Maple syrup urine disease: new insights from a zebrafish model

Nathan B. Roberts

Citation of published version:
Roberts, N. B. (2012). Maple syrup urine disease: new insights from a zebrafish model. In Disease Models & Mechanisms (Vol. 5, Issue 4, pp. 417–418). The Company of Biologists. https://doi.org/10.1242/dmm.010272

Disease Models & Mechanisms 5, 417-418 (2012) doi:10.1242/dmm.010272

Maple syrup urine disease: new insights from a zebrafish model

Nathan B. Roberts[1]

Summary and comment on a recent *Disease Models & Mechanisms* paper entitled 'Mutation of zebrafish dihydrolipoamide branched-chain transacylase E2 results in motor dysfunction and models maple syrup urine disease' (Friedrich et al., 2012).

Maple syrup urine disease (MSUD) is a rare inherited central nervous system (CNS) disorder involving defects in the metabolism of branched-chain amino acids. The amino acids leucine, isoleucine and valine [known as branched-chain amino acids (BCAAs)] are first converted to α-keto acids through a transamination reaction. Next, α-keto acids undergo oxidative decarboxylation through the actions of the mitochondrial branched-chain α-keto acid dehydrogenase (BCKD) complex. The BCKD complex responsible for this reaction is composed of three catalytic subunits encoded by four genes (Friedrich et al., 2012). Eliminating the proper function of the BCKD complex results in an accumulation of α-keto acids and BCAAs in plasma and tissues (Friedrich et al., 2012), resulting in MSUD, which is named after the odor of bodily secretions of those affected by this disease. The abnormal odor of secretions is among the milder symptoms of the disease. The CNS is disrupted by the elevated levels of BCAAs and α-keto acids, resulting in dysmyelination, cerebral edema, dystonia, coma, retardation, psychiatric problems and even death within weeks of birth (Friedrich et al., 2012). MSUD has an autosomal recessive inheritance pattern and affects approximately one in 185,000 people worldwide, with certain communities having a higher incidence of the disease (Skvorak, 2009).

Currently, treatment for MSUD consists of lowering BCAA levels, mainly through a carefully monitored diet. Unfortunately, dietary restriction is not wholly effective, and many of the disease symptoms are still found in individuals whose BCAA intake is monitored (Strauss et al., 2006). A second treatment option involves liver transplantation, which reduces levels of BCAAs and α-keto acids, and lessens CNS damage. However, organ transplantation is wrought with numerous complications and this does not offer a permanent solution (Strauss et al., 2010).

An effective treatment would ideally ameliorate the neuropathological effects of MSUD. Developing such a treatment requires an understanding of the mechanism by which the accumulation of BCAAs and α-keto acids causes CNS damage. To unravel this mystery, researchers are turning to model organisms. Larval-stage zebrafish make an attractive model because they are transparent, have large clutch sizes, develop rapidly outside of the mother and have organ systems that are similar to those in humans (Friedrich et al., 2012). Significantly, the zebrafish locomotor network formation is easily monitored because these animals undergo predictable motor behaviors during development. Thus, this model provides distinct advantages that might further our understanding of CNS disturbances in MSUD.

Wild-type zebrafish larvae typically respond to touch by bending in the shape of the letter 'C'. Mutagenesis screens have been performed to look for mutants with defects in this embryonic locomotive activity. One mutant phenotype, termed accordion, is characterized by tail compression and relaxation along the rostro-caudal axis, beginning around 72 hours post fertilization. Friedrich et al. found that a subset of homozygous recessive mutants, known as Quetschkommode (*que*), expressed the accordion phenotype and had deficits similar to those observed in human MSUD (Friedrich et al., 2012).

que mutants were compared with other subsets of mutants that also had the accordion phenotype. *que* mutants had more severely disrupted swimming, providing evidence of disrupted left-right muscle control. In wild-type animals, swimming is controlled by neuronal firing alternating distinctly between the left and right sides. Upon examination of CNS motor output via extracellular peripheral nerve recordings, it was found that *que* mutants had a noticeable increase in overlap between neuronal output regulating left and right locomotor activity. Notably, the *que* mutants also generally failed to live past 7 days post fertilization, due to failed inflation of the swim bladder (Friedrich et al., 2012).

