Genomic and Epigenomic Instability, Fragile Sites, Schizophrenia and Autism

Cassandra L. Smith*, Andrew Bolton and Giang Nguyen

Molecular Biotechnology Research Laboratory, Departments of Biomedical Engineering, Biology and Pharmacology, Boston University, Boston, MA, USA

Abstract: Increasing evidence links genomic and epigenomic instability, including multiple fragile sites regions to neuropsychiatric diseases including schizophrenia and autism. Cancer is the only other disease associated with multiple fragile site regions, and genome and epigenomic instability is a characteristic of cancer. Research on cancer is far more advanced than research on neuropsychiatric disease; hence, insight into neuropsychiatric disease may be derived from cancer research results. Towards this end, this article will review the evidence linking schizophrenia and other neuropsychiatric diseases (especially autism) to genomic and epigenomic instability, and fragile sites. The results of studies on genetic, epigenetic and environmental components of schizophrenia and autism point to the importance of the folate-methionine-transulfuration metabolic hub that is diseases also perturbed in cancer. The idea that the folate-methionine-transulfuration hub is important in neuropsychiatric is exciting because this hub present novel targets for drug development, suggests some drugs used in cancer may be useful in neuropsychiatric disease, and raises the possibility that nutrition interventions may influence the severity, presentation, or dynamics of disease.

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INTRODUCTION

Genomic instability refers to an increased mutation rate that can take the form of chromosomal abnormalities, translocations, large or small insertions or deletions and base changes. Epigenomic instability refers to perturbed responses of gene regulation to environmental fluctuations. Fragile site regions of the genome have high levels of genetic and epigenetic instability.

In 2003, we reported a link between somatic mutations (genomic instability) and fragile sites and schizophrenia [1]. Later, we reported aberrant epigenetic regulation of genes involved in dopamine metabolism in the synaptic cleft in schizophrenia and bipolar disease brains [2, 3]. Today, there is increasing evidence for genome instability in neuropsychiatric diseases, including an association with fragile site regions. Cancer is the only other disease associated with multiple fragile site regions, and genome instability is a characteristic of cancer. This article will review the evidence linking schizophrenia and other neuropsychiatric diseases (especially autism) to genomic and epigenomic instability and fragile sites.

Schizophrenia and Autism

Schizophrenia and Autism are neuropsychiatric diseases linked to multiple genetic and environmental factors. Like many common illnesses these diseases remain an enigma because there is no single factor or small number of factors that accounts for a large number of patients.

The prevalence of schizophrenia is ~1% worldwide but varies between 0.3 to 2.7% [4]. Diagnosis is based on the appearance and duration of about 30 symptoms divided into positive (e.g. hallucinations (especially auditory are common), negative (e.g. withdrawal, blunted affect etc), and cognitive (executive function). However, symptoms (endophenotypes) and outcome (Fig. 1) vary even in the same family, raising the possibility that several different diseases (i.e. the “schizophrenias”) presenting similar collections of symptoms have been grouped together [5, 6]. These and other observations suggest that a genetic predisposition is not sufficient by itself to cause disease. Further in some cases, the disease appears to be environmentally induced in the absence of detectable genetic predisposition (see below).

Autism is a complex, early onset (typically <5 years of age) lifelong illness that is difficult to diagnose and treat. Autism appears to be multiple diseases that make up autism spectrum disorder (ASD) defined by limits in three behaviors (1) social interactions, (2) communication and imaginative play, and (3) interests and activities. Other symptoms include impaired immunological responses, inflammation (especially in the gut), and oxidative stress [7]. Today, treatments include intensive educational and behavioral interventions with drugs to reduce remaining symptoms.

GENETICS

First-degree relatives of schizophrenia probands have a ~10% probability of becoming ill [8], while ~ 50% of cases
of schizophrenia are spontaneous with no other affected family member [9]. Although variable [10-12], the general belief is that ~50% of monozygotic twins affected with schizophrenia are discordant for the disease, although progeny of both the well and ill discordant MZ twin have the elevated probably (~10%) typical of first degree relatives of ill individuals [13].

Genetic studies have linked many genes and chromosomal regions spread throughout the genome to schizophrenia in different families, but no single or small number of genes accounts for the majority of cases. Common alleles have small effects (e. g. ZNF804) while rare alleles (e. g. NRG1, DTNB1, DAOA and DISC1) have greater effects [14]. A summary of the genes linked to schizophrenia is shown in Table 1. Genes linked to schizophrenia do not affect a single neurobiological system, and include neurotrophic factors (e. g. BDNF, NRG), neuromodulatory receptors (DRD, HTR), members of the synaptic packaging and release machinery (SNAP25), and both inhibitory and excitatory neurotransmitter systems (GRIN, GRIK, GABR). Also, there are genes linked to folate processing (MTHFR) and methylation (e. g. DNMT, COMT) see below.

Except for mitochondrial defects in a subset of patients, no other common genetic or environmental factor, nor is an effective intervention linked to a majority of patients [15]. Clearly, there is a genetic component with multiple genes linked to the disease (for reviews see [16] and [17]). Many genes linked to autism are similar to those linked to schizophrenia and bipolar disorder ([18, 19], http://neuropsych.bu.edu).

**EPIGENETICS**

Epigenetic programming refers to factors that are “epi”, or “on top of” genetic (DNA) sequences and was coined by Waddington in the 1940s to link genes and development [20] (Fig. 2). Epigenetic regulation allows a single genome to code for functionally different cell types and short-term adaptation (for reviews see references [21-25]). In contrast, DNA sequence changes are responsible for long-term adaptation and evolution.

The term “epigenetic programming” is evolving, and today refers to reversible molecular changes to DNA, RNA or proteins (e. g. histones) that regulate gene function but do not involve DNA base changes. Epigenetic changes include DNA methylation, RNA modification (e.g. editing (addition/deletion/change to base sequence), RNA interference) and both histone and non-histone proteins modifications (e. g. methylation, acetylation, phosphorylation, sumoylation, ubiquitination).

Epigenetic programming of chromatin begins shortly after DNA synthesis, although subsequent alterations may occur in response to variety of ordinary or pathological environmental or biological factors. Epigenetic changes occur globally in development, and at specific loci throughout life and in disease states [26-28]. In cancer, the impact of epigenetic modification on gene expression has been studied for some time [29-35].

**DNA Methylation**

DNA methylation is the best-characterized epigenetic factor controlling gene expression (Fig. 3; for reviews see [24, 25, 36-38]). In vertebrates, 4-8% of all cytosines, and 70% of cytosines within the 5’CpG3’ dinucleotide sequence, are methylated. In contrast, 70% of the cytosines at 5’CpG3’ dinucleotide sequences within promoter regions of active genes are unmethylated. There are ~29,000 "CpG islands" (regions rich in 5’CpGs3’) in the human genome 2 sequence. The methylation state of half of these islands regulates mRNA expression. About half of these islands are highly methylated [39]. DNA methyltransferase (DNMT) enzymes are responsible for methylation of CpG sequences [40], with the rate of methylation determined by the availability of DNMTs and their relative affinity for a given CpG site on DNA [41], and other co-factors (see below). Today, no DNA demethylase has been identified.

