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Progress Report: 2009-2010

Research Project "ARSAL and Cerebral Palsy"

Translated from the French by Lindsay Gallimore

Introduction

First described by Dr. Brais' team in 2006, ARSAL, or *Autosomal Recessive Spastic Ataxia with Leukoencephalopathy* is an autosomal recessive disorder with a regional founder effect originating in the Portneuf County of Quebec. ARSAL is a slowly progressive disease with a variable age of onset and progressive neurological symptoms including ataxia, spasticity and other pyramidal signs, with or without dystonia. The locus of this disorder is located on the long arm of chromosome 2 [2q33-34]. The gene responsible for this disorder, *MARS2*, was discovered in recent months (publication in progress). This gene encodes for the protein *mitochondrial methionyl-tRNA synthetase 2*, an important protein for mitochondrial function.

Cerebral palsy (CP) is defined as a movement or motor impairment of central origin, most often spasticity, and with onset most often before the age of 2. There are multiple causes of CP, including: premature birth (the most common cause), perinatal asphyxia, cerebral malformations, etc. There are different types of CP, the most common being the spastic type. The other types are: dyskinetic (also known as athetoid), hypotonic, ataxic and mixed. The hypotonic and ataxic types are rare.

Research Question

While carefully studying the clinical characteristics of patients affected by ARSAL, we have observed that many of them had first been diagnosed with CP in early childhood, typically with a spastic type of CP, typically with an ataxia component. This observation raised the following question: Could the diagnosis of ARSAL explain certain cases of CP without etiology?

Research Methods

We created a pilot study protocol in order to evaluate our hypothesis at a low cost. To test our hypothesis it was necessary to determine if we could explain certain cases of cerebral palsy without an etiology by testing patients for mutations in the *MARS2* gene.

Therefore, with the invaluable support of our associates, we recruited children suffering from CP without an etiology. The children chosen for the pilot project were recruited

from the CP Clinic #1 at the Rehabilitation Centre Marie Enfant (supervising doctor: Marie-Emmanuelle Dilenge).

Our selection criteria for this pilot project were as follows:

1. Confirmed family history of CP
2. Cerebellar type CP
3. Atypical spastic diplegia or atypical spastic quadriparesis (i.e. with cerebellar signs, without a history of prematurity, or with an atypical neurological examination [example: dystonia])

The criteria served to maximize the probability of finding patients suffering from ARSAL. We carried out a detailed clinical evaluation of each of the recruited patients. We then tested these patients for mutations of the *MARS2* gene. By using six assays manufactured by the ABI company to amplify and quantify the targeted gene and its neighbors (three located in the *MARS2 gene*, one in the *HSP60 gene*, one in the *PLCL1* gene), we tested the hypothesis that a certain number of CP patients were affected by ARSAL. The setup and the optimization of the protocol took place in accordance with the manufacturer's instructions (ABI, Applied Biosystems, USA) and in collaboration with the genotyping service from the Génome Québec Innovation Centre. The positive cases were confirmed by standard amplification protocol and gene-specific primers for the rearranging of the *MARS2* coding sequence.

If our hypothesis proves correct, we will test a greater number of patients affected by CP for mutations in the *MARS2* gene, and widen our selection criteria. For this second phase of the project we will need substantial subsidies and we hope to obtain them through such organizations as the Canadian Institutes for Health Research (CIHR).

Preliminary Results

The review of the Marie Enfant Hospital CP Clinic #1 databases revealed that out of the 366 patients in the database, there were 84 children diagnosed with CP without an obvious cause or with a level of handicap that seems disproportionate in relationship to the identified cause. Of these 84 patients, 28 met the selection criteria for the above-mentioned research project. Out of these 28 patients, we find 8 families with 2 affected children and 10 patients with a pure ataxic phenotype or a mixed CP with an ataxia component.

