Common Terminology:

Methyl group: A methyl group is simply a single carbon atom bonded to 3 hydrogen atoms (CH3).

Methylation: Transfer of methyl groups from one chemical to another is called methylation. Essentially any chemical compound that has a *methyl group* as part of its chemical structure is capable of donating it to another chemical that needs it. The chemical that receives the methyl group is "*methylated*". This process of moving methyl groups around is necessary for the functioning of several biochemical reactions such as DNA and RNA synthesis, creatinine generation, immune responses involved in silencing viruses etc.
Role of enzymes: Most of the biochemical reactions in the body operate as cycles that are dependent on one or more enzymes. E.g. *Chemical A* gets converted to *Chemical B*; *Chemical B* in turn gets converted to *Chemical C*. Each of those steps has an enzyme involved that aids in the actual conversion of the first chemical into the second and so on.

In terms of the various pathways that we are addressing, there are several enzymes involved. When these cycles are operating optimally, each chemical moves through the various steps continuously. It is important to remember that while it looks like each of these cycles is occurring in isolation, in reality there are several copies of each of these chemicals being converted into their respective intermediates by several copies of enzymes. It is not a single methionine molecule being converted to SAMe or a single homocysteine molecule being converted to methionine but multiple copies of each by multiple copies of the respective enzymes. I like the analogy I found on one site: start thinking of these not as single chemicals, but buckets full of each, and pumps (the enzymes) to move the chemical from one bucket to the next.
Mutations or Single Nucleotide Polymorphism (SNP): A gene mutation is a permanent change in the DNA sequence that makes up a gene. Mutations range in size from one DNA base to a large segment of a chromosome. A Single Nucleotide Polymorphism or SNP (pronounced "snip"), is a small genetic change, or variation, that can occur within a person's DNA sequence. The genetic code is specified by the four nucleotide "letters" A (adenine), C (cytosine), T (thymine), and G (guanine). SNP variation occurs when a single nucleotide, such as an A, replaces one of the other three nucleotide letters: C, G, or T.

Think of mutations in enzymes as breaks that affect the ability of the enzyme to do its job. Homozygous (++) mutations are ones where both copies of the gene are affected and heterozygous (+-) mutations are the ones where only one copy of the gene is affected. Each of us has two copies of each gene that we inherit from each parent. Some mutations speed up the activity of the enzyme (e.g. CBS upregulation) whereas others slow them down considerably (e.g. MTHFr C677T and A1298C, COMT mutations).

The "slower" mutations create a situation where the bucket cannot be filled, like trying to fill the bath tub with the faucet only open to a drip. The "faster" mutations are like having a hole in the bucket. No matter how fast or slow you fill the bucket, the faster mutations drain out all of the contents of the bucket. This is why the CBS upregulation is such an overriding factor. It will drain the bucket. If the bucket is filling via a slow drip, due to the MTHFr C677T mutation, or methioninesynthasereductase mutations, then having a hole in your bucket will be more of a problem than if you are able to easily refill your bucket because you do not have the slow mutations too.

Note: The following material is intended to be used in conjunction with the pathway diagrams that are posted.

Methylation Cycle: This is the pathway at the far right in the diagrams, it is also known as the **SAM** or **Methionine** cycle. It is so named because of the intermediates involved in the cycle and also because this is the cycle that is responsible for the process of methylation that was described above (adding or removing methyl groups to various chemicals/metabolites and/or reactions). The intermediates or chemicals involved in this cycle are *methionine*, *S-adenosylmethionine* (SAM or SAMe), *S-adenosylhomocysteine* (SAH) and *homocysteine*. It involves the regeneration of methionine from homocysteine. This conversion of homocysteine to methionine occurs with the help of Vitamin B12 (specifically the methyl version of Vitamin b12, methylcobalamin) and 5-methyltetrahydrofolate (folapro), which is an intermediate in the folate cycle. Look at this cycle as starting with methionine, methionine then being converted into the various intermediates such as SAMe, SAH, homocysteine and then ultimately being re-converted into methionine.

Step I: This involves methionine being converted to SAMe in the presence of magnesium (Mg) and ATP (universal energy donor) by the enzyme methionineadenosyltransferase (MAT). SAMe is called the *universal methyl donor* as it is the primary source of methyl groups for most other biochemical reactions including methylation of DNA, RNA, proteins, creatine etc.

Step II: SAMe, once it donates its methyl group to the various reactions, gets converted to SAH.

