PROGRESS REPORT: CLINICAL AND GENETIC STUDIES IN AHC

Principal Investigator: Kathryn J. Swoboda, MD, FACMG
Co-Investigators: Louis Viollet MD, PhD; Sandra Reyna MD; Matthew Sweney MS, MD; Tara Newcomb MS, LCGC.

Funding period July 1, 2012 – June 30, 2013

Specific aims

Aim 1: Ongoing analysis for mutations in ATP1A3, and genotype-phenotype correlation studies

a) Completion of ATP1A3 screening in all patients currently registered in the database, and in newly recruited patients and families

As of September 1, 2013, we have confirmed that 140 sporadic AHC patients have mutations in ATP1A3. In addition, we have confirmed 11 affected individuals in 4 families, including 2 sets of identical twins (4 patients), and 2 families with autosomal dominant inheritance (the original family reported in the Nature Genetics paper has 4 affected individuals, and a new family, referred to us more recently and thus was not included in the Pepsi/ISB whole genome sequencing project has 3 affected individuals). The large majority of the funding received from the foundation necessarily went to efforts related to this first specific aim. The table below indicates the frequency of these mutations among patients in our database so far, and the predicted consequences of the various mutation on protein function.

Table: Heterozygous *ATP1A3* mutations and protein modifications in AHC sporadic and familial cases and their predicted consequence, using PolyPhen for missense mutations, Human Splicing Finder for intronic mutations and Provean for deletions. Previously reported mutations resulting in an AHC phenotype are highlighted in green. Previous mutations reported with RDP phenotype are highlighted in grey. The rest represent novel mutations in ATP1A3 not previously associated with either a phenotype.

c.DNA	Protein	Patient N Sporadic/Familial		Predicted consequence
c.2401G>A	D801N	56	2[1]	probably damaging (0.99)
c.2443G>A	E815K	38		probably damaging (1.00)
c.2839G>A c.2839G>C	G947R	10 1		probably damaging (0.99) probably damaging (0.99)

c.2263G>A	G755S	4		probably damaging (0.99)
c.2431T>C	S811P	3		probably damaging (0.98)
c.2411C>T	T804I	2		probably damaging (0.99)
c.2751_2753delTTG	V919del	2		deleterious (-8.77%)
c.2542+2T>C	splice site	2		splice site broken (-29.76%)
c.1786T>C	C596R	1		probably damaging (1.00)
c.2702G>C	R901T	1		probably damaging (0.99)
c.2314A>C	S772R	1		possibly damaging (0.94)
c.410C>T	S137F	1		probably damaging (1.00)
c.2413G>A	D805N	1		probably damaging (0.99)
c.419A>T	Q140L	1		probably damaging (0.99)
c.2401G>T	D801Y	1		probably damaging (1.00)
c.2302T>C	Y768H	1		probably damaging (0.98))
c.2316C>G	S772R	1		possibly damaging (0.94)
c.2780G>A	C927Y	1		probably damaging (0.98)
c.998G>T	C333F	1		probably damaging (1.00)
c.2317A>C	N773H	1		probably damaging (0.99)
c.2281A>C	N761H	1		probably damaging (0.99)

c.2516T>C	L839P	1		probably damaging (0.99)
c.2264G>T*	G755V*G	1		probably damaging (1.00)
c.977T>G	L326R	1		probably damaging (1.00)
c.972G>C	E324D	1		probably damaging (0.99)
c.2542+1G>A	splice site	1		splice site broken (-29.76%)
c.2303A>G	Y768C	1		probably damaging (0.99)
c.2851G>A	E951K	1		probably damaging (0.99)
c.2305A>C	T769P	1		probably damaging (0.99)
c.2423C>T	P808L	1		probably damaging (0.98)
c.2267G>A	R756H	0	3[2]	probably damaging (1.00)
c.2403T>A	D801E*	0	2[1]	possibly damaging (0.79)
c.821T>A	I274N	0	4[3]	Probably
		140	11	

[1]Affected identical twins with de novo mutations not present in parents; [2]previously unreported family with autosomal dominant inheritance in an affected mother and two children (atypical phenotype); [3]previously reported family with autosomal dominant inheritance (typical AHC phenotype in proband) – confirmed on whole genome sequencing and sanger sequencing

The 2nd family with dominant inheritance listed in the table above has symptoms that represent the emerging spectrum of patients with symptoms that fall between AHC and RDP phenotypes. Our abstract was accepted for presentation at the Child Neurology Society Meeting by our collaborator, Dr. Gyula Acsadi.

b) Genotype phenotype correlation studies in patients with confirmed mutations in ATP1A3. Use of the data previously collected from questionnaires and entered into the database; initial focus on the two most common mutations, which accounting for about half of all those with confirmed mutations.

