Autism Insights

REVIEW

How Could a Gluten- and Casein-Free Diet Ameliorate Symptoms Associated with Autism Spectrum Conditions?

Paul Whiteley, Paul Shattock, Kevin Carr, Malcolm Hooper and Lynda Todd

ESPA Research, The Robert Luff Laboratory, Unit 133i Business and Innovation Centre (BIC), Sunderland Enterprise Park, Wearfield, Sunderland SR5 2TA, UK. Corresponding author email: paul.whiteley@espa-research.org.uk

Abstract: There is a considerable body of experimental evidence suggesting potential efficacy of a diet devoid of gluten and casein in ameliorating some of the core and peripheral symptoms of autism spectrum conditions. Although phenotypic details of best- and non-responders to dietary change remain under investigation, the range of biological mechanisms implicated during intervention is growing. The question of how diet works remains unanswered. We discuss three prospective modes of action used alone and in combination to explain the effects of a gluten- and casein-free diet on autism spectrum conditions focussed on direct or co-morbid consequences of: i) gluten sensitive enteropathy or coeliac disease, ii) food allergy and/or atopic disease, and iii) underlying hyperpermeability of the gastrointestinal tract (leaky gut) and subsequent passage of biologically-active peptide and related species into the central nervous system. No single theory offers a universal explanation for the biological basis of dietary effectiveness despite individual associations with various cases of autism. Impaired intestinal barrier function is a common denominator and represents a promising area for investigation. Furthermore, a number of key points derived from each model offer testable markers for experimental evaluation onwards to ascertaining potential responsiveness to such dietary intervention in autism.

Keywords: autism, autism spectrum, gluten, casein, diet, coeliac disease, allergy, atopy, immune system, gastrointestinal tract, hyperpermeability, leaky gut, gut microflora

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Introduction

Autism spectrum conditions (ASCs) including autism and Asperger syndrome (ASD, MIM 209850) represent a heterogeneous developmental continuum characterised by core problems in areas of communication, social interaction and repetitive or ritualistic behaviours.[1] Diagnosis is exclusively based on observation of presented symptoms alongside an analysis of developmental history and timing of onset. ASCs are disproportionately more common in males[2] and carry a significant probability of additional co-morbidity, particularly for epilepsy or related seizure-type disorders and learning disability.[3] Greater risk for other long-term psychiatric co-morbidity such as depression (MIM 608516) has also been detailed.[4]

The precise aetiology, or rather aetiologies, of ASCs has yet to be elucidated. Genetic factors are generally accepted to play some role in the onset and perpetuation of symptoms, although no unanimous genetic explanation has yet been put forward.[5] Alongside a marked increase in the numbers of cases of ASCs being diagnosed in recent years[6] speculation has turned to a potential role for the environment as being related to at least a proportion of cases[7] (see Fig. 1).

Outside of pharmacotherapy, various management strategies have been put forward in an attempt to overcome the more challenging aspects of the condition and improve quality of life. These have been predominantly focused on the use of psychological and educational interventions.[8] Strategies based on the variable use of nutraceuticals and dietary changes are also increasing in popularity.[9]

The removal of gluten (a combination of the protein fractions gliadin and glutenin derived from various cereal produce) and casein (protein from mammalian milk sources) from the diet of people with ASCs has attracted significant interest from both lay and professional quarters. Experimental evidence for the efficacy of such dietary intervention is accumulating[10–14] although at the current time universal criteria for the identification of best- and non-responders is lacking. Clinical guidelines on the use of such diets for autism remain equivocal[15] on the basis of methodological issues associated with the investigations undertaken so far combined with some evidence of non- or only limited effect.[16,17]

Gluten- and casein-free (GFCF) diets are seen to offer a relatively uncomplicated approach to symptom management compared with some other interventions. Rightly or wrongly, this is based on factors such as the wide-spread availability of gluten- and casein-free alternative foods, being perceived as a dietary change and hence not a pharmacotherapeutic approach, an apparent lack of long-term visible side-effects, and not requiring significant clinical or medical input. Alongside some high profile media reports on the success of such dietary intervention for ASCs, their use is now considered widespread.

Speculation as to the underlying biological mechanisms potentially related to the efficacy or non-efficacy of such diets is plentiful. Assuming that changes are not purely a consequence of a placebo effect or artefact of some other dietary constituent added or removed as a consequence of intervention, several hypotheses have grown up regarding a potential mode of action. These models can be broadly categorised into co-morbidities of: i) gluten sensitive enteropathy or coeliac disease (CD, MIM 212750), ii) food allergy and/or atopic disease, and iii) underlying hyperpermeability of various membranes including the gastrointestinal (GI) tract and subsequent passage of biologically-active species into the central nervous system (CNS). Whilst sharing some degree of overlap, the details for each model are predominantly derived from existing literature from other conditions. Importantly, they offer scientifically testable components which could forward investigations on best- and non-responder phenotypes in ASCs to dietary change.

