The Mary Coleman Lectures on Autism

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INTRODUCTION

The enormous complexity of the human brain is ultimately derived from a finite set of molecular instructions, but those instructions are far from being understood. In the course of trying to understand the neurodevelopmental pathways disrupted in the brain that cause autistic symptoms, the scientific community has come to learn a great deal more about brain function in children in general than was known before. The advent of whole exome/genome sequencing and the technology-driven reduction in the cost of next generation sequencing, as well as the introduction of diagnostic targeted sequencing chips, have resulted in an unprecedented volume of data directly linking patient genomic variability to disorders of the brain. This information has the potential to transform our understanding of psychiatric/neurological disorders such as The Autisms by improving diagnoses, illuminating molecular heterogeneity inside each underlying disease, and identifying new targets for therapeutic treatment. A number of neurobiological mechanisms – mechanism of plasticity in the developing brain, signaling mechanisms, neurotransmitter and hormone as well as immune systems (Estes & McAllister 2015) are all becoming better understood due to studies comparing children with autism with control children. Neuroscientists developing rodent models for the underlying disease entities of The Autisms also have increased our understanding of the central nervous system.

In this paper, we are going to explore the following five questions about The Autisms:

1). Is there such a thing as a single disease called autism with a fully penetrant phenotype albeit including a large spectrum, or is autism a behavior pattern seen in many different, separate, disparate disease entities?

2). Should most children with one of The Autisms have a genomic sequencing to establish or confirm a diagnosis?

3). Why is the ratio of boys to girls in The Autisms 3 to 6 boys to just 1 girl?

4). Why do some children appear to be developing normally and then rather suddenly regress while other children just gradually develop autistic traits?

5). At the present time, what is the best approach with any chance of success of developing a medical therapy for a child with autism?

Autism was first described in the medical literature in the twentieth century but to fully understand the diagnostic implications of this disease we need to start back in the nineteen century. For many centuries, there had been children who were slow to walk, talk and learn and, by the nineteenth century, they were medically labeled as having a disease entity called Mental Retardation.

We start out with two such children who shared a number of characteristics – they had the same major symptom (great difficulty in learning), they were the same age (18 months) when this developmental problem was discovered and they both had a striking feature – protrusion of their tongues, called macroglossia. They were considered examples of children who had a disease entity then called Mental Retardation. (Today we call it Intellectual Disability).

However in London in 1866, a sharp-eyed clinician, Dr. John Langdon-Down, noticed that there were small physical differences between children who were slowed in their developmental milestones and wrote a paper suggesting that they might have different disease entities. He was right – we now know that one patient had an extra chromosome (his disease today is called Down syndrome after Dr. Down) and that the other patient had too little of a hormone from the thyroid gland (his disease today is called infantile hypothyroidism). These children had completely different diseases yet had very similar or identical symptoms. So gradually, during the late nineteenth and early twentieth century, physician researchers slowly stopped publishing long, statistical papers about retarded children all jumbled together in large groups, and began examining and diagnosing each child, one by one. In doing so, they discovered many, many new syndromes. Mostly only the epidemiological papers continued to be written about all the children grouped together, because it was necessary for state planning to know the total number of Intellectual Disability children there were at any one time in a state.

Today, in the twenty-first century, we know there are a large number of disease entities with tongue protrusion at an early age, often seen in those who later exhibit Intellectual Disabilities; macroglossia also can be seen in many children who develop normally. Today we even know that the macroglossia differential diagnosis includes some very rare infants with early childhood autistic features who then recover (Zappella 2015).

It is now almost 150 years since Dr. Down wrote his paper which led clinicians to examine each retarded child as an individual; by now, a large majority of those disease entities in children with developmental cognitive delays have been identified and their etiologies confirmed. This diagnostic process was accelerated during the twentieth century, particularly after Crick and Watson announced the double helical structure of the genetic code of DNA in 1953, Lejeune and Gautier announced that humans had 46 chromosomes in 1958, and the human genome was deciphered in 2000. Techniques were developed to examine the underlying chromosomal and genomic etiology of each disease entity with Intellectual Disabilities. It was discovered that sometimes chromosomal abnormalities were found; in other cases monogenic etiologies were found; these children were studied individually one at a time and the results confirmed in other individuals with the same phenotype. It is now known that many mutated genes, including those on major synaptic signaling pathways, are involved in many cases of Intellectual Disability (Pavlowsky et al 2012). There were other causes, too -- some of these individuals had trauma or infections or toxins or tumors injuring whole pathways in the brain.

The Discovery of Autism

During the twentieth century, another group of unusual children were reported in the psychiatric literature. They were first described by Kanner in 1943 and then by Asperger in 1944. They were characterized by impaired social interaction, impaired verbal and non-verbal communication, and highly

repetitive patterns of behavior and restricted interests. Kanner called the children "autistic", a term he borrowed from Eugene Bleuler, who had coined it to describe the inward, self-absorbed aspects of schizophrenia in adults, although Kanner realized these young children had a quite difference syndrome from schizophrenia. Some of Kanner's patients were mute. Two of the parents of the eleven patients described in Kanner's original paper had occupations exposing them to mercury compounds.

The description in a paper by Asperger with these same characteristics of impaired social interaction combined with highly repetitive patterns of behavior differed from Kanner's description by the relative preservation of linguistic and cognitive development in the children Asperger described. But his paper was ignored by the medical community for the next thirty-five years until 1981 when Lorna Wing got a translation of Asperger's paper from German to English and she published it with her own account. She relabeled the disorder with Asperger's name, calling it "Asperger Syndrome" because she feared that the term he had used in his original paper "autistic psychopathy" might have suggested violent behavior. In an historical sidenote, a recent author has claimed that Asperger also was aware of low functioning patients with these behavior patterns, but published only about the high-functioning individuals because he lived in German-controlled Austria during World War II when the Nazis were euthanizing the "feeble-minded" in the countries they controlled (Silverman 2015).

Today, individuals with both autism and Asperger Syndrome may be combined under the overall name of autism spectrum disorder (ASD) or as having one of The Autisms, a term I prefer. The technical meaning of the word "spectrum' is patients having difference degrees of disability inside a specific, definable single disease entity, which is not true at all of autism. **Autism is not one disease**; autistic patterns of behavior are found in many, many different diseases.