Step III: SAH in turn is metabolized to homocysteine by the enzyme S-adenosylhomocysteinehydrogenase (SAHH). This reaction also generates a chemical called *adenosine*.

Step IV: There are three possible ways homocysteine is removed as an intermediate. One is a reversible reaction that converts homocysteine back to methionine and is dependent on the *folate cycle*. The other is an irreversible reaction that is referred to as the *TransSulfuration pathway*. This involves the conversion of homocysteine into cystathione and its subsequent intermediates. The third involves the methylation of homocysteine into methionine, independent of the folate cycle. Let us take a look at each of them.

a) TransSulfuration Cycle: This cycle entails the irreversible conversion of homocysteine into cystathione by the enzyme cystathione B-synthase (CBS) in the presence of Vitamin B6 and heme as cofactors. Cystathione is in turn converted to cysteine and alphaketoglutarate. The amount of cysteine generated by this process acts as the rate limiting factor for the subsequent products that are generated, i.e. taurine and/or glutathione. If there is excess cysteine generated as a result of the CBS upregulation (mutation that makes the enzyme activity faster than normal), more taurine is generated instead of glutathione. Glutathione is one of the essential antioxidants involved with detoxification in our bodies.

b) Some of the homocysteine goes back up the cycle to regenerate methionine. This process is mediated by the enzyme methionine synthase (MS aka MTR), with the aid of methylcobalamin (Vitamin B12 that has a methyl group as part of its structure). Essentially cobalamin accepts a methyl group from 5-methyltetrahydrofolate (which is an intermediate in the folate cycle) and becomes methylcobalamin. This is where the SAM and folate cycles meet. Think of each of the cycles as independent entities doing their business but each of them are dependent on one another in order to function properly.

Methylcobalamin in turn donates the methyl group it gained to homocysteine and this converts homocysteine back to methionine. Essentially, homocysteine is being re-methylated to methionine.

Once methylcobalamin donates its methyl group to methionine, it becomes cobalamin again. Some of this cobalamin is remethylated into methylcobalamin by the enzyme methioninesynthasereductase (MSR aka MS_MTRR). Think of this as a reaction that is trying to maintain the levels of methylcobalamin. The methyl group necessary for this reaction is donated by SAMe (this reaction is not indicated on the diagram, this is just FYI for those of you wondering what the role of MSR is in the pathway).
Just remember that the methyl group that is donated by methylcobalamin to convert homocysteine to methionine comes from the folate cycle (5-methyltetrahydrofolate). Once methylcobalamin donates that methyl group to homocysteine, it becomes cobalamin which in turn gains a methyl group from SAMe to regenerate some of the methylcobalamin.

c) If that wasn't complex enough, there is yet another reaction that converts homocysteine into methionine (look at the center of the methylation pathway diagram for this reaction). The enzyme involved here is betainehomocysteinemethyltransferase (BHMT). BHMT converts homocysteine into methionine in a reaction independent of the one that is mediated by MS, i.e. the one described before, which involves the transfer of a methyl group from 5-methyltetrahydrofolate to methylcobalamin and from methylcobalamin to homocysteine. Just remember that this step doesn't involve B12 or the folate cycle. In this case methionine is regenerated from homocysteine by the transfer of a methyl group from betaine (TMG or trimethylglycine) to homocysteine. Once TMG loses a methyl group to homocysteine, it gets converted to dimethylglycine (DMG). In turn homocysteine gains a methyl group and becomes methionine.

***This in essence is the Methylation pathway*.**

Folate Cycle: This cycle involves the conversion of tetrahydrofolate (THF) into 5,10-methylenetetrahydrafolate which in turn gets converted to 5-methyltetrahydrofolate (MTHF). MTHF is then converted back into THF.

Dietary folate, or folic acid that you get from your foods, is converted into a product called dihydrafolate (DHF) in the presence of Vitamin B3. DHF is then converted to THF, also with the aid of B3. THF is converted to 5,10-methylenetetrahydrofolate with help from Vitamin B6, P5P and Serine. Essentially THF gains a "methylene" group (different from methyl group) from serine to become 5,10-methylenetetrahydrofolate.
Alternatively folinic acid (5-formyltetrahydrofolate, different from folic acid) is also converted to 5,10-methylenetetrahydrofolate in a reaction occurring simultaneously.