Gluten Sensitive Enteropathy

Coeliac disease (CD) is one of a number of conditions under the heading of gluten sensitive enteropathy. It is a chronic autoimmune disease characterised by a pro-inflammatory immune response to gluten primarily manifesting as bowel-focused symptoms. A loss of oral tolerance to gluten leads to enteropathy of the small intestine and flattening of the mucosal villi limiting the absorption of dietary nutrients. Although the source of continued debate, current screening guidelines for CD are based on serological tests principally including IgA antihuman tissue transglutaminase antibody (anti-tTG) and IgA endomysial antibody (EMA) immunofluorescence testing. Previous assays

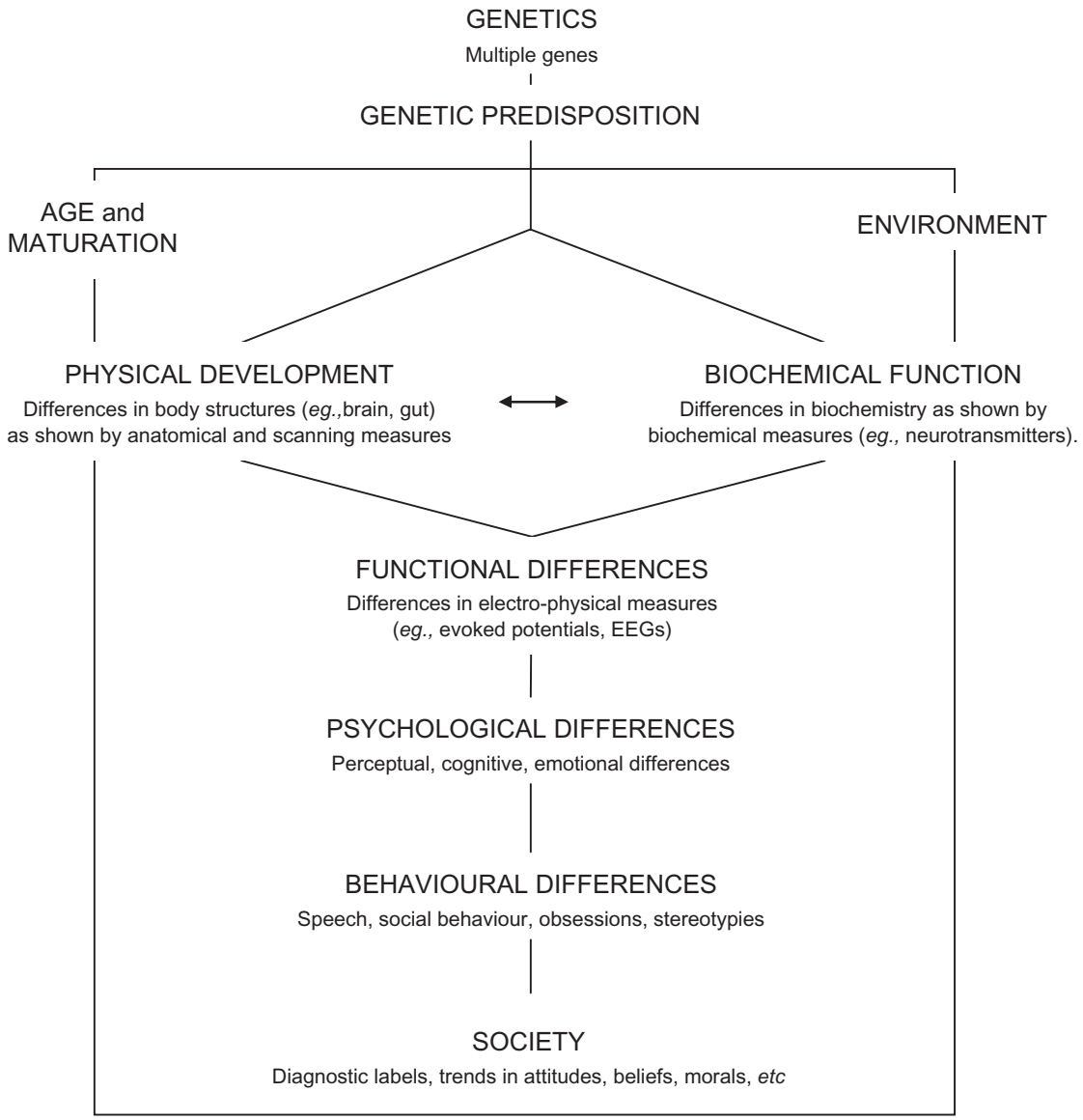

Figure 1. Route map to autism spectrum conditions (ASCs).

based on IgA antireticulin antibody and antigliadin antibodies are no longer considered to be useful markers for accurate diagnosis. Ultimately, the gold standard diagnostic test for CD is through analysis of a small bowel mucosal biopsy based on standardised criteria.

