1. Introduction

Biomedical research increases exponentially and whole areas are often closed fields to many clinicians only a few years after finishing their training. Genomics\(^1\) and proteomics\(^2,3\) to take two examples, have changed out of all recognition over the past decade, while disciplines like genetic epidemiology\(^4\) are only now taking recognizable form.

Many problems face those who try to assimilate research findings into clinical practice - topics seem to become ever more discrete with relevance to clinical practice often seeming remote.

This paper draws together ideas from various research areas and clinical specialities typically regarded as independent and unrelated. One aim is to demonstrate that these topics are fundamentally interrelated and that an interdisciplinary programme of work is required to address and adequately answer many of the questions raised through this recognition of the complexity of the autistic spectrum disorders.

Autism is diagnosed on the presence of overlapping difficulties in communication, social functioning and rigidity of routines (often referred to as the autistic triad of impairments), together with abnormalities of early development. No features are necessary and many combinations are independently sufficient, thus the basis for a clinical diagnosis in two individuals may be very different. It is unsurprising, therefore, that there are a wide range of independently sufficient factors which may result in diagnosis.\(^5,6\) Specific endo- and behavioural phenotypes have begun to emerge, with many diagnostic subgroups such as Asperger syndrome, Rett syndrome, and Childhood Disintegrative Disorder. It is highly unlikely that autistic behaviour will prove to have a common pathogenesis, and equally surprising if any treatment / intervention is successful with the whole autistic population. This paper provides evidence that autism consists of a number of different biological entities. A clearer un-
standing of these conditions is critical to successful intervention and management.

Autism is a condition which seems to arouse complex and often contradictory responses both from those involved in its diagnosis and management1 and not least from parents and affected individuals themselves.8,9 The intention here is to provide access to key research and to allow those reading this material to pursue these ideas further should they wish to, and to draw their own conclusions.

The pharmaceutical industry has highlighted autism as a priority target for drug development.10 The rising prevalence figures have led to autism rivaling Alzheimer’s disease in terms of “illness years”.11

No single model is adequate to explain the causes of autism. The different components of the ‘autistic triad’ seem to be largely independent.12 Happe, Ronald and Plomin using this notion have shown that within the general twin population (using the Childhood Asperger Screening Test with those in the Twins Early Development Study) these three aspects of functioning do not significantly co-vary13 but appear to operate as independent genetic factors.

There are, however, sufficient commonalities to justify the continuing use of the term autism at our current level of understanding. Certain conditions (such as fragile X syndrome, where much of the neurobiology is now well understood) may help to inform our understanding of other types of autistic disorder.

This paper covers general ideas on developmental processes, particularly the relevance of a move from individual to dyadic systems levels of analysis of infant behaviour and development; it provides some general information on autism, developing a model of autism based on the dyadic level of analysis, and how this is amenable to analysis; it discusses the commonalities across those with autism from the perspective of developmental psychopathology; and presents differences between several patterns of autistic presentation. The scope of this review, has been limited, omitting areas such as basic clinical neurochemistry where recent systematic reviews are readily accessible.14

One final caveat concerns the generality of conclusions drawn here concerns ethnic differences in genetic make-up and their possible implications for our understanding of autism. We are aware of ethnic differences in genetic factors which affect a wide range of aspects of human behaviour from athletic prowess,15 to differences in the prevalence of the DRD4-7 allele repeats correlating with prevalence of Attention-Deficit Hyperactivity Disorder.16 Similar ethnic differences may account for inconsistencies in the clinical literature on autistic spectrum disorders as with the recent findings of differences between US and Italian families in the association between PON1 abnormalities and autism24 and the failure to find evidence of an association between the 5-HTT serotonin transporter gene polymorphism and autism in Japanese cases17 despite such findings in other populations.18

2. Social Development and the Neurobiology of the Self and Other

We traditionally draw a boundary for analysis at the level of the individual. In Western thought, this comes from the tradition started by Rene Descartes, of beginning from one’s own personal thoughts and captured by his phrase ‘Cogito ergo sum’ (I think therefore I am). This artificial boundary is ultimately unhelpful in looking at the social as it divorces individual function from its necessary ontogenetic context.

The English Psychoanalyst Donald Winnicott stated that when we try to look at the infant we are inevitably looking at the infant and a caregiver. It is impossible to look at the infant alone.19 This viewpoint is equally valid when we look at the factors in what has come to be called “affective”20 or “social” neuroscience.21 Development in emotional interactions are contingent for each individual on their partner’s emotional communication and cannot be looked at in isolation.22

Parallelling developing interest in the biosocial, the past 25 years have also seen a steady increase in the development of interest in the biological underpinnings to the developmental psychopathologies.23,24

We cannot restrict our study to the biologically bounded individual infant, we have to look at the infant in his/her social context. At the very minimum, this involves the infant and his/her primary caregiver. The process is also a reflexive one - looking at the behaviour of a caregiver outside the context of the dyad is equally limited. Each member requires the other (see Figure 1).

The idea of a pre-formed infant nurtured in the mother’s womb has been present in Western thinking since antiquity. The notion was typically that the infant was a homunculus present in either the egg or in the spermatozoa. For the latter view, the womb simply provided an environment in which the foetus could grow. Early studies by Hartsoeker25 and others seemed, at the time, to convincingly support the view that the infant grew from the sperm.

The complex skills of the newborn are attuned to social interaction with facility for behaviours such as interactional synchrony; imitation; and seeking eye contact on maternal speech (for reviews, see Ref.26,27). The infant is not nearly as well developed in respect of its non-social abilities - for example, visual acuity for social phenomena (as when used in the imitation of tongue protrusion) is far more precise than for non-social stimuli (such as preferential looking).28

Detailed analysis of the infant behaviours seen in such early dyadic processes has been carried out at least since the observations of Dietrich Tiedemann29 over two cen-
turies ago. Observational methods are easier to systematize, operationally define and validate with electronic recording, enabling detailed micro-analysis and inter-rater reliability checks. This approach has been pioneered in the work of researchers like Daniel Stern; Colwyn Trevarthen; and Heidi Als.

