GATM Mutations Cause a Dominant Fibrillar Conformational Disease in Mitochondria—When Eternity Kills

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More than 60 years ago, Luder and Sheldon and then, other nephrologists reported on a rare form of autosomal dominant renal Fanconi syndrome (RFS) that appeared in childhood and slowly evolved toward kidney insufficiency. These clinical features were thus quite distinct from nephropathic cystinosis, the most frequent cause of hereditary (recessive) RFS. Over the years, additional families were described, and the affected locus was identified; however, the causative gene and disease mechanism remained elusive. In this issue of the Journal of the American Society of Nephrology, the paper by Reichold et al. finally defines the mutated gene as GATM with identification of another family with de novo mutation, explains the dominant mode of transmission by an attractive multimerization-based fibrillar molecular model, and further proposes a rational dietary treatment that remains to be tested.

GATM encodes for a nuclear-encoded mitochondrial matrix dimeric enzyme, glycine amidinotransferase (hence GATM; also known as l-arginine:glycine amidinotransferase), catalyzing the penultimate reaction of the creatine biosynthesis pathway. Fortunately, much is known about GATM, including information from crystallographic studies. All patients with GATM disease exhibited a single heterozygous missense mutation at evolutionary conserved proline or threonine residues clustered in a small central stretch representing <5% of the protein. As predicted from dominant transmission, disease-causing GATM mutations did not abolish enzymatic activity. Furthermore, GATM haploinsufficient mice had no significant kidney phenotype, opening the possibility of suppressive intervention.

In kidneys, GATM expression is restricted to proximal tubular cells (PTCs), which house one of the largest mitochondrial compartments in the human body to support the high energy demand for their titanic transport activity. This explained the specific PTC damage related to GATM disease (thus RFS). Remarkably, GATM immunogold labeling of a patient kidney biopsy decorated fibrillar aggregates in mitochondrial PTCs. To address the mechanism of fibril formation by a cell biologic approach, the authors generated stable transfectants in proximal tubular LLC-PK1 cells for tetracycline-inducible overexpression of wild-type human GATM and each of its four known point mutants. Wild-type GATM overexpression had no effect on mitochondria, but all mutants caused a dramatic structural mitochondrial deformation, resembling sickle erythrocytes. Such mitochondria reproduced the immunogold pattern of the patient biopsy. Transfectants elegantly served to show irreversibility of the deformation by aggregates on removal of the tetracyclin inducer, much beyond the normal mitochondrial turnover time. Longitudinal fibrillar aggregation was found to prevent mitochondrial dynamics (fission), trigger reactive oxygen species (ROS) production, and activate the NLRP3 inflammasome.

The paper culminates in proposing an explanation for the propensity for aggregation by molecular dynamic modeling. Normal GATM acts as a homodimer (A:A). All mutations were predicted in silico to generate an additional pathogenic β-sheet–interacting interface (A/A) supporting alternate bead-like elongation, thus converting normal dimers into longitudinal multimers (A:A/A:A/...; i.e., fibrillar aggregates). Incidentally, it is remarkable that, among the 1500 predicted mitochondrial proteins, GATM mutations are so far unique in this behavior. Abnormal β-sheet formation is well known to favor bead-like fibrillar polymerization in α1-antitrypsin affected by homozygous Z mutation (PIZZ) to cause liver cirrhosis and a variety of brain neurodegenerative diseases causing dementia. In PIZZ hepatocytes, bead-like polymers and entangled polymeric aggregates accrue slowly over months/years. Interestingly, unequal exposure to inflammatory episodes explains in part the variable age of cirrhosis onset due to changes in the rate of PIZZ synthesis in the acute-phase response. This indicates that fibrillar disease evolution may be influenced by intervention on monomer synthesis. Dominant mutations of the microtubule-associated τ-protein induce neurofibrillary lesions, which cause frontotemporal dementia and are identical to those prevalent in acquired Alzheimer disease. Most recently, atomic resolution of τ-fibrils, achieved by cryoelectron microscopy, revealed a typical cross-β-structure. Thus, the predicted propensity to aggregation being dominant readily explains the autosomal dominant transmission and the slow progression of GATM disease; it

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places this disease in the family of fibrillar conformational diseases and suggests the possibility of beneficial intervention by decreasing synthesis of the specific protein culprit. Validation of molecular modeling of GATM fibrils by the recently available high-resolution cryoelectron microscopy might be considered.