Appearance of the disease is chiefly governed by a combination of genetic susceptibility (class II human leukocyte antigen, HLA association)[18] coupled with environmental factors (ingestion of gluten in the form of wheat, barley and rye). Various other co-factors including infant feeding schedules and intestinal infections have also been put forward as causative.[19]

An assortment of symptoms are attributed to CD; ranging from significant overt problems manifesting in functional bowel habits (diarrhoea, steatorrhoea) and unexplained weight loss through to clinically silent cases where no apparent symptoms are presented and diagnosis is only suggested via serological testing. The precise mechanism of the disease and specific portion of gluten-containing grains that may trigger symptom onset is still under investigation. Recent advances in immunological assays have suggested that many components of gluten have the ability to stimulate a biological response, although only a small number are immunodominant.[20] Treatment, or

rather management of CD, is by faithful following of a life-long gluten-free diet (GFD).

The true prevalence of CD is difficult to ascertain given the various debates on the type of testing employed and the need for serological screening and biopsy to diagnose. Recent estimates of cases from various international groups have suggested an overall presence near 1% of the population in Western countries.[21] This perhaps underestimates numbers however by failing to take into account the range of clinically silent cases potentially at large. Prevalence data from other, more non-wheat eating countries have indicated lower numbers of cases; increasing with the advancement of gluten-based foods.

Studies examining any association between ASCs and CD have not so far provided unequivocal results. Asperger initially suggested a potential link between the two conditions.[22] Early clinical investigations were subsequently dismissive of any universal link.[23,24] More contemporaneous studies have reignited debate on a potential connection,[25,26] allowing also for reported parental symptomatology indicative of CD and the high heritability of the disease.[27] Screening for CD is not however part of the routine clinical assessment for ASCs; and alongside the problems and ethical issues associated with CD testing (drawing blood samples, undertaking invasive investigations) means no reliable statistics are currently available for rates of co-morbidity.

Various conditions are reported to be potentially co-morbid to untreated CD; some of which have also been detailed in ASCs. Iron deficiency manifesting as anaemia is one such overlapping finding in CD.[28] Dietary iron is absorbed in the proximal small intestine and hence susceptible to the physiological problems caused by CD as a function of location in the gut. Whether occult gastrointestinal blood loss associated with CD is another potential route to anaemia remains a topic of investigation.[29] Iron deficiency as described by measured low serum ferritin concentration has also been reported in ASCs. Latif et al[30] detailed widespread signs of iron deficiency in their cohort; findings complemented by related studies on the effects of iron supplementation for ASCs.[31] Of course such iron deficiency seen in ASCs could also be related to issues of feeding behaviour and poor nutritional intake commonly observed in this group.[32]

A number of psychological conditions have also been linked to gastrointestinal disorders such as CD.[33] Neurological and psychiatric co-morbidity in undiagnosed cases of CD has been reported, including conditions as diverse as depression, dementia and various epilepsy conditions.[34] More developmentally focussed indicators such as Attention-Deficit Hyperactivity Disorder (ADHD) (MIM 143465)—type symptoms have also been displayed in untreated CD.[35] Whilst epilepsy has long been associated with cases of ASCs, other co-morbidities such as depression[36] (particularly alongside a diagnosis of Asperger syndrome) have also been reported. Interestingly, traditional pharmacotherapeutic strategies for tackling such co-morbidities of ASCs have shown only limited evidence of effect following meta-analysis.[37]

Outside of the classical autoimmune response of CD, recent investigations have also suggested changes to gut microbial populations may also be present; additionally dependent on whether a GFD is implemented or not.[38] The types of microbiota implicated in CD are predominantly anaerobic and include *Bacteriodes* and *Clostridium* alongside more facultative anaerobes such as *Staphylococcus* species.

Microecological studies have presented evidence for similar species potentially pertinent to ASCs.[39,40] Whilst GI bacterial populations are to some degree influenced by all manner of environmental factors (diet, medication, etc), clinical evidence on the use of antimicrobials targeting specific bacterial species in ASCs has suggested some efficacy in abating bacterial species with parallel overt behavioural symptom changes noted at least in the short term.[41,42] Information on the use of similar antimicrobials for the treatment of CD is scant.

Allergy and Atopic Disease

Allergy is the body's immune-mediated response to one or more environmental materials. It is characterised by an inflammatory reaction following the production of different isotypes of antibodies (immunoglobulins, Ig). Atopy describes a specific type of Ig response, IgE (type-1 hypersensitivity). All atopic disorders presenting with an IgE mediated response are considered to be allergy but not all allergies are atopic. Allergy predominantly, but not exclusively, first affects the site of exposure to the allergen: most commonly, the skin (eczema, urticaria), the airways

(asthma), the nose (hayfever) or the GI tract (food allergy).

Testing for the presence of allergy and/or atopy is routine. IgE mediated atopy can be identified through a skin prick test, whereby potential target allergens are introduced onto the skin surface alongside positive (histamine) and negative (saline, water) controls. Analysis of specific antibodies in blood can also be undertaken via a RAST (radioallergosorbent) test; a radioimmunoassay technique directly measuring circulating antibodies. Dietetic challenges to potential antigens (eating offending foods to observe response) are also commonly used to confirm the presence of a food allergy.