The number and location of methylated CpG sites in promoter regions usually, but not always, correlates with gene expression in vivo [24, 25, 36, 37, 38, 42]. Usually, dense DNA methylation is associated with irreversible silencing of gene expression, while a strong activator can overcome partial methylation. Partial promoter DNA methylation marks genes that may become unmethylated and expressed, allowing for re-adaptation to a changing micro- or macro-environment (e.g. season, ecological conditions, nutritional habits and demands of different developmental periods (see below)). More complexity in DNA methylation is introduced when the state of CpG sites within genes (i.e. outside the promoter regions) are compared to promoter dinucleotides. Ball et al. [39] show that methylation of CpG sites within genes is correlated with light promoter methylation; hence, gene body methylation appears to correlate with expression.

DNA methylation in promoter regions occurring at 5’CpG3’ dinucleotides within transcription factors
Table 1. Fragile Sites in the Human Genome

| Chr | Locus | Location | R/C | Agent |
|-----|-------|----------|-----|-------|
| 1   | FRA1E | 1p21.2   | C   | Aph   |
| 1   | FRA1M | 1p21.3   | R   | FolA  |
| 1   | FRA1D | 1p22     | C   | Aph   |
| 1   | FRA1L | 1p31     | C   | Aph   |
| 1   | FRA1C | 1p31.2   | C   | Aph   |
| 1   | FRA1B | 1p32     | C   | Aph   |
| 1   | FRA1A | 1p36     | C   | Aph   |
| 1   | FRA1J | 1q12     | C   | 5-Aza |
| 1   | FRA1F | 1q21     | C   | Aph   |
| 1   | FRA1G | 1q25.1   | C   | Aph   |
| 1   | FRA1K | 1q31     | C   | Aph   |
| 1   | FRA1H | 1q42     | C   | 5-Aza |
| 2   | FRA2L | 2p11.2   | R   | FolA  |
| 2   | FRA2E | 2p13     | C   | Aph   |
| 2   | FRA2D | 2p16.2   | C   | Aph   |
| 2   | FRA2C | 2p24.2   | C   | Aph   |
| 2   | FRA2A | 2q11.2   | R   | FolA  |
| 2   | FRA2B | 2q13     | R   | FolA  |
| 2   | FRA2F | 2q21.3   | C   | Aph   |
| 2   | FRA2K | 2q22.3   | C   | Aph   |
| 2   | FRA2G | 2q31     | C   | Aph   |
| 2   | FRA2H | 2q32.1   | C   | Aph   |
| 2   | FRA2I | 2q33     | C   | Aph   |
| 2   | FRA2J | 2q37.3   | C   | Aph   |
| 3   | FRA3B | 3p14.2   | C   | Aph   |
| 3   | FRA3C | 3p24.2   | C   | Aph   |
| 3   | FRA3C | 3p25     | C   | Aph   |
| 4   | FRA4D | 4p15     | C   | Aph   |
| 4   | FRA4A | 4p16.1   | C   | Aph   |
| 4   | FRA4B | 4q12     | C   | BrdU   |
| 4   | FRA4E | 4q27     | C   | Unclas |
| 4   | FRA4C | 4q31.1   | C   | Aph   |
| 5   | FRA5A | 5p13     | C   | BrdU   |
| 5   | FRA5E | 5p14     | C   | Aph   |
| 5   | FRA5B | 5q15     | C   | BrdU   |
| 5   | FRA5D | 5q15     | C   | Aph   |
| 5   | FRA5F | 5q21     | C   | Aph   |
| Chr | Locus | Location | R/C | Agent |
|-----|-------|----------|-----|-------|
| 5   | FRA5C | 5q31.1   | C   | Aph   |
| 5   | FRA5G | 5q35     | R   | FolA  |
| 6   | FRA6C | 6p22.2   | C   | Aph   |
| 6   | FRA6A | 6p23     | R   | FolA  |
| 6   | FRA6B | 6p25.1   | C   | Aph   |
| 6   | FRA6D | 6q13     | C   | BrdU  |
| 6   | FRA6G | 6q15     | C   | Aph   |
| 6   | FRA6F | 6q21     | C   | Aph   |
| 6   | FRA6E | 6q26     | C   | Aph   |
| 7   | FRA7A | 7p11.2   | R   | FolA  |
| 7   | FRA7D | 7p13     | C   | Aph   |
| 7   | FRA7C | 7p14.2   | C   | Aph   |
| 7   | FRA7B | 7p22     | C   | Aph   |
| 7   | FRA7J | 7q11     | C   | Aph   |
| 7   | FRA7E | 7q21.2   | C   | Aph   |
| 7   | FRA7F | 7q22     | C   | Aph   |
| 7   | FRA7G | 7q31.2   | C   | Aph   |
| 7   | FRA7H | 7q32.3   | C   | Aph   |
| 8   | FRA7I | 7q36     | C   | Aph   |
| 8   | FRA8C | 8q24.1   | C   | Aph   |
| 8   | FRA8E | 8q24.1   | R   | DistA |
| 8   | FRA8F | 8q13     | R   | Unclass |
| 8   | FRA8B | 8q22.1   | C   | Aph   |
| 8   | FRA8A | 8q22.3   | R   | FolA  |
| 8   | FRA8D | 8q24.3   | C   | Aph   |
| 9   | FRA9A | 9p21     | R   | FolA  |
| 9   | FRA9C | 9p21     | C   | BrdU  |
| 9   | FRA9B | 9q32     | R   | FolA  |
| 9   | FRA9E | 9q32     | C   | Aph   |
| 9   | FRA9F | 9q12     | C   | 5-Aza |
| 9   | FRA9D | 9q22.1   | C   | Aph   |
| 10  | FRA10B| 10q25.2  | R   | BrdU  |
| 10  | FRA10E| 10q25.2  | C   | Aph   |
| 10  | FRA10G| 10q11.2  | C   | Aph   |
| 10  | FRA10C| 10q21    | C   | BrdU  |
| 10  | FRA10D| 10q22.1  | C   | Aph   |
| 10  | FRA10A| 10q23.3  | R   | FolA  |
| 10  | FRA10F| 10q26.1  | C   | Aph   |
| 11  | FRA11C| 11p15.1  | C   | Aph   |
| 11  | FRA11I| 11p15.1  | R   | DistA |
| Chr | Locus | Location | R/C | Agent |
|-----|-------|----------|-----|-------|
| 11  | FRA11E| 11p13    | C   | Aph   |
| 11  | FRA11D| 11p14.2  | C   | Aph   |
| 11  | FRA11H| 11q13    | C   | Aph   |
| 11  | FRA11A| 11q13.3  | R   | FolA  |
| 11  | FRA11F| 11q14.2  | C   | Aph   |
| 11  | FRA11B| 11q23.3  | R   | FolA  |
| 11  | FRA11G| 11q23.3  | C   | Aph   |
| 12  | FRA12A| 12q13.1  | R   | FolA  |
| 12  | FRA12B| 12q21.3  | C   | Aph   |
| 12  | FRA12C| 12q24    | R   | BrdU  |
| 12  | FRA12D| 12q24.13 | R   | FolA  |
| 13  | FRA13A| 13q13.2  | C   | Aph   |
| 13  | FRA13B| 13q21    | C   | BrdU  |
| 13  | FRA13C| 13q21.2  | C   | Aph   |
| 13  | FRA13D| 13q32    | C   | Aph   |
| 14  | FRA14B| 14q23    | C   | Aph   |
| 14  | FRA14C| 14q24.1  | C   | Aph   |
| 15  | FRA15A| 15q22    | C   | Aph   |
| 16  | FRA16B| 16q22.1  | R   | DistA |
| 16  | FRA16C| 16q22.1  | C   | Aph   |
| 16  | FRA16E| 16q12.1  | R   | Aph   |
| 16  | FRA16A| 16p13.11 | R   | FolA  |
| 16  | FRA16D| 16q23.2  | C   | Aph   |
| 17  | FRA17A| 17p12    | R   | DistA |
| 17  | FRA17B| 17q23.1  | C   | Aph   |
| 18  | FRA18A| 18q12.2  | C   | Aph   |
| 18  | FRA18B| 18q21.3  | C   | Aph   |
| 19  | FRA19B| 19p13    | R   | FolA  |
| 19  | FRA19A| 19q13    | C   | 5-Aza |
| 20  | FRA20A| 20p11.23 | R   | FolA  |
| 20  | FRA20B| 20p12.2  | C   | Aph   |
| 22  | FRA22B| 22q12.2  | C   | Aph   |
| 22  | FRA22A| 22q13    | R   | FolA  |
| X   | FRAXB  | Xp22.31  | C   | Aph   |
| X   | FRAXC  | Xq22.1   | C   | Aph   |
| X   | FRAXD  | Xq27.1   | C   | Aph   |
| X   | FRAXA  | Xq27.3   | R   | FolA  |
| X   | FRAXE  | Xq28     | R   | FolA  |

