The role of brain lipids in the causal model of autism: Re-interpretation of the existing data

Barbara Hall*  

ABSTRACT Autism spectrum disorder (ASD) has been a puzzle and a challenge to scientists for at least half a century, despite considerable scientific efforts. The aim of the present review is to demonstrate significant connections that exist between two parallel, but hitherto unconnected, spheres of research: phenotypic/genotypic studies in autistic individuals and deleterious effects on the brain caused by essential lipid deficiencies. Re-interpretation of the existing data suggests that autism may be a consequence of pre- and postnatal deficiency of docosahexaenoic acid (DHA), a major component of the brain, a lipid essential for its structure and function. This review connects the ASD and DHA databases in a specific pairing of the proximate factors (data from studies in individuals with autism) with DHA deficiency as the ultimate factor. This specific pairing suggests a coherent mechanistic model of the origin of autism and calls for a new, interdisciplinary approach that would connect the two databases in the design of future studies on ASD. The proposed genetic and maternal effects are testable according to the outlined methodology.

INTRODUCTION Autism spectrum disorder (ASD) is a neurodevelopmental condition of high phenotypic heterogeneity and yet unknown aetiology. It is characterised by impaired socialisation, reduced communication and restricted stereotyped activities. The extent of impairments varies between individuals, creating a spectrum of disabilities. It is estimated that just over 1% of individuals worldwide have been diagnosed with ASD, although the apparent dramatic increase in prevalence (approximately five fold between 1994 and 2004) is considered to be due to shifting diagnostic criteria and heightened awareness. This is further supported by the worldwide review of the data up to 2010. Sibling studies support a genetic contribution and heritability of greater than 50%. The number of implicated genes currently stands at 200-1,000, but none is specific for autism. Furthermore, there is a high, 10-50%, discordance among monozygotic twins. This suggests that other modifying factors might influence the clinical outcome. Proximate modifying factors, for example abnormal DNA methylation, have been reported but the ultimate underlying factor is yet to be identified. Thus, at present, ASD is defined by a wide spectrum of symptoms but not by the cause. Majority of scientific efforts have been focused on the genetic aspect of autism but, remarkably, only a few of the genetics-centred studies have taken into account the influence of the molecular
environment in which the genes operate, with no coherent model or practical outcome to date. At the same time, other published evidence has demonstrated a myriad of deleterious effects caused by essential lipid deficiencies, notably on the brain organisation and function. Remarkably, these effects result in phenotypic observations not unlike those reported in autistic individuals. However, none of these studies have been focused on autism.

This causal model brings together the genetic and maternal effects elements. It posits that the genetic components are the ineffective variants of the FADS (fatty acid desaturases) gene cluster and the maternal/environmental influence is a diet poor in DHA and out of balance with respect to the ω6:ω3 fatty acid ratio at the crucial time of pre- and post-natal brain growth. Relative individual contribution of each of these components results in a spectrum of the observed disabilities in individuals with ASD.

The following sections in this paper demonstrate a proposed mechanistic link between each of these observations and DHA. The information is summarised in the pictorial model (FIG 1) in which the data related to DHA (the ultimate causal factor) are paired with those related to the ASD (proximate effect factors).

**DHA AND AUTISM—THE MODEL OF MULTIPLE CAUSALITY AND A SPECTRUM OF DISORDERS**

The model brings together, for the first time, databases related to DHA and to ASD, derived from hitherto unconnected published research. Pairing of the data visualises a mechanistic connection between numerous DHA-related and ASD-related variables, thus creating a multiple causality model, rooted in published experimental data.

**ASD-RELATED OBSERVATIONS, CAUSES OF WHICH REMAIN UNKNOWN**

- Restrictive and repetitive behaviour are widely reported traits associated with ASD.

