

Folate Receptor Autoantibodies and Spinal Fluid 5-Methyltetrahydrofolate Deficiency in Rett Syndrome

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Key words



Abstract

▼
Rett syndrome was associated with low cerebrospinal fluid (CSF) 5-methyltetrahydrofolate (5MTHF) in 42–50% of European patients whereas approximately 93% of the patients from North-America had a normal CSF 5MTHF status. We determined the CSF folate status in Rett patients living in North- and South-Western Europe and measured serum folate receptor (FR) autoantibodies of the blocking type to explain the reduced folate transport across the choroid plexus. Irrespective of their MECP2 genotype and despite normal plasma folate values, 14 of 33 Rett patients (42%) had low CSF folate lev-

els. Blocking FR autoantibodies were found in 8 of the Rett patients (24%), 6 of whom had low CSF folate levels. FR autoimmunity was primarily found within the group of Rett patients with low CSF folate status with a higher incidence in North-Western Europe. In Rett patients from North-America 74 of 76 girls had higher folate values in both serum and CSF than European patients. The food folate fortification in North-America may account for the higher folate levels and may prevent CFD in these Rett patients. FR autoimmunity occurred predominantly in Rett patients from North-Western Europe and may contribute to cerebral folate deficiency (CFD).

Introduction

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After intestinal absorption of food-derived folates, intestinal cells will convert folate compounds to 5MTHF, which represents the predominant and only reduced folate form in plasma which is able to cross the blood-brain barrier. Plasma 5MTHF is actively transported into the spinal fluid compartment by folate receptor (FR) endocytosis at choroid epithelial cells. Approximately 42 to 50 percent of Rett patients from Europe and Israel were previously reported to have a reduced concentration of 5-methyltetrahydrofolate (5MTHF) in the cerebrospinal fluid (CSF) [1–3]. Oral folic acid supplementation restored 5MTHF levels in the spinal fluid with partial clinical improvement and better seizure control [1,3]. The reduced folate in the CSF could not be attributed to abnormalities of the FR1 or FR2 genes and was independent of methyl-CpG binding protein (MECP2) status [1]. A recent study from North America reported that only 2 of 76 female Rett patients had a low spinal fluid 5MTHF level [4].

Serum FR autoantibodies of the blocking type have been associated with the infantile-onset CFD syndrome whereas in age-matched controls and in serum from subjects with central nervous system disease unrelated to cerebral folate deficiency these FR autoantibodies were absent. In vitro studies substantiated the blocking action of these FR autoantibodies that form a complex with the FR at its folate-binding site and thus impairs the binding of 5-methyltetrahydrofolate (5MTHF) to the FR [5]. Recent studies now have confirmed an inverse correlation between the titer of the serum FR autoantibodies and the CSF 5MTHF levels (unpublished results). In order to explain the low CSF folate in 42–50% of Rett patients due to reduced folate transport into the CNS, serum samples from all study participants were analysed for blocking autoantibodies against the folate receptors (FRs).

Methods

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According to the internationally defined clinical criteria for Rett syndrome, thirty-three girls with

received ■■■
accepted ■■■

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Table 1 Age, genotype and laboratory findings in European Rett girls

