, specific to his symptom picture, and supplements, as previously. A movement therapy program ('Move to Learn': Barbara Pheloung) was recommended, together with learning a musical instrument. He has yet to be reviewed following these changes.

### Discussion

Although autism is classified as a psychiatric disorder, there is growing evidence that it is, in fact, a neurological disorder secondary, at least in part, to autoantibody formation. High levels of autoantibodies, up to 60 to 70%, against myelin basic protein (MBP) and neuron axonal filamentary protein have been found in children with autism (Singh, Lin, & Yang, 1998). Various other antibodies to neuronal tissue have also been documented. Antibodies have been found to frontal cortex (Pioplys, Greaves, & Yoshida, 1989), 5HT1A receptors (Todd & Ciaranello, 1985) and cerebellar neurofilament (Plioplys et al., 1989). Antibodies to three cross-reactive peptides have also been found to be raised in children with autism when compared to controls: chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN) (Vodjani et al., 2002). Interestingly, group B streptococcus is a major cause of bovine intramammary infection (Keefe, 1997) and *streptococcal spp* and *enterococcal spp* species have been found to be elevated in large scale studies assessing common pathogens in cow's milk (Daigault et al., 2003).

Streptococcal species have long been known to be associated with neuro-psychiatric disorders, abnormal movements and autoimmune-phenomenon. Scarlet fever, post-streptococcal glomerulonephritis and Sydenham's chorea (SC) associated with rheumatic fever are examples of this. SC typically manifests as the abrupt onset of unwanted repetitive thoughts and behaviours, classically occurring 2 to 4 weeks following an attack of rheumatic fever.

It was noted that prepubertal children with obsessive compulsive disorder (OCD) frequently had exacerbations following group A beta haemolytic Streptococcal infections (GABHS or GAS), with raised streptococcal titres, ASOT (anti-streptolysin-O) and anti-DNAse B, which are antibodies to GABHS toxins and markers of recent GABHS infection. These exacerbations were accompanied by a cluster of comorbid symptoms, including emotional lability, separation anxiety and attentional difficulties (Swedo, 2002). Evidence was found of basal ganglia dysfunction in SC and OCD and also in Tourette's Syndrome (Swedo & Rappaport, 1993). The term 'PANDAS' was coined to describe this phenomenon (Swedo et al., 1998). It was further recognized that chorea, tics and dystonia may also occur as immune-mediated complications of GABHS infection, that females tend to get chorea, while males would tend to develop tics, and that other psychiatric disturbances in addition to OCD, such as generalised anxiety, depression, conduct disorders and hyperkinetic disorders, could also be related to GABHS infection (Dale et al., 2004). In children with Attention Deficit Hyperactivity Disorder (ADHD) (excluding OCD and tics), raised streptococcal titres were found to correlate with the severity of the ADHD, and the raised ASOT correlated with changes in basal ganglia volumes (Peterson et al., 2000). A monoclonal antibody D8/17, a lymphocyte antigen, has been found to be expressed in increased amounts in association with streptococcal antibodies and rheumatic fever, Sydenham's chorea and subgroups of OCD and Tourette's syndrome (Swedo et al., 1997). Raised D8/17 has also been found in patients with autism and correlates with the severity of the compulsive symptoms, further linking streptococcal infections and autism (Hollander et al., 1999). Interestingly, not all patients later diagnosed with PANDAS have a history of streptococcal infection, throat swabs are positive only in some 20%, with positive serology providing evidence of recent infection in other patients (Dale et al., 2004). Antibiotic therapy has been trialed in PANDAS with no apparent success in established cases, (Dale et al., 2004) although antibiotic treatment at the sentinel episode has been successful in both eradication of tonsillar infection and resolution of symptoms (Murphy & Pichichero, 2002).

Streptococcal infections are common in childhood, and positive GBHS serology is also common, in up to 18% of the population (Dale et al., 2004). Overt streptococcal

infection is usual in the history of children prior to the diagnosis of autism, most commonly otitis media. In a recent study of 206 children under the age of three diagnosed with autism, it was found that there was an average of nearly 10 bouts of acute otitis media, and that the children had received an average of 12 courses of antibiotics, over one third of all of these having been given to children under 12 months of age (Fallon, 2005). It has previously been noted that there is a high correlation between the prevalence of otitis media and autism, that an earlier age of onset of otitis media correlates with more severe autism, and an increased incidence of otitis media also correlates with the more severe form of autism (Konstantareas & Homatidis, 1987). However, some studies have found no association with otitis media, perhaps due to the high rate of otitis media in the general paediatric community. Autism incidence is possibly also linked with pneumonia, with one study of 132 children finding that the prevalence of autism fluctuated in four-year cycles in close correlation with hospital admissions for pneumonia and bronchiolitis (Tanoue, Oda, Asano, & Kawashima, 1988). With regard to the microbiology of acute otitis media, aspiration of middle ear fluid analysed by PCR indicates that 75-80% of infections are bacterial, with *Streptococcus pneumoniae* the major bacterial infecting agent (Kilpi, Herva, Kaijalainen, Syrjanen, & Takala, 2001; Post, Preston, & Aul, 1998). Nasopharyngeal carriage of *Strep pneumoniae* is extremely common, especially in young children. In one Australian study, 91% of children between age 6 and 54 months old, became nasal carriers of *Streptococcus pneumoniae* at least once during a five-month period (Hansman & Morris, 1988), with acute sinusitis in the previous three months being the major factor associated with the carriage of *Streptococcus pneumoniae* (Marchisio et al., 2002). Anecdotally, chronic sinusitis is extremely common in children with autism, presenting clinically as chronic mouth breathing, nasal congestion, dark periorbital circles and head-banging or pressing the head.

