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Original article

Single-Cell Transcriptomics Reveals that VDR TaqI/ApaI Risk Haplotypes Impair Remyelination by Disrupting Microglial Oligodendrocyte Crosstalk

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Abstract
The failure of spontaneous remyelination represents a critical therapeutic barrier in multiple sclerosis (MS) and other demyelinating pathologies. While genetic epidemiological studies have consistently linked Vitamin D Receptor (VDR) polymorphisms to disease susceptibility and severity, the specific cellular mechanisms translating these risk haplotypes into regenerative failure have remained unresolved. To deconstruct the demyelinated lesion microenvironment, we utilized high-resolution single-nucleus transcriptomics across distinct VDR genotypic cohorts (Risk vs. Wildtype). We integrated topological intercellular communication modelling to map shifts in local cellular crosstalk and employed computational pseudo-time trajectory inference to evaluate the direct developmental consequences of genetic risk on the complete oligodendrocyte lineage. Our analysis revealed a profound and targeted transcriptomic collapse of the RXRA heterodimer specifically within the resident microglial compartment of the VDR-Risk cohort. This intrinsic receptor uncoupling functionally neutralized the microglial neuroprotective state, resulting in a near-total cessation of Prosaposin (PSAP) secretion. Trajectory inference demonstrated that oligodendrocyte precursor cells (OPCs) deprived of this critical microglial PSAP-GPR37 trophic signaling axis suffered a severe developmental stall, structurally preventing their terminal maturation into functional, myelin-forming oligodendrocytes. These findings shift the pathophysiological paradigm of VDR genetic risk from generalized neuroinflammation to a highly targeted disruption of microglial-oligodendroglial crosstalk. The RXRA-mediated PSAP-GPR37 signaling cascade is identified as a pivotal mechanistic vulnerability, offering a novel, targeted therapeutic pathway to overcome remyelination arrest.
Keywords. Multiple Sclerosis, Remyelination, Vitamin D Receptor (VDR), Microglia-oligodendrocyte Crosstalk.

Introduction

Demyelinating diseases of the central nervous system (CNS), most notably multiple sclerosis (MS), are characterized by the progressive destruction of myelin sheaths and a subsequent failure of spontaneous remyelination [1]. This regenerative block is a critical inflection point in disease pathology; it leaves denuded axons highly vulnerable to irreversible metabolic stress, driving neurodegeneration and long-term clinical disability [2]. While systemic adaptive immune responses heavily drive the initial demyelinating onslaught, the failure to regenerate myelin is increasingly recognized as a localized defect within the resident CNS microenvironment. Overcoming this barrier requires a precise understanding of the complex intercellular interplay required for tissue repair, particularly the critical crosstalk between resident innate immune cells and the oligodendrocyte lineage [3].

Epidemiological and genome-wide association studies (GWAS) have consistently established a profound link between Vitamin D signaling and the susceptibility to demyelinating pathologies [4]. However, while systemic serum 25-hydroxyvitamin D levels are widely investigated, their direct correlation to established neurological disability often yields complex or equivalent clinical outcomes. This was recently comprehensively demonstrated in a large cross-sectional multicenter cohort of Libyan adults with MS, where differences in systemic 25(OH)D were found to be unlikely to correspond to clinically meaningful differences in established disability [5]. This clinical ambiguity underscores a critical need to shift the investigative focus from circulating serum metabolites to the intrinsic functional capacity of the Vitamin D Receptor (VDR) within the resident CNS tissue. VDR is a nuclear transcription factor responsible for mediating the widespread biological effects of Vitamin D.

Specific genetic variants, notably the VDR TaqI and ApaI risk haplotypes, are strongly correlated not only with increased disease incidence but also with severe clinical progression and hampered recovery [6]. Yet, despite decades of genetic evidence, the precise cellular mechanisms by which these VDR risk variants translate into remyelination failure remain critically elusive. It is currently unknown whether these haplotypes simply induce a generalized state of chronic neuroinflammation or if they drive highly targeted, cell-specific signaling defects that actively dismantle the tissue repair cascade [7]. Successful myelin repair is an actively orchestrated developmental program requiring oligodendrocyte precursor cells (OPCs) to proliferate, migrate to the lesion site, and terminally differentiate into mature, myelin-forming oligodendrocytes [1]. This maturation trajectory is heavily governed by resident microglia, which have emerged as the primary rheostats of CNS repair [8].