A paper has now been completed in draft form and is ready to be sent out to our collaborators and coauthors for review prior to the meeting in Rome. We have prepared the submission in the format appropriate for PLOS ONE, which has a policy of open access, so that anyone will be able to download the paper should it be accepted for publication. The paper was somewhat delayed due to ongoing discussions with the international community about which cases we should include and not include in this paper, and getting updated phenotype data on all 140 affected patients has also been a highly time and effort intensive process. The intent of this paper is to summarize the genotypes as described above, and some preliminary observations on genotype/phenotype correlations, investigating in more detail the phenotypes associated with the two most common genotypes, the D801N and the E815K mutations and a review of those patients with catastropic outcomes including death. More than half of all patients in the database have one of the two most common mutations in a hotspot area involving exons 17 and 18 of the gene. The abstract submitted to the meeting in Rome next week, summarizing our more detailed analysis is copied in its entirety below, and this data will be included in the PLOS ONE paper.

Catastrophic Outcomes in AHC Patients with ATP1A3 mutations

Authors: Kathryn J. Swoboda, Marcio Sotero, Louis Viollet, Ben Nelson, Francis Renault, Bernard Echenne, Nathalie Bednarek, Sandra P. Reyna, Matthew Sweney, Tara M. Newcomb, Austin Stevens, Kenneth Silver

Objective: To review catastrophic outcomes in ATP1A3 mutation positive patients from the U.S./International Alternating Hemiplegia of Childhood database and registry.

Methods: We reviewed all patients prospectively enrolled since 1999. Data was obtained from standardized questionnaires, structured interviews with primary caregivers and examination at one of several family meetings, photo and video archives, medical records, consultation with referring physicians and ongoing direct communication with caregivers.

Results: Catastrophic outcomes resulting in death occurred in 10 of 142 mutation positive sporadic AHC patients. Four patients, all female, had a E815K mutation. Age of death ranged from 5 - 21 years; 3/4 were never ambulatory, 4/4 had

confirmed epilepsy, 3 had recurrent status epilepticus and deaths relating to complications thereof. The fourth patient had neurogenic bladder, severe generalized hypotonia, recurrent apnea, progressive scoliosis, bulbar insufficiency and gastrostomy tube dependence due to chronic aspiration, and died of respiratory complications under hospice care. 2 patients had the D801N mutation, with relatively high level of cognitive function at baseline, both females ages 24 and 37 years. 1 had neurogenic bladder, both had epilepsy, and deaths occurred suddenly and unexpectedly, but followed an increase in seizure frequency in the weeks to months preceding death. A 14 yo girl with a G947R mutation died one week post placement of a baclofen pump for status dystonicus. Of 3 remaining patients, one 36 yo female with a G755S mutation had support withdrawn in the setting of refractory status epilepticus ; 2 others, one male, had previously unreported missense mutations; both had history of epilepsy and unwitnessed sudden death.

Acknowledgements: US Alternating Hemiplegia of Childhood Foundation and Association Française de l'Hémiplégie Alternante

c) Ongoing collaboration with physicians, scientists the AHC foundation and other international foundation efforts to prioritize phenotypic data to collect and to confirm consensus on minimal core set.

We have collaborated with the international community to prepare an article summarizing data on disease associated with ATP1A3 mutations – it is to be submitted by Erin Heinzen this week, and she will be announcing this at the Rome meeting. We have established a minimum core dataset we are using to do the phenotype/genotype correlations. We will continue to work to achieve consensus on a more detailed dataset of additional items thought to be the most important for ongoing characterization and followup of patients, particularly with regard to medication exposures and frequency of need for urgent medical interventions and adverse events. However, there has been limited progress to date on this front. This will be critical for future clinical trials.

Aim 2: Further characterization of mutations in ATP1A3 at the RNA and protein level

We have been unable to achieve further progress in this area in large part due to lack of funding and laboratory personnel for this aspect of the project. However, we have actively been collaborating with our colleagues at Vanderbilt to provide them cell lines and reagents from affected patients in the database in order further characterize the most common mutations as to their ATP1A3 expression levels and how these mutations impact cellular function. We did submit an NIH grant to seek further funding for this aim, but unfortunately, the score we received was not sufficient for funding.