Many of these children with autism have comorbidities besides their basic autistic symptoms of impaired social interaction, highly repetitive patterns of behavior and restricted interests and impaired verbal and non-verbal communication. In addition, many had co-morbidities including intellectual disability ranging from mutism to lowered IQs, attention deficit/hyperactivity disorder (ADHD), obsessive compulsive disorder, bipolar disorder, anxiety or depressive disorders. Seizure disorders are more prevalent than in the general population, and statistically found more often in patients with autism who have tall stature (Valvo et al. 2013); in fact, epilepsy that runs in some multiplex autism families may define a separate subgroup of The Autisms (Amiet et al. 2013). When young, one in five of children with autism may have Pathological Demand Avoidance (Gillberg et al. 2015). Their parent's lives may be made miserable by a child's sleeping problems (Silvertsen 2012), self-injurious behavior (Edelson 2015), aggression, severe tantrums (Hodgetts et al. 2013) and eating/gastrointestinal problems. When the children reach adolescence, osteoporosis or catatonia can occur. There are a plethora of *secondary* medical therapies designed to treat these comorbidities, but many are not very successful. The eventual goal for each child is a specific *primary* medical therapy to reverse the underlying mechanism of action of the underlying autistic disease process causing the symptoms in the first place.

Kanner had thought that autism was one disease, but as children with autism began to be tested biochemically, it was puzzling that they did not have consistent biochemistries. One of the first biochemical abnormalities to be tested in a group of children meeting autistic criteria was a neurotransmitter, serotonin, which could be tested in the platelets of the blood. The results showed no consistency -- there were serotonin levels that were elevated in a great many of the patients; on the other hand some were normal and still others were below normal (Schain and Freedman 1961; Goldstein et al. 1976). A large number of biochemicals were then tested and the results were all over the map -- generally found inconsistently between patients (Coleman & Gillberg 2012). In retrospect, several of the most relevant finding from that early period of laboratory testing was the variation in calcium – one out of five children with autism were found to have hypocalcinuria (too little or even no calcium in their urine) (Coleman 1976; Rosenthal, 1985, Palmieri et al 2010)) and abnormalities in purine metabolism in preadolescent children were documented (Coleman 1976; Rosenthal, 1984, Page

& Coleman, 1998, Feron et al. 2015). Later clinical studies also began to back-up the concept of heterogeneity; the presence of different distinct developmental pathways for infants even though they all ended up with the diagnosis of autism spectrum disorders was demonstrated (Chawarska et al. 2014).

It was discovered that a few individuals presented with autistic symptoms because of infections, teratogenic drugs, endocrine syndromes, or brain tumors but these were only a tiny handful of cases. There was no answer for the overwhelming number of cases. A number of studies including comprehensive Scandinavian studies found an estimated heritability rate in autism in monozygotic twins as high as 90%. It was this finding in monozygotic twins combined with the inconsistent biochemical findings which helped push the medical community to begin looking at genetic studies in autism. Soon it became undeniable that autism, like Intellectual Disability with which it often overlapped in clinical presentation, apparently was a series of different diseases with different biochemistries and different mechanisms of action. From an etiological perspective, no disease entity of either pure autism nor pure Asperger's syndrome so far has ever been found. Autism and Asperger's syndrome remain a diagnostically identifiable set of behavioral manifestations seen in hundreds of different disorders. There is no evidence that there is a unique "autism gene." It is important to note that none of the disorders implicated in autism to date by DNA studies are associated with autism in 100% of the cases; none exhibiting a fully penetrant autism phenotype.

Thus, there is answer to the first inquiry -- Is there such a thing as a single disease called autism with a fully penetrant phenotype or is this behavior pattern seen in many different, disparate disease entities? The answer is no, there is no unique disease called autism and yes, autism is a behavior pattern seen in many different, separate, disparate disease entities.

The Genetic Code

Here is a very brief over-simplified review of some of the basics of the genetic code that will be mentioned throughout in this paper. (Bored professionals can skip this section) We start with the 46 human chromosomes, containing our genetic material. They are in pairs, each chromosome containing genetic information from either a father or a mother, with each chromosome having a long arm and a short arm. They are numbered roughly by size. In the male chromosome, the 23rd pair consists of an X and a Y; the female has two Xs. We shall be mentioning chromosomes 2, 5, 7, 15, 17, 18, 22 and the X chromosome in this lecture. Bits of the chromosomes can be missing – they are called deletions or, if tiny, microdeletions. Parts of the chromosomes also may be duplicated – they are called duplications or, if tiny, microduplications. There has been duplication of entire or partial chromosomes, one of the factors in evolution. It is interesting that in some species such as salmon, this process has occurred so recently that four copies of what is essentially the same chromosome can be recognized in 4 separate species of salmon.

DNA has a phosphate-sugar backbone attached to 4 nitrogen-containing bases – A, T G and C. Two of these bases (A - adenine and G - guanine) create purines - a pathway of interest in autism - and the two others (C - cytosine and T - thymine) create pyrimidines. These compounds form base-pairs which creates the double helix. In all cellular organisms, excluding viruses, DNA is the inherited material responsible for the genetic composition of every cell. Each chromosome is made up of double helices of DNA tightly coiled many times around proteins called *histones* that support its structure. This DNA and its associated proteins and macromolecules is collectively known as *chromatin*, which is further packaged along with its associated molecules into a discrete structure called a *nucleosome*. The long strings of DNA contain what is called the *genome,* in the case of homo sapiens it is called the human genome. DNA is a remarkable stable molecule. It is fascinating that the genome of the ancient Neanderthals, who died many thousands of years ago, can still be been replicated in lab studies and are

99.84% similar to the DNA of homo sapiens (Paabo 2014), raising the question of whether the Neanderthals were indeed simply another form of human beings.

The RNA molecules, made from DNA, are copies of DNA except for a slightly different sugar in the backbone and are much less stable, a few RNAs surviving only minutes within the cell. RNA is used for many different purposes as we shall see, and some hold the key to the dysfunction that results in autistic features.

The genetic code is the set of rules by which information encoded within genetic material (DNA decoded by mRNA sequences) is translated into proteins by living cells. Biological decoding links the amino acids reading the mRNA three nucleotides at a time. The code defines how sequences of these nucleotide triplets, called *codons*, specify which amino acid will be added next during protein synthesis. Protein biosynthesis is the process whereby biological cells generate new proteins from DNA; biosynthesis is balanced by the later loss of the cellular proteins via degradation. Having the exactly the right amount of correct proteins in each cell at the right time and place is how the brain operates, as we have learned in some cases with autistic features.