Chr = chromosome; R/C = rare or common; Aph = amphidicolin; FolA = Folic acid; 5-Aza = Azacytidine. Data was compiled from [147, 148] and Genome Database. 1999. Chr = chromosome; R/C = rare/common; Aph = amphidicolin or folic acid; FolA = Folic Acid; 5-AzaC = 5-Azacytidine; BrdU = Bromo-uridine, Unclass = unclassified, DistA = Distamy-cint (http://ncbi.nlm.nih.gov).
recognition sites (e.g. GGGCGG and TACGTCA for factors stimulatory protein 1 (SP1) and cAMP response element protein (CREB), respectively) may decrease expression of genes driven by these factors [25]. Gene activation itself may impact local DNA methylation. For instance, transcription factor (e.g. SP1) binding may interfere with DNA promoter methylation directly [43].

Transcription can be inhibited by proteins that bind directly or indirectly to methylated DNA (see referenced reviews above). One methylated DNA binding family, consisting of the MeCP2, MBD1, MBD2, MBD3, and MBD4 proteins, has a conserved methyl-binding domain (MBD) and binds singly methylated CpG dinucleotides [44]. Another repressor family, all containing a zinc-finger motif, consists of Kaiso protein, which binds CGCGs, the Kaiso binding sequence (KBS; recognition sequence = TCCTGCNA) protein, and the ZBTB4 and ZBTB38 proteins that bind lone methylated CpGs dinucleotides [45].

Epigenetic changes in DNA are correlated with amino terminal histone 3 modifications (methylation and acetylation) (for reviews see [46, 47, 25]; Fig. 3). Promoter regions of expressed genes (i.e., unmethylated regions) have histone 3 lysine-4 methylation (H3K4me) and histone 3 lysine-9 acetylation (H3K9ac) modifications. Promoter regions of unexpressed genes, (i.e. highly methylated regions) have no modification at histone 3 lysine 4 (H3K4) but have histone 4 lysine 9 methylation (H3K9me).

Generally, chromatin codes (DNA and histone) are preserved through mitosis, although reprogramming may occur [48]. During meiosis and early development, complex differential global chromatin reprogramming occurs, some specific for male or female germline and others for development. Some germline epigenetic patterns are inherited [48].

Epigenetic programming imprints some genes to be expression in a parental origin dependent manner [47]. Gene imprinting is proven for ~80 genes, and predicted for ~200...
genes (http://www.geneimprint.com). Most imprinted genes are associated with growth and development. In female cells, epigenetic changes turn off all gene expression from one X chromosome randomly in each cell during early embryogenesis [49]. This insures that chromosome X gene expression levels are similar for female (XX) and male (XY) cells.

Although, epigenetic contributions to cancer phenotypes have been studied for some time, only recently has this area of research begun to impact neurological diseases. We and others have previously reviewed [24, 25, 50, 51] the connection between epigenetic modifications and neurological disease, including the effect of folic acid (a source of methyl groups for epigenetic modifications) metabolism on psychotic symptoms, and the co-morbidity of psychosis with diseases clearly linked to epigenetic changes (e.g. schizophrenia, bipolar disease, autism, Rett's and Angelmen’s/Prader-Willi disease, mental retardation and degeneration (see below)).

GENETIC AND EPIGENETIC REGULATION OF DOPAMINE METABOLISM

The dopamine hypothesis of schizophrenia arose because many anti-psychotic medications used in the treatment of schizophrenia are dopamine receptor antagonists. Oxygen methylation of dopamine by Catechol-O-Methyl Transferase (COMT) appears to be the prominent means of dopamine catabolism after synaptic release in brain regions such as the prefrontal cortex (reviewed in [52]). The 5’ region of the

![Diagram](image-url)

**Fig. (4). Genetic and epigenetic regulation of dopamine metabolism in schizophrenia.** (A) Dopamine released by the pre-synaptic neuron into the synaptic cleft may dock with dopamine receptors on the post-synaptic neuron for downstream signaling; be degraded by MAO or COMT; or be taken back up into the pre-synaptic neuron by binding to DAT. (B) When dopamine degradation is high, for instance, by an increase in COMT activity, dopamine receptors expression is elevated to compensate for low amounts of dopamine in the synaptic cleft. (C) In schizophrenia, the coordinated up-regulation of the dopamine receptors does not exist, or exists at a greatly reduced level.

- dopamine
- BDNF - brain derived neutrophic factor
- COMT - catechol-O-methyl transferase
- DAT - dopamine transporter
- DRD1, 2, 4 - dopamine receptors 1, 2, 4
- MAO - mono amine oxidase
- RELN - reelin
- TH - tyrosine hydroxylase
COMT gene contains methylation sites that are actively regulated. Our experiments [2, 3] studied promoter methylation and gene expression levels in Brodmann Area 46 (DL-PFC) of normal versus neuropsychiatric (schizophrenia and bipolar) individuals (Fig. 4). The results revealed a significant correlation between membrane-bound COMT (MB-COMT) promoter hypo-methylation (especially at SP1 binding sites) and over-expression of the MB-COMT gene product in schizophrenia and bipolar disorder.

The same samples used above were genotyped for a common COMT allele (Val158Met single nucleotide polymorphism (SNP)). The results showed that schizophrenia samples were more likely to have a VAL allele, and less likely to be homozygous for the MET allele than controls. Bipolar patients were more likely to be homozygous for the VAL allele than controls.

The Val158Met polymorphism is known to directly affect the thermostability of the MB-COMT protein. The Met alleles is thermolabile, causing COMT enzyme activity in Met homozygotes to drop to approximately 1/3 the level of Val homozygotes at physiological temperature [53]. COMT hyperactivity (from the Val allele) has been linked to poor working memory as well as disturbed executive function and attention [54-58]. Genetic epigenetic gene expression results showed that dopamine degradation in the synaptic cleft is increased in individuals with schizophrenia because of increased COMT activity or expression.

Additional studies examined the expression and regulation of other genes involved in dopamine metabolism. The results revealed that expression of the dopamine receptor 1 (DRD1) was inversely correlated with MB-COMT expression in all groups, although to a lower level in the patient groups. DRD2 showed the reverse pattern: hypo-methylation of the MB-COMT promoter was nearly always associated with hypo-methylation of the DRD2 promoter and higher DRD2 gene expression levels. However, schizophrenia and bipolar patients show a significantly less decrease in methylation of their DRD2 promoters in response to MB-COMT hypo-methylation.