**ASD-RELATED OBSERVATIONS, CAUSES REMAIN UNKNOWN, THESE ARE PROXIMATE FACTORS**

- Stereotyped behaviour is one of the characteristics of ASD individuals.
- Autism prevalence ratio is 4-5 times greater in males.
- DHA erythrocyte content is significantly decreased in ASD individuals. Rare SNP alleles of FADS genes are associated with lower erythrocyte DHA.
- Are these individuals carriers of the SNP alleles linked to less active FADS enzymes?
- Disorganization of cortical neurones, resulting from disruption of layer formation has been found in the brains of ASDD individuals. Are brains of these individuals DHA deficient?
- Synaptic proteins and receptor defects have been reported in ASD individuals.
- Differentially-methylated, autism-associated DNA regions have been found in autism-discordant monozygotic twins. Are some of these regions the FADS genes?
Greater prevalence of ASD among males has been reported for some decades\(^1\). Currently accepted 4-5-fold male to female prevalence ratio has been confirmed in a report on the so-called “Female Protective Model” which states that the ASD phenotype in females requires a significantly greater number of the SNV (single nucleotide variants) than a similar disorder in males\(^1\).

Recently Ghezzo et al.\(^5\), as well as others previously, have reported erythrocyte membrane alterations in children with autism, expressed as a reduction in the erythrocyte membrane fluidity and alterations in the erythrocyte membrane fatty acid profile. In particular, a decrease in eicosapentanoic (EPA) and docosahexaenoic \(\omega 3\) fatty acids, with a consequent increase in \(\omega 6: \omega 3\) fatty acid ratio has been noted. See (FIG 2) for description of the \(\omega 3\) and \(\omega 6\) fatty acid metabolic pathways.

Genetic studies have reported, among others, defects in synapsin and glutamate receptors genes associated with ASD pathogenesis\(^1\). A pioneering study of brain structure of young children with autism\(^1\) has demonstrated a significant disturbance in the organisation of the prefrontal cortex that had occurred prenatally. Abnormal laminar cytoarchitecture and cortical disorganisation of neurons has been found in frontal and temporal cortical layers of brain tissues from children with autism. Genetic markers point to the dysregulation of layer formation and disruption of neuronal differentiation occurring during gestation\(^1\). This suggests impairment of the normal processes of neuronal migration, differentiation and apoptosis during gestation.

**Figure 2** | Overview of PUFA metabolism illustrating the involvement of FADS1 and FADS2 genes in desaturation steps necessary for the metabolism to DHA. Omega 3 and Omega 6 pathways are indicated, along with known genes (centre rectangles) and dietary sources; reproduced from Mathias et al.\(^33\).
Methylomic analysis of monozygotic twins, discordant for ASD, has demonstrated, for the first time, numerous ASD-associated differentially methylated DNA regions, with significant correlations between DNA methylation and quantitatively measured autistic traits\(^6\). In addition, several studies, referenced in Melnyk et al.\(^7\) have reported DNA hypomethylation in children with autism, suggesting an epigenetic effect of yet undefined origin.

- Prenatal exposure to valproic acid is one of the most frequently studied animal models of ASD induction\(^9\). Recent epidemiological investigation in children exposed to valproic acid (an antiepileptic drug) in utero, points to a significantly increased risk of ASD in exposed humans\(^10\).