Accurate details on the epidemiology of allergy and atopy are difficult to report given the wide range of conditions ascribed to allergy and the multitude of allergens potentially present. On the basis of observed symptoms, estimates of the worldwide prevalence of asthma symptoms for example are reported at between 11%–13% depending on chronological age.[43] Wide variations in symptom report rates have been detailed between different countries and following direct testing for specific allergens related to asthma and wheeze symptoms.[44]

An increase in the numbers of cases of allergy has been documented.[45] This has led to various models being proposed to account for a change in numbers. Among the more widely researched theories to explain increased allergy levels, a primary theme has been the role of hygiene[46] and its subsequent impact on parasitic infestation and GI microflora as potential moderators of allergy.

Co-morbidity of allergy and/or atopy in ASCs has been reported. Based on data derived from the US 2003 National Survey of Children's Heath, Altarac[47] reported increased levels of reported respiratory, skin and food allergy in ASCs compared to controls. Given the strong basis for inheritance in allergy,[48] it is perhaps not surprising that increased levels of allergies have also been reported in parents of children with ASCs compared with controls.[49]

Studies utilising direct measures of allergy also provide some interesting results. Based on antibody isotypes not including IgE (atopy), several groups have reported aberrant findings. Heuer et al[50] found pronounced differences in total IgG and IgM antibodies (low levels) in ASCs compared to controls, concluded as indicative of underlying problems with immune function. This contrasts with the findings of elevated antibodies reported by Trajkovski et al[51] (although reliant on healthy siblings for control samples in this case).

Specific IgA and IgG antibodies to gluten and casein in ASCs have been reported by other groups.[11,52,53] Vojdani et al[52] detailed the presence of IgA, IgG and IgM antibodies against neuron specific antigens that shared structural homology to cow milk protein, implying the possibility of cross-reactivity to different targets. Where specific measures of atopy (IgE) have been conducted, results have been more inconsistent. Heuer et al[50] and other groups[54] found no overall increase in the percentage of atopic ASC cases compared to controls. Magalhaes and colleagues[55] conversely reported IgE levels similar to an atopic control group as being present in teenagers diagnosed with Asperger syndrome.

The role of allergy and atopy in ASCs is complicated. Notwithstanding variations in the populations studied and the possibility of physiological differences according to diagnostic sub-groups,[56] there is general agreement that levels of IgE antibodies, indicative of atopy, whilst present do not seem to place people with ASCs at any greater risk than comparison populations studied. This mirrors similar findings in other potentially co-morbid developmental conditions such as ADHD.[57] In contrast, analysis of other antibody isotypes (IgA, IgG, IgM) in ASCs do indicate a greater frequency of potential disruption; particularly with findings specifically relating to dietary components including gluten and casein as is also seen in conditions such as schizophrenia (MIM 181500) and psychosis.[58]

Hyperpermeability of the Gastrointestinal Tract with Passage of Biologically Active Species

Unlike the conditions already described, hyperpermeability of the GI tract (the so-called "leaky gut") is perhaps more reflective of a clinical finding rather than a disease state in its own right. That being said, it is an important facet of several diseases, some of which have already been mentioned in our review.[59] Type 1 diabetes and inflammatory liver disease have likewise been linked with a propensity towards abnormal intestinal barrier function.

The nature of the wall separating intestinal contents from the rest of the body, and all the potential onward effects is delicate. Movement across the barrier is either through transcellular (through the epithelial cells) or paracellular (between the cells) action. Tight junction (TJ) (zonula occluden) proteins associated with a series of peripheral junctional proteins form the predominant barrier within the paracellular pathway, performing important gate and fence functions[60] (for a good review, see Groschwitz and Hogan[61]).

Measurement of GI permeability is an evolving process. Enteral ingestion of the monosaccharide and disaccharide probes, mannitol and lactulose followed by ratio measurement in urine has been the test of choice for many years. More advanced measures include analysis based on the use of polyethylene glycol (PEG), sucrose excretion and translocation of horseradish peroxidase.

Various mechanisms are thought to affect TJ constitution. As with many bodily functions, maturation of intestinal barrier function occurs during gestation and onwards into childhood. The precise evolutionary reasons for incompletely formed barrier function at birth are numerous but potentially include the benefits derived from first and subsequent early feeding practices. Fractions of casein protein (derived from mammalian dairy sources) for example, have been detected in the plasma of neonatal mammals following first milk intake[62] whilst not present in adult mammals including humans.[63] This led to the assumption that only the infantile intestine appears to be permeable to such compounds and additionally why early infant feeding for example, might produce such a soporific effect. Another primary effector of barrier function are the prostaglandins which have been demonstrated to enhance mucosal repair via mechanisms such as phosphatidylinositol 3-kinase (PI3K) signaling.[64]

Various environmental agents can also affect intestinal barrier function. Specific dietary components can affect integrity[65] and are discussed later. Anaerobic microbiota such as specific *clostridia* species have been shown to increase paracellular permeability and contribute to pseudomembranous colitis.[66] Applications of specific strains of *saccharomyces cerevisiae*[67] and the amino acid glutamine[68] by contrast, have been reported to improve intestinal barrier function. Various viral infections including HIV,[69] measles[70] and rotavirus[71] have been similarly linked to enhanced intestinal permeability.