Babies are born with the ability to ensure that they are likely to be cared for and nurtured by those around them. This social attunement ensures their survival. The infant tailors use of such abilities to the responses obtained from their social environment - the same infant can produce higher levels of tactile and vocal communication to a blind caregiver, while using more animated facial expressions and non-tactile movements with a sighted caregiver.

It is well known that the maturation of the brain in part depends on the quality of its early experience. There are predictable periods of rapid development and predictable periods of development stasis - human social and neural development are non-linear processes.

There is now clear evidence that modifying early experience can enhance brain development in premature human infants. Various mechanisms have been proposed for this process. A critical aspect is that infants are intimately involved in generating and enriching the mutual social experience which drives this process. The effects of factors such as caregiver mental health limit the environment through which this is canalized.

Psychosocial factors are crucial in the neurological development of both the normal and the developmentally different infant.

3. Mirror Neurons and Social Understanding

A recent development in our understanding of the biological mechanisms for social responsiveness comes from our understanding of the neurobiology underpinning both self-referential processes and what have been termed ‘mirror neurons’.

Studies which have looked at activation during tasks that require self-reference and tasks that do not indicate coordination in the activity of areas involved in self-referenced sensory, motor, emotional and proprioceptive processes during the former but not the latter. Similarly, there is now a considerable literature on the brain systems activated during tasks such as imitation and perspective-taking in normal subjects.

Mirror neurons are activated specifically when we are engaging in or thinking of being involved in activities, or, when we observe others engaging in activities and are inferring why an action is being carried out.

“...premotor mirror neuron areas—areas active during the execution and the observation of an action—previously thought to be involved only in action recognition are actually also involved in understanding the intentions of others.”

In fMRI activation tasks in normal controls, recognition of emotion is associated with increased activation in various structures, but particularly in paracingulate, anterior and posterior cingulate and amygdala. In contrast the ability to infer and attribute mental states was associated with increased activation in lateral orbito-frontal cortex, middle frontal gyrus, cuneus and superior temporal gyrus.
4. The Intrinsic Motive Formation (IMF)

Trevarthen and Aitken have developed the idea of a system which is the neural basis to social abilities, that is functional and accessible from birth and is thus not verbally mediated, and that has a high degree of phylogenetic preservation.\textsuperscript{26,41,42}

These papers develop a model which bases affect on a range of neural structures that are functional prior to the development of expressive or receptive language in the infant. These structures, their neurochemistry and their broad roles are detailed in table 2 below. They rely on structures in the limbic system\textsuperscript{43} and the emotional motor system of the vertebrate nervous system. Ergotropic and trophotropic are terms used here to describe respectively 1) effortful behaviours which enable connection with / avoidance of social stimulation in the environment and 2) behaviours which result in physical nurturance / care / procreation.

The discontinuous nature of development is based on the alternating ascendency at any point in time of ergotropic and trophotropic processes.\textsuperscript{35}

5. What about autism?

Kanner’s initial description of autism\textsuperscript{47} was as “a biologically provided disturbance of affective contact”. For Kanner, autism was a condition which was a biological disorder of the individual child.

Today it is clear, through studies of perinatally institutionalized Roumanian children, that although extreme psychological deprivation alone cannot result in autism,\textsuperscript{48} there are autistic-like behaviours in many who are subjected to extreme early deprivation. A proportion of those in such early care fulfill behavioural criteria for autism which ameliorate steadily over time in post-adaptive placement.\textsuperscript{49} It is of course possible that some of those placed in such care were also at biological risk of developing an ASD in any event.

The typical age at diagnosis for autistic disorder in the UK and USA is between 2 and 3 years of age. When identified at this age, studies do not discriminate within the ASD spectrum but have reasonable overall prospective validity.\textsuperscript{50}

The current World Health Organization diagnostic classification system (ICD-10) and the American Psychiatric Association’s system (DSM-IV Tr) have employed similar criteria since 1994, the previous systems having had major differences.\textsuperscript{51,52} There are still differences between accepted measures used in diagnosis and a clear agreed protocol for diagnosis using standardized tools is some way off.\textsuperscript{53} In the clinical context, scales such as the ADI-R and ADOS do not adequately discount other possible conditions, and more broadly based tools such as the DISCO (Diagnostic Interview for Social and Communication Disorders)\textsuperscript{54} may be more helpful for clinical use.
6. Are there any early warning signs of autism which can be reliably identified?

There is a higher rate of pre- and peri-natal risk factors (such as abnormal growth rate both in utero and after birth; enlarged head at birth; bleeding in pregnancy and low Apgar scores) in those who go on to receive an autism diagnosis. Some 5% of the variance in head circumference can be explained on the basis of the HOXA1 A218G G allele.

Collaborative Programs of Excellence in Autism (CPEA) studies are beginning to demonstrate associations, with biomedical factors, for example, between regressive onset autism and Familial Autoimmune Thyroid Disease.

Ongoing prospective studies such as the MoBa Autism Birth Cohort (ABC) study and the CHARGE Study will help to identify early signs of autism, perhaps linked to specific biobehavioural phenotypes.

7. Are there differences in Early Motor development in those who go on to receive a diagnosis?

Philip and Oznat Teitelbaum have analysed early videotapes of children who were subsequently diagnosed with of autism. Differences in early motor develop-
ment - particularly in early motor abilities like rolling over and protective reflexes and in facial expressions such as the ‘Moebius mouth’ (typically an indication of a problem with cranial nerves VI and VII) were apparent. This work compared ASD children to earlier descriptive accounts of normal development. This is not an ideal basis for clinical comparison as the children were not drawn from comparable populations.

In a study of home movie recordings of subsequently diagnosed autistic children, Adrien and colleagues described a number of behavioural abnormalities in the first two years of life - abnormalities of eye contact, limited and variable emotional expression, attention deficits and limited initiation of communication, together with abnormalities of motor development.