The human genome has 3 billion nucleotides (DNA bases). It is indeed a very complex allelic architecture. Thus the problem ahead to find the underlying genetic mutations for each child with autism in their genome is quite an overwhelming, challenging task. The genome has many other variations besides substituting one nucleotide for another. These include deletions, duplications, insertions, transpositions, inversions and other rearrangements. Small duplications or deletions, called copy number variations, are very common in the human genome and a number o them of them have been implicated in The Autisms.

Human DNA is thought to contain around 25,00 distinct genes; however surprisingly they take up less than 2% of the human genome. Originally it was believed that “one protein, one gene”. However the number of potential protein species turned out to be much larger because of the splicing mechanism. Introns may be removed to produce an mRNA protein and there can be variations so that this alternative splicing can result in quite different proteins arising from a single protein-coding region, a significant cause of the physiological variation between cells in different tissues, even though they have identical DNA structures. This is one factor to keep in mind since, when planning a therapy for a child with autism, we often make DNA diagnoses from blood cells, inferring that that protein is identical in the brain, which is not necessarily the case.

As though 3 billion nucleotides and 25,000 genes weren’t enough of a challenge, it has been found that the brain has 85-100 billion neurons that are connected by a neural network, called the connectome or the wiring diagram of the brain. Modern genomic technologies have begun to facilitate the discovery of relevant genes that affect this neural network. In autism, there are no big diseases with lots of patients, like Down syndrome (trisomy 21) as found in Intellectual Disability. In the case of autism, when you enter this field, you enter the world of rare diseases, where huge numbers of different genes are affected. However it is possible that, in spite of the large numbers and heterogeneity of autism candidate genes, many of these genes theoretically might converge into a series of interconnected functional modules that possibly could be someday targeted therapeutically (Pinot et al. 2014, Hormozdian et al. 2015). Some clinicians characterize individuals with autism into two clinical groups -- as having either a more classic type of autism (beautiful children with virtually no stigmata) or as having syndromic autism, where facial and other bodily symptoms are obviously affected, but this distinction is not holding well as more genomic data is gathered.

Rare inherited mutations and even small *de novo* copy number variations (Poultney et al. 2013) are both significant contributors to autism etiology. One study sequencing 44 candidate genes for mutations in 2446 probands discovered 27 *de novo* events in 16 genes, 59% of which were predicted to truncate

proteins or disrupt splicing; this one study alone reported that 6 genes (*CHD8, DYRK1A, GRIN2B, TBR1, PTEN and TBL1XR1*) may account for an estimated 1 percent of all sporadic cases (O'Roak et al. 2012). Since then many, many more candidate genes have been identified. Including copy number variants, another study predicted that coding *de novo* mutations contribute to about 30% of all simplex diagnoses (Iossifov et al. 2015). Because of the extreme heterogeneity in The Autisms, it is known that only a fraction of autism genes have been discovered (Cukier et al. 2014).

Another complication to interpreting a mutation in an individual with autism is the reality that many different mutations can be identified inside the same gene, often causing different levels of clinical presentation. The *CHD8* gene, for example, associated with one autism subtype of macrocephaly has 20 independent mutations, some found after the original paper was published (Bernier et al. 2014). This is not an uncommon pattern; the longer a disease is studied with genetic techniques, the greater variety of the locations of mutations inside the causative gene is found.

To the clinician struggling to find the answer for their patients, there is literally an avalanche of genomic data in each case, making it quite a challenge to disentangle causation versus correlation versus irrelevant findings. Criteria that can by used are 1) identifying the same mutation in a number of other patients with similar clinical presentation, 2) identifying variants not found in geographically matched controls, or 3) the fact that the gene codes for a protein which involves amino acids that were evolutionarily conserved.

A great many of the mutated genes discovered so far in autism are important genes in neurodevelopment – that is genes involved in the formation of the brain *in utero*. They are involved in the proliferation, migration, guidance targeting and final connectivity of the neurons of the brain as can be seen in a picture of the genes involved in the (mis)development of the prefrontal cortex (Schubert et al. 2014). Other genes disrupted in autism encode proteins for synaptic formation, transcriptional regulation and chromatin-remodeling pathways (De Rubeis et al. 2014). In particular, perturbed synaptogenesis has been found in a number of different disease entities.

Examples of Diseases with Autistic Features.

To illustrate the role of genetics in diagnosing individuals with one of The Autisms, we have selected one of the relatively common disease entities (by relatively common we mean that up to half of 1% of individuals with autism meet the criteria); this disease is called the Angelman syndrome. Somewhere between 40% to 80% (depending upon the study) of these Angelman children have autistic features. They were once called the Happy Puppet syndrome, actually an insulting label to apply to a child; they were called that because they often had a happy disposition including unprovoked laughter combined with a mild spasticity making them stiff. But this syndrome is no joke -- it is a very serious syndrome with a severe level of intellectual disability and speech impairment. One of the major characteristics of Angelman syndrome is the unusual developmental profile in which cognitive abilities are stronger than receptive language skills that in turn are stronger than expressive language. The young children are hypotonic; most do not speak or say only a few words. They may have widely spaced teeth and mouthing behaviors. The children wake up multiple times during the night, making their parents miserable. Seizures occur and are hard to control; even children without seizures have abnormal EEGs. These children are often attracted to water. On imaging studies, they have a thin corpus callosum.

The Angelman syndrome is thought to be caused by the lack of expression of the maternally inherited *UBE3A* gene in the brain; 70 % of individuals have a large deletion in the fifteenth maternal chromosome on its long arm -- in the 15q11-q13 region which contains the *UBE3A* gene. Mutations in *ATP10A*, a maternally inherited neighboring gene, may contribute to increased severity of the Angelman syndrome in the deletion cases. In 10% of the patients, the maternally inherited *UBE3A* gene may be mutated

alone. About 5% of the patients have an imprinting defect and about 2% have a paternal chromosome 15 uniparental disomy, a less severe form of the Angelman syndrome. Studies in mice deficient in Ube3a suggest that this gene is necessary for maintaining plasticity during experience-dependent neocortical development (Yashiro et al. 2009).

Although suppression of the *UBE3A* gene is considered the *sine qua non* of Angelman syndrome (Williams et al. 2010), only about 90% of the Angelman syndrome patients have the identifiable *UBE3A* molecular defect. So what causes the syndrome in the other 10%, who could be called Angelman syndrome mimics (Tan et al. 2014).