Proposed permeability of the GI tract accompanied by passage of biologically-active dietary-derived compounds lies at the heart of many contemporary explanations for the success of dietary intervention in ASCs.[72] Based on the anthropological observations of Panksepp[73] examining the developmental effects of long-term exposure to opioid compounds such as morphine on animals, the opioid-excess theory describes a series of processes indicative of an underlying metabolic disorder in some cases of ASCs to account for the various symptoms linked to the conditions.

The theory can essentially be split into two parts; i) the presence of biologically active peptide species derived from dietary proteins and ii) the presence of an abnormally permeable GI tract. Evidence for each process is derived from direct studies of ASCs coupled with analyses of alternate conditions.

Peptides are molecules formed following the joining of one or more amino acids coupled by a peptide bond. They can be endogenously or exogenously derived and are present in every living cell, possessing a variety of different functions and activities including neuromodulation and nociception. Dietary proteins consist of long chains of amino acids. During normal digestion, proteins are broken down by enzymatic degradation into intermediate smaller chains of amino acids (peptides) and onwards to constituent amino acids.

The relationship between peptides, health and disease is long and complex; a thorough overview is outside the scope of this review. Elevated levels of endogenous opiates (endorphins) have been reported in psychiatric conditions such as schizophrenia. Peptide fractions isolated from patients with schizophrenia were found to bind to opioid receptors and induce hyperactivity and mania in rats.[74] Findings of similar endorphins present in ASCs have also been described.[75–77] Peptides with opiate activity mimicking endogenous peptides are also known to originate from specific dietary proteins including gluten[78,79] and casein.[80,81] Such casomorphin peptide sequences encrypted in intact milk proteins are known to have physiological effects akin to opiates such as prolonging GI transit time[82] and exerting an anti-diarrhoeal

action through enhancement of water and electrolyte absorption.

The transport of biologically active peptides and other compounds across the GI tract and/or Blood-Brain-Barrier (BBB) to exert an effect in ASCs represents the second stage of the hypothesis. It has long been known that medium and low molecular weight peptides (even intact proteins) can cross the GI tract[83] and under certain circumstances, are detectable in various biological mediums. More recent evidence indicates that various milk-derived peptides will also cross the intestinal mucosa intact.[84]

Direct measures of the permeability of the GI membrane in ASCs have revealed hyperpermeability in some cases[85–87] although not universally.[88] Importantly however the study by Robertson et al[88] which reported no overall abnormal permeability did not exclude participants who were already following special diets (see later text). Ancillary findings of impaired cysteine-sulphur metabolism potentially leading to aberrant sulphation of membrane mucoproteins[89–91] provides important secondary confirmation and potential clues as to the type of permeability that may be present.

The precise cause of increased permeability in ASCs is more contentious. The identification of the urinary compound *trans*-indoly1-3-acryloylglycine (IAcrGly)[92,93] (see Fig. 2) has been suggested to show linkage to GI membrane integrity.

IAcrGly, an abnormal metabolite of the essential amino-acid tryptophan, has been linked to hyperpermeability through the proposed action of a precursory compound, indole-3-acrylic acid (IAcrA). The flat, planar geometry of IAcrA is hypothesised to have a deleterious effect on physiological membranes by way of its intercalation into phospholipid bilayers.[72] Whilst a universal connection to ASCs is unlikely for IAcrGly,[94] investigations have suggested a role for the compound in cases associated with functional GI disturbance.[95]

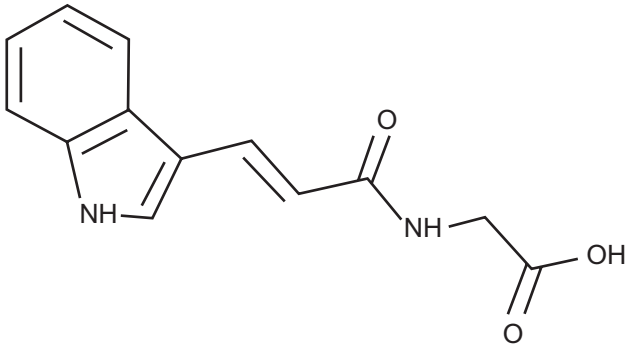

Figure 2. Chemical structure of *trans*-indolyl-3-acryloylglycine (IAcrGly). **Formula:** $C_{13}H_{12}N_2O_3$ (accurate monotopic mass = 244.0848 u, where u = unified atomic mass units).

Hyperpermeability as a result of the GI pathology described in a sub-group of ASCs is another potential route. Multiple reports have documented wide ranging gastrointestinal disturbances in subgroups of ASCs. Reports have demonstrated gastritis (inflammation of the stomach lining),[96] eosinophilic colitis[97] and oesophagitis,[98] ileal and colonic lymphoid nodular hyperplasia,[99,100] and reflux oesophagitis;[101] all indicative of chronic inflammation of the GI tract. Such GI pathology has been linked to immune dysfunction, and has in the most part been suggested to be distinct from other more recognised inflammatory bowel diseases[102] despite some opinion to the contrary.[103]

Discussion

Reports of changes to the behavioural presentation of autism-related core and peripheral symptoms following introduction of a GFCF diet have persisted for many years. No single observation or theory has so far universally explained why exclusion of such foods should, or should not, so positively affect the behavioural presentation of the various manifestations of ASC. Starting from a point that a GFCF diet can positively affect some cases of ASCs, our review of the mechanisms potentially explanatory of efficacy infers three important issues to be fundamental.