Osterling and Dawson (1994) on an analysis of first birthday party videotapes were able to discriminate subsequently diagnosed autistic children on various criteria including showing objects to others, orienting to their own name, looking at others faces and pointing. This suggested that there were potential social risk features which were evident well before the 18 month age at which the CHAT screening was done. Later research by the same group has again found variation in early presentation, with a subgroup failing to manifest obvious behavioural signs of early developmental risk.

Sandra Maestro and colleagues have examined early videotapes of 40 such children and found clear evidence of abnormalities within the first year of life in 87.5% (35) with a small subgroup (the remaining 12.5%) showing no behavioural differences. This group used a standardized rating scale of early behaviour - the Behavioural Summary Evaluation Scale.

8. Are there structural or functional differences in the autistic brain?

Gross anatomic and cytoarchitectonic differences in autism are now well recognized. Neuroanatomical differences being reported in many structures including the amygdala; more generally of medial temporal lobe structures including amygdala, hippocampus and entorhinal cortex. The elegant and detailed neuropathology studies of Bauman and Kemper have shown a range of anatomical differences, particularly in prefrontal cortex; limbic and cerebellar structures. There may be subtle regional reductions in hippocampal volume.

There are reported differences in the structure of the cerebellum in some cases, associated with reduced numbers of Purkinje cells. There may be a causal link between these abnormalities and autistic symptomology. Similar features are described in the “cerebellar cognitive affective syndrome” (CCAS).

In CCAS, a group of patients with frank neurological damage confined to the cerebellum, a wide range of non-motor functions are impaired which include blunting of affect; dysprosodia; executive dysfunction and working memory problems.

A number of studies suggest that the brain is typically larger in autism at birth (albeit with a high degree of overlap with the general population), with a subsequent slowing in brain growth compared to normal.

Some studies suggest underconnectivity between brain areas, involving abnormal development of the corpus callosum and of connectivity between dorsolateral prefrontal cortex and hippocampus or anterior cingulate, ventral striatum and anterior thalamus.

One study has suggested differences in the functional activation of the primary language areas in able autists with reduced activation in Broca’s area (left inferior frontal) and increased activation in Wernicke’s area (left temporal) during language processing tasks. Another study showed abnormal fMRI activation of the amygdala and fusiform gyrus to cartoon faces and reduced activation to normal faces in one autistic subject obsessive about cartoon characters when compared to normal controls.

9. What about early social communication in autism?

Baron-Cohen, Allen and Gillberg (1992) studied 41 children at high-risk for developing autism (siblings born into families with previously diagnosed autistic offspring). At 18 months, these children were screened using a tool called the Checklist for Autism in Toddlers (CHAT). This was administered partly by observation and partly by parental questioning. Abnormalities were found in eye contact, pretend play, protodeclarative pointing (pointing to show), social play and social interest in the four children who went on to receive a diagnosis of autism. The first three features were seen in all subsequently diagnosed cases. This was a prospective screening study in a group known to be at high risk of developing autism so could not be used to infer the likely occurrence of such features as risk factors in the general population. A population study was required to ascertain false positive and false negative rates (how many children would be classified as high-risk who would subsequently develop normally and how many subsequently affected children would be missed).

In a subsequent population based study, the team screened 16,235 children at 18 months from 10 health districts in South London. Their initial screening identified 10 children who were predicted on their CHAT scores to be likely autistic children. All were found to be autistic when followed up several years later.

In the followup screening, a further 40 cases who had passed the CHAT were identified. Thus 80% of their subsequently diagnosed cases had had normal eye contact, protodeclarative pointing and/or play at 18 months. These early prognostic indicators did not seem to be as
useful as initially thought either because they are of limited sensitivity or because there is a group who show later emergence of autistic behaviour. It would be interesting to know how many of the high-risk children in the original 1992 paper have gone on to show any ASD features in subsequent years.

A number of subsequent studies have used refinements of the CHAT scale such as the M-CHAT\textsuperscript{88,89} and the CHAT-23\textsuperscript{90} in attempts to improve sensitivity and specificity.

As indicated above, various groups have examined early video material of subsequently diagnosed children. To date, this source of data is limited as it is retrospective, is not systematically collected, and is collected by parents. These factors introduce a number of biases - socioeconomically more affluent families are more likely to collect this type of material; selective recording, as parents of those with an ASD are more likely to exhibit similar difficulties and could thus choose to record different types of material - the child’s interest in mobiles rather than in people, for example. Video material being collected in some of the prospective research studies now underway will allow this material to be validated.\textsuperscript{59}

Initiatives are underway to try to develop earlier screening tools such as the SEEK which has been trialled for 8 month screening focussing on aspects of early behaviour such as sleep rhythm; eating rhythm; social interaction; eye contact; body contact and bodily tonus.\textsuperscript{91}

10. Are there differences in Mirror Neurons in Autism?

There has been speculation over possible mirror neuron defects in autism for some time\textsuperscript{92} and evidence is emerging of anatomical differences in the areas implicated in mirror neuron function.\textsuperscript{93} These differences are, however, part of more general differences also reflected in other brain areas involved in emotional recognition and social understanding. A somewhat different perspective suggests a general problem of underconnectivity between cortical areas.\textsuperscript{81}

Several studies address mirror neuron function in autism and related disorders, documenting functional differences in both Asperger syndrome\textsuperscript{94} and ASD.\textsuperscript{95}

There is a fundamental problem in “intentional attunement” in autism.\textsuperscript{96} There appears to be a lack of coordination in the activity of areas involved in sensory, motor, emotional and proprioceptive processes.\textsuperscript{97} A reduction in spindle cells (also known as Von Economo neurons) may be involved in the empathy deficits seen in autism. These cells, found in the anterior cingulate are involved in emotional understanding\textsuperscript{98} and function abnormally in autism.\textsuperscript{99}