Beyond their canonical role in the phagocytic clearance of myelin debris, microglia must transition into a highly specialized, neuroprotective state to secrete essential trophic factors that stimulate OPC differentiation [9]. Because Vitamin D signaling is known to be a potent modulator of microglial plasticity, we hypothesized that VDR risk haplotypes fundamentally impair this critical microglial oligodendroglia axis, starving progenitor cells of the localized trophic support

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strictly required for terminal maturation. To resolve the mechanistic gap between genetic susceptibility and remyelination failure, we utilized high-resolution single-nucleus transcriptomics to systematically deconstruct the demyelinated microenvironment. By isolating the distinct cellular compartments associated with specific VDR genotypic cohorts, we sought to map the internal transcriptomic vulnerabilities and model the corresponding shifts in intercellular communication networks. Furthermore, we employed computational pseudotime trajectory inference to evaluate the direct developmental consequences of this genetic risk on the complete oligodendrocyte lineage. Through this integrated approach, this study aims to pinpoint the exact molecular breakdowns linking VDR risk haplotypes to regenerative arrest, ultimately identifying novel signaling vulnerabilities that could be targeted to overcome the remyelination block.

Methods

Data Acquisition and Study Cohort

To understand how the VDR TaqI and ApaI risk haplotypes disrupt remyelination, we designed a dual-omics approach that integrates human single-nucleus RNA-sequencing (snRNA-seq) with myeloid-specific expression quantitative trait loci (eQTL) data. First, we obtained transcriptomic profiles of human white matter tissue from the NCBI Gene Expression Omnibus (GEO accession: GSE118257) [10]. We selected this landmark dataset because it provides high-resolution sequencing of both the microglial compartment and the full oligodendrocyte lineage. The patient cohort captures a diverse spatial spectrum of demyelinating pathology, including healthy control tissue alongside multiple sclerosis (MS) lesions at various stages of progression (normal-appearing white matter, chronic active, chronic inactive, and actively remyelinating lesions).

A major challenge in single-cell transcriptomics is that standard workflows frequently miss intronic and synonymous variants, such as the VDR TaqI (rs731236) and ApaI (rs7975232) polymorphisms, due to technical dropout. To overcome this limitation, we used an eQTL framework to build a transcriptomic proxy. We sourced functional genomic mapping profiles from the Myeloid Cells Genomics Assembly (MiGA) consortium [11]. This database maps exactly how specific genetic variants alter baseline gene expression in human myeloid cells. By combining these two datasets, we successfully linked the downstream transcriptomic signature of the VDR risk alleles directly to the single-cell expression space. Because our study relies entirely on the secondary computational analysis of publicly available, de-identified data, it is exempt from institutional review board (IRB) ethics approval. We conducted all data acquisition and analysis in strict adherence to the open-science declarations and data use agreements provided by the primary authors.

Single-Cell RNA-Seq Pre-processing and Quality Control

We processed the raw expression matrices and performed comprehensive quality control (QC) using the Seurat R package (v.4) [12]. Because human post-mortem brain datasets are particularly susceptible to ambient RNA contamination and variable nuclear degradation, we applied stringent filtering thresholds to isolate only high-quality nuclei for downstream analysis. First, we initialized the Seurat object, retaining only genes expressed in at least three individual cells to filter out uninformative, low-expression transcripts immediately. We then evaluated the overall complexity and health of each nucleus.

We retained nuclei expressing between 500 and 6,000 unique molecular features, successfully eliminating low-quality reads (often corresponding to cellular debris) and extreme outliers. Additionally, we excluded any cell where mitochondrial transcripts accounted for more than 10% of the total read count, as elevated mitochondrial fractions typically indicate nuclear lysis or cellular stress induced during tissue dissociation. Following quality control, we addressed differences in sequencing depth across the individual nuclei using global-scaling normalization. We applied Seurat's LogNormalize function, which scales the feature counts of each cell by a standard factor of 10,000 and applies a natural-log transformation. To focus our computational resources on the genes driving the biological differences within the tissue, we utilized a variance-stabilizing transformation (VST) to identify the top 2,000 highly variable genes. These genes, exhibiting high cell-to-cell variation relative to their average expression, formed the mathematical foundation for all subsequent dimensionality reduction and clustering steps.