First, although ASCs are recognised to exhibit predominantly behavioural symptoms, the various modes of action presented for any dietary effect imply an underlying organic pathology to symptoms, at least co-morbid in some cases. Whilst a physiological explanation as a function of genes and environment may seem to be a well understood contemporary fact of ASCs, significant efforts continue into the formulation of psychological models of the condition for example, very often with little or no reference to the biological processes that have been described.

Second, assuming that efficacy of dietary intervention is due to diet and not merely an artefact of methodology or some other system, food as an envi-

ronmental trigger factor must be related to symptoms, either directly or again through some co-morbid condition. Diet as a mediator of physical health is well recognised in modern medicine; diet as an influencing variable of behaviour and development is perhaps not so well understood. ASC as a generic diagnosis has not yet been found to be protective against other diseases or conditions present in the general population including disorders related to dietary components. As such the presence of underlying food allergy or intolerance cannot be ruled out as appearing alongside symptoms. The fact however that diet is seemingly able to so profoundly affect overt symptom presentation and developmental course in some cases of ASCs, would imply a more fundamental relationship to be present. Whilst behaviours associated with ASCs may not be the only feature to be affected by dietary components,[104] the specific relationship proposed to cereal and dairy-based foods appears quite unique amongst childhood developmental conditions.

Third, much of the research included in this review point to a potentially interesting concept: an extraintestinal influence on the brain and behaviour far beyond notions such as anxiety and "butterflies in the stomach". Hadjivassiliou and colleagues documented the presence of several different neurological presentations of untreated CD and gluten sensitivity including peripheral neuropathy and ataxia.[105] Studies of patients with psychotic disorders and schizophrenia have also indicated some potential merit in the removal of foods containing gluten, in relation to an improvement in psychiatric symptoms[106,107] and in one report, also showing corresponding changes to cerebral blood flow.[108] Data from similar dietary studies in ASCs point to comparable behavioural findings as being present. The debate continues as to whether GI pathology in ASCs is merely a consequence of intellectual disability (as is often associated with ASCs) or whether potential environmental factors are at work behind pathology. It is almost certain however that such GI pathology will, if present, impact on GI function with potential onward effects manifesting in behaviour including issues such as a pain response.

Given the heritability factors independently cited in ASCs and conditions such as CD, potential shared genetic overlap is a reasonable place to start looking. Only few studies have analysed the prevalence of CD-linked DQ8 and DQ2 heterodimers specifically in ASCs. Kemperman et al[109] reported positive genotypes for one or other heterodimer in approximately 35% of participants with an ASC. de Magistris et at[87] reported on the presence of these alleles during screening in their study of GI permeability in ASCs; finding that approximately one-third of relatives in their cohort were positive. In both studies, no participants were diagnosed with CD on the basis of subsequent negative serology. Similarly, specific studies of any overlap between the potential genetic markers of allergy and ASCs are difficult to find. Shared genetic linkage of autoimmune disorder and ASCs through specific alleles of the HLA system (HLA-DR4) has however been reported.[110]

The picture is therefore incomplete and potentially influenced by several confounders. Many if not all of the conditions included in our review show some spectral heterogeneity in terms of symptom presentation. ASCs demonstrate significant diversity on the basis of expressed symptoms described as degrees of functioning. CD likewise demonstrates variation based on clinical presentation (typical, atypical, silent). Allergy by the same token shows similar spectral traits as witnessed for example by range of severity of symptoms in peanut allergy.[111]

Drawing on the various results for conditions outside of ASCs, specific threads of data could provide important information on the nature of effect from use of a GFCF diet for autism. Hyperpermeability of the GI tract whilst a finding in its own right is a common denominator to many conditions described in our review. Increased intestinal permeability has been associated with several paediatric conditions[112] and has long been known to occur alongside CD and other active small bowel conditions such as Crohn's disease (MIM 266600).[113] Patients with CD are for example, well known to present with increased antibody titers to foodstuffs other than gluten, explicable by altered intestinal permeability.[114]

Current opinion on the role of leaky gut in ASCs is undecided[115] despite some credible evidence of involvement. In support, a degree of compatibility is present between findings from ASCs and other conditions as illustrated by: a) the presence of intestinal hyperpermeability in patients and first-degree relatives in CD and other bowel conditions[59,116] and ASCs;[87] b) the presence of various GI pathology in ASCs akin to inflammatory bowel disease supportive of abnormal membrane function; c) the increased

