Role of Multiple Vitamin D-Related Polymorphisms in Multiple Sclerosis Severity: Preliminary Findings

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Abstract: Background: Multiple Sclerosis (MS) is a multifactorial disease whose pathogenesis is the result of interaction among genetic, epigenetic, and environmental factors. Among these, a role for vitamin D hypovitaminosis has emerged in recent decades. Vitamin D levels are influenced by both environmental and genetic factors. Single nucleotide polymorphisms (SNPs) in genes codifying for molecules involved in vitamin D metabolism have been associated with an increased risk of developing MS. However, few studies assessed the association of such SNPs with the severity of the disease. The aim of this observational study was to evaluate the potential association among vitamin D status, MS severity, and vitamin D-related SNPs, alone or in combination. Methods: In a cohort of 100 MS patients, we genotyped 18 SNPs in the following genes: NAD synthetase 1, CYP2R1, vitamin D binding protein, vitamin D receptor, Retinoid X Receptor-α, KLOTHO, CYP24A1, and CYP27A1. Serum 25(OH)D3 levels were measured by high-performance liquid chromatography. Genotyping was performed by real-time polymerase chain reaction or PCR-RFLP. Results: We did not find any association between SNPs, alone or in combination, and MS severity. Conclusion: In this study, we make an initial evaluation of the possible influence of several SNPs in vitamin D-related genes on MS severity.

Keywords: genetic; prognosis; severity; SNP; MS

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system, characterised by focal lesions of primary demyelination and neurodegeneration. It is a multifactorial disease whose pathogenesis could be a result of the interaction among environmental, genetic, and epigenetic factors. Among these, a role for vitamin D has been proposed.

Vitamin D is a secosteroid, which plays pleiotropic functions. Beyond its well-known role in calcium homeostasis, it is an important regulator of the immune system [1,2]. Accordingly, low vitamin D levels have been involved in the pathogenesis of several autoimmune diseases, including MS. A relationship between hypovitaminosis D and MS susceptibility has been hypothesised since the late nineties. In recent decades, several observational studies worldwide showed an inverse association between vitamin D levels...
and MS risk [3,4]. Notably, vitamin D levels are influenced by both environmental and genetic factors. Accordingly, the potential role of single nucleotide polymorphisms (SNPs) in genes codifying molecules involved in vitamin D metabolism in MS susceptibility has also been evaluated, achieving contrasting results [5]. Less investigated is the role of such SNPs in the MS severity.

In this observational study, we evaluated the potential association among vitamin D-related SNPs, vitamin D status, and MS severity. Specifically, we evaluated 18 SNPs in the following genes: NAD synthetase 1 (NADSYN1), CYP2R1, vitamin D binding protein (VDBP), vitamin D receptor (VDR), Retinoid X Receptor-α (RXR-α), KLOTHO, CYP24A1, and CYP27A1 (Table 1).

Table 1. Characteristics of vitamin D-related SNPs.

| Gene       | Chromosome | SNP                | Ancestral Allele | Substitution Allele |
|------------|------------|--------------------|------------------|---------------------|
| NADSYN1    | 11         | rs3829251          | G                | A                   |
|            |            | rs7944926          | G                | A                   |
|            |            | rs12785878         | G                | T                   |
| CYP2R1     | 11         | rs10766197         | G                | A                   |
|            |            | rs10741657         | G                | A                   |
| VDBP       | 4          | rs7041             | G                | T                   |
|            |            | rs4588             | C                | A                   |
| VDR        | 12         | rs1544410 (Bms-I)  | B                | b                   |
|            |            | rs7975232 (Apa-I)  | A                | a                   |
|            |            | rs731236 (Taq-I)   | T                | t                   |
|            |            | rs2228570 (Fok-I)  | F                | f                   |
| RXR-α      | 9          | rs9409929          | G                | A                   |
|            |            | rs12004589         | G                | A                   |
| KLOTHO     | 13         | rs9536314          | T                | G                   |
|            |            | rs1207568          | G                | A                   |
| CYP24A1    | 20         | rs2762939          | G                | C                   |
|            |            | rs2248137          | G                | C                   |
| CYP27A1    | 2          | rs17470271         | T                | A                   |

NADSYN1, NAD Synthetase 1; VDBP, Vitamin D Binding Protein; VDR, Vitamin D Receptor; RXR-α, Retinoid X Receptor-α; ICV, Initial Codon Variant.

