KAT6A SYNDROME: DEFICIENCY OF A HISTONE ACETYLTRANSFERASE AS THE CAUSE OF MILD TO SEVERE MITOCHONDRIAL DISEASE

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Introduction

The MOZ (**MO**nocytic leukemia **Z**inc finger, or MYST3) protein, KAT6A, is a histone lysine acetyltransferase, for which *de novo* haploinsufficiency was identified in 2015 as the cause of a multiple malformation, developmental disabilities syndrome. Principal clinical findings in KAT6A syndrome include facial dysmorphisms, cardiac defects, mild to severe developmental disabilities, and profound speech impairment, often diagnosed as autism. Because histone acetylation is strongly influenced by mitochondrial regulation of cytoplasmic acetyl-CoA levels, it was not surprising to learn that several children with KAT6A syndrome had been thought on initial evaluation to have a mitochondrial disorder. This observation prompted further study of mitochondrial metabolism in KAT6A syndrome and its link to specific systemic disease in affected patients.

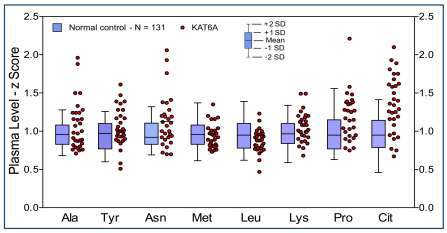
Methods

Based on metabolic findings in two children with KAT6A syndrome that suggested mitochondrial dysfunction, a standard set of laboratory tests was established for future referrals and used in the evaluation of 28 children and adults with *KAT6A* mutations, 26 truncating and 2 missense. The laboratory tests included plasma amino acid levels measured at 4 to 5 hours fasting; plasma levels of vitamin E, Coenzyme Q10, total homocysteine, and lactate; and routine CBCs and chemistry profiles. Amino acid levels were converted into normalized ratios-to-mean for analysis. By definition, the mean normalized ratio-to-mean is 1.00, with most diagnostically important amino acids having standard deviations for normalized ratios of 0.12 to 0.16, based on 131 controls.

Results

The most common amino acid abnormalities were increased levels of citrulline (mean z-score 1.28), asparagine (mean z-score 1.24), with other variably increased or decreased amino acid levels, such as alanine and proline, commonly present in mitochondrial diseases (fig 1).

Fig 1: KAT6A – Plasma Amino Acid Levels



Other laboratory findings consistent with mitochondrial dysfunction were low or borderline-low levels of CoQ10 and vitamin E and mildly increased levels of lactate. However, whereas only 1 of 23 subjects under age 10 had a combination of amino acid and other metabolic abnormalities consistent with mitochondrial oxidative (free radical) damage, 4 of 5 subjects over age 10 had signs of moderately severe mitochondrial disease, suggesting cumulative mitochondrial oxidative damage. Treatment with a mitochondrial antioxidant cocktail in severely affected patients or with only carnitine + pantothenic acid in several more mildly affected children led to improved motor and cognitive abilities and a decreased number of infections in most subjects. In the few patients who had been treated for more than a year, most of the amino acid abnormalities present before treatment were absent or had improved.

General Health and Development

Except for two adults studied, most patients treated with either L-carnitine + pantothenate or a full mitochondrial antioxidant cocktail, such as MitoSpectra®, were found by quantitative measures to have improved motor and language skills, which continue to improve in most patients. Patients who were treated with a full mitochondrial antioxidant cocktail also had a decreased number and shorter duration of viral infections, a commonly observed effect of treatment with a mitochondrial antioxidant cocktail, most likely because of down-regulation of the inflammatory response.

Methionine Metabolism and Bone Marrow Dysfunction

Despite having normal absolute plasma methionine levels by laboratory norms, 3 of 6 patients with a normalized methionine level of 0.80 or less had other evidence of systemic methionine deficiency (macrocytosis with high B12 and folate levels and a low total homocysteine level) and improved clinically and biochemically with methionine supplementation. In one 6-year-old boy with transfusion-dependent chronic pancytopenia and short telomeres (hyper-methylated DNA) in peripheral lymphocytes, marrow function rapidly returned to normal with oral methionine supplementation.