frequency of functional bowel problems in cases of ASCs;[117,118] d) reduction in intestinal hyperpermeability in CD[119] and ASCs[87] following the introduction of a GFD (potentially explanatory of the intestinal permeability results reported by Robertson et al);[88] e) the presence of mucosal eosinophil (white blood cell active in allergic disease) populations in CD[120] and ASCs[102] with a corresponding reduction of infiltrate for those on the GFD; and f) the variable presence of decreased bone cortical thickness in CD[121] and ASCs[122] potentially explicable by malabsorption. Hyperpermeability of the GI tract is also an essential part of the opioid-excess hypothesis of ASCs and in particular enhancing the passage of dietary-derived opioid peptide and similar species to the CNS. The cornerstone of the opioid-excess theory is the assertion that either endogenously or exogenously, dietary-derived peptide species, exert a pharmacological effect on the CNS. Comparisons of studies utilising a GFCF diet or administration of opiate antagonists such as naltrexone for ASCs show some overlapping effects particularly on measures of attention.[12,14,123] Importantly, naltrexone also shows promise as a treatment for specific inflammatory bowel conditions.[124]

For many years, several groups have reported the isolation and detection of proteins and peptides using various methods in people with ASCs[125–128] and related conditions such as Rett syndrome (MIM 312750).[129] The methods used to detect such compounds have primarily included immunoassay and chromatographic separative methods such as High Performance Liquid Chromatography (HPLC). Detecting specific peptides in blood is susceptible to various methodological difficulties such as extensive protein binding[130] and aggregation with other peptides.[131] Analysis of urine is less disposed to such problems; such that urinary peptide content will to some degree be reflective of circulating blood/plasma content. Various controversies continue to occur, as to the finality of the urinary findings[132] reported and whether the compounds detected are dietary-derived opioid peptides[133–135] or in fact other relevant markers.[136,137] The problems primarily stem from the technologies originally employed not being of sufficient analytical power to provide a definitive answer.

Hyperpermeability of the GI tract may also present onward effects to other membrane reliant systems. Inflammatory bowel diseases have for example, been reported to affect various other organs outside the site of inflammation. This includes the Blood-Brain-Barrier (BBB), allowing an increased passage of low molecular weight compounds through the barrier.[138] GI peptides[139] and other exogenous compounds[52,140] have similarly been suggested to traverse regions of the BBB. The specialised system of endothelial cells that constitute the BBB similar to the GI membrane provide important gate and fence functions, restricting the passage of compounds to the brain both physically (via TJs) and chemically (enzyme barriers), whilst continually furnishing the brain with the required nutrients for functioning. Direct support for additional abnormal BBB permeability in ASCs is still sparse due to the difficulties in measuring porosity of this partly physical—partly biochemical membrane.

Taken as a whole, the opioid-excess hypothesis is complex, drawing on several areas of study. Many elements of the model still require substantially greater, and more controlled study despite indications of potential association and effect.

Research into the connection between hyperpermeability of the GI tract, ingestion of gluten and use of a GFD is also of great potential interest to ASCs. As per our review, various psychological co-morbidities are known to be present and potentially alleviated following removal of gluten from the diet in CD;[141] the assumption being that psychological effects were the result of corresponding mechanisms that also alleviate the physical symptoms. Although no study identified thus far has plotted dietary efficacy of a GFD or GFCF diet in ASCs according to behaviour and any underlying GI conditions, several associated observations may be pertinent. Hsu et al[142] reported an improvement in anthropometric measures (height and weight) following adoption of the GFCF diet alongside improvements in cognitive and behavioural measures. Whiteley et al[12] detailed reports of withdrawal-type behaviours following initial adoption of a GFD for ASCs and during transient periods of dietary non-compliance. Similarly, Whiteley et al[14] detailed a propensity towards a plateau in achieved positive outcomes following several months on a GFCF diet. Whilst speculative, all these observations could be indicative of underlying physiological changes akin to the other conditions detailed in this review. Normalisation of growth parameters is a common feature following treatment of CD with a

GFD[143] likely as a result of the partial abatement of the malabsorptive state. Direct comparisons between ASCs and CD on the behavioural measures of GFD challenge or inclination of potential plateau effects is not possible given the different focus of the two conditions. Where a GFD is not fully adhered to however, greater intestinal permeability has been noted in comparison to dietary compliant participants. Similarly, use of GFD in CD does not lead to total reversal of intestinal permeability issues.[119]

The relationship between allergy and/or atopy and ASCs is equally complex. In this review, we have focused on the role of the humoral (antibody-mediated) immune response as opposed to the cell-mediated response. Based however on the available research evidence, both aspects of immune function are thought be involved in specific phenotypes of the condition.[144] On balance, the evidence presented tends to favour a potential role for non-IgE mediated allergy as potentially playing a part in some cases of ASCs rather than any significant influence from atopy.[145] Other related areas such as the role of mast cells in allergic and non-allergic immune response whilst beyond the scope of this paper also deserve attention.[146]

Alongside the overlap and similarities detailed in our review, it is worthwhile highlighting some of the various differences present between ASCs and the conditions described alongside other areas of controversy. Poorer life outcomes have been argued to be more frequent for males in general[147] particularly with regards to the appearance of various developmental conditions. Relative risk of ASCs is much greater for males over females[2] even when allowing for issues such as potential under-diagnosis in females. By contrast, females are at greater risk of developing CD compared to males.[148] The caveat to this gender difference being the suggestion that males are more likely to present with greater malabsorption associated with their CD than females.[149] Details of any sex disparity in allergy or more specifically, food allergy is slightly more difficult to determine. Meta-analysis of data has shown differing rates of food allergy according to age; where in children, males were more likely to present with atopy compared with adults where roles were reversed.[150]

Whether this implies maturational and hormonal changes are involved in the allergy process remains under investigation.