Research was then performed to uncover the genetic defect in *que* mutants. First, using positional cloning, it was found that *que* is located on chromosome 22. To further elucidate the genomic location, single nucleotide polymorphism (SNP) markers were used to determine a specific 0.36 cM interval. Using genomic databases and by sequencing nearby genes, the identity of *que* was found to be dihydrolipoamide branched-chain transacylase E2 (*dbt*), which encodes a component of the BCKD complex. *dbt* is predicted to be 493 amino acids and is ~78% identical to its human counterpart. The mutation proved to be a single nucleotide substitution (G to A) in a splice donor site. Reverse transcriptase (RT)-PCR showed that *que* mutants have abnormal splicing of the *dbt* gene, causing inclusion of the entire sixth intron, which contains several stop codons, thereby resulting in truncation of the protein by 224 amino acids (Friedrich et al., 2012). The relevance of this protein defect is supported by findings that truncations of the DBT protein at a similar amino acid position in humans result in severe MSUD (Herring et al., 1992; Friedrich et al., 2012). Furthermore, blocking translation of *dbt* resulted in phenotypes that were similar to those of *que* mutants (Friedrich et al., 2012).

Experiments examining *dbt* mRNA expression revealed that it is enhanced in the brain and gut several days post fertilization (Friedrich et al., 2012). Analysis of free amino acid levels showed that *que* mutants had

[1]Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA
e-mail: nbroberts@crimson.ua.edu

significantly higher levels of BCAAs: levels of valine, isoleucine and leucine were increased by 688, 788 and 1006%, respectively. *que* mutants also had significantly decreased levels of other amino acids and only 24% of the glutamate levels found in wild-type fish, a finding that was confirmed using anti-glutamate antibodies (Friedrich et al., 2012). A reduced level of neurotransmitters is not new to our understanding of MSUD, however; in mouse models of MSUD, reduced levels of neurotransmitters were correlated with motor behaviors that mimicked the human disease (Zinnanti et al., 2009).

This study not only provides a valuable new model of MSUD, but also furthers our understanding of disease etiology by providing clues as to how truncations of a subunit of the BCKD complex result in the accumulation of BCAAs and in abnormal CNS motor function. Importantly, Friedrich et al. have established easily assayable phenotypes for MSUD in their model; in particular, monitoring amino acids and glutamate levels provides a read-out with direct relevance to the disease. In the future, morpholinos can be used to identify other genetic factors that are important in MSUD. Finally, this new model provides the opportunity to carry out rapid, large-scale screening to uncover additional disease factors or test small-molecule therapeutics.

There are many questions outstanding about MSUD. In particular, although the authors uncovered decreased levels of glutamate in their zebrafish model, the nature of the disease in humans suggests a more complex neuronal disruption. Notably, dystonia (uncontrolled muscle contractions) in individuals with MSUD suggests increased activity at the neuromuscular junction (Breakefield et al., 2008), rather than reduced activity, which is at odds with the findings of Friedrich et al. (Friedrich et al., 2012). Perhaps the disease involves the cholinergic system, an idea supported by findings that some human dystonias are linked to disruption of the cholinergic system (Breakefield et al., 2008). Thus, although mysteries remain, the use of this zebrafish model will undoubtedly help to solve them.

REFERENCES

Breakefield, X. A., Blood, A. J., Li, Y., Hallet, M., Hanson, P. and Standaert, D. G. (2008). The pathophysiological basis of dystonias. *Nat. Rev. Neurosci.* **9**, 222-234.

Friedrich, T., Lambert, A. M., Masino, M. A. and Downes, G. B. (2012). Mutations of zebrafish dihydrolipoamide branced-chain transacylase E2 results in motor dysfunction and models maple syrup urine disease. *Dis. Model. Mech.* **5**, 248-258.

Herring, W. J., McKean, M., Dracopoli, N. and Danner, D. J. (1992). Branched Chain acyltransferase absence due to an Alu-based genomic deletion allele and an exon skipping allele in a compound heterozygote proband expressing maple syrup urine disease. *Biochim. Biophys. Acta* **1138**, 236-242.

Skvorak, K. J. (2009). Animal models of maple syrup urine disease. *J. Inherit. Metab. Dis.* **32**, 229-246.

Strauss, K. A., Mazariegos, G. V., Sindhi, R., Squires, R., Finegold, D. N., Vockley, G., Robinson, D. L., Hendrickson, C., Virji, M. and Cropcho, L. et al. (2006). Elective liver transplantation for the treatment of classical maple syrup urine disease. *Am. J. Transplant.* **6**, 557-564.

Strauss, K. A., Wardley, B., Robinson, D., Hendrickson, C., Rider, N. L., Puffenberger, E. G., Shellmer, D. and Moser, A. B. (2010). Classical maple syrup urine disease and brain development: principles of management and formula design. *Mol. Genet. Metab.* **99**, 333-345.

Zinnanti, W. J., Lazovic, J., Griffin, K., Skvorak, K. J., Paul, H. S., Homanics, G. E., Bewley, M. C., Cheng, K. C., Lanoue, K. F. and Flanagan, J. M. (2009). Dual mechanism of brain injury and novel treatment strategy in maple syrup urine disease. *Brain* **132**, 903-918.