How do fibrils escape clearance to cause disease? Protein aggregation is a central issue in biology and disease. Appropriate formation of intra- and extracellular fibrils has been remarkably tamed by evolution (e.g., to generate cystoskeletal and collagen fibers). In the cytosol, labile actin-based microfilaments and tubulin-based microtubules undergo permanent remodeling by assembly at the “plus” end and depolymerization at the “minus” end (unless stabilized by drugs, such as taxol). Also, in the secretory apparatus, premature intracellular collagen fibril formation is prevented by the addition of transient, extracellularly cleavable globular termini at the two ends of the newly synthesized triple helix. Unwanted aggregation of cytosolic proteins into irreversible fibrils is normally prevented by chaperone proteins, such as heat-shock proteins, and aggregates are normally cleared by the proteasome, selective macroautophagy, or chaperone-mediated autophagy. In the endoplasmic reticulum of PIZZ hepatocytes, fibrils preserve the folding of individual monomers, which prevents recognition by chaperones and thus, evades proteolytic clearance. Nuclear-encoded mitochondrial matrix proteins are first assembled by free cytosolic ribosomes and remain unfolded thanks to specific cytosolic chaperones until they cross the double mitochondrial membrane by unidirectional transport systems; only after that do they fold (and can dimerize). As in their bacterial ancestors, mitochondria contain folding chaperones (e.g., mtHSP70) and proteases competent to clear misfolded (e.g., aged) proteins. Alternatively, misfolded ubiquitinated mitochondrial matrix proteins can be retrotranslocated into the cytosol for degradation by the proteasome. It can, however, be suspected that, like in PIZZ proteins, preservation of mutated GATM monomer structure in bead-like polymers prevents their recognition by chaperone and proteolytic clearance, allowing for fibril extension and aggregation. Furthermore, large mitochondrial fibrils should obviously not be eligible for retrotranslocation into the cytosol. As a final quality control, mitochondria constantly rejuvenate by fission/fusion, and defective mitochondria are disposed of by mitophagy. However, when GATM mutant mitochondria are rigidified by transversal fibril bundles, fission is prevented. By analogy to “frustrated phagocytosis,” which occurs when extracellular preys are too large to be engulfed, huge mitochondria deformed by longitudinal GATM fibrils could predictably no longer be wrapped by the autophagosome membrane, causing “frustrated autophagy.” Consequences easily follow. Healthy mitochondria keep ROS production under control by their complex electron transfer system. Conversely, aging mitochondria release abundant ROS, which trigger the inflammatory status as documented here.

What are new perspectives? The paper by Reichold et al. stresses how important in-depth genetic analysis of unexplained causes of RFS is, including identification of de novo mutations. This will allow, in particular, a better estimate of GATM disease frequency, which is especially justified if effective treatment can indeed be proposed. As is the case for other human enzymes in biosynthetic pathways downregulated by the end product, this paper further shows that creatine supplementation to wild-type mice at equivalent acceptable doses for humans significantly decreases GATM expression and thus, might slow down disease progression (analogous to the control of inflammatory status in PIZZ patients). Whether creatine would prevent fibril formation in the transgenic LLC-PK1 cells could not be tested, because the endogenous promoter was swapped with the tetracycline-inducible promoter. A knock-in animal model (e.g., by CRISPR/Cas9 technology) is eagerly awaited to not only further investigate pathogeny in vivo (in particular, natural course and disease adaptations that might open unexpected avenues) but also, test effectiveness of creatine supplementation.