Also, the promoter methylation state of the RELN gene was significantly linked to Val158Met genotype. All schizophrenics and control subjects possessing a Val/Val genotype had a hyper-methylated RELN promoter and a decrease in RELN gene expression. This is consistent with results [59, 60] that hyper-methylation of the RELN promoter and subsequent low expression of the reelin gene in the frontal lobes is correlated with schizophrenia.

The fact that control subjects more strongly downregulate DRD1 expression and upregulate DRD2 expression when they possess a hypo-methylated MB-COMT promoter suggests that a mechanism exists for regulation of synaptic dopamine at the transcriptional level. Coordinated regulation was absent or decreased in neuropsychiatric patients. More recent unpublished data has detected aberrant methylation of the DAT1 and DRD4 promoters, but not the NRG1, HTR2A or NOS1 promoters, in samples from schizophrenic brains versus control subjects. The results suggest that aberrant synaptic dopamine metabolism in the schizophrenia/bipolar brain through genetic or epigenetic causes may contribute to disease pathogenesis.

Other groups have also examined methylation deficits in schizophrenia. For example, the methyltransferase DNMT1 is up-regulated in the inhibitory inter-neurons of schizophrenia patients (reviewed in [61]). DNMT1 up-regulation is suggested to induce hyper-methylation and down-regulation of RELN and the GABA synthesizing enzyme GAD67 in prefrontal inter-neurons of schizophrenia patients. Woo et al. [62] and Costa et al. [61] speculated that down-regulation of the NMDA receptor subunit NR2A in these neurons may stem from hyper-methylation after DNMT1 up-regulation. RELN controls the surface expression of two other NMDA receptor subunits (NR2B and NR1, [63]) suggesting a possible deficit in NMDA receptors in the inter-neurons of schizophrenics. This supports the “NMDA hypofunction theory of schizophrenia” developed from observations that NMDA receptor antagonists, PCP and ketamine, both induce schizophrenia-like symptoms. In addition, the SOX10 (sex-determining region Y-box containing gene 10) gene, an oligodendrocyte specific transcription factor with a large CpG promoter island, is hyper-methylated and down-regulated in the prefrontal cortex (BA10) of schizophrenia patients [64].

GENOMIC INSTABILITY

Our initial research on schizophrenia focused on monozygotic twins. The goal was to understand disease discordance: how does one monozygotic twin avoid illness, and how do both the ill and well twin passed the same elevated genetic predisposition to progeny [1]. The specific aim was to identify, clone and sequence the expected small number of somatic changes present in monozygotic twins discordant for disease, and then do further studies to determine whether any differences were related to disease occurrence/presentation. The research targeted anonymous (CAG)n because these sequences are unstable and located within a number of genes linked to schizophrenia (e.g. [65-67], Fig. 5). The experiments examined anonymous restriction length polymorphism (RFLPs) of PCR amplicons containing (CAG)n repeating and adjacent sequences in lymphocytes using a method developed by us called Targeted Genomic Differential Display (TGDD) [68]. TGDD is similar to differential display [69], but examines subsets of DNA sequences sharing a targeted sequence.

Unexpectedly, a statistically significant high level of RFLP variability around (CAG)n was detected in monozygotic twins discordant for schizophrenia (Fig. 6). Twin pairs concordant for the disease had greater variability than controls, but for this small sample size this variability did not reach statistical significance. Assuming all the twin pairs were monozygotic (i.e., began life with identical DNA), RFLP variability must reflect somatic mutation rates after twinning. Hence, the results showed that a high somatic mutation rate was associated with schizophrenia, especially in monozygotic twins discordant for disease.

Evidence supporting the idea include that schizophreni is linked to genome instability. Cytogenetic observations of increased chromosome aneuploidy in brain cells from individuals with schizophrenia [70, 71] and other neurological diseases including autism, ataxia-telangiectasia [72, 73], Alzheimer’s disease [72], Down syndrome, Edwards syndrome, Patau syndrome, Parkinson’s disease, spinal muscular
atrophy, mental retardation, Turner syndrome, psychiatric disorders associated with trisome X and Klinefleter syndrome, and 47,XYY karyotype (reviewed in [74-77]). Other evidence (reviewed in [1]) is the skewed (CAG)n repeat distribution in schizophrenia (Fig. 5), and the inverse correlation of disease with some cancer (reviewed in [78]).

More recently, genome wide scanning of SNPs in association studies revealed an elevated rate of copy number variation (CNV) in schizophrenia [79-82], and a number of other neuropsychiatric diseases such as autism, mental retardation, bipolar disease, Rett syndrome, Tourette’s syndrome, Prader-Willi/Angelman syndrome etc. (e.g. [18, 83], for review see [84]). Clearly, genomic instability is linked to neurological disease.

FRAGILE SITES

Fragile sites are regions of the genome that are prone to mutation and epigenetic changes; hence, hot spots for genomic instability. A fragile site is defined as unstable DNA stretch that appears as a gap or break on metaphase chromosomes (Fig. 7A) when DNA replication of dividing cells is partially inhibited by incubation in culture medium deficient in folic acid or containing Bromodeoxyuridine (BrdU), distamycin, 5 azacytidine, or aphidicolin [85, 86].

Fragile sites are unusual chromosomal abnormalities because, although heritable, they appear only in a subset of cells, and usually only occur when induced. There are 119 known fragile sites (Tables 1 and 2), spread throughout the genome classified as common or rare based on frequency in the population (greater or less than 5%, respectively).

The first identified and best studied example of the association between fragile sites and mental illness is Fragile X syndrome. Fragile X syndrome is associated with transcriptional silencing of either FMR1 or FMR2 (Fragile X mental retardation genes 1 and 2) on chromosome X (for review see [87]). Silencing of FMR1 or FMR2 is accompanied by hyper-methylation of the (CGG)n expansion within fragile sites FRAXA at Xq27.3 or FRAXE at Xq28, respectively. The number and methylation status of the (CGG)n repeating sequences influences the expression of the fragile X mental retardation genes. The FRAXA and FRAXE promoter sites behave similarly. For FRAXA sites, well individuals have 7 to 50 (CGG)n repeating sequences (with a mode of 30). Mental retardation occurs, and the fragile site becomes visible under folate deficient conditions, when the repeat number exceeds 230 and becomes hyper-methylated. Repeat numbers can reach up to 2000. Numbers between 50 and 200 are un-methylated and considered “pre-mutations”, but carriers may have symptoms other than mental retardation [88]. Schizophrenia is linked to several fragile sites (Table 4), some of which are unique (e.g. [89]). Neurological diseases and cancers [90, 91] are linked to specific sites as well (Table 3).

Cells from schizophrenia patients grown in the absence of folate present a greater overall number of fragile sites per metaphase than controls [92, 93]. These results may indicate that schizophrenia patients may have a greater sensitivity to folic acid deficiency, or a higher number of fragile sites with borderline expansion (e. g. see (CAG)n repeats in schizophrenia above).

Most fragile sites are mapped only to the low-resolution chromosomal cytogenetic band level; ~15 fragile sites are characterized at the sequence level. One site appears to be ~3 million base pairs (bp) in size and contains 10 genes and multiple repeat sequences. Rare folate sensitive sites like FRAXA are composed of the expanded simple trinucleotide repeat (CCG)n while some contain other interspersed repeats
(e.g. LINE) or AT-rich sequences (e.g. common fragile sites are linked to AT-rich sequences). Replication of repeating sequences, or any sequence that deviates from the mean G+C level, can stress metabolism because the DNA replication machinery requires a different ratio of deoxynucleoside triphosphates (i.e. the ratio of G+C vs A+T).