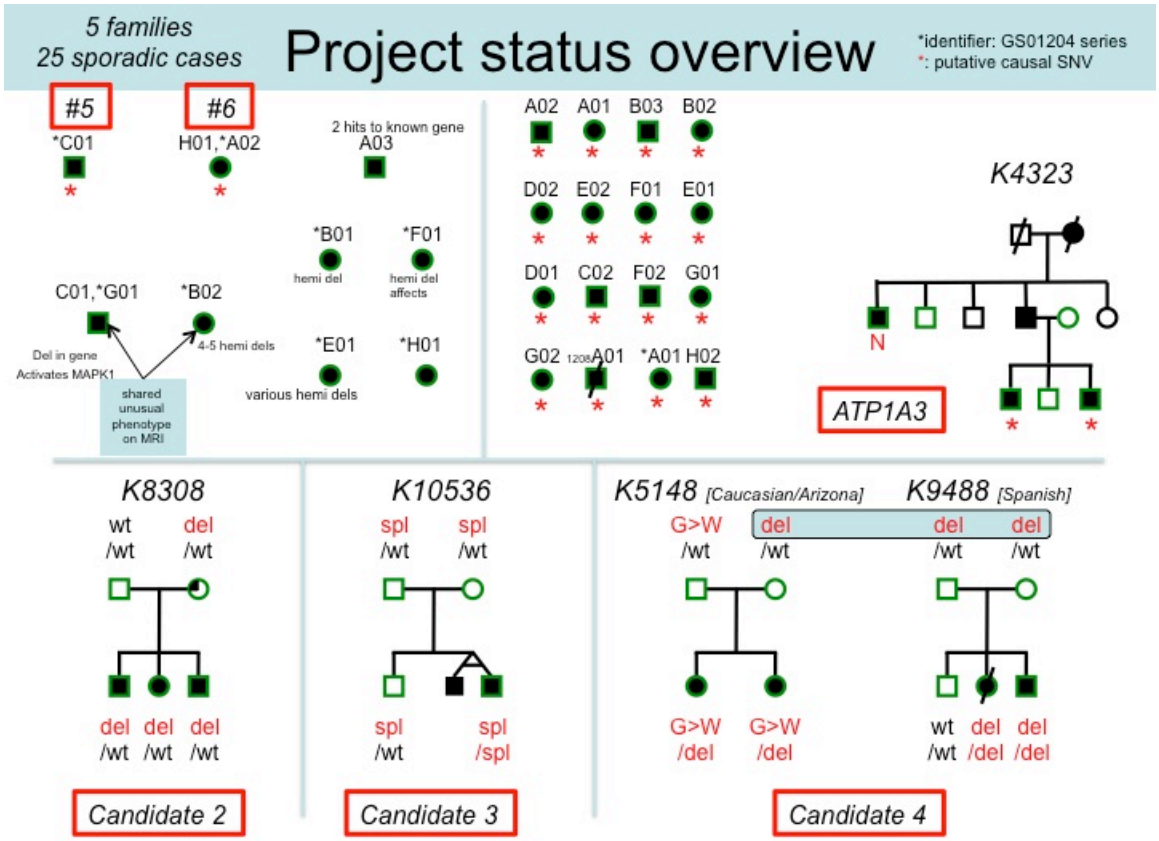
Aim 3: Ongoing analysis of whole genome data in familial and sporadic cases to identify additional causative gene(s) in patients without mutations in ATP1a3

a) Screening of the candidate gene hits from WGS in a selected panel of non-ATP1A3 mutated patients

b) Secondary more extensive screening of the candidate genes selected in all the other patients

c) Confirmation of promising hits via functional and expression studies.

We have continued to work closely with our ISB collaborators Gustavo Glusmann and Mary Brunkow at ISB. Below is a screenshot summarizing further candidates we've identified that we continue to work on, from a powerpoint presentation from a recent conference call. We are pursuing additional studies in a half dozen candidates, and in the most promising case, we have two families who appear to share mutations in a second candidate. However, virtually nothing is known about this gene as yet.



Aim 4: Collaborate with pharmaceutical companies and the international community to develop a discrete plan for moving forward with clinical trials in AHC. First proposed project: secure approval for flunarizine use in children with AHC.

We have been communicating regularly colleagues at Marathon/Paragon Pharmaceuticals, and we have had a series of calls to help facilitate their goal, which is to obtain FDA approval for Flunarizine. We have also been involved in discussions about clinical trial designs to better understand the proper dosing in pediatric patients, and what will be necessary in order to help ensure that this compound becomes more readily available in the U.S for our patients. I will be flying to Washington D.C. on November 7th, to meet with them on November 8th to prepare for the FDA meeting, which is scheduled for November 9th.