2. Material and Methods

2.1. Study Population

This was an observational, retrospective study performed at the University of Palermo, Italy. Patients with MS were enrolled at the Unit of Neurology, Department of Biomedicine, Neurosciences, and Advanced Diagnostics.

Diagnosis of MS was made by an experienced neurologist and based on a previous history of the disease, physical examination, cerebrospinal fluid analysis, and magnetic resonance imaging findings, according to revised McDonald criteria [6]. The neurological status of patients was assessed using Kurtzke’s Expanded Disability Status Scale (EDSS). The progression of disability was assessed using the Multiple Sclerosis Severity Score (MSSS) [7]. The annualized relapse rate (ARR) was calculated in the year prior to the genotyping. At enrolment, all patients did not have clinical or radiological relapse or remission and had not been subject to corticosteroid treatment for at least one month. Patients were excluded if they did not provide informed consent or had other autoimmune diseases.
2.2. Biochemical and Genetic Analyses

All laboratory analyses, including vitamin D status evaluation and genotyping, were performed at the Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, Department of Biomedicine, Neurosciences, and Advanced Diagnostics.

We selected 18 SNPs in genes coding for molecules involved in the metabolism of vitamin D. The characteristics of all SNPs investigated are summarised in Table 1.

SNPs in NADSYN1, CYP2R1, CYP24A1, CYP27A1, RXR-α, and KLOTHO genes were analysed by real-time allelic discrimination TaqMan assay on a 7500 real-time polymerase chain reaction (PCR) system (Applied Biosystems, Monza, Italy), as previously described [8–11]. SNPs in VDR and VDBP genes were assessed by PCR followed by restriction fragment length polymorphism (RFLP) analysis, as previously described [12,13]. Vitamin D status was evaluated by measuring serum 25-hydroxy-vitamin D3 [25(OH)D3] levels using high-performance liquid chromatography (HPLC).

2.3. Statistical Analysis

Statistical analysis was performed by SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and R Language v.3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). Quantitative variables were expressed by the median and interquartile range (IQR), while categorical variables by relative frequencies. Association between polymorphisms was evaluated by Fisher’s Exact test. Univariate analyses to evaluate the association between MSSS and vitamin D levels were performed by Kruskal–Wallis and General Linear Model (GLM). The analysis was also separately conducted on RRMS subgroup only. Multivariate analysis was performed by GLM, using a continuous dependent variable (MSSS or vitamin D) and binary predictors (form of the disease and/or polymorphisms). A new variable called “number of polymorphisms” was created, using, for each polymorphism, 0 as WT and 1 as the presence of polymorphism. Correlation between number of polymorphisms (n) and MSSS, or vitamin D, was evaluated by Spearman’s rank correlation. Considering that the IQR of the new variable was 10–13, we have arbitrarily selected the two groups with the most extreme values, n ≤ 9 and n ≥ 14 polymorphisms (about 15% of patients in each group). These groups were compared for difference in MSSS score or vitamin D levels by the non-parametric Mann–Whitney test.

3. Results

We included a total of 100 MS patients, of whom 82 had RRMS form and 14 SPMS form. Demographics, clinical and biochemical characteristics, as well as allele frequencies of studied polymorphisms, are shown in Table 2. At the univariate analysis, the association between dependent variables (MSSS or vitamin D) and predictors (demographics, clinical variables, and genotypes) was further evaluated. Form of the disease (p = 0.002) and CYP24A1 rs2762939 were found to be significantly associated with MSSS (Table 3); whereas all NADSYN1 gene polymorphisms (p values ranging from 0.024 to 0.047) and CYP24A1 rs2248137 (p = 0.026) were found to be significantly associated with vitamin D levels (Table 3). Post-hoc comparisons (between homozygosity for the ancestral gene, heterozygosis, and homozygosis for the substitution gene) were not statistically significant, if taking into account Bonferroni’s correction.