Patient	Age years	MECP2 genotype	Serum folate nmol/L*	CSF 5MTHF nmol/L §	Folate Ratio CSF/serum	Autoantibodies pmol FRs/ml	AED
1	13.5	normal	47	15	0.32	1.22	none
2	4.3	normal	16.5	12	0.72	1.5	E
3	5.7	normal	22.5	30.9	1.3	1.63	none
4	3.8	R106W	ND	14.1	–	2.04	none
5	13.1	P152R	33.22	12.5	0.37	0.3	none
6	13	dup 763–775	13.2	17	1.28	1.78	none
7	4	del exon 2–3	19	45	2.36	negative	V
8	6	del G 483	29.24	68	2.32	negative	none
9	6	normal	ND	92	–	negative	none
10	16	R294X	ND	68	–	negative	C
11	19	normal	8.8	38	4.31	negative	C+R
12	11	normal	ND	73	–	negative	V+E
13	3	S229L	34.7	40	1.15	negative	V
14	5	R306C	45	64	1.45	negative	C
15	2	R255X	15.1	47	3.13	0.29	none
16	2	normal	62.2	65	1.04	negative	V
17	2	Y141X	40	69	1.72	negative	none
18	4	P302H	9.4	34	3.6	negative	V
19	10	R306C	9.9	33	3.3	negative	C+R
20	5.6	R294X	20.15	46	2.28	negative	none
21	15.5	K39fsX43	14.49	42	2.89	negative	V+C
22	10.9	R133C	ND	41	–	negative	none
23	4.9	R294X	22.8	43	1.88	negative	none
24	7.1	R168X	17.21	40	2.32	negative	V+C
25	3.4	normal	14.94	57	3.81	negative	none
26	9.3	normal	27.18	48	1.76	0.34	none
27	6.4	normal	19.93	42	2.10	negative	none
28	5.6	normal	21.06	45	2.13	negative	none
29	16.3	R168X	20.38	54	2.64	negative	V+C
30	2.8	T158M	19.70	58	2.93	negative	none
31	15.7	normal	12.45	46	3.69	negative	V+C
32	3.7	R294X	40.54	42	1.03	negative	none
33	6.6	T158M	25.59	61	2.38	negative	V

Patient 1–8 from Northern Europe and patient 9–33 from Southern Europe. ND = not determined

*Serum folate for healthy controls with a mean at 29 and range 8.3–45 nmol/L

§ CSF folate values below the reference range have been indicated by bold numbers. Reference range of CSF 5MTHF for healthy controls from Northern Europe, 0–1 years 64–182 nmol/L, 2–4 years 63–111 nmol/L, 5–10 years 41–117 nmol/L, 11–16 years 41–117 nmol/L, > 16 years 41–117 nmol/L

Reference range of CSF 5MTHF for healthy controls from South-Europe, aged 0–1 years 63–129 nmol/L, 2–3 years 44–122 nmol/L, 4–18 years 42–81 nmol/L

Abbreviation for anticonvulsant drugs: V: Valproate; C: Carbamazepine; R: Clonazepam; E: Ethosuximide

a classical Rett phenotype were selected for this study [6]. Complete genetic analysis of the MECP2 gene revealed abnormalities in 21 of the 33 patients. Epileptic seizures were found in 15 of 33 Rett patients, for which anticonvulsant drugs were administered. All patients whose serum and red blood cell folate levels were normal, underwent a spinal tap to determine the concentration of 5MTHF in the CSF. Eight girls with Rett syndrome lived in North-Western Europe (patient 1–6 in Germany; patient 7, 8 in Belgium), while 25 patients resided in Southern Europe (patients 9–19 in Spain; patients 20–33 in Portugal) (Table 1). Age-related references for 5MTHF in the CSF have been established for both populations from North-Western and South-Western Europe [5, 7].

To determine the cause of reduced 5MTHF in the CSF, serum samples were analyzed for the presence of blocking autoantibodies against the FRs using previously reported methods [5]. Statistical analysis used the Fisher exact test to compare the incidence of low CSF folate and FR autoimmunity among the two groups from North-Western and South-Western Europe, and to study the association of FR autoimmunity with CSF folate status.

The Fisher exact test was also applied to compare the incidence of cerebral folate deficiency (CFD) between Rett patients from Europe and North-America, and to determine the incidence of FR autoantibodies in Rett patients in Europe and healthy controls. Statistical analysis used the analysis of variance for independent samples to compare the serum and CSF folate values between the European Rett population, North-American Rett population and healthy controls. The analysis of variance was also used to compare the CSF folate levels between Rett girls residing in North-Western, South-Western parts of Europe and healthy controls. Data have been used from a study on North-American Rett girls and from the available data on serum folate extracted from the American National Health and Nutrition Examination Survey 1999–2000 [4, 8].