Citrate Deficiency and Gastrointestinal Dysmotility

Many KAT6A patients had a history of severe constipation and/or acute attacks of abdominal pain, often diagnosed as gastrointestinal dysmotility or intestinal pseudo-obstruction, and four young KAT6A children had at least one episode of intussusception. Because pseudo-obstruction in classical mitochondrial diseases is associated with low plasma citrate levels (R. Kelley, unpublished) oral citrate, 2 – 3 meq/kg/d, was given to several patients with dysmotility, all of whom responded with elimination or reduction of episodes of abdominal pain and/or a return to a normal stooling pattern.

Discussion

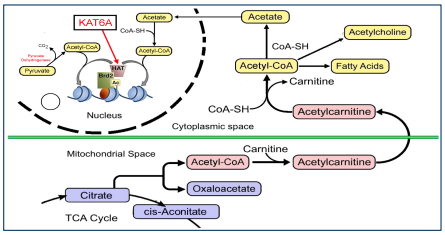
Histone acetylation is a dynamic process that responds to and regulates mitochondrial function. Although quantifying gene expression is today the most common way to study gene regulatory effects in disorders of histone modification, our results show the important and almost immediate clinical benefits of planned metabolic studies. Such studies can reveal the causes of medical problems in patients whose clinical problems are usually attributed to consequences of clinically more obvious, structural prenatal developmental abnormalities rather than to ongoing metabolic disease.

Mechanism of Action of Carnitine in KAT6A

The almost immediate clinical improvement caused by treatment of a then undiagnosed KAT6A child with a mitochondrial antioxidant cocktail first pointed to the possible effect of carnitine treatment in KAT6A. This is because CoQ10 and vitamin E in a mitochondrial cocktail require at least 3 months to reach therapeutic levels, whereas the metabolic effects of carnitine are almost immediate. Indeed, rapid gains in motor abilities and a reduction in autistic behaviors with carnitine treatment alone have since been observed in many KAT6A children, for whom we at first recommended treatment with only carnitine and pantothenate (to increase CoA levels), unless there were laboratory signs of mitochondrial oxidative damage or medical complications attributable to mitochondrial disease. Although it is possible that carnitine treatment would prevent the apparent age-dependent progression to frank mitochondrial disease in KAT6A, most parents today opt to treat with a full mitochondrial antioxidant cocktail as soon as the diagnosis of KAT6A deficiency is made.

Although the mechanism by which carnitine mediates its clinical effects in KAT6A is unknown, increasing the cellular free carnitine level would be expected to increase cytoplasmic levels of acetyl-CoA (fig 2) in a disorder in which synthesis of the major source of extramitochondrial acetyl-CoA, citrate, is limited. However, an intranuclear form of pyruvate dehydrogenase (PDH) was recently found and shown to be activated by carnitine in the same way as mitochondrial PDH. Therefore, nuclear PDH might generate most intranuclear acetyl-CoA for histone acetylation (PMID 24995980).

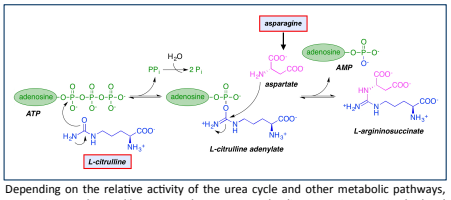
Fig 2: Effects of Carnitine on Levels of Acetyl-CoA



Abnormal Amino Acid Metabolism

The pairing of increased levels of asparagine and citrulline is an uncommon finding in mitochondrial diseases but has an easily understood origin, in that impaired synthesis of citrate in a subset of mitochondrial diseases leads to an accumulation of aspartate and asparagine, of which only asparagine can be accurately quantified in plasma. In support of this mechanism, the serum citrate level was found to be low in all 5 KAT6A patients in whom it was measured.

Fig 3: Conversion of Asparagine to Citrulline

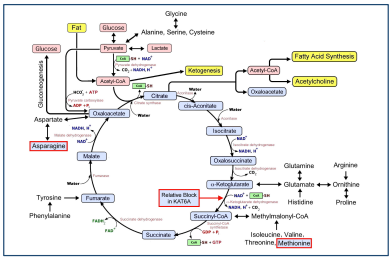


Depending on the relative activity of the urea cycle and other metabolic pathways, asparagine can be readily converted to aspartate, leading to an increase in the level of citrulline, as shown in fig 3, rather than the usual preferential synthesis of argininosuccinate. This possible direct link between hypercitrullinemia in KAT6A and the diet-influenced activity of the urea cycle could explain the variability in the levels of asparagine and citrulline in these patients. Further study of citrulline levels in KAT6A individuals is needed to determine the optimal timing for measuring plasma amino acid levels for monitoring treatment response.