11. Prevalence of Autistic Spectrum Disorders

With a few notable exceptions there are major limita-

12. Outcome in Autistic Spectrum Disorders

There are a number of reviews of outcome in autistic spectrum disorders.\textsuperscript{106} Speech and language problems at 2.5 years appear to be a strong predictor of subsequent neuropsychiatric disorder (ADHD or ASD) but not to be syndrome specific with regard to prognosis.\textsuperscript{107} Diagnoses such as Rett syndrome and Childhood Disintegrative Disorder have poorer developmental outcome and a degenerative timecourse. Certain medical co-morbidities such as epilepsy are also associated with increased morbidity and mortality.\textsuperscript{108}

13. Do we have a model for how social understanding in autism takes place?

Various models of the social deficits in autism have been proposed. I will briefly elaborate on one psychological model which has clear parallels to the interesting findings on mirror neuron dysfunction described above.\textsuperscript{81}

The process of normal interpersonal interaction relies on conveying information and on internal models to process and interpret the feelings, intent and likely future actions of self and other. Where aspects of the process are impaired — through sensory or motor deficit or through a lack of an internal model of self / other - interaction will be affected.

In autism, it appears that the ability to interpret others is selectively impaired.

Such a deficit has implications for therapy. A range of interventions can be viewed as operating by simplifying B’ in Figure 3 to make interaction with the autistic individual easier to understand. The ‘Option’ approach, for example, provide a situation where the other person becomes easier to interact with through providing a situation in which the autistic child can successfully interact using the belief that A = B’. Structured approaches such as Applied Behavior Analysis (ABA) simplify the behaviour of others by the use of limited vocabulary, frequent
repetition and the use of the same repertoire by all significant caregivers (thus making B' more predictable).

The common factor underpinning successful early intervention approaches appears to be making the world appear simpler and more predictable. Other approaches can introduce patterning and predictability using methods from music therapy or by introducing ‘rhythmic intention’ (see: Jaqueline Robarts section on music therapy in).

14. Is Autism a Genetic Condition?

The view that autism is largely a single but polygenic genetic condition came from early twin studies that appear to support this view. Bailey et al found a 60% MZ concordance for narrowly defined autistic disorder and a 0% concordance in DZ pairs. High and stable concordance rates in identical twins and lower rates in non-identical twins were used to argue for a polygenic model. Increasingly, general objections have been raised to the view that MZ-DZ concordance differences are adequate to address such issues. Various objections to twin data specifically in autism research have been raised including the higher rate of autistic traits in male twins when compared to non-twin controls, and the suggestion in some studies that twinning may be an associated risk factor per se; but see also:  

Concordance appears to have fallen in identical twin pairs and risen in non-identical twin pairs, leading one group to draw the following conclusion:

“Our data suggest that heritability estimates from previous studies may have overestimated the role of genetics and underestimated the role of environmental factors in the etiology of autism.” (Croen, Grether & Hallmayer 2002)

A recent advance in our conceptualization of neurodevelopmental disorders in general has been the recognition of ‘Behavioural Phenotypes’. Genetic analysis is starting to provide answers to why particular individuals are affected and may benefit from particular approaches. There are also recommendations from a number of quarters concerning the importance of identifying ‘syndromal’ autism, or pathogenesis specific clinical presentations which help to identify patients who warrant further laboratory and imaging studies.

There are many recognized clinical conditions which are associated with ASDs at a greater than expected frequency. There are also a large number of gene markers which from linkage studies are over-represented in the autistic population but where we are currently unsure of their functional significance (see Ref. for a discussion of many of the weaker associations).

A wide range of conditions which have been described in individuals with ASD diagnoses have distinct behav-
ional and physical phenotypes, mostly associated with developmental disability/learning problems. Many are linked to other co-morbid problems such as ADHD symptomology; sensory deficits; immune dysfunction or epilepsy.

The principal conditions are listed here:
15q11-q13 duplication; chromosome 2q deletion; XYY syndrome; 10p terminal deletion; 45,X/46,XY mosaicism; 22q13 deletion syndrome; Aarskog syndrome; Adenylosuccinate lyase deficiency; Adrenomyeloneuropathy; Angelman syndrome; Apert syndrome; ARX gene mutations; Autism secondary to lymphoproliferative syndrome (ALPS); Bannayan-Riley-Ruvalcaba syndrome; Basal cell nevus syndrome; Biedl-Bardet syndrome; CATCH 22; CHARGE syndrome; Coffin-Lowry syndrome; Coffin-Siris syndrome; Cohen syndrome; Cole-Hughes macrocephaly syndrome; Congenital adrenal hyperplasia; Cowden syndrome; De Lange syndrome; DiGeorge syndrome; Down syndrome; Duchenne’s disease; Ehlers-Danlos syndrome; Fragile X syndrome; Fragile X permutation (partial methylation defects); GAMT deficiency (guanidinoacetate methyltransferase deficiency); Goldenhar syndrome; HEDADD syndrome; Hyper IgE syndrome with autism; Hypomelanosism of Ito; Hypothyroidism; Joubert syndrome; Kleine Levin syndrome; Lujan-Fryns syndrome; macrocephaly/autism syndrome also defined above as Cole-Hughes macrocephaly syndrome; Moebius syndrome; Myhre syndrome; Neurofibromatosis type 1; Noonan syndrome; Ornithine carbamyltransferase deficiency; Oculocutaneous albinism; Orstavik 1997 syndrome; Phenylketonuria; Pituitary deficiency; Prader-Willi syndrome; Proteus syndrome; Rett syndrome; Rett syndrome (Hanefeld variant); Rubenstein-Taybi syndrome; Schindler’s disease; Smith-Lemli-Opitz syndrome; Smith-Magenis syndrome; Sotos syndrome; Steinert myotonic dystrophy; Timothy syndrome; Tourette syndrome; Trichothiodystrophy; Tuberous sclerosis; Turner’s syndrome; Unilateral cerebellar hypoplasia syndrome; Velo-cardio-facial syndrome (also see CHARGE syndrome); Williams syndrome; Xeroderma pigmentosa.