Results



Compared to North-American Rett patients and healthy European controls, the 33 Rett girls from Europe had a statistically

Table 2 Comparison of folate levels in serum, 5MTHF levels in CSF and serum FR autoantibodies among Rett patients from Europe, North America and healthy controls

Analyte	European Rett (S1)	American Rett (S2)	European Controls (S3)	American Controls	Statistical test	Significance
number of patients	33	70	28	1586		
age (years)	2–19	2.7–20.2	1.9–19	3–19		
serum folate						
mean	24.36	38.14	24.72	39.38	F-value = 7.62	p = 0.00080
range	8.8–62.2	5.4–103.1	8.3–45	10.2–68.6	S1 vs. S2 S1 vs. S3 S2 vs. S3 t-test (&) = 3.71	p < 0.01 NS p < 0.01 p < 0.01
CSF folate						
mean	45.53	72.6	82 *	75.7	F-value = 21.879	p < 0.0001
range	12–92	27–136	44–181	40–150	S1 vs. S2 S1 vs. S3 S2 vs. S3	p < 0.01 p < 0.01 NS
number of patients with CFD	14	2			Fisher test (§) p = 0.0000	significant
FR antibodies						
number	8	unknown	0		Fisher test (†) p = 0.011	significant
mean titer	1.25					
range	0.29–2.04					
incidence of FR antibodies for low vs normal CSF folate	6/14 vs 2/19	unknown			Fisher test p = 0.037	significant
anticonvulsant drugs in CFD vs normal CSF folate	7/14 vs 8/19				Fisher test p = 0.250	NS

* CSF folate data derived from 99 healthy controls;

S1: Sample representing European Rett patients

S2: Sample representing American Rett patients

S3: Sample representing European healthy controls

§: Fisher test between European and American Rett patients

†: Fisher test between European Rett patients and healthy controls

&: t-test for comparison between healthy European and American controls

lower 5MTHF value in their CSF (analysis of variance F-value = 21.879 and $p < 0.0001$; ● **Table 2**).

In 14 European Rett patients (10 subjects with MECP2 gene abnormalities and 4 with a normal genotype), the concentration of 5MTHF in the CSF was below the lower reference limit of healthy controls (● **Table 1**). However, in 19 patients (11 subjects with an abnormal MECP2 genotype and 8 with a normal genotype), the 5MTHF concentration in the CSF was within the normal control range.

Compared to 28 healthy control subjects with no FR autoantibodies, the serum of 8 out of 33 Rett patients from Europe contained blocking FR autoantibodies (a mean of 1.25 pmol FR blocked/ml of serum; range 0.29–2.04; significant Fisher exact test with probability $p = 0.011$). The serum of 6 of the 14 patients with a low folate concentration in the CSF contained autoantibodies against FRs. In contrast, the serum of only 2 of the 19 patients with normal CSF folate levels contained low titers of the FR autoantibodies (patients 15,26). Comparison of the groups of Rett patients with low CSF folate and those with normal CSF folate, showed a significantly higher incidence of FR autoantibodies for the Rett patients with low CSF folate (● **Table 2**; significant Fisher exact test $p = 0.037$). It should be stressed that the serum of 8 out of 14 Rett patients with low CSF folate, did not contain FR autoantibodies (patients 7, 1, 3, 8, 19, 22, 24, 32;

● **Table 1**). The use of anticonvulsant drugs in 15 of 33 Rett patients was not found to exert a statistically significant effect on their folate status (● **Table 2**).

The 8 Rett patients from Northern Europe (patient 1–8 from Germany and Belgium) showed a higher incidence of CSF 5MTHF deficiency and FR autoantibodies than the 25 Rett patients from Southern Europe (patients 9–33 from Spain and Portugal; ● **Table 3**) with a calculated Fisher exact probability at $p = 0.005$ and $p = 0.037$ respectively. The serum folate values between patients from Northern and Southern Europe showed no statistical differences, but comparison of the CSF folate values between Northern Europe, Southern Europe and healthy controls showed the lowest CSF folate values for Rett patients from Northern Europe, followed by moderately lowered CSF folate values for Rett patients from Southern Europe (analysis of variance $F = 20.92$ and $p < 0.0001$; ● **Table 3**).

The serum folate levels in Rett patients from North-America (mean value: 38.14 nmol/Liter; range 5.4–103.1) were significantly higher than the levels in European Rett patients (mean value: 24.36 nmol/Liter; range 8.8–62.2), and healthy European controls (mean value: 24.72; range 8.3–45) using the analysis of variance for three independent samples ($F = 7.62$ and $p = 0.000802$). Serum folate levels from healthy North-American girls were also significantly higher than healthy European controls (● **Table 2**).