5,10-methylenetetrahydrofolate is then converted to 5-methyltetrahydrofolate (MTHF) aka "Folapro" by the enzyme methylenetetrahydrafolatereductase (MTHFr) with the aid of NADH, B2 and ATP.

MTHFr: The MTHFr enzyme has multiple functions. However we are concerned with two of the roles it plays with respect to autism and these pathways. The first one being its involvement in the generation of MTHF within the folate cycle and the second being its ability to drive the conversion of BH2 to BH4 (BH4 cycle). Mutations in the part of the eznyme that is involved in the folate cycle are characterized as the "C677T" mutation and this mutation slows down the activity of the

enzyme. This means MTHF production will be affected. Why does the amount of MTHF (folapro) matter? Because if you remember from the previous discussion on the Methylation pathway, MTHF is the compound that donates the methyl group to cobalamin which in turn donates it to homocysteine to regenerate methionine. If we have less of the MTHF to begin with, there will be less of it to go around to regenerate THF in the folate cycle, and to transfer methyl groups to regenerate methionine in the Methylation cycle. So this will affect not only the Folate cycle but also the Methylation cycle. Remember the Folate and Methylation pathways meet to transfer methyl groups and any breaks preceding that transfer will affect the functioning of both of the pathways.

As mentioned before MTHFr has a dual role in terms of these pathways. While it is driving the folate cycle in one direction, it is also driving a reverse reaction on the other side. This reaction is the conversion of BH2 to BH4. Mutations that affect this part of the enzyme are characterized as "A1298C" mutation.

BH4 cycle: Tetrahydrobiopterin (BH4) is essential for normal central nervous system functioning. It is an essential factor or cofactor for the enzymes in the biological pathways necessary for synthesizing catecholamines (dopamine, noradrenaline/norepinephrine) and indolamines (serotonin and melatonin), as well as for all three isotypes of nitric oxide synthases (NOS in the Urea cycle). BH4 is a cofactor for tyrosine and trytophan hydroxylase, the enzymes involved in catecholamine and indolamine synthesis respectively. The rate of BH4 formation determines the rate of production of these important neurotransmitters, because BH4 happens to be the rate limiting factor here. How much of it is present affects the ability to synthesize neurotransmitters like dopamine, norepinephrine, serotonin etc. and also affects the outcome of the Urea Cycle.

Tyrosine (amino acid) is converted to dopamine through a series of reactions involving the enzyme dihydroxyphenylalaninereductase (DHPR). Dopamine can be further metabolized to norepinephrine by the enzyme dopamine-b-hydroxylase. Dopamine and norepinephrine can also be metabolized by the enzyme monoamine oxidase (MAO) to 3,4,-dihydroxyphenylacetic acid or the enzyme Catechol-O-MethylTransferase (COMT) to 3-methoxytyramine. Action by both enzymes results in the formation of homovanillic acid (HVA or 3-methoxy-4hydroxy-phenylacetic acid) and VMA.

Serotonin (5-HT) is synthesized from the amino acid tryptophan in two steps catalyzed by the enzymes tryptophan hydroxylase and L-amino acid decarboxylase. Serotonin is metabolized by monoamine oxidase (MAO) to 5-hydroxyindoleacetic acid (HIAA).

Serotonin can also be methylated (first acetylated i.e. addition of acetyl group and then methylated) to form Melatonin. The enzyme involved in the acetylation of serotonin to form N-acetylserotonin is serotonin-N-acetyltransferase. N-acetylserotonin is methylated (addition of methyl group) to form melatonin by the enzyme HydroxyIndole-O-MethylTransferase.

The A1298C mutation in the MTHFr enzyme affects the conversion of dihydrobiopterin (BH2) to tetrahydrobiopterin (BH4). Less amounts of BH4 will therefore put a strain on the conversion of trytophan to serotonin and tyrosine to dopamine. This will lead to low levels of neurotransmitters such as dopamine, norepinephrine, serotonin and melatonin.
In addition the activity level of the COMT enzyme will further affect the levels of dopamine and norepinephrine.