Dimensionality Reduction and Cell Type Subsetting

To map the global cellular landscape of the dataset, we first subjected the 2,000 highly variable genes to linear dimensionality reduction using Principal Component Analysis (PCA). We evaluated the statistical significance of the principal components and selected the top 20 dimensions to capture the majority of the biological variance while minimizing technical noise. Using these dimensions, we constructed a K-nearest neighbor (KNN) graph and clustered the cells using the Louvain algorithm. For visual representation and topological analysis, we embedded the data into a two-dimensional space using Uniform Manifold Approximation and Projection (UMAP) [13]. Because our central hypothesis focuses exclusively on the interaction between the immune and myelinating compartments, retaining the entire brain cellular milieu (such as neurons, astrocytes, and endothelial cells) would introduce unnecessary computational burden and transcriptomic noise. Therefore, we performed targeted subsetting to isolate only our populations of interest. We identified and extracted microglia using established canonical markers, including HEXB, P2RY12, and CX3CR1. Similarly, we mapped and isolated the complete oligodendrocyte trajectory, capturing oligodendrocyte precursor cells (OPCs) highly expressing VCAN and PDGFRA, committed oligodendrocyte precursors (COPs), and mature myelinating

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oligodendrocytes characterized by MAG, MOG, and PLP1 expression. After extracting these specific clusters, we discarded the non-target cells and ran a secondary round of normalization, variable feature selection, and scaling on the newly focused Seurat object. This strict isolation strategy ensured that our downstream differential expression and cell-cell communication models were driven purely by the biological crosstalk between microglia and the oligodendrocyte lineage, free from the background signaling of other neuroglial cell types.

eQTL Integration and VDR Risk Haplotype Scoring

Standard single-nucleus RNA sequencing generally lacks the resolution to directly genotype intronic or synonymous polymorphisms, such as the VDR TaqI and ApaI variants, due to technical dropout and 3' bias. To overcome this critical limitation, we developed a transcriptomic proxy scoring system by integrating functional eQTL data. We first queried the MiGA consortium dataset to identify a specific network of downstream genes whose baseline expression in human myeloid cells is significantly altered in the presence of the VDR risk alleles. Rather than relying on the expression of the VDR gene alone, which is subject to high dropout rates, we used this broader downstream gene network to capture the functional activity of the pathway. Using these validated downstream targets, we applied Seurat's Add Module Score function to calculate a composite expression signature score for each microglial cell [14]. This algorithm calculates the average expression levels of our target gene set on a single-cell level, subtracted by the aggregated expression of randomly selected control gene sets, thereby controlling for variable sequencing depth and cell complexity. To establish our comparative cohorts for downstream analysis, we applied a median-split threshold to these module scores. We classified cells exhibiting scores below the median as the VDR Risk group, representing suppressed VDR signaling analogous to the TaqI/ApaI risk haplotype. Conversely, we categorized cells scoring above the median as the VDR Wildtype group, representing baseline or healthy VDR activity. This robust stratification strategy provided the pseudo-genotypic labels required to assess how the risk haplotype shifts cellular behavior.

Differential Gene Expression and Pathway Enrichment

To map the exact transcriptomic shifts driven by the VDR risk profile, we performed differential gene expression (DGE) analysis between our computationally defined VDR-Risk and VDR-Wildtype microglial subsets. We executed this comparison using the non-parametric Wilcoxon Rank Sum test implemented in Seurat. To ensure we isolated biologically meaningful changes rather than statistical artifacts, we applied strict significance thresholds. We defined genes as significantly differentially expressed if they demonstrated an absolute log2 fold change (log2FC) greater than 0.25 and a Benjamini-Hochberg adjusted p-value less than 0.05. Once we established the core list of up- and down-regulated genes associated with the VDR risk signature, we translated these molecular alterations into functional biological insights through pathway enrichment analysis. We utilized the clusterProfiler R package [15] to query our differentially expressed gene sets against the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. During this functional annotation, we specifically focused on pathways essential to the remyelination cascade. We evaluated the enrichment of terms related to microglial phagocytosis of myelin debris, lipid metabolic processing, and inflammatory polarization. We considered biological pathways to be significantly enriched using a stringent false discovery rate (FDR) threshold of $q < 0.05$. This allowed us to objectively determine whether the genetic risk profile functionally stalled microglia in a pro-inflammatory or metabolically inactive state.