Table 3 Comparison of folate levels in serum, 5MTHF levels in CSF and FR autoantibodies between Rett patients from Northern and Southern Europe

Analyte	Northern Europe (S1)	Southern Europe (S2)	Controls (S3)	Statistical test	Significance
number of patients	8	25	99		
serum folate					
mean	25.8	23.88		t-test t=0.33	NS
range	13.2–47	8.8–62.2			
CSF folate					
mean	26.8	51.52	82	F-value = 20.92	p<0.0001
range	12–68	33–92	44–181	S1 vs. S2	p<0.05
				S1 vs. S3	p<0.01
				S2 vs. S3	p<0.01
incidence of low CSF folate	7/8	7/25		Fisher test p=0.005	significant
incidence of positive FR antibodies	6/8	2/25		Fisher test p=0.037	significant

S1: Sample representing European Rett patients

S2: Sample representing American Rett patients

S3: Sample representing European healthy controls

An interesting finding was that among the 12 European Rett patients with a normal MECP2 genotype, four patients had low CSF folate levels with FR autoimmunity as contributing factors in 3 of these 4 patients (patients 1–3).

Discussion

We have identified autoantibodies against the FRs in 24% of 33 Rett patients from Europe. This finding suggests a mechanism for the low CSF concentration of 5MTHF and the accompanying CFD-like features in these Rett patients. In contrast, a subgroup of Rett patients with low CSF folate and most Rett patients with normal CSF folate levels lack these autoantibodies. Furthermore, two Rett patients with low titers of autoantibodies had normal CSF folates (patients 15,26). Our study also showed a higher incidence of CFD and FR autoimmunity among the group of Rett patients residing in North-Western Europe, when compared to those living in Southern Europe. The lowest CSF folate values associated with FR autoantibodies were found in the Rett patients living in Northern Europe.

It is likely that Rett syndrome patients with these autoantibodies in their serum cannot transfer the 5MTHF into the CSF because the autoantibodies block the binding of folate to the FR expressed on the choroid plexus and thereby prevent the transfer of folate to the CNS. Our previous study showed that the CSF of Rett patients with low folate contained a normal concentration of immunoreactive folate receptor protein that did not bind radiolabelled folate [1,2]. These soluble non-functional FR proteins in CSF could represent autoantibody-FR complexes released into the spinal fluid after cleavage from their GPI-anchor attachment to the choroid plexus epithelial membranes. Our observation of circulating FR autoantibodies of the blocking type in three patients with a Rett phenotype, low CSF folate and a normal MECP2 genotype may point to a variant of the classical Rett phenotype that presents as a CFD-like syndrome. However, the finding of low CSF folate in 1 of the Rett patients with a normal MECP2 genotype and absence of FR autoimmunity suggests that other unknown aetiologies can contribute to the low CSF folate status in the Rett phenotype.

The geographical differences for FR autoimmunity and CFD between North-Western and South-Western Europe may be explained by nutritional differences with the higher consumption of milk and milk products in Northern Europe. Bovine FR

has high amino acid sequence homology with the human FR [9] and may be the source of the antigen. Cross-reactivity of the antibodies generated against the soluble bovine FR with the human FR antigen may provide a basis of our hypothesis on FR autoimmunity, which was proposed as a possible mechanism for the observed FR autoimmunity in children with the infantile-onset CFD syndrome [5]. The lower incidence of cerebral folate deficiency in the Rett population of North America may be explained by the folate food fortification and the folate/betaine trial, where the participants stopped folate at least 30 days or 1 week prior to lumbar puncture [4]. After folate is incorporated into cells, it takes more than 90 days before these folate reserves decrease [10]. Therefore, if folate supplementation is interrupted for less than 100 days before a lumbar puncture, the results have to be interpreted with caution. Comparison of the data from America and Europe has confirmed the significantly higher serum and CSF folate levels present in the American Rett population. A comparison of the incidence of the FR autoantibodies in the two patient populations from both continents would be an interesting issue to address in order to explain these conflicting results.

Our finding of low CSF folate in 42% of Rett patients suggests that folate transport across the choroid plexus may be blocked. The presence of serum FR autoantibodies in some of these patients provides an explanation for their low CSF 5MTHF. However, in Rett patients where the CSF/plasma folate ratio remains normal other mechanisms such as an increased folate turnover due to an increased utilization or catabolism of 5MTHF or other metabolic abnormalities have to be considered [10].

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