**CORRESPONDING DHA-RELATED OBSERVATIONS**

- Deficits in behavioural tasks of learning and increased stereotyped behaviour have been reported in non-human primates raised on DHA-deficient diet\(^11\).
- Availablility of DHA (conversion rate from α-linolenic acid to DHA) in humans (Fig 2) has been reported to be 2.5-4 fold lower in males compared to females\(^12,13\).
- Erythrocyte DHA content is the function of DHA dietary supply and the activity of rate-limiting enzymes, FADS 1 and FADS 2 (Fig 2). A consistent and significant association has been found between genetic polymorphism of rare SNP alleles of the FADS genes with lower amounts of erythrocyte DHA\(^14,15\).
- DHA-depleted maternal diet resulted in reduced synaptic plasticity in the offspring brain in mice in-vivo. This deficit in synaptogenesis could be reversed by subsequent DHA supplementation in vitro. DHA supplementation in embryonic mouse neuronal cultures promoted synaptic protein expression, particularly that of synapsins and glutamate receptors\(^16\).
- Dietary DHA modulated the large-scale organisation of the rhesus macaque brain. Low level of dietary DHA intake was associated with decreased connectivity and impairment of distributed cortical networks\(^17\).
- Dietary DHA deficiency altered neurogenesis in the embryonic rat brain, with significantly reduced thickness of the cortical plate and under-development of the hippocampus and dentate gyrus, suggesting impaired post-mitotic cell migration\(^18\).
- Dietary DHA influenced global DNA methylation in the placenta in rats\(^19\).
- Prenatal DHA supplementation modulated gene methylation in human infants\(^20\).
- Dietary DHA influenced gene expression in humans\(^21\) and rats\(^22\).
- In the rat autism model, DHA supplementation has been shown to play a neuroprotective role, mitigating deleterious effects of valproic acid\(^23\).

On the basis of the above evidence it is proposed that autism is a consequence of pre- and post-natal DHA deficiency resulting from the combination of ineffective variants of the fatty acid desaturase gene cluster and the DHA-poor molecular environment. The support for this proposal is outlined in the following sections.

**DOCSAHEXAENOIC ACID AND THE BRAIN** Overwhelming evidence demonstrates the irreplaceable role of DHA not only in the structure but also the function of the brain, from the cephalopods to humans. Human brain is essentially a fatty tissue with approximately 60% of its dry mass being highly specialised phospholipids and polyunsaturated fatty acids (PUFAs). In the human brain DHA is the most abundant ω3 fatty acid (11% of the brain dry matter), specifically concentrated in membrane lipids of the brain grey matter\(^24\). The number of synaptic contacts in human brain is estimated in trillions and in synaptic membranes DHA constitutes up to 35% of fatty acids. It plays a vital structural role by providing a necessary lipid domain in which specialist proteins perform their functions\(^25\). It is indispensable for the maintenance of trans-membrane receptors that support synaptic transmission and cognitive functions, while activating, among others, the energy-generating metabolic pathways that affect molecules such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF1)\(^26\).

DHA is involved in the expression of several hundred genes in the brain through direct effects on transcriptional modulators(27). It has been demonstrated that the dominant functional role of the docosahexaenoic acid in the neural tissue is due to its quantum mechanical properties. Its structure, containing six methylene-interrupted double bonds, allows quantum tunnelling of the π electrons, resulting in speed and precision of neurotransmission\(^25\). Among its many functions DHA has been shown to modulate gene methylation and gene

---

**hypothesis**
expression in human infants. For a comprehensive review of the role of DHA in the brain see Liu et al. Despite the abundance of DHA in the blood-brain barrier through the recently-identified major facilitator superfamily domain containing MFSD2A protein. The strongest indication to date that human brain cannot function without DHA is found in a recent report of cases of lethal microcephaly caused by an inactivating MFSD2A mutation. This is the first evidence of a lethal mono- genic disease linked to transport of DHA in humans.

However, DHA is a difficult molecule to acquire. Its biosynthesis in man is subject to metabolic and genetic restrictions and to dietary influence. Especially affected are individuals with specific SNPs (single nucleotide polymorphisms) who are defective in their capability to synthesise their own DHA. Such individuals may be at risk of compromising their pre-and post-natal brain development when confronted by a DHA-restrictive diet.

**PROVENANCE OF DHA—METABOLIC RESTRICTIONS** (Fig 2) outlines metabolic pathways leading to the formation of docosahexaenoic acid, in the context of metabolism of the polyunsaturated fatty acids. PUFAs are defined by the position of the first double bond from the methyl end (ω) of the fatty acid. This double bond position remains unchanged when the fatty acids are metabolised, giving rise to fatty acid “families”, such as the ω6 and the ω3, that are of particular interest in the context of brain structure and function. Two fatty acids, each 18 carbon atoms long, are essential for animals and must be obtained from plant diet. These are: linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3), carrying two and three methylene-interrupted double bonds, respectively. These dietary essential fatty acids are metabolised in animals in the series of reactions involving chain elongation and desaturation, with two enzymes playing a key, rate-limiting role: the Δ6 and the Δ5 desaturase.