One of the first reported mimics was the Phelan-McDermid syndrome, an autism syndrome (Phelan & McDermid 2012) thought to be due to insufficiency of the *SHANK3* gene. *SHANK* genes code for scaffold proteins located in the post-synaptic density of glutamatergic synapses (Leblond et al. 2014). *SHANK3* also is often missing in patients with the 22q13.3 deletion syndrome, although not in every case. The children with the Phelan-McDermid syndrome mimic the global developmental delay with absent or minimal speech, the seizures and the mouthing behavior. Like Angelman syndrome, they have a thin corpus callosum. However they usually have large ears and hands and dysplastic toenails, not typically associated with the Angelman syndrome. Although the gene involved in this syndrome is the *SHANK3* gene on the long arm of the 22nd chromosome, neighboring genes such as *MAPK81P2* may influence the severity of the syndrome. A double-blind, placebo-controlled crossover design has shown that there may be a therapy (insulin-like growth factor-1) for these children that can reverse synaptic plasticity and motor learning defects (Kolevzon et al. 2014) in this disease.

Another autism syndrome that is an Angelman mimic is a deletion in the long arm of the 2nd chromosome at 2q23.1 causing the *MBD5* Haploinsufficiency Syndrome, which has been reported to initially been diagnosed as an Angelman-like syndrome in many affected individuals. As its name describes, the deletion leads to haploinsufficiency of the gene *MBD5*. Again there is global developmental delay, absent speech in most although a few children eventually become able to speak in short sentences. There are seizures and EEG abnormalities. However, these children can become microcephalic, which is not typical of Angelman syndrome.

The chromosome 17q21.31 deletion syndrome, called the Koolen-de Vries syndrome, is another Angelman mimic. This condition resembles the Angelman syndrome in that the cognitive development appears to be more advanced than the language development and the young children are hypotonic. They also are friendly and happy, sometimes laughing easily or frequently. Seizures are common. Haploinsufficincy of gene *KANSL1* is thought to cause the syndrome (Koolen et al. 2012) but neighboring genes may modulate the phenotypic severity of the disease.

I think you get the idea of these mimics, but I'll just briefly mention two other syndromes which can be mistaken for the Angelman syndrome in certain cases. These cases are well-defined monogenetic syndromes. There is the Pitt-Hopkins syndrome caused by loss-of-function mutations in gene *TCF4* on chromosome 18q21.1 (Whalen et al. 2012). Also there is the Christianson syndrome found only in males; this is an Angelman-like syndrome (Schroer et al. 2010) that is caused by loss of functional mutations in gene *SLC9A6* on the X chromosome at Xq26.3.

No one yet knows how many autistic syndromes have a genomic error that can be identified, but we already know there are close to 200 and speculation has gone as high as up to a thousand loci (Pinto et al. 2014). So to even list the current ones would take a great deal time. Instead we'll list the far fewer disease entities discovered so far in Asperger syndrome. (Betancur & Coleman 2013).

Why is it so important to discover the gene or genes that are mutated in any individual patient with autism or Asperger syndrome? Because the essential first step to reverse any disease is to understand

its failed *mechanism of action*, which is revealed by the gene mutations. So far there is no medical treatment available yet for Angelman syndrome and only one of its mimics, the Phelan-McDermid syndrome, has a research medical therapy available at this time for patients. In other cases, the progress of developing medical therapies for children is well underway in three other diseases with a subgroup of patients with autistic features – in tuberous sclerosis (Trans & Zupanc 2015), fragile X syndrome and Rett syndrome (Wang et al. 2015). We have begun.

So we now have an answer to the second inquiry -- Should children with one of The Autisms have a genomic sequencing to establish or confirm a diagnosis? The long-term answer in most cases is yes, since genomic screening is now entering an affordable range. These are life-long illnesses and each family should be able to know what appears to be wrong with their child's genome so they and their physician can be alert to any new developments in the field that might affect their child.

Epigenetics

The conventional 'one gene, one protein' model has turned out to be an oversimplified view of how proteins are formed. There exists a second genetic system at the cellular level that regulates gene expression; it is called the epigenome. We shall spend a bit of time on this subject because deciphering how the epigenome works is likely to make major contributions in the future to autism research. Every cell in the body carries the same genome (with a few rare exceptions) but the epigenome changes with cell and tissue type, as can be identified by epigenetic marks. Epigenetics is the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequences. Interestingly this regulation of gene expression can be passed on to a person's progeny even though these effects are not due to changes in the nucleotide sequence of the gene -- that is, epigenetics reveals the long-term alterations of DNA function that do not involve changes in the DNA sequence itself. That epigenetic effects can be passed on to the next generation is suggested by a new study of the children of German Jews who lived through World War II – children born after the war share with their parents evidence of DNA epigenetic methylation of *FKBP5*, a gene related to stress and anxiety (Yehua et al. 2015).

Numerous noncoding RNA (ncRNAs) classes have now been identified and are implicated in central nervous system function. The interrogation of noncoding regulatory regions in sequencing studies is hindered primarily by the massive size of the noncoding genome and the present limited data on its functionality in the brain.

Epigenetics offers a key missing link in the dynamic interplay between experience and the genome in sculpting neuronal circuits and neuronal properties, an openness and plasticity of the social brain (Meloni 2014). The genome and epigenome operate together. Based on studies of human pluripotent stem cells, the neural development of neural differentiation regulatory networks in embryos can be recapitulated *in vitro* and epigenetic priming at transition factor binding sites can be demonstrated (Ziller et al. 2015). It is already clear that some neurobehavioral phenotypes can be epigenetically controlled by non-coding RNAs (Kocerha et al. 2015).

A fixed genome can respond in a dynamic way to a changing environment and produce different phenotypes from a single genome. The metabolites needed for DNA methylation and histone modification are extraordinarily sensitive to environmental feedback and nutrient stress. For example, different nutrition, via its impact on DNA methylation, is responsible for the different phenotypes between a sterile worker and a fertile queen honeybee (Kucharski et al. 2008). Another example of the epigenetic results of nutrition has been seen in humans. The so-called *Hongerwinter* was a six-month period during which food deliveries to the people of the northern Netherlands were restricted by the German Nazi occupying forces. This produced a humanitarian disaster. By April 1945, it was estimated

that 20,000 people had died as a result of malnutrition; many expectant mothers survived on between 400 and 800 calories a day. However, the fact that this brief famine struck a reasonably well-nourished population allowed an almost unique opportunity for later scientists to study the effects of malnutrition on a group of children conceived around that time. In this case, a group of almost 300 adults in their late 50s, all of whom had been exposed to the famine in the first or second trimester of their mother's pregnancy, were given genetic and mental tests, and the results compared to those of similarly aged people. As adults, these individuals were shorter and had many more general health problems, including neurological and psychiatric problems. They were found to have a different molecular setting of methylation, an epigenetic effect (Tobi et al. 2014). In mice, it has been shown that changes in the dietary glycemic index during prenatal and developmental periods has a significant impact on ASD behaviors (Currais et al. 2015).

