Involvement of the epilepsy gene *TBC1D24* in resistance to oxidative stress

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**Équipe de recherche**
Philippe Campeau, M.D.
Médecin généticien, CHU Sainte-Justine
Professeur adjoint de clinique, Département de pédiatrie, Université de Montréal
Axe de recherche : Maladies musculosquelettiques et réadaptation

**Coordonnées**
[pm.campeau@umontreal.ca](mailto:pm.campeau@umontreal.ca)
Centre de recherche du CHU Sainte-Justine
3175 Chemin de la Côte-Ste-Catherine
Montréal, Qc, H3T 1C5

**Responsable de la supervision du stagiaire**
Justine Rousseau, Associée de recherche

**Description du projet**
Mutations in TBC1D24 cause DOORS syndrome (Deafness, Onychodystrophy, Osteodystrophy, mental retardation and Seizures), myoclonic epilepsy and early infantile epilepsy. We have previously reported 11 patients from 9 unrelated families with DOORS syndrome\(^1\). TBC1D24 has been implicated in the regulation of synaptic vesicle trafficking and proper neurotransmitter release\(^2\)\(^3\). Recent studies have also highlighted a role of TBC1D24 in the oxidative stress response, resulting in neuroprotection\(^4\). To characterize the mechanisms that are responsible for the phenotype observed in patients harboring TBC1D24 mutations, we identified proteins that

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interact with TBC1D24 using mass spectrometry. We found many proteins implicated in the regulation of intracellular and mitochondrial calcium (Ca2+) homeostasis like SERCA2, LETM1, MAIP1 and FUNDC1\(^5\) \(^6\). Another candidate, ARL6IP5, has been shown to increase the expression of Nrf2 upon neuronal damage\(^7\), a master regulator of the antioxidative response\(^8\).

Seizure activity is known to induce the production of reactive oxygen species (ROS), which eventually compromise cell survival\(^9\). Mitochondrial Ca2+ overload, as well as impaired oxidative stress response, can contribute to the accumulation of ROS, both of which could be regulated by TBC1D24. Sulforaphane, an activator of Nrf2, is in clinical trials for the treatment of Autism Spectrum Disorder\(^10\), and could be considered in treating TBC1D24 associated disorders as it has emerged as a potential therapeutic target for epilepsy\(^11\)\(^11\). We hypothesize that TBC1D24 is implicated in calcium homeostasis and plays a role in the regulation of ROS accumulation, which could protect against seizure induced cell damage.

First, we will confirm the interaction of TBC1D24 and the identified interacting proteins by co-immunoprecipitation as well as their sub-cellular localization by confocal microscopy using specific markers for different organelles. Next, we will generate TBC1D24 knockout SH-SY5Y neuronal cell lines using Crispr-Cas9 and inducible cell lines expressing either WT TBC1D24 or the mutants found in DOORS patients, and also generate iPSCs-derived neurons from TBC1D24 patient cells. Using these models, we will measure changes in Ca2+ homeostasis in response to surges in cytoplasmic Ca2+ using fluorescence microscopy. Secondly, we will measure ROS by FACS upon acute and/or prolonged Ca2+ release. Thirdly, we will monitor the expression of select downstream target genes regulated by Nrf2, and also determine the effect on other genes by RNAseq. Finally, we will determine in vitro if Sulforaphane can protect against some of the consequences of TBC1D24 mutations. This will open the door to therapeutic tests in mice, and perhaps one day in patients.

**Mots clés**
Épilepsie, stress oxidatif, génétique.

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\(^7\) Zhao X et al., JWA antagonizes paraquat-induced neurotoxicity via activation of Nrf2. Toxicol Lett. 2017 Aug 5;277:32-40.