COMT: Catechol-O-MethylTransferase is the enzyme involved in the metabolism of dopamine

and norepinephrine into subsequent compounds such as HVA and VMA. The rate of activity of COMT will determine how fast these neurotransmitters will be broken down. Mutations in the COMT enzyme (COMT ++ or COMT +-) actually slow down the activity of the enzyme. Normal COMT activity (no mutations) is depicted as COMT --
Mutations in the COMT enzyme will slow down the breakdown of dopamine, therefore individuals who are COMT ++ or +- have higher (as in good) levels of dopamine compared to COMT -- individuals who are rapidly draining their dopamine stores. This condition is further exacerbated if the individual has the A1298C mutation (++ or +-) because their dopamine levels are low to begin with (remember these individuals have less BH4, so less dopamine gets made).
Undermethylators: Dr.Amy categorizes individuals who are COMT -- as undermethylators. One of the ways the COMT enzyme breaks down dopamine is by using a methyl group donated by SAMe (remember it is the universal methyl donor). Therefore a COMT -- individual will be in constant need of methyl groups as they are rapidly metabolizing dopamine. This puts a strain on the Methylation cycle as the demand on SAMe for methyl groups is increased. Think of this as COMT constantly demanding methyl groups from SAMe. If there are issues in the Methylation cycle or Folate cycle that affect the levels of SAMe (which in turn is dependent on the levels of methionine), there will be less methyl groups to begin with and even less to go around. It is like a domino affect. A break or strain in one cycle has a ripple effect on the rest as they are all co-dependent. Less methyl groups -> less methylation -> less RNA/DNA/protein synthesis/heavier viral load due to lack of methylation etc.

Overmethylators: These are the individuals who are COMT ++. Mutations make the COMT enzyme slower, so it will not break down dopamine as rapidly. Since it is slower in metabolizing dopamine, its demand for methyl groups is also reduced. Subsequently there is less of a strain on SAMe for methyl groups. So there will be relatively more methyl groups available for other biochemical reactions and to go around the various cycles.

Remember these are just relative terms. An undermethylator is low in methyl groups and an overmethylator has a higher store of them, in comparison. Remember one of the reasons we are in this predicament with autism is because we have problems with methylation, regardless of the ***under*** or ***over*** status.

In addition the strain on the BH4 cycle, the amount of BH4 will also affect the functioning of the Urea cycle. BH4 is the rate limiting factor for the Urea cycle. Two molecules of BH4 are necessary to drive the Urea cycle. One molecule will in turn generate *peroxynitrite* and if the individual has no BH4 left, *super oxide* is formed. Peroxynitrite and super oxide in combination cause damage to neurons when they accumulate in excess. . Peroxynitrite is a potent oxidant, which is capable of DNA strand scission (breaks open the bonds that keep the two DNA strands bound in a double-helix) , and nitrating tyrosine, all of which wreak havoc on the nervous system, especially a developing nervous system as in children. The ability to detoxify superoxide is facilitated by the enzyme superoxidedismutase (SOD).

Urea Cycle: Urea is the chief nitrogenous waste of mammals. Most of our nitrogenous waste comes from the breakdown of amino acids. Breakdown of amino acids results in the production of ammonia (NH3). Ammonia is a toxic compound that is converted into its safer counterpart urea, by enzymes in the liver. Urea is then eliminated by our kidneys*. Essentially the urea cycle involves the conversion of ammonia into urea with the help of the intermediates listed below.*

Arginine from our diet or from protein metabolism is converted to ornithine and *urea* by the

enzyme Arginase. Ornithine is then converted to citrulline by ornithine transcabamoylase. This is the reaction on the far left side of the pathway diagram. Citrulline is converted back to arginine. This cycling of Arginine through the various intermediates is what converts ammonia to urea. Arginine is also required for the production of Nitric Oxide (NO) by the enzyme nitric oxide synthase (NOS or eNOS). This reaction is dependent on the levels of BH4 available from the BH4 cycle. Remember two molecules of BH4 are needed to generate Citrulline and NO. One molecule of BH4 will in turn generate peroxynitrite and if there is no BH4, super oxide is formed. If we do not have enough BH4 to go around because of the A1298C mutation, we are going to have trouble with ammonia. Because ammonia is dangerous to the body, any BH4 we have is going to be used to try to get rid of the ammonia rather than to be making neurotransmitters like serotonin and dopamine. Furthermore mutations in the NOS (eNOS) exacerbate the situation as they will affect the synthesis of NO. NO is needed for several functions including secretion of certain hormones, addressing inflammation, killing pathogens etc.

In essence if the limited supply of BH4 puts a strain on the functioning of this pathway, excess ammonia will accumulate as there is not enough BH4 to help convert it to urea. In addition the lack of BH4 also creates damaging free radicals like peroxynitrite and super oxide (SOD is needed to detoxify superoxide).