The ω6 series leads from linoleic acid to arachidonic acid (20:4ω6) and beyond, to the 22:5ω6 fatty acid. The ω3 series begins with α-linolenic acid and its final product is DHA, 22:6 ω3. Both metabolic series share the same the Δ6 and the Δ5 desaturase enzymes and this introduces a competition for the desaturation: high dietary intake of linoleic acid suppresses accumulation of DHA and vice versa.

Humans obtain DHA either through conversion of the plant α-linolenic acid, or ready-formed from the diet. The conversion pathway is tortuous and slow, involving a number of desaturating and elongating steps described in (Fig 2). Notably, the final step from DPA to DHA requires import, metabolism and export from peroxisomes. Only about 1% of α-linolenic is converted to DHA in humans. The most effective way of obtaining DHA is directly from the diet, the richest source being fatty fish and seafood. DHA is absent from plants.

**GENETIC INEQUALITY SPECTRUM** The inefficiency of the conversion pathway is not the only difficulty in humans. Further problem arises from the metabolic inequality brought about by the human genetic polymorphism, leading to unequal effectiveness of the endogenous DHA synthesis.

The fatty acid desaturases, Δ6 and Δ5 (Fig 2), are encoded by FADS1 and FADS2 genes, respectively. These genes are localised as a cluster, with the genes oriented head to head on the human chromosome 11q12-q13.1 (National Centre for Biotechnology Information). Some 500 single nucleotide polymorphisms (SNPs) have been annotated for this region in humans. These polymorphisms have been shown, in numerous studies, to influence the fatty acid composition of plasma, erythrocyte membrane and breast milk lipids. These findings are significant because blood EPA and DHA biomarkers are considered to be reliable indicators of the brain tissue DHA status. In general, current data support the conclusion that the minor alleles lead to lesser expression of FADS1 and FADS2, resulting in low levels of endogenously produced DHA. In these individuals, serum DHA concentrations are determined by the dietary supply of preformed DHA, primarily from fish.

**DHA-RESTRICTIVE DIET** Tissue accretion of DHA is further restricted by diet high in the ω6 linoleic acid, because metabolism of the ω6 and ω3 fatty acids uses the
The role of brain lipids in the causal model of autism: Re-interpretation of the existing data.

Hall

same FADS-encoded enzymes. In animals, high tissue content of the 22:5 \( \omega-6 \) fatty acid is a sign of \( \omega-3 \) fatty acid deficiency\(^{15} \). It is considered that for the optimal DHA accretion in the developing brain the ratio between dietary linoleic and \( \omega-6 \)-linolenic acids should not exceed 2:1\(^{12} \). Such ratio is thought to have been present in early human diet. Furthermore, fossil evidence demonstrates the importance of seafood consumption in early human evolution when freshwater or marine sources of protein constituted between 10-50% of the diet\(^{16-24} \). However, due to high consumption of vegetable oils by modern man, the linoleic acid intake is estimated to exceed 10 times that of \( \omega-6 \)-linolenic acid on average\(^{11} \), with restricting effect on endogeneous synthesis of DHA, due to the competition for the \( \Delta6 \) and the \( \Delta5 \) desaturases. Recent studies conducted in healthy school children in several European countries have shown blood levels of EPA and DHA to be below the minimum recommended for good cardiovascular health in adults\(^{21,25,26} \).

