Study on Genetic Variants Associated to Multiple Sclerosis by Exome Sequencing in a High Prevalence Family

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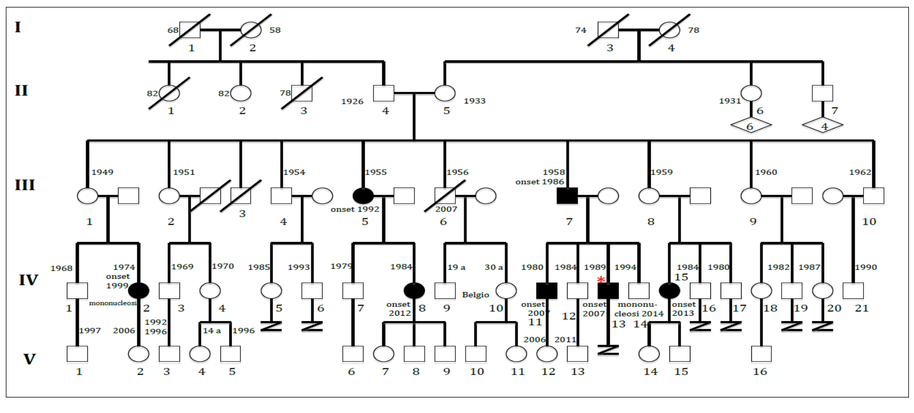
Introduction:

The genetic component of the Multiple Sclerosis (MS) has been studied to date mainly through Genome Wide Association Studies, which confirmed the involvement of genetic factors in the etiopathogenesis of the disease.
Despite the literature data confirm the involvement of the genetic component in MS, mechanisms that may be responsible for disease onset have not been elucidated yet. While in sporadic cases for the onset there seems to be necessary a combination of genetic causes and exposure to environmental factors, in some families the high prevalence of the disease seems to suggest that the genetic causes is predominant on the environmental one.
By this study, we aim to shed light on the genetic component of etiopathogenesis of MS by performing whole exome sequencing (WES) in a high prevalence family. This strategy is based on sequencing by NGS only exon regions instead of whole genome, allowing both an additional lowering of the costs and a decreasing of the times necessary for a study. The greatest strength of this method is that in exome regions reside about 85%[1] of the variants attributed to mendelian diseases. Due to these characteristics, the WES has successfully already been used in this area to study other disorders but up to date, due the need of the identification of a high prevalence family or a large number of affected individuals, studies published on MS conduced by WES are only three. By WES we aim to identifying rare gene variants that may shed light on genetic processes that could lead to MS onset.
We expect, therefore, to have a deeper understanding of the regulatory mechanisms that can be altered and of the variants that can lead to such alteration, thus enlightening genetic processes leading to MS onset also in sporadic patients.

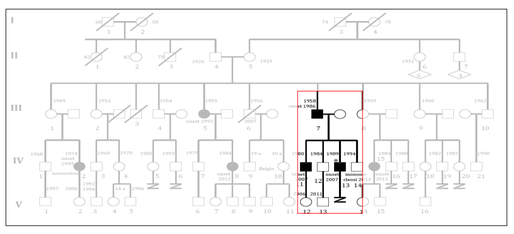


In this family the disease appears in the 3rd generation, where there are 2 patients on 9 members. In the 4th generation are present 5 patients on 22 members. In the 5th generation all members are below the typical age of onset of the disease and there are no patients in this generation. Overall we can assess that there are 2 immune carriers and 7 patients and we can assume that the penetrance of variants is incomplete. Penetrance: 7/9 = 0.78=78%.

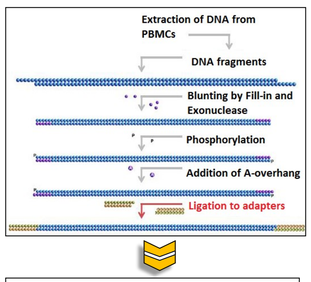
Methods:

FINDING FAMILY TRIO:

The family tree has been studied to identify patients more suitable to join the study. We considered three affected member: a father and two sons

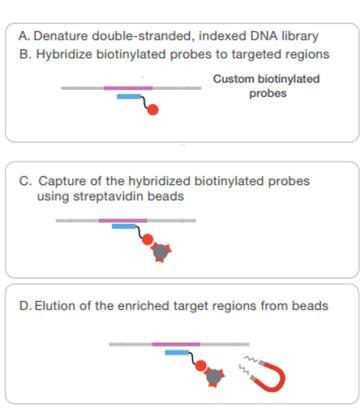


LIBRARY PREPARATION:

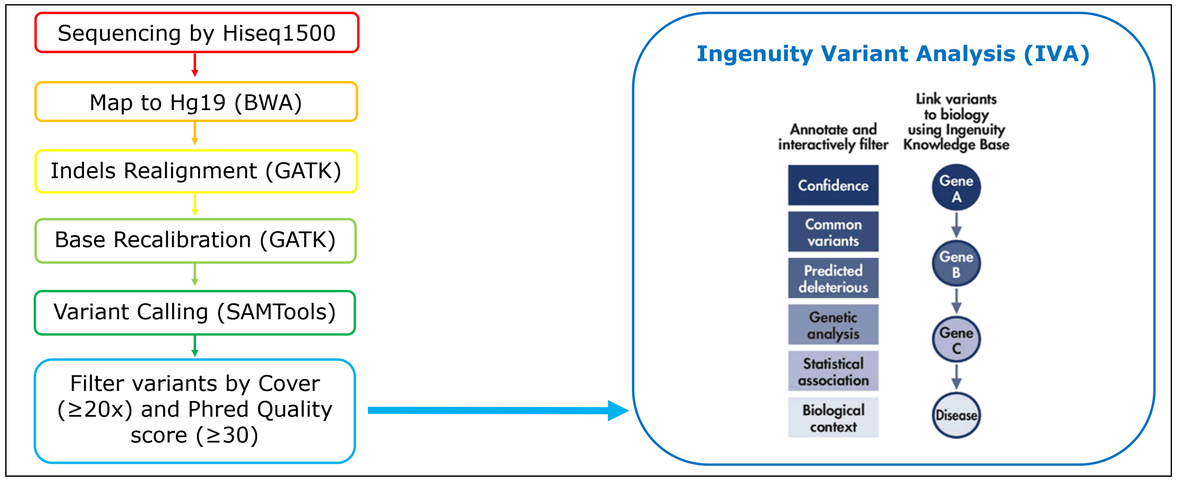


By electrophoresis were selected fragments about 70bp. Then, they were extracted by MinElute PCR Purification Kit and amplified by PCR

EXOME ENRICHMENT:



SEQUENCING AND DATA PROCESSING:



Finding Variants and Filtering Strategies:

By IVA we selected those variants shared between donors and filtered out rare variants with a global allele frequency 2% and non-deleterious ones obtaining a **Shared Variants List**. Then we selected from the Shared Variants List all those variants that potentially can induce to a phenotypic manifestation: homozygous variants, compound heterozygous variants, hemizygous variants and haploinsufficient variants obtaining **Variants with a Phenotypic Manifestation List** where we investigated which pathway could be altered and which genes were affected by strongly deleterious variants (PolyPhen2≥0.95 and SIFT≤0.05). Lastly, we also analyzed, in Variants with a Phenotypic Manifestation List, variants affecting genes that interact with **Environmental Risk Factors**. For this purpose we compared the list of genes with most recent Virus Mentha database to find altered genes that interact with EBV and performed an analysis by String to find genes related to Vitamin D pathway.

Results:

SHARED VARIANTS:

We found 1483 variants on 856 genes shared between the three patients.
We firstly investigated whether any gene affected by variant was yet associated to MS by previous Genome Wide Association studies.
We found variants on 4 genes already related to the MS by comparing our genes list with gwascentral's database (www.gwascentral.org):
PLCL2, TCF7 PRKRA, and **MPV1IL2**.
We also investigated which genes were affected by strongly deleterious variants. Results are similar to those obtained in Variants with a Phenotypic Manifestation List: see next box.

*(Reactome: P-value=0.000171) (Wikipathways: P-value=0.007035)

VARIANTS WITH A PHENOTYPIC MANIFESTATION:

832 variants on 288 genes having a phenotypic manifestation were found.

Two pathways potentially related to MS appeared to be possibly altered by such variants[2].
TLR pathway*[1] which could be altered by 2 variants that affect both alleles of **TLR6** (rs199766026 and rs376295385) and 1 on **TLR10** (rs111829929) and NOTCH pathway*[2] which is affected by variants laid on **NOTCH1** and **NOTCH2**. These probably deleterious variants may affect the entire pathway which could be altered also by other found variants laid on 5 proteins of the same.

Moreover, we found 3 genes with strongly deleterious variants potentially associated to MS onset in this family[3-4].
We found 6 damaging variants (rs76869766, rs1052975, rs62133127 rs620207, rs150628522, rs140753993) on both alleles of **LILRA6**, a gene that is supposed to **control inflammatory responses** and **limit autoreactivity**[5].
We found 2 deleterious variants (not in dbSNP) affecting **NFkB2** predicted to alter efficiency of **transcription factors** binding.
Lastly, we found 2 variants on both alleles (not in dbSNP) affecting **STIM2**, which regulates **IL10 production**.

*[1](Reactome: P-value=0.001304) (Wikipathways: P-value=0.0005412) *[2](Wikipathways P-value=0.005623) (KEGG P-value=0.006057)

ENVIRONMENTAL RISK RELATED:

We identified 11 variants affecting genes that interact with EBV. Most interesting affected genes are NFKB2, DARS and IPO5.
Variants that affects **NFkB2** could lead to the loss of interaction with **BRRF1**, an EBV's gene that enhances **lytic infection** by BRLF1.
DARS interacts with **EBNA-LP** important for the **B-cell immortalization**. We also found variants on other genes (PCCA, RPL12, SET and TBP) that interact with EBNA-LP.
IPO5 interacts with viral protein **EBNA-1**, essential for suppression of spontaneous **lytic reactivation** during latent infection status.
By String analysis, we detected one gene, **CDK11B**, involved in Vitamin D pathway as it interacts with VDR. The gene presents 1 deleterious homozygous variant that could alter interaction with VDR and Vitamin D immunity modulation.

Conclusions: Using exome sequencing approach we found new variants potentially associated with MS in a high prevalence family.

References:
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