Additionally, we evaluated the associations between polymorphisms and vitamin D in the RRMS subgroup. However, in this subgroup no polymorphism resulted to be associated with vitamin D levels. Polymorphisms found to be associated in the whole sample in the RRMS subset were found to be closed to statistical significance but with p > 0.05 (0.06–0.1). This discrepancy between significances found in the subset and in the whole sample is possible due to reduced sample size, hence reduced statistical power.
Table 2. Characteristics of the study population (n = 100).

| Variable | Demographics | Clinical | Genotype | Biochemical |
|----------|--------------|----------|----------|-------------|
| Sex, (%) | M: 25%       | RRMS: 82%| NADSYN1  | Vitamin D, µg/L (median, IQR) 20 (16–25) |
|          | F: 75%       | SPMS: 14%| rs3829251, (%) GG 75%, GA 25% |
| Age, years (median, IQR) | 39 (32–47) | Other: 4% | rs7944926, (%) GG 43%, GA 52%, AA 5% |
| Form of the disease, (%) | RRMS: 82% | | rs12785878, (%) GG 5%, GT 51%, TT 44% |
| EDSS, (median, IQR) | 2.3 (1.4–5.0) | CYP2RI rs10766197, (%) GG 19%, GA 44%, AA 37% |
| MSSS, (median, IQR) | 3.34 (1.45–5.48) | CYP2RI rs10741657, (%) GG 59%, GA 4%, AA 37% |
| ARR, % | 0: 22%       | VDBP rs7041, (%) GG 38%, GT 43%, TT 19% |
|          | 1: 41%       | VDBP rs4588, (%) CC 63%, CA 31%, AA 6% |
|          | 2: 29%       | VDR FOK-I, (%) FF 34%, FF 47%, ff 19% |
|          | 3: 5%        | VDR BSM-I, (%) BB 22%, Bb 48%, bb 30% |
|          | 4: 3%        | VDR APA-I, (%) AA 29%, Aa 55%, aa 16% |
|          |              | VDR TAQ-I, (%) TT 33%, Tt 47%, tt 20% |
|          |              | RXR-α rs9409929, (%) GG 48% GA 44% AA 8% |
|          |              | RXR-α rs12040589, (%) GG 80%, GT 20% |
|          |              | KLOTHO rs9536314, (%) TT 75%, TG 23%, GG 2% |
|          |              | KLOTHO rs1207568, (%) GG 69%, GA 28%, AA 3% |
|          |              | CYP24A1 rs2762939, (%) GG 52%, GC 42%, CC 6% |
|          |              | CYP24A1 rs2248137, (%) GG 17%, GC 45%, CC 38% |
|          |              | CYP27A1 rs17470271, (%) TT 12%, TA 51%, AA 37% |

Multivariate analysis was performed considering only variables found to be associated at the univariate analysis. To this aim, MSSS was considered a continuous dependent variable, while form of the disease (RRMS vs. all others) and the CYP24A1 rs2762939 gene polymorphism (GG + GC vs. CC) were taken as binary independent variables. The analysis showed that the form of the disease ($p = 0.006$), but not the CYP24A1 rs2762939 gene polymorphism ($p = 0.072$), is an independent predictor of MSSS (adjusted R squared
for the model 0.70). Same results were obtained considering the GG vs. GC + CC for the CYP24A1 rs2762939 gene polymorphism.