One of the classes of non-coding RNAs, the microRNAs (miRNAs) are ubiquitously expressed throughout the brain and are thought to govern many major neuronal pathways, including many CNS synaptic functions and glutamate signaling. Pleiotropic ncRNAs, including miRNAs, can target large numbers of genes and signaling pathways simultaneously (Bartel 2009; Nam et al. 2014). Many other different ncRNAs also are implicated in brain function; long intergenic RNAs (lncRNAs) and natural antisense transcripts (NATs); all have been reported to have regulatory activities in the brain. Regions of the brain expressing enhancer RNAs (eRNAs) are enriched for genetic variants associated with The Autisms (Yao et al. 2015). There also are other ncRNAs, such as NATs, which hybridize a limited and subset of candidates (Faghihi & Wahlestedt 2009). All classes of ncRNAs are possible targets for future therapies – what is quite interesting is that the pleiotropic ones possibly could affect more than one disease entity; the more targeted ncRNAs might be limited to a single disease based upon the mechanism of action of that particular disease.

miRNAs function by several different known mechanisms. One is the process of RNA interference, an action of miRNAs to alter RNA stability and translational efficiency. They can modify ribosomal RNA as well as affect alternative splicing and regulatory mechanisms mediated by RNA-RNA interactions.

DNA methylation, the addition of a methyl group to a DNA base often can silence gene expression. Given its crucial function as a regulation of gene expression, methylation has been defined as the “prima donna” of epigenetics. Methylation of cytosine nucleotides is a well understood epigenetic mechanism and can be identified in collections of CpG dinucleotides that often occur upstream of genes. DNA methylation during spermatogenesis is an active process, which makes it susceptible to errors that can be propagated to subsequent generations (Milekic et al. 2015). Monozygotic twins discordant for autistic traits may demonstrate the role of DNA methylation *in utero* (Wong et al. 2013).

Prominent among other epigenetic mechanisms is histone modification. This is the post-translational modification of histones, especially by methylation and acetylation of specific lysine residues on histone tails. These alterations change the association of histones into nucleosomes, and their subsequent interaction with DNA to form higher-order chromatin structures, chromatin remodeling. This can affect the expression of certain genes and also be transmitted from one cell to its progeny, in a similar way to cytosine methylation.

A third major epigenetic mechanism, nucleosome remodeling, has been implicated in human developmental and Intellectual Disability disorders. Nucleosome remodeling is driven primarily through nucleosome remodeling complexes with specialized ATP-dependent enzymes. These enzymes directly interact with DNA or chromatin structure, as well as histone subunits, to restructure the shape and organization of nucleosome positioning to ultimately regulate gene expression.

Epigenetic marks include DNA methylation and hydromethylation, covalent histone modifications, chromatin folding and ncRNAs. A number of examples of diagnosed disease entities with one of The

Autisms as well as cases of idiopathic autism have been shown to have DNA methylation, chromatin remodeling, altered ncRNAs and also imprinting (Coleman & Gillberg 2012).

There is a strong history of mutations in GABA receptor genes being involved in neurological diseases, particularly the epilepsies. In addition, a substantial number of variants and mutations have been found in GABA receptor genes in patients with autism, suggesting potential links between the GABA receptors and diseases of The Autisms.

In the central nervous system, the processing from a primary transcript to a precursor and then mature miRNA requires standard miRNA biogenesis machinery; this includes Dicer, Drosha, DGCR8 and argonaute proteins. In mice, it has been shown that miRNAs are formed in part by processing of pre-miRNAs locally within dendritic spines (Lugli et al 2005; Glanzer et al. 2005). Synaptic stimulation then leads to local processing of pre-miRNAS in proximity to the synapse (Smalheiser 2008). A cohort of a number of different miRNAs are located within the synapse and can directly affect neurogenesis throughout development from the embryo to the adult. They also are involved in neurotransmission, learning and memory.

This processing from a primary transcript to a precursor and mature microRNAs requires standard microRNA biogenesis machinery, which includes the enzyme Dicer (encoded by the *DICER1* gene) and other proteins. Functional knockdown of Dicer, which processes the pre-miRNA to its mature transcript, leads to reduced neuronal size and branching as well as aberrant axonal pathfinding. MicroRNAs and long non-coding RNAs and their processing genes are susceptible to regulation by well-established signaling modulators such as BDNF, CREB, calcium and calcium-responsive neurotransmitters.

Recently it was reported that Dicer activates proteolytic cleavage under conditions of elevated calcium levels. This is quite interesting because it has been reported that six children with autism had calcium levels significantly elevated in their temporocortical gray matter of the central nervous system on autopsy (Palmieri et al 2010). Abnormal levels of intracellular Ca(2+) appear to be associated with some types of autism (Krey & Dolmetsch 2007) (Qiu & Cheng 2010). Genetically there is a list of nine proteins encoded by calcium-related genes found to be involved in autism; mutations in those genes all result in abnormal calcium homeostasis in the patients (Napolioni et al. 2011). Although the mechanism of action and locations in the cell involved in each gene mutation are different, they each have the same result -- amplifying Ca (2+) signaling. During early stages of brain development, abnormalities of Ca (2+) signaling have been described (Zou et al. 2015). As early as 1976, one in five children with autism had already been found to have a paucity of calcium levels in the urine, suggesting that their bodies might have selectively moved extra calcium to the brain. Extra brain calcium raises the question of increased microRNA biogenesis, a factor in brain epigenetics.

<u>Why do more Boys than Girls suffer from a disease entity presenting as one of The Autisms?</u>

A typical finding in surveys of the autism disorders is a ratio of 3 to 6 males for every 1 female patient (Ellefsen et al. 2007). There are endless papers that study a group of children with autistic features that have all of the cases mixed together in an attempt to answer that sex ratio question. For example, there are papers that show sex differences in the corpus callosum of boys compared to girls (Nordahl et al. 2015). Several papers have tried to show that there is a female protective effect (Mottron et al. 2015); but further study can not confirm this hypothesis and have shown this theory is incorrect (Messinger et al. 2015).

