Functional consequences of mutations in *ATP1A3* causing alternating hemiplegia of childhood

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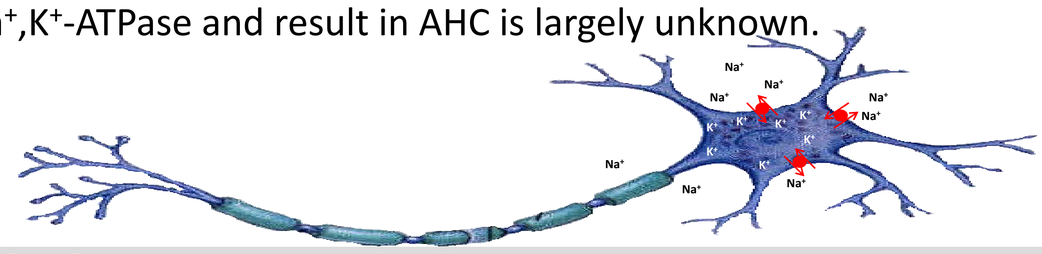
Introduction

De novo mutations in the ATP1A3 gene are associated with alternating hemiplegia of childhood (AHC). The ATP1A3 gene encodes the Na^+,K^+-ATPase α3-subunit, which together with the β-subunit forms a functional ion pump. This pump is located in neural cells, where it transports three sodium ions out and two potassium ions into the cell. How these ATP1A3 mutations affect Na^+,K^+-ATPase and result in AHC is largely unknown.

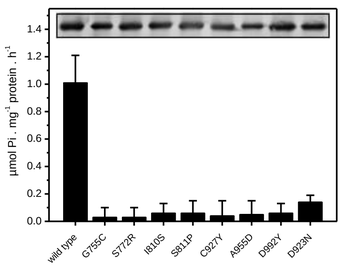


Objective

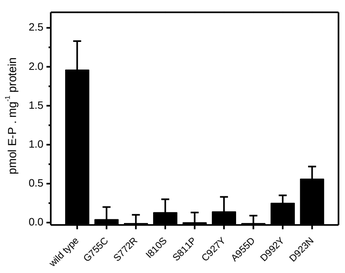
In this study we want to reveal how the ATP1A3 mutations affect the Na^+,K^+-ATPase function. This information is essential for understanding the variation in symptoms observed in AHC patients.

Material and Methods

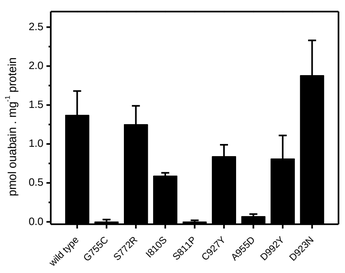
Mutations identified in AHC were introduced in the human ATP1A3 sequence (Na^+,K^+-ATPase α3-subunit). Next, the DNA of the α3-subunit and the β1-subunit were introduced in a baculovirus that was used for expression of the Na^+,K^+-ATPase subunits in a human cell line (HEK293). Isolated membrane fractions containing the recombinant Na^+,K^+-ATPases were used in the functional Na^+,K^+-ATPase studies described below.



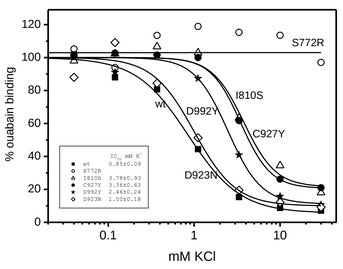
Equal expression of wild type and mutant Na^+,K^+-ATPase α3-subunits is shown on a Western blot.
ATPase activity will show if the affected pumps can consume energy.



ATP phosphorylation will show if the affected pumps can bind ATP and attain the phosphorylated conformation (E1P).



Binding of the specific inhibitor ouabain will show if the affected pump can attain the conformation (E2P) that is essential for ouabain binding.



The ouabain-K^+ antagonism will show if the affected pump is able to bind K^+ (E2K conformation).

Results

Effects of ATP1A3 mutations on Na^+,K^+-ATPase function

Mutation	overall function: ATPase activity	partial reactions: ATP phosphorylation	partial reactions: Ouabain binding	partial reactions: Ouabain - K^+ antagonism
Wild type	++++	++++	++++	++++
S137Y *	-	-	++	+
D220N *	++++	++++	++++	++++
I274N *	-	-	-	nd
G775C	-	-	-	nd
S772R	-	-	++++	-
D801N *	-	-	++++++	-
I810S	-	-	++	++
S811P	-	-	-	nd
E815K *	-	-	-	nd
C927Y	-	-	+++	++
A955D	-	-	-	nd
D992Y	-	-	+++	+++
D923N#	+	+	++++	++++
G947R	-	-	-	nd

* These mutations have been analyzed previously: Weigand *et al* Alternating Hemiplegia of Childhood mutations have a differential effect on Na^+,K^+-ATPase activity and ouabain binding. Biochim Biophys Acta. 2014 Jul;1842(7):1010-6.
# RDP mutation. nd not determind.

The ATP1A3 mutations can be divided in four groups:
1 Similar to wild type Na^+,K^+-ATPase.
2 Decreased ATPase activity.
3 No ATPase activity but able to bind ouabain.
4 No ATPase activity and no ouabain binding.

The Na^+,K^+-ATPase mutations in group 3 possess a great variety in K^+ sensitivity.

Discussion and Conclusion

- The ATP1A3 mutations could be divided into four groups.
- All AHC mutations inactivate Na^+,K^+-ATPase activity. (D220N might not be causal)
- Only the rapid-onset dystonia-parkinsonism (RDP) mutation shows some residual ATPase activity, which might indicate that mutations that retain some activity cause RDP, whereas those that inactivate the pump cause AHC (further research has to prove this hypothesis).
- Comparing the identified groups with patients symptoms might reveal a relationship between ATP1A3 mutations and the severity of disease.

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