Table 3. Univariate analysis to predict MSSS and vitamin D levels.

| Independent Variable | MSSS          | Vitamin D  |
|----------------------|---------------|------------|
| Sex                  | $p = 0.133$   | $p = 0.604$|
| Age                  | $p = 0.764$   | $p = 0.500$|
| Form of the disease (RRMS vs. all others) | $p = 0.002^*$ | $p = 0.294$ |
| MSSS                 | $p = 0.146$   |            |
| NADSYN1 rs3829251    | $p = 0.860$   |            |
|                      | $p = 0.024^*$ |            |
|                      | GG 22         |            |
|                      | GA 19         |            |
| NADSYN1 rs7944926    | $p = 0.816$   |            |
|                      | $p = 0.025^*$ |            |
|                      | GG 24         |            |
|                      | GA 19         |            |
|                      | AA 24         |            |
| NADSYN1 rs12785878   | $p = 0.920$   |            |
|                      | $p = 0.047^*$ |            |
|                      | GG 24         |            |
|                      | GT 19         |            |
|                      | TT 24         |            |
| CYP2R1 rs10766197    | $p = 0.888$   | $p = 0.771$|
| CYP2R1 rs10741657    | $p = 0.667$   | $p = 0.732$|
| VDBP rs7041          | $p = 0.952$   | $p = 0.954$|
| VDBP rs4588          | $p = 0.651$   | $p = 0.310$|
| VDR FOK-I            | $p = 0.461$   | $p = 0.397$|
| VDR BSM-I            | $p = 0.632$   | $p = 0.322$|
| VDR APA-I            | $p = 0.297$   | $p = 0.992$|
| VDR TAQ-I            | $p = 0.192$   | $p = 0.185$|
| RXR-α rs9409929      | $p = 0.404$   | $p = 0.086$|
| RXR-α rs12004589     | $p = 0.085$   | $p = 0.392$|
| KLOTHO rs9536314     | $p = 0.412$   | $p = 0.590$|
| KLOTHO rs1207568     | $p = 0.170$   | $p = 0.171$|
| CYP24A1 rs2762939    | $p = 0.042^*$ | $p = 0.987$|
|                      | GG 4.55       |            |
|                      | GC 2.44       |            |
|                      | CC 0.53       |            |
|                      | GG vs. CC $p = 0.041$ (not significant if considering Bonferroni’s correction) | |
| CYP24A1 rs2248137    | $p = 0.763$   | $p = 0.987$|
| CYP27A1 rs17470271   | $p = 0.061$   | $p = 0.607$|

NADSYN1, NAD Synthetase 1; VDBP, Vitamin D Binding Protein; VDR, Vitamin D Receptor; RXR-α, Retinoid X Receptor-α; ICV, Initial Codon Variant. * $p$ statistically significant.

Multivariate analysis for vitamin D levels was also conducted. Since NADSYN1 rs3829251 and rs7944926 were significantly associated ($p < 0.001$), only NADSYN1 rs3829251
and rs12785878 and CYP24A1 rs2248137 were included in the multivariate model. Only NADSYN1 rs3829251 was found to be an independent predictor of vitamin D levels ($p = 0.025$), while NADSYN1 rs12785878 ($p = 0.786$) or CYP24A1 rs2248137 ($p = 0.387$) were not associated.

We further performed sub-analysis recoding allele variables (3 levels: homozygosis for the ancestral gene (0), heterozygosis (1), homozygosis for the substitution gene (2)) into binary variables: in the 1st sub-analysis 0 + 1 vs. 2; in the 2nd sub-analysis 0 vs. 1 + 2. For all polymorphisms investigated, the groups were compared for EDSS, MSSS, and ARR (only comparisons with a minimum sample size for each group of 5 patients were considered). In the first sub-analysis, no association was found; in the second sub-analysis an association was found between EDSS and CYP24A1 rs2762939 ($p = 0.024$).

A score summing up all polymorphisms (using 0 as WT and 1 as the presence of polymorphism) was calculated. The median (IQR, min–max) number of polymorphisms was 11 (10–13, 7–16). Number of polymorphisms did not correlate with MSSS (rho = 0.051, $p = 0.676$) or with vitamin D levels (rho = –0.046, $p = 0.681$). Accordingly, patients with more polymorphisms ($n \geq 14$) did not show significantly higher MSSS or vitamin D levels than patients with fewer polymorphisms ($n \leq 9$), with $p = 0.526$ and $p = 0.917$, respectively.