Instead there is a much clearer answer to why there are more boys than girls with autism, a phenomenon seen in many other pediatric neurological and psychiatric diseases. The answer is that each child put into these conglomerate studies has a different disease entity with a different disease

mechanism of action. Although many disease entities occur in both sexes, each individual disease has its own male/female ratio; some are 50:50, but many are not. A number of prominent diseases, such as the fragile X syndrome have a strong male predominance. Some disease entities with autistic features are almost completely male (up to 99%) such as the *MECP2* duplication syndrome and the dysmaturational/Tourette autism syndrome, also known as the Zappella syndrome. In contrast, only a very small handful of disease entities with autistic features are found primarily in females. Recently a 2015 paper by Mandy and Tchanturia is the latest to confirm what Christopher Gillberg predicted in 1983 -- girls and women with eating disorders are likely to have an unrecognized form of autism that predates the onset of their eating problems.

One mechanism that causes disease entities to be more often seen in boys than girls is that boys have only one X chromosome, while girls have two. If a boy has a gene with a mutation on his single X chromosome, he may not have another copy of that gene to compensate for the altered gene on his Y chromosome, and this can cause a genetic disorder; conditions that are inherited in this way are called X-linked disorders, which have been documented in a certain cases of autism. These disease entities usually combine Intellectual Disability with features of autism. One listing of genes mutated in autism on the X chromosome was made and listed 44 such genes (Betancur & Coleman 2013); since then at least 17 new mutations of genes on the X chromosome involving autism have been found in individuals with one of The Autisms (Cukier et al. 2014; Ardisonne et al. 2015).

So the answer to the third inquiry -- Why is the ratio of boys to girls in The Autisms 3 to 6 boys to 1 girl? – appears to be the sex ratio of the underlying specific disease entities of the patients selected for any given study, some with diseases which have overwhelming male clinical presentations.

Why do some patients regress while others do not?

We are beginning to understand why some patients with autistic features show a gradual presentation with symptoms while others appear to have a normal development and then have a rather sudden regression to autistic features, usually between 18 to 36 months of age. We know from adult neurology (Escott-Price et al. 2015), where it is better worked out than in pediatric neurology, that in monogenic diseases, the precise age of onset may be determined by the contribution from the presence of one or more often common polygenic alleles of relatively low effect that happen to be present in that particular patient. In other words, one or more other alleles with other developmental tasks may become functional at that specific age and combined with the essential monogenic mutation together precipitate the presentation of the autistic phenotype. These other alleles do not have even necessarily have to be risk factors for one of The Autisms; they may be common polygenic alleles present in that individual; in that sense each patient may be unique. It could even be argued that because common polygenic alleles are used for population identification studies, this may be a factor in why some forms of monogenic autism present earlier or are more frequent in certain countries.

So the answer to the fourth inquiry -- Why do some children appear to be developing normally and then rather suddenly regress while other children just gradually develop autistic traits? – appears to be related to both the presence of other alleles in that particular child as well as to the developmental timing when a particular mutated gene is signaled to begin to produce its protein in significant amounts.

Why finding the exact mechanism of action causing a genomic disease is so important

A basic principle of medicine is that the ideal way to reverse a disease is to identify its exact mechanism causing the symptoms and then find the ways to reverse that mechanism of action. Usually the mechanism of action can be determined by by identifying exactly which gene is mutated, most easily in classic Mendelian cases. However, in many cases unfortunately it is more complicated than that. There is evidence of multigene contributions to many of The Autisms. One example of this complexity is the

FMR1 gene. The pathological mechanism causing the fragile X syndrome in children with autism is altered protein synthesis-dependent plasticity due to excess messenger RNA translation. Understanding this mechanism has led to the development of therapies (Dolen & Bear 2009) (Scarf et al. 2015). But if the mutation of the *FMR1* gene does not clinically present until late adult life, it causes a different syndrome called the fragile X-associated tremor/ataxia syndrome. The mechanism of action in these cases appears to be a toxic RNA gain of function -- and I happy to report a paper has just come out (Hukema et al. 2015) showing for the first time the potential of disease reversibility for these adult patients.

The imbalance between glutamatergic and GABAeric synapses was postulated in the past to be involved in the etiology of autistic symptoms. Glutamate signaling through NMDA receptors on the surface of spines is necessary for the plasticity of excitatory receptors. Deregulation of glutamate signaling is seen in a number of different disease entities with autistic subgroups – fragile X, PTEN hamartoma syndrome, and tuberous sclerosis. Genome dosage changes in the 15q12 region represent one of the most common chromosomal changes in autism and this region harbors three GABA receptor genes. Now an anti-NMDA receptor encephalitis has been reported in a boy of 33 months of age who fits diagnostic criteria for regression in autism (Scott et al. 2014). This type of encephalitis is well studied and a treatable autoimmune disorder, found usually in women and older children. Although this case in a young child does help support the glutamateric/GABA imbalance hypothesis of autism, it is still just one case. However what is particularly interesting about this case is that anti-NMDA receptor antibodies often develop during or after herpes simplex encephalitis. Herpes simplex encephalitis is one of the major types of the many different kinds of encephalitis that is been reported to have autistic symptoms, sometimes even in older children. This case raises the question of whether this could be why herpes encephalitis patients become autistic? However it is important to keep in mind about this new single anti-NMDA receptor encephalitis case with autistic symptoms is that we have so many, many other cases of this encephalitis of older ages, that **do not** have autism but sometimes can have a variety of psychiatric symptoms (there is a least one case diagnosed as schizophrenic).

Knowing the precise mechanism of action is the essential first step in the development of successful therapies in any brain disease. However additional knowledge is also needed, since the metabolic processes are defined not only by which enzymes are present and the extent to which they are produced but by physical interactions between the enzymes, interactions with regulatory proteins, as well as activation or inactivation by small molecules that might be present.

To the clinician, there is literally an avalanche of genomic data, making it quite a challenge to disentangle causation versus correlation versus irrelevance in each case. There have been attempts to define subgroups (Amiet et al. 2013). And there have been so many unexpected and not understood findings as we study the genomes of these children. One recent surprise was a study by Yuen et al. (2015) in a study of whole genome sequencing in 85 quartet families, which studied *de novo* and rare inherited single-nucleotide and structural variations in genes. Very surprisingly, they found that approximately 70% of affected siblings in the same family carried *different* mutations thought to be autism-relevant.

So regarding the answer to the fifth inquiry -- At the present time, what is the best approach with any chance of success of developing a medical therapy for a child with autism? -- there are no final answers. However, uncovering the mechanism of action of the underlying disease of the child with autistic traits is an important first step. Theoretically, as seen both by the genetic and epigenetic literature, it may someday be even possible to devise a more generalized therapy which helps several underlying diseases at the same time, but that possibility is still quite far away.

