Site-specific phosphorylation and caspase cleavage of GFAP are new markers of Alexander disease severity

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Abstract: (Limit 600 words)

Alexander disease (AxD) is a fatal neurodegenerative disorder caused by mutations in glial fibrillary acidic protein (GFAP), which supports the structural integrity of astrocytes. Over 70 GFAP missense mutations cause AxD, but the mechanism linking different mutations to disease-relevant phenotypes remains unknown. We used AxD patient brain tissue and induced pluripotent stem cell (iPSC)-derived astrocytes to investigate the hypothesis that AxD-causing mutations perturb key post-translational modifications (PTMs) on GFAP. Our findings reveal selective phosphorylation of GFAP-Ser13 in patients who died young, independently of the mutation they carried. AxD iPSC-astrocytes accumulated pSer13-GFAP in cytoplasmic aggregates within deep nuclear invaginations, resembling the hallmark Rosenthal fibers observed in vivo. Ser13 phosphorylation facilitated GFAP aggregation and was associated with increased GFAP proteolysis by caspase-6. Furthermore, caspase-6 was selectively expressed in young AxD patients, and correlated with the presence of cleaved GFAP. We reveal a novel PTM signature linking different GFAP mutations in infantile AxD. All Alexander disease patients develop GFAP aggregates, but the type of mutation they have in the gene for GFAP does not predict how their illness will progress. The age of onset of disease, for example, can vary between less than one year old to more than 70 years old. Battaglia et al. sought to understand how GFAP aggregates form in the cells of Alexander disease patients. One way that GFAP can be altered in the cell is by a process called phosphorylation. Enzymes called kinases add phosphate groups to GFAP, which can regulate the protein's activity, stability and interactions with other proteins.Battaglia et al. found high levels of phosphorylation at one specific site in the GFAP protein in people who had very early onset of Alexander disease. This phosphorylation was not related to any particular mutation in the gene for GFAP. An added phosphate group at this location in the protein made GFAP more likely to be broken into two pieces by an enzyme called caspase-6. One of the breakdown products is already known to play a role in aggregation. Young patients with Alexander disease had high levels of GFAP breakdown products and caspase-6. The phosphorylated protein and this enzyme were found to accumulate in astrocyte aggregates.

Biography: (Limit 200 words)

Rachel A Battaglia developed his research work in Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, United States, MD; Adriana S Beltran developed his research work in Department of Pharmacology, University of North Carolina, Chapel Hill, United States; Samed Delic also developed his research work in Departments of Nutritional Sciences and Veterinary and Biomedical Science and Center for Molecular Immunology and Infectious Disease, Pennsylvania State University, University Park, PA;

About University: (Limit 200 words)

University Park is Penn State University's main campus, where thousands of undergraduates live in well-equipped residence hall complexes. The iconic Old Main building and its clock tower sit behind a huge lawn with walking paths. Beaver Stadium is Penn State Football's home, while the Bryce Jordan Center hosts concerts and basketball games. The 1860s Berkey Creamery offers dozens of ice cream flavors

Importance of Research: (Limit 200 words)

Considerable progress has been made toward understanding the mechanistic roles of specific nutrients in the function of leukocytes of rodent models and in human cell lines. In particular, the discovery of cell signaling networks by which nutrients regulate the differentiation and phenotype of regulatory leukocytes has been an important development. The translation of these mechanistic results in ways that improve outcomes in human diseases has been limited. Aside from the obvious inability to control the experimental environment, the complex nutritional context of at-risk human populations presents a daunting challenge. For example, although research with rodents typically examines deficiencies of a single nutrient in a diet in which all other nutrient amounts are optimal, human diets may commonly be lacking in multiple nutrients and simultaneously have certain nutrient excesses. Consequently, a key research priority is the need to examine interactions between essential nutrients with the immune and related systems to determine if they have additive, synergistic, facilitating, or unpredictable effects relative to an individual's nutritional status.In human populations, genetic and epigenetic differences likely account for important variations in the response of the immune system to nutrient fortification. Reliance on inbred mouse species housed in highly controlled environments may not be the most relevant model for understanding the implication of these genetic interactions in humans. New approaches for model systems that more closely duplicate the dietary, genetic, and hygienic realities of human populations should be considered.

References: (Limit 10 to 16

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. . Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.

2. Mayosi BM, Lawn JE, van Niekerk A, Bradshaw D, Abdool Karim SS, Coovadia HM. Health in South Africa: changes and challenges since 2009. Lancet 2012;380:2029–43.

3. Creswell J, Raviglione M, Ottmani S, Migliori GB, Uplekar M, Blanc L, Sotgiu G, Lonnroth K. Tuberculosis and noncommunicable diseases: neglected links and missed opportunities. Eur Respir J 2011;37:1269–82

4. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham-McGregor S, Katz J, Martorell R, et al. . Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013;382:427–51.

5. Garmendia ML, Corvalan C, Uauy R. Addressing malnutrition while avoiding obesity: minding the balance. Eur J Clin Nutr 2013;67:513–7

6. Camps J, Garcia-Heredia A. Introduction: oxidation and inflammation, a molecular link between non-communicable diseases. Adv Exp Med Biol 2014;824:1–4.

7. Hernandez-Aguilera A, Rull A, Rodriguez-Gallego E, Riera-Borrull M, Luciano-Mateo F, Camps J, Menendez JA, Joven J. Mitochondrial dysfunction: a basic mechanism in inflammation-related non-communicable diseases and therapeutic opportunities. Mediators Inflamm 2013; 2013:135698.

8. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, et al. . Inflammatory disease processes and interactions with nutrition. Br J Nutr 2009;101(Suppl 1):S1–45.

9. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.

10. Gordon AH, Koy A. The acute phase response to injury and infection: the roles of interleukin 1 and other mediators. Amsterdam: Elsevier; 1985.

11. Gruys E, Toussaint MJM, Landman WJM, Tivapasi M, Chamanza R, van Veen L. Infection, inflammation and stress inhibit growth: mechanisms and non-specific assessment of the processes by acute phase proteins. In: Wensing T, editor. Production diseases in farm animals. 10th annual conference, 1998. Wageningen (The Netherlands): Wageningen