4. Discussion

In this study, we assessed the hypothesis that the presence of vitamin D-related SNPs could influence MS severity. The main finding of our study can be summarised as follows: (i) the form of the disease is an independent predictor of MSSS, as expected; (ii) among all investigated SNPs, only the CYP24A1 rs2762939 is significantly associated with MSSS; (iii) all SNPs of NADSYN1 and rs2248137 of CYP24A1 are associated with decreased vitamin D levels, with rs3829251 being independently associated at the multivariate analysis; (iv) the simultaneous presence of multiple SNPs is not associated with the disease severity. To the best of our knowledge, this is the first study that evaluates the cumulative effect of vitamin D-related SNPs on MS severity. Indeed, each patient enrolled harboured at least seven SNPs. Specifically, we selected SNPs in genes codifying for molecules with an important role in the vitamin D pathway. The NADSYN1 gene codifies for an enzyme that catalyses NAD synthesis, a coenzyme involved in 25(OH)D synthesis and hydroxylation. CYP2R1 and CYP27A1 catalyse the reaction of 25-hydroxylation of vitamin D, leading to the production of 25(OH)D. CYP2R1 is located in the liver and represents the major contributor to vitamin D 25-hydroxylation. Also, CYP27A1 is a hepatic enzyme and participates to the hydroxylation of vitamin D but to a lesser extent than CYP2R1. VDBP is fundamental for vitamin D and related metabolites transport in the circulation and, consequently, regulates vitamin D availability to target cells [14]. Vitamin D exerts its biological function through the interaction with the intracellular heterodimer complex consisting of VDR and RXR-$\alpha$. After binding the active form of vitamin D, namely 1-$\alpha$ 25-dihydroxyvitamin D (1-$\alpha$,25(OH)$_2$D), the complex migrates to the nucleus where it interacts with Vitamin D Responsive Element (VDRE) located in the promoter region of several genes. Finally, Klotho is an important regulator of vitamin D homeostasis. We chose to evaluate SNPs in the above-mentioned genes because they were previously associated with altered vitamin D status. Although the investigated SNPs have been associated with MS susceptibility [8,10,12,13,15–17], they do not seem to affect disease severity, except for CYP24A1 rs2762939, according to our findings. This is the first study revealing a possible role of CYP24A1 genetic alteration in MS severity. Recently, Malhotra et al. showed that another SNP in the CYP24A1 gene, namely the rs2762943, is associated with MS susceptibility but not with severity [18].

Overall, most studies failed to find an association between polymorphisms in several genes, including vitamin D-related ones, and MS severity [19–23]. Thus, the contribution of genetics to MS severity remains elusive.

It can be postulated that mechanisms that predispose individuals to develop MS could diverge from those that drive disease progression. The contribution of genetics to MS severity is still far from being elucidated. Indeed, several mechanisms could be involved...
in the disease progression, including the effect of treatments, environment, and stochastic processes. It is plausible that common genetic variants exert only a weak influence on disease progression.

Our study has some limitations. We included a small number of MS patients, and we did not perform a longitudinal evaluation. Moreover, since for some polymorphisms very few subjects displayed the ancestral or the substitution allele, a lack of association with MSSS or vitamin D could be possibly due to the limited statistical power. The strength is that we evaluated 18 vitamin D-related SNPs for each patient.