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DISCLOSURES

None.

REFERENCES

Greater Burden of ESRD among Immigrants: Kwa nini?

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At this time in our global history, when immigration policies are under frequent review, an understanding of the health status of immigrants has become increasingly important. In this issue of the *Journal of the American Society of Nephrology*, Perl et al. provide the first comprehensive report of the epidemiology of dialysis-dependent ESRD (ESRD-D) among immigrants compared with long-term residents. They conducted their study in Ontario, Canada, where universal health care coverage, including maintenance dialysis, begins shortly after an immigrant arrives. The authors found that the prevalence and incidence of ESRD-D differed significantly by world region and country of birth, with immigrants to Canada who were from sub-Saharan Africa and the Caribbean having the highest ESRD-D risk. To understand how best to address these risk differences, careful consideration of exactly why certain immigrant groups might have a greater burden of ESRD-D is imperative—or to state it in Swahili, kwa nini?

The factors influencing the risk of ESRD-D among immigrants likely include those related to the country of origin, the influences that informed the decision to immigrate, and the new country itself (Figure 1). Perl et al. found the greatest prevalence of ESRD-D to be among immigrants from countries likely to have high prevalence of *APOL1* risk variants given their predominant African ancestry. If this was the primary driver of greater risk of ESRD-D for these individuals, one might expect their kidney failure rates to mirror those seen in their country of origin. Unfortunately, for many low- and middle-income countries (including several in sub-Saharan Africa and the Caribbean), data on ESRD prevalence are limited, and dialysis is not widely accessible. Thus, estimates of ESRD-D are incomparable with what might be seen in countries with broader availability of dialysis. For example, Perl et al. found a prevalence of ESRD-D of 159.2 per 100,000 population for immigrants from Sudan, but in 2015, only 20 per 100,000 population were treated with hemodialysis in Sudan.

If genetic risk was the key explanatory factor for the differences in ESRD-D in Ontario, it would also be expected that long-term Canadian residents with similar ancestry or race/ethnicity as a proxy would have comparable rates of ESRD-D. Perl et al. note that a limitation of their study was the lack of data on race/ethnicity among long-term Canadians to allow for this comparison.

The motivation for immigration and the ability to immigrate may each play an important role in determining ESRD-D risk among immigrants. Younger, more physically robust individuals may be more likely to pursue moving to another country than older and/or frail persons. Consistent with this, Perl et al. found a 4.6-year (31%) difference in mean age between immigrants and long-term residents with ESRD-D. Socioeconomic status is also likely a strong determinant of ability to immigrate. Perl et al. found that immigrants from Somalia, designated by the United Nations to be a least developed country, had the highest prevalence and the second highest incidence of ESRD-D. Although many immigrants from least developed countries, such as Somalia, may have very low incomes, they may be more financially stable than others in their country of origin, leading to their opportunity for mobility. Notably, the average age of immigrants with ESRD-D in Ontario was 62.4 years old, which is >7 years older than the average life expectancy for Somalis in Somalia, who, on average, live to only 55 years old.

Because of limited access to dialysis in many low- and middle-income countries, individuals who are able may move to a new country in pursuit of long-term care of their kidney disease. For those able to pass the immigration process (including screening for communicable diseases and demonstration of value to the workforce), moving to countries such as Canada, where universal health care is available, would be attractive. Therefore, populations of immigrants with prevalent ESRD-D might be enriched with individuals with relatively better overall health than long-term residents with ESRD-D due to the “healthy immigrant effect.” Whether this leads to immigrants’ better survival on dialysis is worthy of further study.

The incident ESRD-D findings by Perl et al. argue that factors related to similarities and differences between the country of origin and the country immigrated to affected risk of ESRD-D. Compared with persons who immigrated to Ontario from Western nations, immigrants from East Asia, South Asia, Latin America, the Caribbean, and sub-Saharan Africa nini?