BDNF polymorphism methylation: risk factor of disease progression in Multiple Sclerosis

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OBJECTIVES

Brain-Derived Neurotrophic Factor (BDNF) is a member of the neurotrophin growth factor family. A single-nucleotide polymorphism (SNP) has been identified in the human BDNF gene, which results in a single-nucleotide substitution that leads to an amino acid substitution from valine to methionine in position 66 (Val66Met) leading to an impaired intracellular trafficking and decreased depolarization and secretion of BDNF [1]. In Multiple Sclerosis (MS), the polymorphism Val66Met has been correlated with alteration of cognitive performance and measures of brain atrophy [2], with ambiguous results. Accumulating evidences suggest the involvement of DNA methylation in the regulation of BDNF expression. The aim of the present study was to assess in blood samples of MS patients the potential correlation between methylation status of CpG site near BDNF-Val66Met polymorphism and the severity or progression of disease, aiming at using the methylation of this site as a biomarker of the disease progression.

METHODS

We recruited 136 MS patients (88 women and 48 men) with mean age 38,32 ± 12,49 years, mean disease duration 138,98 ± 90,47 months and onset mean age 33,26 ± 10,51 years (Table 1 and 2).
The MS patients included in this study were genotyped for the BDNF Val66Met polymorphism at nucleotide 196 (G/A) using a "high resolution melting" technique [3]. For each patient we quantitatively measured the methylation level of cytosine included in the exonic CpG site that can be created or abolished by the Val66Met BDNF polymorphism.
Furthermore we analyzed the clinical history of each patient and determined the time elapsed since the onset of the disease and an EDSS score of 6.0.

	Total Patients		Val/Val Patients		Val/Met Patients	
	Average	SD	Average	SD	Average	SD
Age (years)	44,4	12,58	44,24	12,81	43,78	12,34
Lenght of disease (months)	138,98	90,47	152,00	86,44	149,47	96,88
EDSS score	3,0	2,2	3,1	2,2	2,8	2,0

	Total Patients		Val/Val Patients			Val/Met Patients		
	N	%	N.	absolute %	relative %	N.	% assoluta	% relativa
Patients	136	/	82	60%	/	54	40%	/
Therapy								
IFNβ-1a im	19	14%	8	6%	9%	11	8%	21%
IFNβ-1a sc	37	27%	24	18%	30%	12	9%	23%
IFNβ-1b sc	12	9%	8	6%	10%	4	3%	9%
Glatiramer Acetate	18	13%	8	6%	10%	10	7%	15%
Fingolimod	14	10%	10	7%	11%	4	3%	9%
Natalizumab	7	5%	3	2%	4%	4	3%	7%
Immunosuppressive agents	15	11%	11	8%	14%	4	3%	9%
No Therapy	15	11%	10	7%	12%	5	4%	7%
Sex								
Men	48	35%	32	24%	67%	16	12%	33%
Women	88	65%	50	37%	57%	38	28%	43%
Type of MS								
RR-MS	88	65%	48	35%	54%	41	30%	48%
SP-MS	44	32%	29	22%	69%	12	10%	31%
PP-MS	4	3%	3	2%	75%	1	1%	25%

Table 1 and Table 2: characteristics of the sample of patients

RESULTS

In our sample the distribution of the Val66Met polymorphism is approximately 59,5% for the Val/Val genotype and 40,5% for the Val/Met genotype; we haven't found any Met/Met genotype. We found that the majority of the Val/Val patients had a methylation level between 60% and 90%, while the majority of the Val/Met population had a methylation level above 90% (p<0,001) (figure 1).
When the endpoint of a EDSS score of 6 was taken into account, 6 events were observed in subjects carrying the Val/Met genotype (incidence rate: 0.009/year/person) and 20 events were observed in Val/Val subjects (incidence rate: 0.021/year/person) (p=0.052); when the sample was stratified according to the median methylation, (high methylation [>79%]; low methylation [<79%]), we observed 19 events in subjects with low methylation (incidence rate: 0.027/year/person) and 7 events with high methylation (incidence rate: 0.008/year/person) (figure 2); the difference in incidence was statistically significant (p=0.004). The Cox's proportional hazards model showed an increased risk in subjects with low methylation (HR=6.46; 95%CI=1.432-29.160; p=0.015) and an increased risk associated with age (HR=1.05; 95%CI=1.003-1.010; p=0.036).

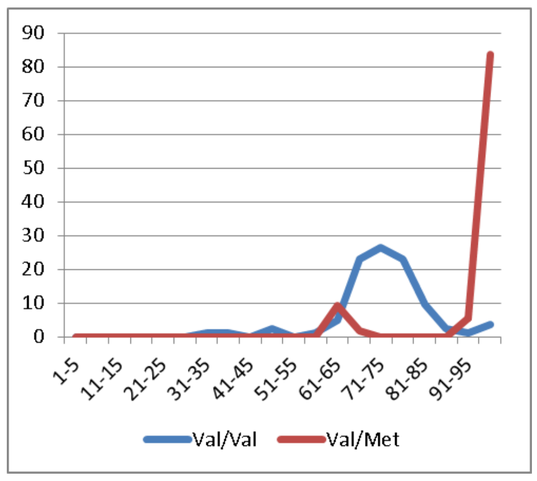

Figure 1: distribution of the percentage of methylation of BDNF gene in Val/Val patients (blue) and Val/Met patients (red)

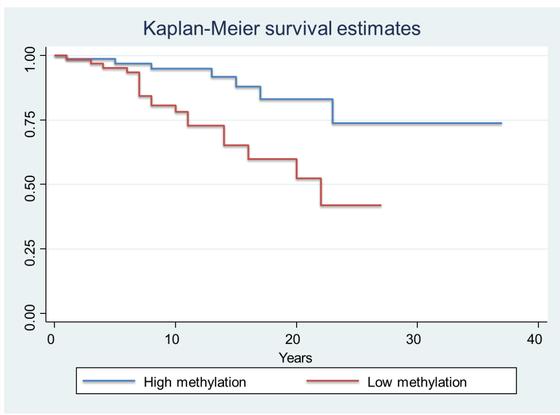

Figure 2: Kaplan-Meier plot with ending point of reaching an EDSS score of 6,0. In blue the patients with a high methylation percentage of BDNF , in red the patients with a low methylation one

CONCLUSIONS

The reduction of BDNF gene methylation could be seen as a protection mechanism for the brain. In patients with high disease progression the hypomethylation of the BDNF gene could increase the secretion of the protective neurotrophin. Moreover, with the progressive increase of the age, a progressive decrease of the brain functional reserve also occurs. So epigenetic modifications, as hypomethylation of the BDNF gene, could be an organism response to limit the brain functional reserve loss. Our study suggests that the percentage of **methylation of the BDNF gene could be used as a prognostic factor for disease progression toward a high disability in MS patient**

REFERENCES

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