In conclusion, in this study, we first evaluate the possible influence of several SNPs in vitamin D-related genes on MS severity. Although we did not find any association between investigated SNPs and MS progression, further studies are required to confirm such findings.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Bivona, G.; Agnello, L.; Ciaccio, M. Vitamin D and Immunomodulation: Is It Time to Change the Reference Values? *Ann. Clin. Lab. Sci.* 2017, 47, 508–510. [PubMed]
2. Bivona, G.; Agnello, L.; Bellia, C.; Iacolino, G.; Scazzone, C.; Lo Sasso, B.; Ciaccio, M. Non-Skeletal Activities of Vitamin D: From Physiology to Brain Pathology. *Medicina* 2019, 55, 341. [CrossRef] [PubMed]
3. Scazzone, C.; Agnello, L.; Bivona, G.; Lo Sasso, B.; Ciaccio, M. Vitamin D and Genetic Susceptibility to Multiple Sclerosis. *Biochem. Genet.* 2021, 59, 1–30. [CrossRef]
4. Elkama, A.; Karahalil, B. Role of gene polymorphisms in vitamin D metabolism and in multiple sclerosis. *Arh. Hig. Rada Toksikol.* 2018, 69, 25–31. [CrossRef] [PubMed]
5. Ruiz-Ballesteros, A.I.; Meza-Meza, M.R.; Vizmanos-Lamotte, B.; Parra-Rojas, I.; de la Cruz-Mosso, U. Association of Vitamin D Metabolism Gene Polymorphisms with Autoimmunity: Evidence in Population Genetic Studies. *Int. J. Mol. Sci.* 2020, 21, 9626. [CrossRef] [PubMed]
6. Thompson, A.J.; Banwell, B.L.; Barkhof, F.; Carroll, W.M.; Coetzee, T.; Comi, G.; Correale, J.; Fazekas, F.; Filippi, M.; Freedman, M.S.; et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* 2018, 17, 162–173. [CrossRef]
7. Roxburgh, R.H.; Seaman, S.R.; Masterman, T.; Hensiek, A.E.; Sawcer, S.J.; Vukusic, S.; Achiti, I.; Confavreux, C.; Coustans, M.; le Page, E.; et al. Multiple sclerosis severity score: Using disability and disease duration to rate disease severity. *Neurology* 2005, 64, 1144–1151. [CrossRef]
8. Agnello, L.; Scazzone, C.; Lo Sasso, B.; Ragonese, P.; Milano, S.; Salemi, G.; Ciaccio, M. CYP27A1, CYP24A1, and RXR-α Polymorphisms, Vitamin D, and Multiple Sclerosis: A Pilot Study. *J. Mol. Neurosci.* 2018, 66, 77–84. [CrossRef]
9. Scazzone, C.; Agnello, L.; Ragonese, P.; Lo Sasso, B.; Bellia, C.; Bivona, G.; Schillaci, R.; Salemi, G.; Ciaccio, M. Association of CYP2R1 rs10766197 with MS risk and disease progression. *J. Neurosci. Res.* 2018, 96, 297–304. [CrossRef] [PubMed]
10. Scazzone, C.; Agnello, L.; Sasso, B.L.; Ragonese, P.; Bivona, G.; Realmuto, S.; Iacolino, G.; Gambino, C.M.; Bellia, C.; Salemi, G.; et al. Klotho and vitamin D in multiple sclerosis: An Italian study. *Arch. Med. Sci.* 2019, 16, 842–847. [CrossRef]
11. Scazzone, C.; Agnello, L.; Lo Sasso, B.; Salemi, G.; Gambino, C.M.; Ragonese, P.; Candore, G.; Ciaccio, A.M.; Giglio, R.V.; Bivona, G.; et al. FOXP3 and GATA3 Polymorphisms, Vitamin D3 and Multiple Sclerosis. *Brain Sci.* 2021, 11, 415. [CrossRef] [PubMed]
12. Agnello, L.; Scazzone, C.; Lo Sasso, B.; Bellia, C.; Bivona, G.;Realmuto, S.; Brighina, F.; Schillaci, R.; Ragonese, P.; Salemi, G.; et al. VDBP, CYP27B1, and 25-Hydroxyvitamin D Gene Polymorphism Analyses in a Group of Sicilian Multiple Sclerosis Patients. *Biochem. Genet.* 2017, 55, 183–192. [CrossRef] [PubMed]
13. Agnello, L.; Scazzone, C.; Ragonese, P.; Salemi, G.; Lo Sasso, B.; Schillaci, R.; Musso, G.; Bellia, C.; Ciaccio, M. Vitamin D receptor polymorphisms and 25-hydroxyvitamin D in a group of Sicilian multiple sclerosis patients. *Neur. Sci.* 2016, 37, 261–267. [CrossRef] [PubMed]
14. Bouillon, R.; Schuit, F.; Antonio, L.; Rastinejad, F. Vitamin D Binding Protein: A Historic Overview. *Front. Endocrinol.* 2020, 10, 910. [CrossRef] [PubMed]