A number of overgrowth syndromes with associated neurodevelopmental difficulties have been reported, which may also present with autistic/autistic-like symptomology.

These include the following:
Weaver syndrome (this may be synonymous with Sotos syndrome also now localized to 5q35); Simpson-Golabi-Bechmel syndrome Type 1; hemihyperplasia; Sturge-Weber syndrome; and PEHO syndrome (Progressive Encephalopathy with Edema, Hypsarrythmia and Optic Atrophy).

In addition to the described clinical phenotypes which have been reported in association with autism, there are many genes associated with autistic diagnosis through linkage studies. Such studies have been carried out through the analysis of large case series or of archived genetic material. The reported associations with LOD scores of 2.2 or greater are tabulated below (data adapted from Ref.1,5,119,121 for justification of this cutoff, see Ref.122). There are currently few areas of overlap between linkage sites and associated conditions—the homeobox sites DLX1/2 and HOXB1 on chromosomes 2 and 17 and the serotonin transporter SLC6A4 on chromosome 17 currently looking the most promising.

Gene mapping of complex disorders such as autism is proceeding rapidly. Understanding the biological and behavioural mechanisms by which these have effects in the process poses greater difficulties. It is the epigenetic regulation of gene function that is core to our understanding of mechanism.

Associations between such linkage sites and specific behavioural phenotypes are coming to light. A recent paper suggests a link between a chromosome 2q linkage site - D2S116 and delayed onset of phrase speech in autistic disorder (MLS 2.86), while MLS in the total autistic disorder group is only 1.3.

Known genetic causes account for around 12% of cases. Recent findings on the prevalence of factors such as DCHR7 mutations, and a recently described variant of the MET receptor tyrosine kinase suggest that further genetic factors may be important in a higher proportion of cases. Based on the epidemiological data so far collected, perhaps in as many as 65% of cases. The majority of such factors are not screened in routine clinical practice. It seems likely that the vast majority of those with autistic disorders will be found to have pathogenically relevant genetic factors. Given what we know of the few conditions that have been investigated, many genetic differences seem likely to vary by ethnicity.

The extent to which genetic factors increase susceptibility to environmental effects and the extent to which these are sufficient in the absence of any environmental factors is, for most, an open question. Environmental exposures may result in autistic behaviour primarily in genetically susceptible individuals.

15. Do we know of non-genetic biological causes of autism?

There are apparent peaks for exposure to environmental stressors at 25-28 weeks post conception associated with a heightened likelihood of the development of autism.

The recent rise in reported prevalence (see Ref.102,145 for discussion), the changes in reported MZ/DZ concordance rates (see above), increased susceptibility to autistic traits in male twins compared to the general population, and public concerns over various environmental exposures have led to a developing research focus.
A number of large studies are now underway which should help to address and resolve the issues, such as the CHARGE study. That environmental factors may compound a genetic vulnerability - for example, that various PCBs can multiply glutamate based potentiation five fold *in vitro* is clearly a plausible factor. A recent, well-controlled Australian study found that by age 5 years the risk of ASD following neonatal encephalopathy was 5% as opposed to 0.8% in a randomly selected control group.

There are many findings suggestive of an association between oxidative stress and autism, particularly in cases associated with loss of previously acquired language skills.

### Table 2 Gene positions with linkage to ASD diagnoses

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<th>Chr</th>
<th>Position</th>
<th>Marker (at or near)</th>
<th>Scan</th>
<th>MLS Group Candidate (multipoint LOD score)</th>
<th>Gene Locus</th>
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16. Teratogenic Exposures

Sodium Valproate, one of the safest antiepileptic agents, is a compound that acutely interrupts neural migration in animal models, and when taken during pregnancy it can result in autism. In a recent series, 8.9% of those exposed in utero to sodium valproate were subsequently diagnosed with autistic disorder or Asperger syndrome.\(^{152}\)

As many epileptic women may conceive whilst on medication and may be unaware of the potential risk it is important that this is part of pre-conceptual advice to women of child-bearing age on sodium valproate prophylaxis. The lesser risks associated with other antiepileptic medications may be an important consideration for epileptic women wishing to have children.

Misoprostal is an abortifascient which, in babies subsequently carried to term, results in an iatrogenically induced cranial nerve VI and VII palsy. It is associated with the subsequent diagnosis of autistic disorder in approximately 1/3 of cases.\(^{153}\) The effects of misoprostal are equivalent in nature and extent to those seen in the genetic form of Moebius syndrome. In both conditions, there is a significant association of the neurological features with autistic disorder.

Thalidomide, a medication which was used in Europe as treatment for ‘morning sickness’ during pregnancy also resulted in a significant number of autistic children, if taken during a critical time window of pregnancy (20-24 days post-conception), also resulting in associated ear and digit anomalies in those affected by autistic disorder and suggesting a critical time window in embryogenesis.\(^{154,155}\)

17. Viral pathogenesis

Animal models demonstrate the biological plausibility of viral pathogeneses of ASD.\(^{156}\)

In autism, exposure to a range of viral agents has been suggested as having played a possible causal role, including Cytomegalovirus;\(^{157}\) Herpes encephalitis;\(^{158}\) Human immunodeficiency virus;\(^{159}\) Rubella\(^{160}\) and Measles (SSPE and OCDD).\(^{161}\)

It is important to stress that research in this area is not robust. Demonstration of cases where viral exposure and autism co-occur does not prove causality without greater knowledge of factors such as community levels of exposure and ASD prevalence within such communities.

18. Can a knowledge of biological differences in pathogenesis have relevance to management?

There are many disparate approaches being advocated for the biological management of ASDs, some of which have become the subject of much controversy - secretin and chelation therapies being two amongst many such ‘alternative/adjunctive’ approaches. A plausible theoretical basis for interventions can often be constructed, but there is only limited / anecdotal evidence of abnormalities in the parameter of interest in the autistic population and even less of clinical benefit from addressing such differences (see Ref.106,162,163 for reviews).