We calculated that ~70% of the human genome was devoid of fragile sites by determining what percent of the genome, at the cytogenetic band level, was linked to one or more fragile sites (Fig. 7B). Our preliminary analysis [1] using chromosome abnormalities and genes linked to schizophrenia (reported in [94] and [95], respectively) found that ~70%, rather than the expected ~30% (X², p = 0.001), co-localize to regions of the genome having fragile sites.

More recent studies by us reviewed 387 genetic studies from the literature that identified 111 unique genes linked to schizophrenia (Fig. 8). Of the 111 genes, 58 co-localized with at least one fragile site at the Giemsa band level (df = 1, χ²=14.227, p <0.0001; Odds Ratio = 2.92). Moreover, a significant number of rare (CCG) containing fragile sites co-localized with the sample of genes (df = 1, χ²=5.67, p < .025; Odds Ratio = 2.285). More detailed and updated information will be provided elsewhere.

Expansion of repeating sequences within fragile sites is accompanied by local hyper-methylation (i.e. FRAXA and FRAXE) and the appearance of fragile sites in vitro.
Table 3. Neurological Diseases Associated with Specific Fragile Sites. Gene Names for Abbreviations are Shown in Table 4

| Fragile Site | Associated Gene(s) | Neurological Disease                      |
|-------------|--------------------|------------------------------------------|
| FRA2A       |                    | Mental retardation/schizophrenia          |
| FRA2B       |                    | Autism                                   |
| FRA4F       | GRID2              | Tremor/Ataxia                            |
| FRA6A       |                    | Autism                                   |
| FRA6E       | PARK2              | Autosomal Juvenile Parkinsonism           |
| FRA6F       | LAMA4              | Schizophrenia                            |
| FRA7I       | CNTAP2             | Tourette's                               |
| FRA9F       |                    | Schizophrenia                            |
| FRA11B      | CBL2               | Jacobsen’s Syndrome                      |
| FRA12A      | DIP2B              | Autism / Mental retardation              |
| FRA13A      | NBEA               | Sporadic Autism                          |
| FRA15A      | RORA               | Tremor/Ataxia, Imbalance                 |
| FRA1A       | FMR1               | Fragile X Mental Retardition / FRA1A Tremor Ataxia |
| FRA1C       | IL1RAPL1, DMD      | Mental Retardation associated with complex glycerol kinase deficiency |
| FRA1E       | FMR2               | Fragile X Mental Retardition (mild)      |
| Global FS Expression | ATR   | Seckel syndrome                         |

Table 4. Summary of Genes Linked to Schizophrenia and Fragile Sites

| GENE | FRAGILE |
|------|---------|
| NAME | ALIAS   | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|---------|----------|---------|------|---------|
| GSTM1 | glutathione S-transferase M1 | 1p13.3 | |
| GRIK3 | glutamate receptor ionotropic | 1p34-p33 | |
| HTR6 | 5-hydroxytryptamine (serotonin receptor type 6) | 1p36-p35 | FRA1A | 1p36 |
| RHD | Rhesus blood group D antigen | 1p36.11 | FRA1A | 1p36 |
| MTHFR | 5 10-methyltetrahydrofolate | 1p36.3 | FRA1A | 1p36 |
| SCZD9 | schizophrenia disorder 9 | 1q21-q22 | FRA1F | 1q21 |
| SYT1 | Synaptotagamin X1 | 1q21.2 | FRA1F | 1q21 |
| KCNN3 | potassium intermediate/small c | 1q21.3 | FRA1F | 1q21 |
| RGS4 | regulator: g-protein signaling 4 | 1q23.2 | |
| IL10 | interleukin 10 | 1q31-q32 | FRA1K | 1q31 |
| DISC2 | disrupted in schizophrenia 2 | 1q32.1 | |
| DISC1 | disrupted in schizophrenia 1 | 1q42.1 | FRA1H | 1q42 |
| CHROMOSOME 2 | | |
| NOGO | RTN4 | reticulin 4 | 2p13-p14 | FRA2E | 2p13 |
| IL1B | interleukin 1 beta | 2q14 | |
| NR4A2 | nuclear receptor subfamily 4, group A, member 2 | 2q22-23 | FRA2K | 2q22.3 |
| CTLA4 | cytotoxic T-lymphocyte-associative protein | 2q33 | FRA2I | 2q33 |
| NAME | ALIAS | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|-------|----------|---------|------|---------|
| GRM2 | GRM2 | glutamate receptor metabotropic 2 | 3p21.31 | |
| CCK  |       | cholecystokinin | 3p22-p21.3 | |
| GRM7 | GRM7 | glutamate receptor metabotropic 7 | 3p26.1-p25.1 | |
| CHL1 CALL | | cell-adhesion molecule with homology to L1CAM | 3p26.1 | |
| DRD3 |       | dopamine receptor D3 | 3q13.3 | |

## CHROMOSOME 4

| NAME | ALIAS | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|-------|----------|---------|------|---------|
| GABRB1 | GABRB1 | gamma-aminobutyric acid (GABA) receptor, beta 1 | 4p12 | |
| CCKAR |       | cholecystokinin A receptor | 4p15.1-p15.2 | FRA4D | 4p15 |
| DRD5 |       | dopamine receptor D5 | 4p16.1 | FRA4A | 4p16.1 |

## CHROMOSOME 5

| NAME | ALIAS | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|-------|----------|---------|------|---------|
| GDNF |       | glial cell derived neurotrophic factor | 5p13.1-p12 | FRA5A | 5p13 |
| SCZD1 |       | schizophrenia disorder 1 | 5q11.2-q13.3 | |
| Homer 1 | Homer homolog 1 (Drosoph) | 5q14.2 | |
| HTR4 |       | 5-hydroxytryptamine (serotonin) receptor 4 | 5q31-q33.2 | FRA5C | 5q31.1 |
| GABRB2 |       | GABA A receptor, beta 2 | 5q34 | |
| H2 rec | HRH2 | histamine H2 receptor | 5q35.3 | FRA5G | 5q35 |
| DRD1 |       | dopamine receptor D1 | 5q35.1 | FRA5G | 5q35 |

## CHROMOSOME 6

| NAME | ALIAS | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|-------|----------|---------|------|---------|
| NQO2 |       | NADPH hydrogenase quinone 2 | 6pter-q12 | FRA6C/A/B | 6p22.2/23/25.1 |
| NOTCH4 |       | Notch homolog 4 (Drosophila) | 6p21.3 | |
| TNFA |       | Tumor necrosis factor alpha | 6p21.31 | |
| HLA | HLA-A | major histocompatibility complex , class I, A | 6p21.3 | |
| TNXB |       | tenascin XB | 6p21.3 | |
| DTNBP1 |       | dystrobrevin binding protein 1 | 6p22.3 | |
| SCZD3 |       | schizophrenia disorder 3 | 6p23 | FRA6A | 6p23 |
| SCAI |       | spinocerebellar ataxia 1 (oliv) | 6p23 | FRA6A | 6p23 |
| CB1 | CNR1 | Cannabinoid receptor 1 | 6q14-q15 | FRA6G | 6q15 |
| SCZD5 |       | schizophrenia disorder 5 | 6q13-q26 | FRA6D/E | 6q13,q26 |
| HTR1B |       | 5-hydroxytryptamine (serotonin) receptor 1B | 6q13 | FRA6D | 6q13 |
| Fyn kinase | FYN | FYN oncogene related to SRC, FGR, YES | 6q21 | FRA6F | 6q21 |