15. Simon, K.C.; Munger, K.L.; Xing, Y.; Ascherio, A. Polymorphisms in vitamin D metabolism related genes and risk of multiple sclerosis. *Mult. Scler.* 2010, 16, 133–138. [CrossRef]

16. Martinez-Hernandez, A.; Perez-Guerrero, E.E.; Macias-Islas, M.A.; Nava-Valdivia, C.A.; Villagomez-Vega, A.; Contreras-Haro, B.; Garcia-Ortega, Y.E.; Espana-Guerrero, Y.; Gallardo-Moya, S.G.; Gamez-Nava, J.I.; et al. Polymorphisms CYP2R1 rs10766197 and CYP27B1 rs10877012 in Multiple Sclerosis: A Case-Control Study. *J. Immunol. Res.* 2021, 2021, 7523997. [CrossRef]

17. Cancela Diez, B.; Pérez-Ramírez, C.; Maldonado-Montoro, M.; Carrasco-Campos, M.I.; Sánchez Martín, A.; Pineda Lancheros, L.E.; Martínez-Martínez, F.; Calleja-Hernández, M.A.; Ramírez-Tortosa, M.C.; Jiménez-Morales, A. Association between polymorphisms in the vitamin D receptor and susceptibility to multiple sclerosis. *Pharm. Genom.* 2021, 31, 40–47. [CrossRef]

18. Malhotra, S.; Midaglia, L.; Chuquisana, O.; Patsopoulos, N.A.; Ferrer, R.; Giralt, M.; Fissolo, N.; Gil-Varela, E.; Lunemann, J.D.; Montalban, X.; et al. The CYP24A1 Gene Variant rs2762943 Is Associated with Low Serum 1,25-Dihydroxyvitamin D Levels in Multiple Sclerosis Patients. *J. Neuroinflammation*, 2021. Preprint. [CrossRef]

19. Čírný, D.; Michalík, J.; Kurča, E.; Dobrota, D.; Lehotský, J. FokI vitamin D receptor gene polymorphism in association with multiple sclerosis risk and disability progression in Slovaks. *Neurol. Res.* 2015, 37, 301–308. [CrossRef]

20. George, M.F.; Briggs, F.B.; Shao, X.; Gianfrancesco, M.A.; Kockum, I.; Harbo, H.E.; Celius, E.G.; Bos, S.D.; Hedström, A.; Shen, L.; et al. Multiple sclerosis risk loci and disease severity in 7125 individuals from 10 studies. *Neurol. Genet.* 2016, 2, e87. [CrossRef]

21. Kalincik, T.; Guttmann, C.R.; Krasensky, J.; Vaneckova, M.; Lelekova, P.; Tyblova, M.; Seidl, Z.; De Jager, P.L.; Havrdova, E.; Horakova, D. Multiple sclerosis susceptibility loci do not alter clinical and MRI outcomes in clinically isolated syndrome. *Genes Immun.* 2013, 14, 244–248. [CrossRef] [PubMed]

22. Jackson, K.C.; Sun, K.; Barbour, C.; Hernandez, D.; Kosa, P.; Tanigawa, M.; Weideman, A.M.; Bielekova, B. Genetic model of MS severity predicts future accumulation of disability. *Ann. Hum. Genet.* 2020, 84, 1–10. [CrossRef] [PubMed]

23. Sadovnick, A.D.; Traboulsee, A.L.; Zhao, Y.; Bernales, C.Q.; Encarnacion, M.; Ross, J.P.; Yee, I.M.; Criscuoli, M.G.; Vilarino-Güell, C. Genetic modifiers of multiple sclerosis progression, severity and onset. *Clin. Immunol.* 2017, 180, 100–105. [CrossRef] [PubMed]