Origin of Methionine Deficiency in KAT6A

Although methionine is one of the least abundant amino acids in human protein, constituting only 1.2% of amino acids in human milk, it is heavily utilized and recycled as a methyl group donor. For example, almost half of methionine imported into mitochondria is used for the synthesis of creatine, with the balance entering many other anabolic pathways, such as the synthesis of RNA and DNA nucleotides. However, in mitochondrial diseases, typically those with moderate to severe oxidative damage, methylmalonyl-CoA becomes one of the few available substrates for making succinyl-CoA to maintain citric acid cycle oxidative metabolism and anaplerosis (fig 4). As a result, the methionine carbon skeleton is catabolized at a much higher-than-normal

Fig 4 Amino Acid Metabolism and the Citric Acid Cycle

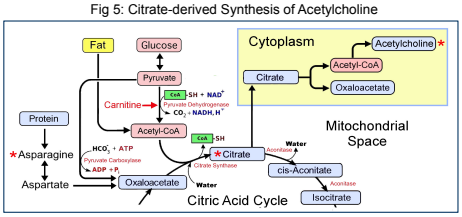


rate, causing methionine depletion, a condition mimicking vitamin B12 deficiency except for having a low rather than high total homocysteine level. Surprisingly, however, the plasma methionine level in KAT6A usually is not low or even relatively low when methionine is deficient, because an important adaptation to systemic methionine deficiency is down-regulation of the hepatic transsulfuration pathway as a way to conserve methionine for export to other tissues. As a result, methionine deficiency as the cause of blood dyscrasias and neurological deterioration in KAT6A syndrome and other mitochondrial diseases is often missed.

Citrate Deficiency and Gastrointestinal Dysmotility

Acetylcholine is the primary postganglionic neurotransmitter of the gastrointestinal tract, synthesized via acetylation of choline by acetyl-CoA. Because acetyl-CoA for acetylcholine synthesis is derived from cytoplasmic citrate, mostly exported from mitochondria, deficient synthesis of citrate, which characterizes a subset of mitochondrial disorders, causes dysmotility due to a deficiency of acetylcholine (fig 5).

Fig 5: Citrate-derived Synthesis of Acetylcholine



However, supplementation with oral citrate can restore GI motility to normal, as was the experience for KAT6A children with GI complaints consistent with intestinal dysmotility. Although a hamartoma or other small bowel malformation can be the leading point for intussusception, no intestinal anomalies were detected at the time of intussusception reduction.

Conclusions and Observations

1. Most patients with truncating mutations of *KAT6A* have signs of mitochondrial dysfunction, which is the apparent cause of bone marrow failure and multiple gastrointestinal complaints, such as intestinal dysmotility and, possibly, intussusception.

2. Treatment of KAT6A children and adults with a mitochondrial antioxidant cocktail leads to improved verbal and motor abilities and reduces the frequency and severity of infections.

3. Identifying mitochondrial dysfunction as the cause of several serious medical complications of KAT6A and has allowed the discovery of effective metabolic treatment for previously unexplained macrocytic anemia and bone marrow failure (methionine) and several manifestations of intestinal dysmotility (citrate).

4. KAT6A is only one of multiple disorders of histone modification known or predicted to affect mitochondrial metabolism that should be similarly studied to identify treatable complications of mitochondrial dysfunction.

References

1. Tham E, Lindstrand A, Santani A, et al. Dominant mutations in *KAT6A* cause intellectual disability with recognizable syndromic features. Am J Hum Genet. 2015 96:507-13. **PMID 25728777**

2. Arboleda VA, Lee H, Dorrani N, et al. *De novo* nonsense mutations in *KAT6A*, a lysine acetyl-transferase gene, cause a syndrome including microcephaly and global developmental delay. Am J Hum Genet. 2015 96:498-506. **PMID 25728775**.

3. Thomas T, Corcoran LM, Gugasyan R, et al. Monocytic leukemia zinc finger protein is essential for the development of long-term reconstituting hematopoietic stem cells. Genes Dev. 2006; 20:1175-86. **PMID 16651658**.

4. KAT6A Foundation: http://www.kat6a.org/about-kat6a/