Genome-wide methylation array reveals epigenetic drift and epivariations in ALS

Brusati Alberto [1], Ratti Antonia [2,3], Calzari Luciano [4], Peverelli Silvia [2], Tiloca Cinzia [2], Carbone Erika [4], Silani Vincenzo [2,5], Ticozzi Nicola [2,5], Gentilini Davide [1,4]

1 – Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy.
2 – Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy.
3 – Department Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.
4 – Bioinformatics and Statistical Genomics Unit,IRCCS Istituto Auxologico Italiano,Milan,Italy.
5 – Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy.

Introduction

During the last decades, our knowledge about the genetic architecture of sporadic ALS has significantly increased. However, besides the recognized genetic risk factors, also the environment is supposed to have a role in disease pathogenesis. Epigenetics, reflecting the direct consequences of the interaction between genes and environmental risk factors, may play a role in the development and progression of ALS. A recent large epigenome-wide association study (EWAS) in blood identified differentially methylated positions mapping to 42 different genes, involved in cholesterol biosynthesis and immune-related pathways [1].

Objectives

The aims of this study could be divided into the following 4 tasks:

1. The investigations of the role of epigenetics in sporadic ALS progression and development.
2. The calculation of stochastic epigenetic mutations (SEMS) and the validation of epigenetic drift at global and gene level.
3. The identification of regions enriched in SEMs, also known as epivariations.
4. The validation of the previous epigenetic signature and its relationship with toxic compounds.

Methods

We performed a genome-wide DNA methylation analysis in whole peripheral blood on an Italian cohort of 61 sporadic ALS patients and 61 healthy controls, sex- and aged-matched. SEMs were calculated following the criteria already validated by literature [2]. Epigenetic drift was globally assessed using a multivariate regression model, taking account of several covariates such as principal components, age and gender. At gene level, epigenetic drift was estimated using the SKAT rare variants test. Epivariations were estimated following the standard procedure previously described by our laboratory [3]. Exploratory and differential analysis were obtained using the R package RnBeads adopting all the stardards procedure of quality control. Meta-analysis was conducted using the METAL package and all the available public datasets. Gene set enrichment analyses (GSEA) were estimated by the ShinyGO app using a fold discovery rate (FDR) threshold of 0.01.

Results

We found an increased global epigenetic drift in ALS cases compared to controls. At gene level, 700 genes resulted significantly different between cases and controls. GSEA on these results additionally revealed an enrichment in the neurotrophin pathway. Intriguingly, we found an association between the age at onset (AAO) and the burden of SEMs in enriched neurotrophin genes. Moreover, for the first time, we calculated regions enriched in stochastic epigenetic mutations (SEMs), also named epivariations. Interestingly, we identified 153 genes showing epivariations uniquely enriched in ALS cases compared to controls, 88 of which expressed in cerebral structures. By a meta-analytical approach, we also conducted a GSEA on significant concordant probes with those obtained in the previous EWAS on ALS. By using the resulting genes, we validated the previous epigenetic signature and investigated the relationship with toxic compounds according to the Toxicogenomic Database.

Conclusions

Overall, our study reinforces the evidence that epigenetics may contribute to the pathogenesis of ALS and that epigenetic drift may be a useful diagnostic marker. Further research is needed to determine the role of epivariations in the identified candidate genes.

Bibliography

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