It is now known that bacteria exist in biofilms, that is, aggregates adherent to mucosa and surrounded by mucus slime, a state that allows organism persistence (Costerton, Stewart, & Greenberg, 1999). Biofilms are a sessile, high density consortia of bacterial cells, glued together by an exopolymeric matrix. Biofilms serve as a bacterial refuge against predation, whether against antibodies, immune cells or antibiotics, provide greater resistance to exogenous stressors and provide an accumulation of nutrients at the biofilm surface. Bacteria change their metabolism once in a biofilm, to produce and excrete the 'slime' that cements them together and to produce toxins (Matz & Kjelleberg, 2005). The reduced oxygen tension, limitation of nutrient flow and sessile quiescent state of the bacteria is thought to explain the high level of antibiotic resistance (Anderl, Zahller, Roe, & Stewart, 2003).

Otitis media and sinusitis are associated with persistent bacterial infection that is typically culture negative and resistant to antibiotics. Biofilm has recently been demonstrated to be common in otitis media and sinusitis, with 46 out of 50 middle ear mucosa aspirates from children with otitis media demonstrating biofilm in one study, and also to be present in conjunction with serous otitis media or recurrent otitis media (Hall-Stoodley et al., 2006). Adherent biofilms have been also been demonstrated in adenotonsillar disease in children (Al-Mazrou & Al-Khattaf, 2008). *Streptococcus pneumoniae* and *Enterococcus faecalis* are among the many bacteria now known to exist in biofilm. It has been estimated that some 80% of chronic bacterial infections involve biofilms (Hall-Stoodley et al., 2004).

The use of antibiotics in the treatment of biofilm infection has proven unsuccessful, explaining the high recurrence rates of otitis media post antibiotics, some two to six times that of placebo (Cantekin, McGuire, & Griffith, 1991). In addition, the antibiotic treatment of otitis media increases the nasopharyngeal colonisation of the non-pneumococcal alpha haemolytic *Streptococcus* at two months, and antibiotic resistance in these strains is becoming very common (Ghaffar et al., 2002). It is well-known antibiotics alter gastrointestinal flora, and this change is thought to be the reason for the TH2 immune system shift, with tendency to antibody formation, documented post antibiotics (Oyama, Sudo, & Sogawa, 2001).

Streptococcal colonisation is common in various parts of the body. As previously mentioned, *Streptococcus pneumoniae* normally colonises the nasopharynx in 20 to 40% of children. *Streptococcal sp* have long been known to be commonly found in the stomach and duodenum, as a result of passage from the upper respiratory tract (Chapman, 1946). In prepubertal girls, vulvovaginitis (*Streptococcal spp* colonisation is common, with vulvovaginitis GABHS seen only in this age group (Welsh, Howard, & Cook, 2004). In pregnant women, GBHS vaginal swabs are positive in one third (CDC, 2002).

In a retrospective, multicentre, comparative study of the faecal microbial flora of 86 autistic patients and 117 control subjects (Butt, Emms, Cosford, Duff, & Patterson, 2006), a consistent pattern of loss of *E coli* with corresponding overgrowth of *Enterococcus* and *Streptococcus* was seen. Overgrowth of these species in the colon indicates a significant overgrowth higher in the duodenum and ileum, where streptococcus and enterococcus respectively typically can be found. As the microflora of the gut, in particular the small intestine, is now believed to be the major determinant of immune system function (Guaner & Malagelada, 2003) due to host-microbial cross-talk (Bourlioux, Koletzko, Guarner, & Braesco, 2003; Isolauri, Kankaanpaa, Arvilommi, & Salminen, 2001), this significant shift in microflora could perhaps be responsible for some of the immune abnormalities noted in autism.