## CHROMOSOME 7

| NAME | ALIAS | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|-------|----------|---------|------|---------|
| DDC | DDC | dopa decarboxylase (aromatic L-amino acid decarboxylase) | 7p11 | FRA7A | 7p11.2 |
| NPY |       | Neuropeptide Y | 7p15.1 | |
| GRM3 |       | glutamate receptor metabotropic fact. 3 | 7q21.1-q21.2 | FRA7E | 7q21.2 |
| RELN |       | reelin | 7q22 | FRA7F | 7q22 |
(Table 4). Contd.....

| NAME     | ALIAS       | FUNCTION                          | ADDRESS   | SITE   | ADDRESS |
|----------|-------------|-----------------------------------|-----------|--------|---------|
| NRG1     |             | neuregulin 1                       | 8p21-p12  |        |         |
| SCZD6    |             | schizophrenia disorder 6           | 8p21      |        |         |
| PPP3CC   |             | protein phosphotase 3              | 8p21.2    |        |         |
| FDZ3     |             | frizzled homolog 3                 | 8p21      |        |         |
| DPYSIL2  |             | human dihydroppyrimidinase-related protein 2 | 8p21-p22 |        |         |
| OPRS1    | OPRS1       | opioid receptor, sigma 1           | 9p13.2    |        |         |
| DBH      |             | dopamine beta-hydroxylase (dop)    | 9q34      |        |         |
| GRIN1    | NMDA        | glutamate receptor ionotropic      | 9q34.3    |        |         |
| SCA8     |             | spinocerebellar axia protein 8     | 10q23.3-24.1 | FRA10A | 10q23.3 |
| VMAT2    | SVMT        | solute carrier family 18 (vesicular monoamine), member 2 | 10q25 | FRA10B/E | 10q25.2 |
| PAX6     |             | paired box gene 6 (aniridia k)     | 11p13     | FRA11E | 11p13   |
| BDNF     |             | brain-derived neurotrophin fac     | 11p13     | FRA11E | 11p13   |
| TPH1     |             | tryptophan hydroxylase             | 11p15.3-p14 | FRA11D | 11p14.2 |
| TH       |             | tyrosine hydroxylase               | 11p15.5   |        |         |
| cPLA2    | HTATIP2     | HIV-1 Tat Interactive Protein 60kDa | 11q13     | FRA11A/H | 11q13.3/13 |
| GRIA4    |             | glutamate receptor ionotrophi      | 11q22     |        |         |
| DRD2     |             | Dopamine receptor D2               | 11q23     | FRA11B/G | 11q23.3 |
| HMBS     |             | hydroxymethylbilane synthase       | 11q23.3   | FRA11B/G | 11q23.3 |
| B3GAT    |             | beta-1, 3-Gluconyltransferase-1    | 11q25     |        |         |
| NR2B     | GRIN2B      | glutamate receptor, ionotropic, N-methyl D-aspartate 2B | 12p12  |        |         |
| NTF3     | NT3         | neurotrophin 3                     | 12p13     |        |         |
| B37      | DRPLA       | dentatarubral-pallidolysian atrophy (atrophin-1) | 12p13.31 |        |         |
| PAH      |             | phenylalnine hydroxylase           | 12q22-24.2 | FRA12C/E/D | 12q24/24.13 |
| PLA2     |             | phospholipase A2, group IB         | 12q23-q24.1 | FRA12C/E/D | 12q24/24.13 |
| NOSI     |             | nitric oxide synthase 1 (neuro)    | 12q24.2-q24.31 | FRA12C/E | 12q24 |
| DAO      | DAOA        | d-amino acid oxidase               | 12q24     | FRA12C/E/D | 12q24/24.13 |
| CAGR1 ***|             | mab21-like 1 (c. elegans)          | 13q13     | FRA13A | 13q13.2 |
| HTR2     | HTR2/HTR2a  | 5-hydoxytryptamine (serotonin) receptor | 13q14-q21 | FRA13B/C | 13q21-q21.2 |
| SCZD7    |             | schizophrenia disorder 7           | 13q32     | FRA13D | 13q32   |
| G72      | DAOA        | d-amino acid oxidase activator     | 13q34     |        |         |
| NAME          | ALIAS                        | FUNCTION                                           | ADDRESS       | SITE   | ADDRESS       |
|---------------|------------------------------|----------------------------------------------------|---------------|--------|---------------|
| NPAS3         | neuronal pas domain protein 3| 14q12-q13                                          |               |        |               |
| HERC2         | hect domain and RLD2         | 15q13                                              |               |        |               |
| CHRNA7        | cholinergic receptor nicotinic| 15q14                                              |               |        |               |
| SCZD10        | schizophrenia disorder 10    | 15q15                                              |               |        |               |
| GRIN2A        | glutamate receptor, ionotropic 2A| 16p13.2                                          |               |        |               |
| SLC6A4        | serotonin transporter       | 17q11.2-q12                                        |               |        |               |
| ACE           | angiotensin I converting enzyme| 17q23                                             | FRA17B        | 17q23.1|               |
| IMPA2         | inositol(myo)-1(or 4)-monophos| 18p11.2                                          |               |        |               |
| SCA6          | calcium channel, voltage dependent, P/Q type, alpha 1A subunit| 19p13.2-p13.1| FRA19B | 19p13  |               |
| APOE          | apolipoprotein E             | 19q13.2                                            | FRA19A        | 19q13  |               |
| DNMT          | DNA methyltransferase 1      | 19q13.2                                            | FRA19A        | 19q13  |               |
| PRNP          | prion protein (p27-30) (Creutz)| 20pter-p12                                         | FRA20B        | 20p12.2|               |
| SNAP-25       | synaptosomal-associated protein 25kDa| 20p12-p11.2| FRA20B/A | 20p12.2/11.23|               |
| CHGB          | chromogranin B (secretogranin 1) | 20pter-p12                                         | FRA20B        | 20p12.2|               |
| COMT          | catechol-O-methyltransferase | 22q11.21                                           |               |        |               |
| SNAP29        | synaptosomal-associated protein| 22q11.21                                         |               |        |               |
| PCQAP         | PC2 (positive cofactor 2 mult)| 22q11.2                                           |               |        |               |
| PRODH/DGCR6   | DiGeorge Syndrome critical region, gene 6| 22q11.21                                         |               |        |               |
| UFD1L         | ubiquitin fusion degradation 1| 22q11.21                                          |               |        |               |
| ZN7F4         | zinc finger protein 74 (Cos52)| 22q11.21                                          |               |        |               |
| APOL-4        | apolipoprotein L-4           | 22q11.2-13.2                                       | FRA22A/B      | 22q12.2/13|               |
| APOL-2        | apolipoprotein L2            | 22q12                                              | FRA22B        | 22q12.2|               |
| SYN3          | synaptin 3                   | 22q12.3                                            |               |        |               |
| TIMP3         | tissue inhibitor of metalloprot.3| 22q12.3                                          |               |        |               |
| YWHAH         | tyrosine 3-monooxygenase/tryp| 22q12.3                                            |               |        |               |
| APOL-1        | apolipoprotein L1            | 22q13.1                                            | FRA22A        | 22q13  |               |
| SYNGR1        | synaptotygin 1               | 22q13.1                                            | FRA22A        | 22q13  |               |
| CYP2D6        | cytochrome P450 family 2 sub| 22q13.1                                            | FRA22A        | 22q13  |               |
Genomic and Epigenomic Instability, Fragile Sites, Schizophrenia