Those who take a strictly ‘Evidence-Based’ approach have to admit that there is a paucity of adequate evidence for all approaches, whether conventional or alternative, and a laissez-faire approach is easy to adopt and justify. This situation should not be used to argue for maintenance of a status quo, but rather to stimulate adequate research (adequate in the sense of being sufficient to affect clinical practice if it were to demonstrate positive effects).

The UK Medical Research Council Research Review\(^{164}\) highlighted a range of potentially promising lines for further research in the area of dietary treatments. There is now an extensive literature on gastrointestinal problems in autism.\(^{165,166}\) As the various biological factors involved in gastrointestinal function are identified, as with the MET receptor tyrosine kinase,\(^{143}\) the nature and role of such involvement will be clarified.

A number of differences between those with ASDs and the control populations against which they are often matched may be important in interpreting any biological findings which are reported. For example, creatinine levels are often used as a standard reference against which abnormalities in other biochemical products are compared. Recent research has indicated that creatinine levels may be significantly reduced in the ASD population.\(^{167}\) This difference may be genetic in origin - a GAMT deficiency,\(^{168}\) for example, would lead to low levels of creatine, and consequently to lowered creatinine levels. Such a difference may be important in interpreting the results of studies such as the recent paper on differences in urinary porphyrin levels that may be of clinical relevance.\(^{169}\) Results should be reported making allowances for and pointing out any such group differences found.

18.1. Abnormalities of Tryptophan Metabolism

As early as 1986 there was published research suggesting a link between abnormal tryptophan metabolism and severity of problems in children with autism.\(^{170}\) This group demonstrated that free plasma tryptophan levels were elevated in autistic children compared to both child and adult controls and that levels correlated positively in their autistic sample (n = 37) with both activity level and rated severity of psychiatric symptomology. Subsequently, McDougle and colleagues have demonstrated that by depleting tryptophan in adult autistic subjects they could induce marked changes in behaviour - significant increases in behaviors such as whirling, flapping, pacing, banging and hitting self, rocking, and toe walking could...
be induced (p < .05) and these were not seen in case-matched controls.\textsuperscript{171}

A potential pathological role for abnormal tryptophan metabolism in the development of autism and epilepsy has been described.\textsuperscript{172} One aspect of abnormal metabolism of tryptophan could be the induction of cortical dysplasia, predisposing to a higher risk of both autism and epilepsy.

A recent genetic study has shown a polymorphism of the tryptophan 2-3 dioxygenase gene in a significant subgroup of autistic patients from 196 multiplex families,\textsuperscript{173} effectively blocking one catabolic pathway for tryptophan metabolism to kynurenine and increasing the availability of tryptophan for the production of serotonin. This suggests that a subgroup may be centrally hyperserotonergic.

Differences in the brain expressed form of tryptophan hydroxylase (TPH2) have also been reported as occurring at a modestly elevated level in a subgroup of the autistic population.\textsuperscript{174} This suggests that there may be a further subgroup who are centrally hyposerotonergic.

These genetic differences in tryptophan metabolism may account for the variable response reported in the clinical literature to SSRIs.\textsuperscript{175}

As tryptophan is the dietary precursor to a wide range of biologically active substances such as serotonin, melatonin, and kynurenine, the role of this metabolic pathway in autism deserves more detailed analysis.

18.2. Methylmalonic Acidaemia, Vitamin B12 (methylcobalamin) and cobalt levels

Methylmalonic acidaemia (MMA) was first described in children in 1967 and had previously been described in adults with pernicious anaemia. It results from a deficiency of either methylmalonyl-CoA mutase (the principal factor in converting Methylmalonyl-CoA to Succinyl-CoA) or of adenosine cobalamin, an essential cofactor derived from vitamin B12 (cobalamin). MMA is a branched-chain organic aciduria like Maple syrup urine disease (MSUD), isovaleric acidemia (IVA) and propionic aciduria (PA).\textsuperscript{176}

There are well recognised neurological-psychiatric sequelae of impaired intracellular synthesis of adenosylcobalamin and methylcobalamin (cobalamin C disease) which is an inborn error of metabolism.\textsuperscript{177}

Vitamin B-12 deficiency can arise for various reasons including mother adhering to a vegan diet during pregnancy and while breastfeeding,\textsuperscript{178} or through strict adherence to a vegan diet.\textsuperscript{179}

A genetic basis to some forms of vitamin B-12 dependent methylmalonic acidaemia has now been identified.\textsuperscript{180} Imerslund-Gräsbeck disease is an autosomal recessive problem with B12 absorption, coupled with proteinuria.\textsuperscript{181}

Abnormalities of methylation are described in autism and, when present, can be ameliorated by the use of a combination of folic acid, betaine and methylcobalamin.\textsuperscript{182}

Metabolic endophenotypes\textsuperscript{183} may be associated with transmethylation and transsulfuration of methionine, demonstrating abnormal rates of allele frequency or gene-gene interactions in the following:

- Reduced folate carrier (RFC 80G > A);
- Transcobalamin II (TCN2 776G > C);
- Catechol-O-methyltransferase (COMT 472G > A);
- Methylene tetrahydrofolate reductase (MTHFR 677C > T and 1298A > C);
- Glutathione-S-transferase (GST M1).

18.3. Abnormal Sterol Metabolism and the case of SLOS

Abnormalities of short chain fatty acid metabolism have been hypothesised as being involved in some forms of autistic spectrum disorder. Abnormal long chain acyl-CoA dehydrogenase (LCAD), for example has been found in an autistic patient with consequent defects in the beta-oxidation of branched and unsaturated fatty acids, with reduced synthesis of omega-3 DHA and abnormal cholesterol metabolism.\textsuperscript{184}

Bell has described abnormal erythrocyte cell membrane phospholipids - in particular reduced levels of highly unsaturated fatty acids (HUFAs) in an ASD sample, specifically after cold storage, suggesting a possible more rapid breakdown of these compounds in those with ASDs and particularly in those who presented with regressive autism than in those with ‘classical’ autism.\textsuperscript{185}

One small study\textsuperscript{186} has shown an increased level of amino-glycerophospholipids in the plasma of 14 autistic children compared to their sibling controls, also suggesting abnormal erythrocyte membrane composition.