We contacted the parents of these 28 patients (20 families). 13 patients (10 families) accepted the invitation to participate in the study, which is 50% of the contacted families. Table 1 shows the date of birth, gender and type of CP of the 13 patients who agreed to participate in the study. As can be seen, we recruited 5 male patients and 8 female patients. Of the 13 recruited patients, 8 present with ataxia, either pure or associated with

spasticity or dystonia (61.5%). Table 2 summarizes the perinatal history of the patients. 6 out of the 13 patients show a completely normal pregnancy and birth history (46%). 3 patients were premature (23%). These patients were included in the study because the degree of neurological dysfunction was out of proportion to the degree of prematurity and/or because they had a sibling affected by CP. 2 patients (15%) showed a certain degree of perinatal asphyxia, to a degree which, once again, was considered insufficient to explain the gravity of the neurological signs and symptoms. Finally, the 2 remaining patients (15%) presented complications during pregnancy or birth, but which did not cause asphyxia or prematurity. No other cause could be identified to explain the CP of these patients.

Table 3 presents the preliminary findings of the genetic study of the *MARS2* gene in 11 out of the 13 recruited patients, as well as for one control subject. The results show that 5 patients present duplications for 3 of the tested probes. 5 of the 11 tested patients showed deletions of 1 or all of the 4 tested probes. These results are promising, but will have to be confirmed with the help of other genetic techniques. Furthermore, we must test the 2 patients for whom we do not currently have results.

Discussion

The preliminary findings suggest that 10 of the 11 cases of CP tested by the Taqman CNV method (ABI company) fulfilling the selection criteria for a pilot group are carriers of mutations in the *MARS2* gene. The obtained results must be validated and confirmed by alternative methods, by PCR and sequencing. We put forth the hypothesis that these mutations cause problems in the expression of the protein. When the results are confirmed, we will have to pursue the research project and recruit more patients. To do this, we will try to recruit patients from the CP Clinic #2 at the Rehabilitation Center Marie Enfant, as well as patients throughout the province of Quebec with the help of cooperating neuropediatricians and pediatricians. If the obtained results are again promising, we will expand our selection criteria in order to include more patients. To do this, we will have to request funds from organizations such as the CIHR. Perhaps the ARSAL phenotype is still more variable than initially believed? Perhaps ARSAL can explain more cases of CP than previously thought?

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Table 1 : Demographics and CP Subtype

# Patient	# Family	Date of Birth	Sex	Type of CMD
1	1	2005-08-17	M	Mixed ataxic - Spastic diplegia
2	2	1989-08-04	F	Mixed ataxic - Spastic diplegia
8	6	2001-07-23	M	Ataxic
9	6	1996-02-07	M	Mixed ataxic - Dyskinesic
10	7	2006-03-17	F	Spastic quadriparesis
11	7	1997-07-23	F	Spastic diplegia
12	8	2002-12-21	F	Mixed spastic quadriparesis - Dyskinesic
16	11	1991-11-16	M	Ataxic
17	11	1993-10-12	F	Ataxic
21	14	1994-08-12	M	Spastic diplegia
22	15	1992-01-06	F	Spastic diplegia
24	17	2000-04-09	F	Mixed ataxic - Spastic quadriparesis
25	18	1995-10-26	F	Mixed ataxic - Spastic diplegia

Legend:

CP: Cerebral Palsy

F: female

M: male

Table 2 : Perinatal Clinical Data

# Patient	Pregnancy	Birth and neonatal period
1	N	N
2	N	Shoulder dystocia, asphyxia
8	N	N
9	N	N
10	N	Prematurity (33 weeks)
11	N	Prematurity (31 weeks)
12	N	Prematurity (27 weeks)
16	N	N
17	N	N
21	N	N
22	HTN at end of pregnancy	N
24	N	Placenta abruptio, asphyxia
25	Twin pregnancy	Umbilical cord prolapse, NO neonatal encephalopathy

Legend:

HTN : hypertension

N : Normal

Table 3 : Preliminary Genetic Results

#	PLCL1	MARS2				HSPD1	COQ10
	Hs01068343_cn	Hs02277482_cn	Hs02141409_cn	Hs01848862_cn	Hs02550138_cn	Hs00304988_cn	Hs00506215_cn
1	2	2	2	2	2	2	2
2	2	5	4	2	3	2	2
8	2	2	2	1	2	2	2
9	2	2	2	1	2	2	2
10	2	2	2	1	2	2	2
11							
12	2	3	4	2	3	2	2
16							
17	2	4	4	2	3	2	2
21	2	4	3	2	3	2	2
22	2	2	2	1	2	2	2
24	2	3	3	2	3	2	2
25	1	1	1	1	1	1	1
CTRL	2	2	2	2	2	2	2

Duplication
Deletion
No genetic data

Legend:
CTRL : control