Neural Networks

Although a large number of gene defects have been found studying individuals with autism, the commonality of the autism phenotype – so consistent that Kanner thought it was one disease – suggest that these genetic defects may act through a specific, limited set of convergence pathways. This convergence of symptoms may be assessed at many levels, ranging from the connectome, (the neural networks of brain circuitry) to various molecular pathways. It is an overwhelming task in view of the genetic complexity underpinning these diseases.

In the case of the connectome, we are searching for functional connectivity neural networks; that is, distributed brain regions interacting to perform a specific neural function. It appears that virtually every part of the CNS, from the brain stem to the frontal lobe, has been found to be involved by one research paper or another in an autistic network. A basic question to ask is are we looking for networks for the social brain in general or are we looking for one or more separate pathways, such one for communication, another for impaired social interaction and a third for rigid or repetitive behaviors (Happe et al. 2006). Brain connectivity datasets comprise networks of brain regions connected by anatomical tracts or by functional associations. (There is a non-technical introduction to complex network analysis of brain connectivity in a paper by Rubinov & Sporns 2010).

So far most brain connectivity studies have been imaging studies, using functional MRI (fMRI), PET, diffusion tensor imaging, etc. Alteration of functional connectivity networks in autism (Kleinhans et al. 2008, Mason et al. 2008, Rudie et al. 2012) studying such domains as face processing and theory of mind has been one factor in our present understanding the social brain and its functional connectivity networks. For example, one study limited to a mix of high functioning individuals differentiated those people from controls regarding hyperconnectivity of the salience network, composed of the anterior insular cortex and anterior cingulate cortex (Uddin et al. 2013). However the neuroimaging findings in autism spectrum disorders have been quite mixed and at times contradictory due to the vast genetic and phenotypic heterogeneity characteristic of the disorder and thus has become of limited value (Hernandez et al. 2015). One factor making it much more complicated is the realization of that not just gray matter may be involved, but there are also widespread abnormalities of white matter development in autism, including the case of two brothers with heterozygous deletions within 16q22.3-q23.1 which includes the gene *FA2H* (Scheid et al. 2013).

Potential Convergent Molecular Pathways

Regarding molecular pathways inside cells, it appears that there is an important “happy medium” of signaling in the CNS; either loss or gain of function of a protein can have opposite effects on synapse numbers and neuronal activity, and yet autistic features may present on both sides of these reciprocal syndromes. Examples of this phenomenon are seen in deletions and duplications of 5q35, 7q11.23, 15q11-13, 17p11.2, 22q13.3 and the gene *MECP2* on the X chromosome.

Such considerations add to the already great complexity of identifying the signaling pathways altered in autism that have the potential of contributing to convergent molecular pathways. Using *de novo* mutations, two distinct modules of autism with Intellectual Disability have been reported by Hormozdiari et al. (2015) – one a module of 80 genes involved in chromatin remodeling and another module of 24 genes associated with synaptic functioning /calcium signaling. Many such molecular studies are well underway using duplications, deletions and mutated genes trying to find possibly related signaling pathways located inside brain cells, even suggesting certain mutations occur at neurodevelopmental time points critical for autism. (Willsey et al. 2013; Pinto et al. 2014). Synaptic genes are a promising area, such as those coding for subunits of synaptic ion exchange, such as calcium or sodium, as well as the clustering of potassium channels; others code for the synaptic scaffolding proteins and ubiquitination pathways (Berg & Geschwind 2012). Synaptogenesis is one of several categories, such as axon outgrowth, cell-cell adhesion, GTPase signaling and down-regulation of several markers of GABaergic interneurons that extend composite pathway analysis. One study, using the

technique of protein interactome, revealed interactions between *SHANK3* and *TSC1*, both known autism genes (Sakai et al. 2011). A specific protein interaction module strongly enriched with autism candidate genes has been reported. (Ji et al. 2014). One study in rodents connected some autistic features to a known canonical neural pathway, the kinase (MEK)ERK signaling pathway and other pathways (Lanz TA et al. 2013). Just beginning to be understood are the multiple molecular assemblies of RNAs and proteins and how they might contribute to disease.

Remember the Angelman syndrome described earlier which was due to a mutation of a gene in 15q11-13. 15q11-13 comprises a complex region encompassing many genes and is associated with several different presentations of autism. One of the most common of these etiologies is the opposite of the deletion found in Angelman syndrome; it is the maternally inherited duplication of that region which has been found in about 1% of autism cases. One of those genes, *CYFIP1*, has been found to be upregulated in post-mortem brain studies of duplication patients (Oguro-Ando et al. 2015) and this gene regulates elements of the mTOR cascade known to affect the fragile X syndrome, some PTEN syndromes and tuberous sclerosis, raising the possibility of some type of control of a convergent pathway (PI3K-Akt-TSC-PTEN-mTOR). Since this pathway leads to cellular proliferation and there is a clinical macrocephaly subgroup in The Autisms, this might be a very early glimpse of how we possibly might correlate molecular pathways with the macroscopic findings in neural networks. But connecting the information from these different levels of analysis will be a huge, formidable task ahead.

Some of these pathways are also affected in Epilepsy, Intellectual Disability (Pavlowsky et al. 2012) and Tourette syndrome – all comorbidities seen in patients with one of The Autisms. The high comorbidity of autism and Intellectual Disability is well established, as is the comorbidity of autism and epilepsy. Possible explanations include either different neurological diseases sharing common neurodevelopmental pathways or direct genetic disease comorbidity. However the meaning of alterations in some pathways apparently leading to autistic symptoms is far from clear since some of these signaling pathways may also be affected in adult psychiatric disease and even cancer.

Induced Pluripotent Stem Cells

An important new way to study The Autisms is the use of induced pluripotent stem cells (iPSCs) derived from patients. Because there is evidence of abnormal communication already present in the earliest stages of brain development (Boersma er al. 2013), this research approach is particularly important. Another advantage is that there are numerous studies in the field of autism, relying on rodent or murine models, where a different or even opposite finding is revealed when comparing the animal study to the human cases (Frazier et al. 2015). Working from human sources is more likely to hold up in the long run.

One study that used iPSCs was derived from human nasal olfactory cells which included nine adults with severe autism plus two adults with no or mild cognitive abilities. Besides the dysregulation of several genes already associated with The Autisms, this research project identified a new candidate gene, *MOCOS,* a gene that creates an enzyme involved in purine metabolism. Ablation of MOCOS was responsible for abnormal neurotransmission phenotypes and, when MOCOS was only partially knocked down, there was a reduced synaptogenesis (Feron et al. 2015). Another research project was based on human skin cells from 4 families with at least one member with macrocephaly, a well documented subgroup. Using transcriptome and gene network analyses, the study revealed upregulation of genes involved in cell proliferation, neuronal differentiation and synaptic assembly as well as an accelerated cell cycle and overproduction of GABergic inhibitory neurons. Using RNA interference, the overproduction of the transcription factor *FOXG1* was shown to be responsible for the overproduction of GABAnergic neurons (Mariani et al. 2015).