In a retrospective case analysis of 50 children with diagnosed ASD, 29 had been tested for streptococcal serology (Cosford, 2007). Of those 29, 13 were positive (44.8%), with either ASOT >200iu/ml and /or antiDNAse B >100 iu/ml, (RR ASOT <200 iu/ml, antiDNAse B <100 iu/ml), indicating recent infection with GABHS. This compares with the community rate of nearly 20% (Dale et al., 2004). However, only 3 of the 13 (23.1%) who tested positive had any known history of GABHS disease, that is, tonsillitis, impetigo or scarlet fever; a further 8 of the 13 (61.5%) had a known history of streptococcal disease: otitis media, sinusitis or pneumonia, all *Streptococcus pneumoniae*, which is an alpha-haemolytic streptococcus. Of the same group of 50 children, 16 had been tested by stool analysis (Bioscreen laboratory Melbourne), and 14 were found to have overgrowth of streptococcus and/or enterococcus: of the group of 13 children with positive serology, 9 had been tested with stool analysis, and all 9 were positive. The streptococcus grown were usually alpha- haemolytic streptococcus, as found in nasopharyngeal aspirates post antibiotic treatment for otitis media, with beta-haemolytic streptococcus seen only rarely.

Gastrointestinal inflammation has been well documented in children with autism. Duodenitis and related reductions in duodenal enzymes is apparently common in these children (Horvath, Papadimitriou, Rabsztyrn, Drachenberg, & Tildon, 1999). DPPIV is also a duodenal enzyme. DPPIV is a proline endopeptidase, a tissue enzyme produced in the duodenum that hydrolyses bonds containing proline, and necessary for the degradation of gluten and casein, the predominant proteins in wheat and dairy (Shattock, Hooper, Waring, 2004). Dipeptidyl-peptidase IV is also CD26 lymphocyte cell surface marker and has numerous biological functions in eukaryotes, including involvement in T-cell activation as CD26, cell adhesion, digestion of proline-containing peptides in the kidney and intestines, HIV infection and apoptosis, and regulation of tumorigenicity in certain melanoma cells. (Boonacker & Van Noorden, 2003) The streptococcal enzyme streptokinase (SK) is itself highly immunogenic and binds to the DPPIV enzyme more strongly than gluten or casein (Vodjani, Panghorn, Vodjani, & Cooper, 2003), with the potential for streptococcal overgrowth in the duodenum to interfere with the degradation of gluten and casein and impact on the immune system. Indeed, the binding of SK to DPPIV results in autoantibody formation to DPPIV itself (Cuchacovich et al., 2002), peptides, such as milk butyrophilin, and tissue antigens, may result in neuroimmune dysregulation and autoimmunity (Vodjani et al., 2004), such as has been demonstrated in autism. There is also suggestion that DPPIV function may be reduced in the intestine in children with autism (Shanahan, Venturini, Daiss, & Freidman, 2000). Soluble CD26 in the plasma was found to be only

insignificantly different in one small study (Hunter, O'Hare, Herron, Fisher, & Jones, 2003), indicating that this deficiency may be primarily localised in the intestine. There is mounting evidence of the presence of caseomorphin and gluteomorphin, casein and gluten metabolites and other opioid peptides in the urine, (Alcorn et al., 2004; Cade, 2000; Reichelt, 1990) which together with the response of some children to GFCD diets, (Knivsberg, Reichelt, Høien, & Nødland, 2002) as is the case with Child A, further supports this possible connection.

As previously mentioned, antibiotic therapy for established cases of PANDAS has not been found effective. In this case, the overgrowth of streptococcus and enterococcus, in conjunction with the reduction in lactobacilli, resulting in the demonstrated increased intestinal permeability is possibly the cause of the abnormal immune responses. Microbial cross-talk with the cellular components of the gut-associated lymphoid tissue is now recognized to be the major determinant of immune system function (Macpherson & Harris, 2004). Probiotics have been demonstrated to help restore intestinal microflora towards normal, reduce levels of colonic *Clostridia sp* (McFarland, 2006), reduce intestinal inflammation and down-regulate over-expressed immune responses, possibly due to improved intestinal barrier function leading to decreases in antigen translocation (Majamas & Isolaurie, 1997). The change in diet to include increased fruit and vegetables and reduced processed foods ('Primitive diet') with resultant reduced acid load and altered nutrients to the microflora would have further altered their distribution. It is possible that the use of probiotics in conjunction with other nutrients, therefore, altered gut-based immune responses, thus resulting in the symptomatic improvement seen in this case.

## Conclusion

It has been suggested that CNS infection early in life may trigger autoantibodies that cross react with CNS antigens, with the age at initial infection determining the ultimate phenotype. In autism, the infection could be in utero or early in the postnatal period, whereas in PANDAS and Sydenham's chorea, the infection could be later in life. Children with autism frequently have recurrent early infections consistent with *Streptococcus* existing in biofilm state in the ears and sinuses, which can travel through the stomach to the small intestine, where it could establish a biofilm state in the duodenum. This case study, supported by the other studies mentioned, raises the possibility that gastrointestinal streptococcal overgrowth triggers autoimmune responses that cross-react with CNS antigens, presenting as a diagnosable psychiatric disorder, and that this response is able to be modified by alterations in diet and nutrition, together with probiotic supplementation.

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N.B. Child A was seen in private clinic. In accordance with RACGP requirements for publication of a case history, all data has been de-identified. The mother has given permission for the use of this case history for publication.