A small double-blinded group-matched randomized placebo-controlled pilot study of omega-3 fatty acids in autistic children provides evidence for a significant effect on hyperactivity and stereotypy in this population.\textsuperscript{187}

Smith-Lemli-Opitz syndrome (SLOS) is a defect in the final stage of cholesterol biosynthesis, due to an inborn error of the gene for 3 beta-hydroxysteroid Delta7-reductase (DHCR7). It results in multiple congenital anomalies.\textsuperscript{188}

One well constructed study has found a high rate of autistic behaviour in those with SLOS.\textsuperscript{189} A further recent study found approximately 3/4 of a sample of children with SLOS qualified for an ASD diagnosis on parental interview, direct observation and a diagnostic behaviour checklist.\textsuperscript{190}

From unselected blood samples from multiplex autistic families, although none demonstrated cholesterol levels consistent with a severe abnormality of sterol metabo-
GABA is converted from and screened for the two most common DHCR7 mutations, which together account for some 65.2% of Polish cases. This study found an incidence of SLOS of some 1 in 2,300 to 1 in 3,937. This suggests SLOS is one of the most common recessive genetic disorders, and, if replicated, would make it the most prevalent genetic basis to autism, exceeding the prevalence in fragile-X.

In a study of a genetic mouse model of SLOS similarly lowered levels of serum and tissue cholesterol were found with impaired response to glutamate by frontal cortical neurons. A more recent mouse model of SLOS produced mice with the following structural CNS differences - commissural deficiencies, hippocampal abnormalities, and, perhaps most interestingly, hypermorphic development of serotonergic neurons. The same group have gone on to look at the immunohistochemistry of these differences, particularly as they affect commissural and hippocampal axonal guidance pathways.

18.4. Glutamate and Gamma-Amino Butyric Acid

Glutamate and Gamma-amino butyric acid (GABA) have received limited attention in the study of autistic spectrum disorders. Researchers have only recently begun to investigate the role of these factors in the pathogenesis and development of autism and the potential implications for treatment.

Glutamate is the principal excitatory amino acid transmitter found in vertebrate nervous systems. An imbalance between excitatory and inhibitory transmitter systems provides a reasonable theoretical model for certain presentations of autism. The physiological effects of glutamate are ubiquitous. GABA is converted from glutamate by the enzyme glutamic acid decarboxylase (GAD). Pyridoxal phosphate (vitamin B6) is an essential co-enzyme in this process. GAD is the rate-limiting step in the synthesis of GABA and reduction in GAD activity would thus lead to excess levels of glutamate. There are some preliminary results suggesting that autism may be associated with abnormal GABA A receptor genes on chromosome 4 and with a defect in the metabotropic glutamate receptor 8 at chromosome 7q13.

Examination of brain levels of GAD in five autistic and eight control subjects at postmortem found that this enzyme was reduced by 48–61% in parietal and cerebellar areas of the brain in individuals with autism compared to controls. As the “excitotoxic” effects of glutamate interfere with neuronal development, and have their maximal effect during the second year of human life, it seems plausible that such a difference in glutamate metabolism may be implicated in the brain differences reported in the ASDs. A further recent study of adult autistic serum levels of glutamate also provides support for the involvement of abnormal glutamergic neurotransmission in autism.

In addition to being the period of maximal effects of glutamate on neural maturation, the middle of the second year is the time when parents typically first note concerns over development in subsequently diagnosed autistic children.

A further postmortem study of 10 autistic and 23 group matched control brains found a range of neurogenetic differences, particularly of interest here being that mRNA levels of excitatory amino acid transporter 1 and glutamate receptor AMPA 1 were elevated, specifically in cerebellum, while AMPA type glutamate receptor density was significantly reduced, also selectively in cerebellum.

The interactions between glutamate, GABA, and the other neurotransmitter systems involved in brain development such as serotonin and dopamine are complex and not yet fully understood (see Ref.15,207 for recent reviews of the neurochemistry).

Various environmental factors such as chronic stress increase circulating glutamate levels, activating N-methyl D-aspartate (NMDA) receptors and increasing likelihood of cell death. Anti-glutamergic agents are receiving increasing attention for their potential role in the management of autism (see Ref.210 for review). Glutamatergic overactivity is also associated known to be associated with increased risk of seizures. It is well recognized that seizure problems are more common in the autistic population, with more severely impaired individuals having a greater likelihood of also having seizures, however the potential link between these various aspects has yet to be systematically explored.

Researchers have found abnormalities of cellular development in the limbic system and cerebellum at postmortem consistent with abnormal antenatal development of these structures.

We now have evidence for differences in the GABA receptor pathways (as indicated by the abnormal GAD levels reported above), in the ionotropic glutamate receptor system (as indicated by the AMPA 1 differences above) and metabotropic glutamate receptors (as...
we will see in the following section).

Glutamate abnormalities appear to occur in autism at a higher than expected rate. These may be primary, and genetic in origin, driven by secondary factors such as anxiety, or a compound of such effects.

18.5. Fragile-X syndrome and autism (AFRAX)

Recent developments in AFRAX provide perhaps the most exciting developments in biological understanding of ASD, linking genetics, proteomics and neurochemistry to the effects of the biological environment on development.