CONCLUSION—LINK BETWEEN DHA AND ASD

CAUSAL CHAIN OF GENES, ENVIRONMENT AND PHENOTYPE The large body of published evidence demonstrating the damaging effect of DHA deficiency on foetal brain development, have important repercussions for the development of the human brain during its most intense growth period, spanning the last two trimesters of pregnancy and the first months post-partum. Key processes, such as neuronal migration in the cerebral cortex, axonal and dendritic differentiation and apoptosis have been shown to require DHA as one of the factors in the neurodevelopmental outcome\(^{12} \).

Fetal and postnatal supply of DHA to the child is the result of interaction between the genetic FADS variant of both mother and child and the nutritional DHA status of the mother, beginning with the pre-gestation period and continuing through lactation\(^{14} \). Therefore, the foetal supply of DHA does not only depend on the DHA content of maternal diet during gestation but also on the DHA content of maternal stores before pregnancy.

Important maternal (epigenetic) effects have been reported in a recent study by Dominguez-Salas\(^{16} \). For the first time in humans it has been shown how the nutritional status of the mother during early pregnancy modulates DNA methylation, causing persistent and systemic changes in human metastable epialleles. It is plausible, therefore, that the insufficient DHA supply to the foetus, resulting from reduced foetal and maternal FADS activity and low maternal supply of DHA followed by the infant’s postnatal diet low in DHA, may lead to the published observations on disorganisation of the neocortex area of the brain tissues from children with autism, specifically in the region known to contain the Theory of Mind neurons, activity of which seems to be defective in ASD individuals\(^{15} \). In addition, DHA deficient diet has been shown to negatively impact behavioural tasks of learning and to increase stereotyped behaviour in non-human primates, both being characteristics of ASD individuals\(^{12} \). Finally, the new information on differentially methylated regions associated with ASD in autism-discordant monozygotic twins is relevant in the context of the role played by maternal food intake in child’s gene methylation, itself in turn influenced by DHA.

In conclusion, there is a persuasive body of evidence of importance of maternal diet just as crucial for neurodevelopmental studies to take into account both the maternal pre-pregnancy nutritional status and the genetic metabolic variants of the mother and the child. This inter-disciplinary review demonstrates the need for well-designed studies, including multiple biomarkers that link metabolites, co-factors and genes of different pathways with objective behavioural outcomes – to be conducted in children with autism spectrum disorders. Such combined approach may offer a possibility of prevention of ASDs through dietary intervention in genetically vulnerable individuals.

TESTING OF THE MODEL Autism is a spectrum with different aetiologies and not all of them may be DHA-related. However, this is the first evidence-based coherent and testable model of ASD that combines the known genetic, maternal and phenotypic factors. Like all models, it is an approximation of reality and must be verified through testing. The following is a suggested testing strategy, highlighted as text in red in (FIG 1). Brain tissues studied by Stoner (2014) or similar future studies should be analysed for the fatty acid composition as reported by Coti Bertrand (2006). The methyliconic analysis results of monozygotic ASD-discordant twins, as reported by Wong et al. (2014), should be checked for the methylation status of the FADS genes. Individuals with ASD and their mothers should be SNP’s genotyped for the FADS alleles, combined with detailed history of DHA dietary intake, as in studies by Koletzko et al. (2011).

CONFLICTS OF INTEREST

Author declares no conflicts of interest.
The role of brain lipids in the causal model of autism: Re-interpretation of the existing data.

Dr. Barbara Hall, Fellow of the Royal Society of Biology, is a freelance scientist and managing director of Sureconsult Ltd UK. Following a career in academic research at the universities of Stockholm and London, she worked in industry as a toxicologist at Procter & Gamble UK and L’Oreal Paris. She has published in pediatric, dermatology, and toxicology journals and in The Lancet, in addition to articles in the national press. She has lectured at universities in Belgium, Holland, France and the U.K., as well as giving public lectures, the most recent being at the Royal Society of Chemistry in London.

REFERENCES


15Kie L and Innis SM. Genetic variants of the FADS1-FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. The Journal of Nutrition 138: 2222-2228 (2008).


20Kulkami A, Danat K, Kaler A, Sable P Chavan-Gautam Pijoshi S. Effects of altered