In the end, in the strictest sense, social cognition is about understanding other people including their mental, emotional, psychological status and behaviors (Lieberman 2007). So, although imaging and molecular research of groups of patients with autism give us some parameters regarding future study of the social brain, there may be no substitute for studying the gene mutations and regulations causing each individual disease with autistic features or each undiagnosed individual patient with autism, one by one, in order to find the precise functional connectivity networks of the social brain that they directly affect.

Conclusion

One cannot talk about autism without mentioning the heroic parents who raise these children. To the professionals, they are our teachers. We who work in this field are in awe of the strength, creativity and especially of the endurance of these parents in developing ways of helping their children. I think, for example, of the Swedish mother, Karin Stensland Junker, who wrote The Child in the Glass Ball.

The important best study of prevalence trends in The Autisms is the 2015 study by the Gillberg group (Lundstrom et al. 2015).

We have reviewed these 5 concepts about autism today:

1). Autism or Asperger are not diseases in themselves – they are the behavioral results of altered neural pathways in the brain that are found in a great many different disease entities. None of the disorders implicated in autism/Asperger by genomic studies are associated with autism in 100% of the cases to date; none exhibit a fully penetrant autism phenotype. This limitation needs to be kept in mind as we try to discover the neural pathways that lead to the behavioral phenotype.

2) Autistic features are caused by both genetic and epigenetic errors, sometimes including risk across generations (Frans et al. 2013). Identifying these relevant mutations in each individual with autism is the first step toward finding a successful medical therapy for that child. These are life-long diseases where maximum information is needed.

3) There are more boys than girls with autism because there are many more underlying diseases with a male predominance.

4) Molecular studies indicate why some disease entities with autistic features have a pattern of normal development followed by regression.

5). The majority of patient with one of The Autisms remain undiagnosed in 2015. Several reasons for this include the fact that gene mutations and the copy number variation is so diverse in humans that it is not yet fully outlined, as well as our gradual realization that epigenetics, barely begun to be studied, may play a larger role than we had anticipated. We are entering a new age of medicine, where separate diseases may be first identified and labeled by their genomic error rather than clinical criteria.

We do not yet know enough to be sure of which approach to use to create a primary medical therapy for most individual children with diseases including autistic features, but figuring out the mechanism of action of the underlying disease in each child, as revealed by genomic studies, undoubtedly is an important first step. We already know that there probably will be many different primary therapies for the many different diseases, depending upon the mechanisms of action in each disease; as mentioned above, research therapies for children for 4 different diseases are already underway. A possible long-term goal is to try to find single medical therapies for children who belong to certain subgroups of autism that are grouped together by similar signaling pathways.

Regarding the future of research on The Autisms, briefly here are some ideas.

1). Identification in the neonatal period or very early infancy of the underlying diagnosis of the baby is very important. Clinical criteria are indistinct at birth for all kinds of pediatric brain diseases – intellectual disability and even cerebral palsy are not immediately apparent at birth and certainly the symptoms of autism are not readily apparent. Studies of this problem of early identification of autism

are underway (Cohen et al. 2012). One of the earliest clinical clues in autism may be the quality of the pattern of early spontaneous movements, general movements, in the first five months of life (Einspieler et al. 2014). Since it is known that very early intervention both educationally and medically can sometimes ameliorate or even reverse lifelong cerebral and behavioral dysfunction which would otherwise occur in many of these disease entities, a future emphasis needs to be placed on the development of more extensive neonatal and very early medical screening.

2). Regarding medical therapies that may be developed in the future, think out of the box. For example, in the case of Intellectual Disability, certainly if ever there was a disease which seemed hopeless for medical treatment, it would be Down Syndrome. How could one possibly compensate for all the genetic errors of an entire chromosome – such the whole extra 21st chromosome in Down syndrome? Yet recently a promising research solution has been devised that eliminates the entire extra 21st chromosome from the body, turning it into a chromosome 21 Barr body (Jiang et al. 2013). In the case of autism, 40 years ago when everyone thought autism was just one disease, a large double-blind study established the presence of multiple disease entities in patients diagnosed as having autism (Coleman 1976). Accurate diagnosis, whether of syndromic cases or of still undiagnosed idiopathic cases, is a basic initial step to a successful therapy.

To develop a therapy, first the unique pathophysiology of each genetic disease requires an in depth understanding of natural history and the mechanism of action causing the phenotype. Each proposed disease therapy requires us to answer a series of questions: Which cells? What proteins? How much? How toxic? How long? And what side-effects? (Lee & Davidson 2011). In spite of the many different diseases presenting with autistic features, delineation of the areas of neural network and molecular convergence may offer eventual hopeful approaches to a series of medical therapies that target more than one disease. For example, in *in vivo* animal studies using rapamycin, it has been demonstrated that upregulation of autography can have a beneficial effect on quite a few different unrelated neurological diseases, including one with autism.

3). Lifelong care planning is a priority. In the United States, 50,000 individuals with autism transit into adulthood (past 21 years of age) each year. The goal should be integration into a regular workforce by asking employers to use their knowledge of their own business to specifically create a job for a person with one of The Autisms based on the strengths and limitations of that particular individual. At this time, the overwhelming majority of the adult people with Asperger syndrome in the United States are unemployed.

We'll end on a lighter, positive note by mentioning the small minority of cases documented to recover from autism – these include a few cases from early intervention educational programs, those who had neonatal or very early institutionalization, some cases of Landau and Kleffner syndrome and a few other early onset epilepsies, intrauterine rubella, blindness and the dysmaturational syndrome with familial tics, also known as the Zappella syndrome (Zappella 2002, 2012). However many such recovered children continued to be in need of some support educationally from a neurodevelopmental and sometimes from a medical point of view (Olsson et al. 2015). In the Zappella syndrome, virtually all patients are boys (99%) with a history of Tourette syndrome in their families. These boys regress with loss of language usually starting around 18 months meeting classic criteria for autism as well as beginning to develop early-onset Tourette tics. They have no physical stigmata and all laboratory tests are within normal limits. However, these boys gradually recover, with or without interventional therapy, and reach normal or quasi-normal abilities by five to six years of age and are able to attend a normal school.

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