Fragile-X syndrome (FXS) results from a triplet gene expansion at chromosome Xq27.3 of the FMR1 (Fragile-X Mental Retardation 1) gene coding Fragile-X Mental Retardation Protein (FMRP) (see Ref.215). FXS is caused by inactivation of the gene and the reduction or absence of FMRP. The transcription process is partly controlled by Nrf-1 and Sp1 and is not regulated by DNA methylation alone.216 The CGG (cytosine-guanine-guanine) sequence involved in production of FMRP repeats some 6-55 times in the general population, in mothers of affected individuals the same sequence copies some 55-230 times (previously called a ‘pre-mutational’ expansion). In diagnosed individuals some 230 to 1000 copies are typically seen.217 The condition affects the X chromosome in boys who will have inherited their Y chromosome from their father and their affected X chromosome from their mother. De novo mutation and uniparental isodisomy with patrilineal inheritance are possible but neither has been reported to date.

The full mutation form of the Fragile-X expansion is reported as affecting some 1 in 3,717 to 1 in 8,918 of the caucasian male population.218 There appear to be ethnic differences in prevalence - the Afro-American male population being reported as having a prevalence rate of 1 in 1,289 to 1 in 2,545.219 A recent Taiwanese study found only one query positive and 6 premutational cases in 10,046 neonates.220 With a full Fragile-X mutation, 21% of all cases, and 25.9% of males exhibit autistic behaviour and an inverse correlation between severity of autistic symptomology and level of FMRP.221

FMRP is localized in dendrites and reduced in synapses by activation of the glutamate receptor mGluR5.222 mGluR5 can be detected during embryonic development,223 and is most prominent in zones of active neurogenesis.224 Its expression rises through the first two postnatal weeks then falls.225

Neuroanatomically FXS results in an excess of long, thin dendritic spines seen in both human CNS autopsy tissue from affected individuals226 and knockout mouse CNS tissue.227 Dendritic anomalies are seen in various disorders associated with learning problems, with the nature, location and extent of these defects seemingly disorder-specific.228,229 In Fragile X, the effects of the FMRP defect is to produce an abnormality of dendritic spine morphology.230 In certain brain areas, expression of FMRP mRNA is regulated by brain-derived neurotrophic factor.231

FXS has a well recognized physical and behavioural phenotype.232 Seizure problems affect 10-20% of cases, with complex partial seizures being most common.233 FXS is associated with autism at well above chance levels.234,235 Cases where autism has been found in children with pre-mutational expansions236 strengthen the idea of a common underlying pathophysiology to both the Fragile-X-specific physical and behavioural phenotype and autism. This may lead to better understanding of the neurobiological mechanisms that produce and maintain autistic symptomology.

Three recent developments have stimulated interest in Fragile-X syndrome:237

1) The recognition that grandfathers of those affected by Fragile X syndrome often presented in later life with Fragile-X-associated tremor/ataxia syndrome (FXTAS) (see Ref.238 for review), the condition has also been reported in carrier sisters who have preferential activation of the premutation X allele.239

There is also a possible link between pre-mutational expansion and risk of autism with several cases being reported - this suggests that pre-mutational expansions may not be benign but convey risk in pre-mutational males.

Recent studies have shown that inclusion of untranslated FMR1 messenger RNA results in the inclusion bodies seen in the glial cells and neurons in the hippocampus and cerebral cortex of pre-mutation male.241

2) There is strong evidence for premature ovarian failure in female carriers of a pre-mutational expansion.242 There is a particular neuropsychological profile in some female carriers with problems of selective attention.243 There is, in addition, a small effect of carrier status and of the size of premutation expansion on verbal IQ in female carriers, explaining some 4% of the variance.244 and

3) Following on from the previous section, the main effect of the fragile-X expansion is interference with metabotropic glutamate receptor-coupled pathways (specifically those involving mGluR5).245 The mGluR5 theory was first proposed in 2004246 and has quickly established support from animal and human studies. A recent finding is that mGluR5 receptors respond to glutamate preferentially, but also to cysteine, aspartate and asparagine.247

mGluR5 antagonists such as 2-methyl-6(phenylethynyl)pyridine (MPEP) can, in vitro at least, block many of the effects resulting from absence of FMRP.248

Brain areas, including the cerebellum and limbic system have high numbers of glutamate receptors. Researchers have theorized that overactivity of glutamate
may result in "excitotoxicity" and cause aberrant neuronal development. In mice, FMR1 deletion affects the development of various neural structures with abnormal mGluR1 dependent long term depression in hippocampus and in cerebellum. If the glutamatergic system is hyperfunctional, it is likely that neuronal growth and connectivity would be damaged during critical periods of early development.

There is currently much interest in the development of pharmacological treatments targeted at addressing both the abnormal dendritic formation seen at postmortem in the FRAX brain and also in the knockout mouse model of human FMR1. There is exaggerated long term depression of responses caused by the absence of feedback inhibition of mGluR5-induced dendritic translation by FMRP. Long term depression eliminates alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors from the synapse. The interactions in this system are complex, involving a range of other factors including DARPP-32 phosphorylation which is regulated by glutamate.

There appear to be multiple roles for FMRP. From the heterogeneity of expression of FMRP granules in response to oxidative stress and the variability of co-localization of FMRP and T-cell internal antigen (TIA-1), a stress granule marker protein.

In the drosophila dfmr1 knockout model, many behavioural abnormalities mirror those seen in human fragile X patients. Of potential importance is that many of these differences can be corrected through the use of glutamate antagonists. Certain antagonists such as MPEP show reversal of the fragile X physical and behavioural phenotype in animal knockout models and may hold promise in human treatment.

The role of mGluR5 in learning and memory has been extensively studied. From the animal research to date, there are two splice variants - mGluR5a and mGluR5b, with the b variant predominating in the adult brain, and mGluR5 mRNA being found predominantly in hippocampus, amygdala, caudate-putamen and cortex.

There has been a steadily growing interest in metabotropic glutamate receptors as a potential target for pharmacotherapy.

The biology of glutamatergic differences in Fragile-X syndrome is now becoming well understood. The results of treatment studies in animal models are highly encouraging. Human trials of an mGluR5 antagonist (STX107) are to begin in the USA in 2007 (see: Seaside Therapeutics).

19. References


181. Grässbeck R. Intersubjectivity, Affective Neuroscience and ASD