Chronological age of symptom onset may also be an important factor. Diagnosis of an ASC by current clinical descriptions is given only when symptoms present before 36 months of age.[1] Whereas diagnosis of ASC is due to the overt behavioural presentation of the condition, diagnosis of CD and allergic disease are slightly more problematic given that some cases will present with little or no overt symptoms. Outside of too early an introduction of gluten to the infant diet, the majority of diagnoses of CD are generally in adulthood. The numbers of cases of ASC, CD and food allergy in childhood have all been suggested to be increasing in agreement with rates of other similar chronic conditions.[151,152] The precise reasons for such a rise have not been established; also whether there may be shared factors associated with such increases.

Attempts to disentangle the various elements involved in dietary intervention for ASCs potentially highlight many important forms of bias to be present. As in any similar form of intervention, placebo effects accounting for dietary efficacy cannot yet be ruled out. Alongside removal of gluten and casein-containing foods, the likely consequent use of alternative fare and the introduction of various vitamin and mineral supplementation regimes to counteract any resultant deficiencies, mean that nutritional intake is likely to change and could also exert an effect. Removal of gluten for example, including products such as bread and pasta is likely to result in a decrease in carbohydrate intake. Reducing dietary carbohydrates via the specific carbohydrate diet (SCD) as a means of intervention for ASCs is already being used by some people[153] following reports of reduced disaccharidase and glucoamylase activity.[101] The question therefore is whether removal of dietary gluten and casein may be indicative of a more complex problem in carbohydrate and protein digestion and metabolism.

In summary, autism spectrum conditions form a heterogeneous group and are known to place affected persons at increased risk of various co-morbidities alongside core symptoms. Such heterogeneity implies that universal constants to describe the underlying pathology are not going to be easily reconciled. The growing acknowledgment of ASC phenotypes,[154] based on various symptom combinations, provides greater scope to any explanations of the condition and subsequent would-be intervention options. A phenotype potentially based on the effectiveness of

a GFCF diet provides a tantalising opportunity for research to delve into any underlying pathology. At the same time it must be recognised that identification of such a phenotype will inevitably verify the notion that not everyone with an ASC benefits from such dietary intervention. With the hope that such dietary intervention does not succumb to the so-called 'tomato effect'[155] (rejection of an effective treatment because it does not fit established models) it offers many possibilities. Principal among them is the promise of identification of a subset of people with ASCs where such dietary intervention may improve symptoms, developmental outcome and quality of life including management of pain potentially attributable to the presence of co-morbid GI disorder.

Opinion

On the basis of the evidence reviewed, several areas merit further investigation in ASCs as to the potential efficacy of a GFCF diet. Particular attention is required on how the various results may combine and whether any specific combinations of findings provide information on best- and non-response to dietary intervention:

1. Measurement of gastrointestinal (GI) function to ascertain the presence of any increased permeability and to what extent permeability is present,
2. Screening for specific GI pathology where abnormal functional bowel habits are present and/or behaviour indicates potential pain response to GI symptoms,
3. Screening for the presence of underlying gluten-sensitive enteropathy or coeliac disease via serological and halotype analyses (with biopsy if merited),
4. Screening for the presence of low ferritin levels indicative of iron deficiency or anaemia,
5. Screening for the presence of food allergy (IgG, IgM) or atopy (IgE) specifically to gluten or casein,
6. Screening for the presence of elevated levels of urinary IAcrGly.

Glossary

ADHD, Attention Deficit Hyperactivity Disorder; anti-tTG, IgA antihuman tissue transglutaminase; ASC, Autism Spectrum Condition; BBB, Blood-Brain-Barrier; CD, Coeliac disease (also written as celiac disease); CNS, Central Nervous System; EMA, IgA endomysial antibody; GFCF, Gluten- and casein-free; GFD, Gluten-free diet; GI, Gastrointestinal; HIV, Human Immunodeficiency Virus; HLA, Human Leukocyte Antigen; HPLC, High-Performance Liquid Chromatography; IAcrGly, *trans*-indoly1-3-acryloylglycine (sometimes referred to as IAG); IAcrA, indole-3-acrylic acid; Ig, Immunoglobulin; PI3K, Phosphatidylinosital-3-kinase; RAST, Radio-allergosorbent; SCD, Specific Carbohydrate Diet; TJ, Tight Junction.

Disclosures

PW, PS, KC, MH and LT undertake commercial analysis of urine samples from people with ASCs. PW, KC and MH are directors of ESP A Research Ltd, involved in research into the use of gluten- and casein-free dietary intervention for people with ASCs. PW, PS and KC are directors of analutos Ltd which provides commercial separative and mass spectrometric analytical services. PS is a parent of man with an ASC.

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