Table 4. Contd…..

| GENE | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|----------|---------|------|---------|
| IL2RB | interleukin 2 receptor beta | 22q13/13.1 | FRA22A | 22q13 |
| BZRTP | benzodiazapine receptor (peripheral) | 22q13.31 | FRA22A | 22q13 |
| WKLI | megalencephalic leukoencephalopathy with subcortical cysts 1 | 22q13.33 | FRA22A | 22q13 |

X CHROMOSOME

| GENE | FUNCTION | ADDRESS | SITE | ADDRESS |
|------|----------|---------|------|---------|
| HTR2C | 5-hydorxytryptamine (serotonin) receptor 2C | Xq24 | | |
| L1CAM | L1 cell adhesion molecule | Xq28 | FRAXE/F | Xq28 |

Studies were obtained from the National Institute of Health’s database linking specific genes to schizophrenia at http://www.geneticassociationdb.com. In addition, a Pubmed search using the keywords “gene AND schizophrenia” yielded more unique studies. The genes found using these two methods were then searched more exclusively using the keywords “gene name” AND schizophrenia in order to more thoroughly assess whether at least one positive association was found between a gene and schizophrenia. Genes are organized by chromosomal locations, and appear in bold when co-localizing with a chromosomal fragile sites. The co-localizing fragile site name and address is shown. More information can be found at http://schizogad.bu.edu.

Fig. (8). Genomic distribution of genes, chromosomal regions, and chromosomal abnormalities linked to schizophrenia vs fragile sites. These results were obtained by cataloguing genes linked to schizophrenia from a Pubmed search (http://www.ncbi.nlm.nih.gov) using the words “schizophrenia" AND "genes", "genetic studies", or "chromosomal abnormalities". The genomic regions that contain a fragile site was determined from a Pubmed search using the words "fragile sites". The genome "real-estate" of each locus and all the fragile sites was taken as the highest known chromosome banding resolution. Negative controls consisting of (a) all human genes and (b) genes tested but not found to be associated with schizophrenia did not have any preferential association with fragile sites.

Certainly, genes in fragile sites regions in the brain may be impacted in vivo when individuals are folate malnourished during development. In adults, DNA replication in the brain occurs in the dentate gyrus and olfactory bulb, hence folate deprivation could impact neurogenesis during all periods of life, perhaps transiently increasing the severity of disease.

In summary, fragile sites are more frequent in schizophrenia and co-localize with schizophrenia-linked genes. Fragile sites are sensitive to conditions that interfere with DNA replication, including folate deficiencies. Schizophrenia is linked to folate metabolism genetically (e.g. through hypoactive polymorphisms in genes that directly affect folate processing (e.g. MTHFR, MTR – see meta-analysis in [96])) and through epigenetic studies (see above) and environmental studies (see below).

ENVIRONMENTAL FACTORS AND SCHIZOPHRENIA

Some environmental factors linked to schizophrenia during early development are listed in Table 5. No factor is sufficient by itself to induce disease. Family history, CNS damage, bereavement, and rubella infection increase the odds ratio most for disease. Paternal age and nutrition, well-documented factors linked to schizophrenia, provide important clues for understanding the biochemistry of schizophrenia. Further, the metabolic links can be used to postulate a role for other environmental components in disease (see below).

Paternal Age

Since 1958, many studies have implicated paternal age as an environmental factor influencing the occurrence of schizophrenia (e.g. [97-99]). For instance, Malespina et al. [97] reported a three-fold increase in the incidence of schizophrenia in progeny of fathers over the age of 50 years (Fig. 9). Today, the association with maternal age is unclear. Paternal and maternal age are linked to autism [100].

The paternal age connection implicates changes to paternal germline DNA in some cases of schizophrenia because DNA is the sole paternal biological contribution to progeny. Paternal aging is linked to diminished semen quality [101] and fertility [102], increases in sperm DNA damage (e.g. [103-105]) spontaneous abortions [105, 106], birth defects [106, 107] and single base changes in rare autosomal dominant diseases [108-110]. For instance, mutations in DF1 fibroblast growth factor receptor (FGFR3) are linked to Achondroplasia. Mutations in FGFR2 are linked to Apert, Crouzon, and Pfeiffer syndrome (PS), although some PS mutations may occur in FGFR2. Mutations in the lamina A
(LMNA) gene are linked to Progeria, while mutations in REarranged during transfection (RET) are linked to multiple endocrine neoplasia (MEN2A MEN2B) and medullary thyroid carcinoma (MTC).

Base substitutions account for all but progeria mutations in LMNA. The majority of mutations are transitions, (C to T) although some transversions (C to G) occur in a single dinucleotide CpG sequence. However, neither the number of replication cycles nor the observed mutation rates [110-113] accounts for the exponential rather than linear increase in disease as a function of paternal age. Hence, it was suggested that these mutations confer a selection growth advantage to sperm. Lower and more linear-like increases as a function of paternal age are observed for a number of other rare autosomal dominant diseases such as neurobromatosis, bilateral retinoblastoma, Treacher Collins syndrome, multiple exostoses, and Sotos syndrome [108, 112, 113], as well as Down syndrome, neural tube defects, congenital cataracts, and reduction defects of the upper limb [105, 107].

**Nutrition**

Under-nutrition (general caloric or protein deficiency) and malnutrition (deficiencies in specific elements, e.g. folic acid, zinc, copper, etc.) occur worldwide and are the most common diseases of childhood and prenatal life. Moderate to severe under-nutrition occurring prior to 2 years of age is associated with persistent behavioral and cognitive deficits that resist nutritional rehabilitation [114]. Pregnant mothers exposed to famine [115, 116] or malnourished (e.g. for folate deficiencies [117]) have an increased risk for children with schizophrenia. Maternal exposure to nutritional insults leads to persistent physiological and biochemical effects on the offspring [118-121]. Nutritional factors that have been linked to schizophrenia and autism, like folate deficiency, can impact both genetics (DNA damage and fragile site expression) and epigenetics (DNA methylation via folic deficiency) in affected individuals. Generally, the specific mechanism(s) by which nutritional deficiencies produce these birth defects are unknown.

**Folic Acid**

The importance of folic acid in preventing birth defects (e.g. neural tube defects including spina bifida) is well known, although the mechanism of disease induction is not understood [122]. Less well known is that fact that folic acid deficiencies are associated with a number of neurological diseases (e.g. [123, 124]) including schizophrenia and mood disorders [125-129], and are common in patients with psychopathology [130]. Furthermore, genes specifying proteins involved in folate metabolism are associated with schizophrenia and mood disorders as well as autism and other neuropsychiatric diseases [131].

Folic acid provides methyl groups to form S-adenosyl-methionine (SAM, see below), the universal intracellular methyl donor during methylation reactions such as those important in epigenetics.

**Folic Acid Metabolism**

At the molecular level, folic acid deficiencies have the potential to disrupt nucleic acid metabolism, processes that require energy (i.e. ATP or NAD, GTP), activated nucleotide precursors (ribo - and deoxyribo- nucleoside triphosphates, e.g. DNA replication and RNA transcription), or SAM (or folate directly) for methylation (Fig. 10). Abbreviated schemes of de novo synthetic pathways for ribo- and deoxyribo- nucleoside triphosphate synthesis are shown in Fig. (11). Folate derivatives are required by thymidine synthase

| Factor                  | Odds Ratio |
|-------------------------|------------|
| Place/time of birth     |            |
| Winter                  | 1.2        |
| Urban                  | 1.5        |
| Infection              |            |
| Influenza              | 2.0        |
| Respiratory            | 2.2        |
| Rubella                | 5.2        |
| Poliovirus             | 1.1        |
| CNS                     | 4.0        |
| Prenatal               |            |
| Famine                 | 2.0        |
| Bereavement            | 6.2        |
| Flood                  | 1.8        |
| Unwantedness           | 2.4        |
| Maternal depr          | 1.8        |
| Obstetric              |            |
| Rh incompatibility     | 2.8        |
| Hyoxia                 | 3.0        |
| CNS damage             | 7.0        |
| Low birth weight       | 1.6        |
| Pre-eclampsia          | 2.5        |
| Genetics               |            |
| Family history         | 9.7        |

**Table 5. Odds Ratio of Genetics and Environmental Factors Linked to Schizophrenia. Adapted from [149]**

**Fig. (9). The effect of paternal age on schizophrenia.** The data shows a linear increase in the incidence of schizophrenia and paternal age, and a three-fold increase for children of fathers over the age of 50. Figure is adapted from [97].

![Number of births vs paternal age](image)

Although some transversions (C to G) occur in a single dinucleotide CpG sequence. However, neither the number of replication cycles nor the observed mutation rates [110-113] accounts for the exponential rather than linear increase in disease as a function age; hence, it was suggested that these mutations confer a selection growth advantage to sperm. Lower and more linear-like increases as a function of paternal age are observed for a number of other rare autosomal dominant diseases such as neurobromatosis, bilateral retinoblastoma, Treacher Collins syndrome, multiple exostoses, and Sotos syndrome [108, 112, 113], as well as Down syndrome, neural tube defects, congenital cataracts, and reduction defects of the upper limb [105, 107].
Folate is an essential nutrient that is required in the synthesis of nucleic acid, s-adenosyl methionine (SAM) and amino acids. Further, synthesis of these monomers and their incorporation into polymeric molecules most times requires activated nucleosides like ATP, NAD and GTP whose synthesis depends on folic acid intermediates. Hence, the synthesis of DNA/RNA and SAM is heavily dependent on folic acid. (Figure adapted from http://www.tcd.ie/ IUBMB-Nicholson/pdf/29.pdf).

that converts dUMP to dTMP, and for two steps in the purine biosynthetic pathway to make IMP; hence impacting ribo and deoxyribo purine synthesis.

Folate participates in the methionine cycle to synthesize S-adenosyl methionine (SAM). SAM is the second most used cofactor in the cells after ATP (Fig. 12). SAM is used by >100 methyl transferases that act on DNA, RNA, proteins (e. g. DNA methyl transferase DNMT (for review see [40])), histone methyl transferases (HMT), and small molecules (e.g. COMT), and for the synthesis of polyamines that stabilize DNA.

In the methionine cycle, a methyl group from folate is use by the enzyme, Methionine Synthase (MS), to convert homocysteine (HCY) to methionine. Alternatively, Betaine...
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Homocysteine Methyl Transferase (BHMT) regenerates methionine from HCY using a methyl group from betaine (choline). Dietary and regenerated methionine reacts with ATP to generate SAM, while HCY is the product of de-methylated (via methyl transferases) and de-adenylated SAM.

Besides being used to reform methionine, HCY may be directed towards the trans-sulfuration pathway to produce the amino acid cysteine, and the primary intracellular antioxidant, glutathione (GSH). The enzyme MS covalently adds a methyl group to the dopamine D4 receptor (DRD4), which transfers the methyl group to lipopolysaccharides. In mammals, folate, methionine, and vitamins B6, B9 and B12 required by these pathways, must be obtained from the diet or intestinal bacteria. Methionine may also be obtained from degradation of proteins.

Homocysteine is a key intermediate used for SAM metabolism, is required for the synthesis of GSH; hence, dopamine metabolism, DNA replication and epigenetic marking are linked to oxidative stress.

The brain is especially sensitive to oxidative stress. Oxidative stress (hypoxia) is linked to schizophrenia directly (Fig. 9), is a common consequence of obstetric complications linked to schizophrenia [132], and a potent inducer of fragile sites and genomic rearrangements [133]. Hence, oxidative stress through the transsulfuration pathway is linked to DNA metabolism, and epigenetic marking. For instance, increased oxidative stress can direct HCY toward GSH production rather than SAM production, impacting many processes in vivo.

Nutrition is critical for maintaining the folate-methionine-transsulfuration hub because vitamins B6, B9 (folate) and B12, and the amino acid methionine must be obtained from the diet. Other factors listed in Fig. (9) can impact the folate-methionine-transsulfuration hub. For instance, winter births are associated with times of food scarcity [134], and many times bereavement and depression are accompanied by reduced food intake. Infection or inflammation increases metabolites requirements such as those needed for DNA replication, or transcription.

Aberrant folate metabolism in schizophrenia has been demonstrated in a number of studies, for review see [135, [Fig. (12)]].
Ablerrant folate metabolism has been detected in autistic patients. In an impressive series of experiments, James et al. [138-141] detected aberrant levels of metabolic markers for the folate-methionine-transulfuration hub in patients and their mothers. For instance, decreased levels of methionine cycle (e.g. methionine, SAM, S-adenosylhomocysteine (SAH), adenosine, and HCY), and trans-sulfuration pathway (e.g. cystathionine, cysteine and total glutathione (oxidized (GSH) + reduced GSSG)), metabolites were detected. Also reported was an increase in other methionine cycle (e.g. SAM, adenosine) and transsulfuration (e.g oxidized glutathione) pathway metabolites. In 2006, James et al. [138] linked SNPs in genes within the folate cycle (in the reduced folate carrier (RFC), methylentetrahydrofolate reductase (MTHFR), the methionine cycle (COMT), and the transsulfuration pathway (glutathione-S-transferase (GST) to autism. In a preliminary study, James et al. [141] demonstrated that a nutritional treatment regime (supplementation with methylcobalamine (methylated vitamin B6), and folic acid) improved but did not normalize abnormal metabolite blood values. An analysis of the effect of nutritional supplementation on disease symptoms was not measured, although anecdotal improvements were reported.

CONCLUSION

In summary, genetic and environmental components of schizophrenia and other neuropsychiatric diseases point to the importance of the folate-methionine-transulfuration pathway. This idea is exciting because this hub presents novel targets for drug development, and may lend themselves to nutrition interventions.

Folate supplementation has been successful in the prevention of spina bifida and related abnormalities. Similar therapies may reduce risk and severity for neuropsychiatric disease. Faulty DNA replication and epigenetic marking during brain development and adult neurogenesis may impact occurrence, presentation and dynamics of neuropsychiatric disease. Simply providing excess folate may not be useful (see [142]).

Reed and colleagues [143-146] have developed a dynamic model of the interaction of the folate and methionine cycles at the protein level. The Reed model is consistent with published data but does not yet include the entire folate-methionine-transulfuration hub, nor has the model been tested experimentally. However, this model is a beginning, and reminds us that an understanding the complex, dynamic behaviors of metabolic pathways are required to developed individualized nutritional and/or medical interventions in patients.

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