Intercellular Communication Analysis

To investigate how the VDR risk haplotype disrupts the coordinated signaling required for remyelination, we modelled the intercellular communication networks using the CellChat R package [16]. We restricted our interaction modelling to the curated CellChatDB.human database, focusing specifically on secreted signaling factors and direct cell-cell contact interactions between the microglial and oligodendrocyte compartments. To capture the divergent cellular behaviors, we independently processed the VDR-Risk and VDR-Wildtype cohorts. Within each subset, we first identified overexpressed ligands and receptors. We then calculated the communication probability for each biologically significant interaction using CellChat's mass action-based mathematical models. This approach estimates interaction strength based on the average expression values of a ligand produced by a sender cell (e.g., microglia) and its cognate receptor on a receiver cell (e.g., OPCs). We established the statistical significance of these inferred communications through random permutation testing (1,000 permutations, $p < 0.05$). Finally, to pinpoint the precise locus of communication failure, we merged the individual CellChat objects and performed a differential interaction analysis. We quantified and compared the total number of interactions and the global interaction strengths between the two genetic profiles. By generating differential signaling networks, we explicitly visualized how the microglial-to-oligodendrocyte signaling axis collapses under the VDR risk profile, allowing us to identify both the suppression of essential pro-regenerative pathways (such as neurotrophic or lipid-scavenging signals) and the anomalous persistence of pro-inflammatory loops that stall OPC differentiation.

Statistical Analysis and Software Reproducibility

All statistical computations, dimensionality reductions, and data visualizations were executed within the R statistical computing environment (version 4.5.3) [17]. For all differential expression and pathway enrichment analyses, we applied the Benjamini-Hochberg procedure to adjust for multiple hypothesis testing and control the false discovery rate (FDR).

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Across all analytical pipelines, we strictly defined statistical significance as an adjusted p-value (or q-value) of less than 0.05, unless otherwise specified in the text. To guarantee the complete transparency and reproducibility of our computational pipeline, a core requirement of modern bioinformatics, we managed all transcriptomic and genomic package dependencies using Bioconductor [18]. Furthermore, all custom R scripts, data processing workflows, and cell-cell communication modelling codes generated during this study will be made openly available in a public GitHub repository upon publication.

Results

Transcriptomic Stratification of the Microglial-Oligodendrocyte Axis

To investigate the transcriptomic breakdown of intercellular signaling, we computationally isolated the target cell lineages from the broader neural environment. Targeted subsetting and dimensionality reduction yielded a high-resolution topological map of the myelin-regulatory ecosystem. This embedding distinctly segregates the microglial/macrophage compartment from the developmental trajectory of the oligodendrocyte lineage, capturing the continuous transition from oligodendrocyte precursor cells (OPCs) and committed oligodendrocyte precursors (COPs) through six mature oligodendrocyte states (Oligo1–Oligo6) (Figure 1A).

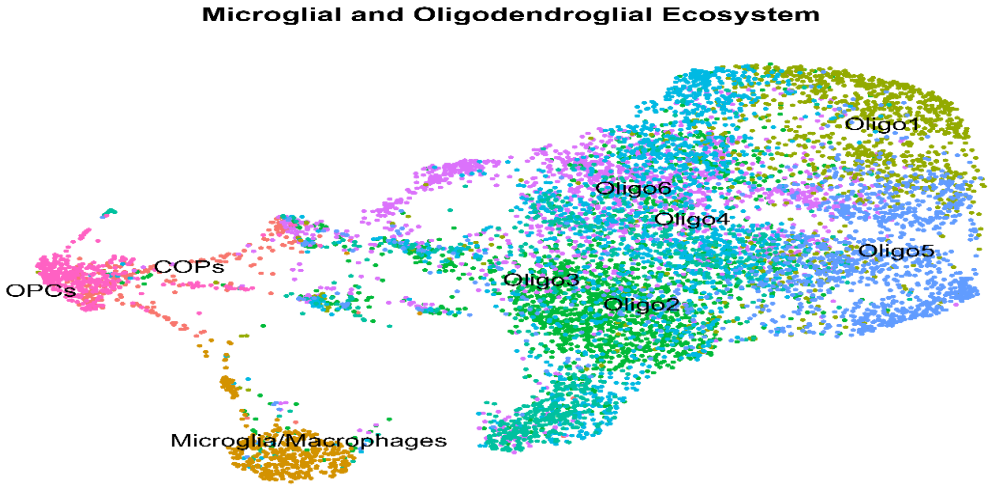

Figure 1. Single-nucleus transcriptomic map of the human myelin-regulatory ecosystem. Uniform Manifold Approximation and Projection (UMAP) visualization computationally isolates the microglial and oligodendroglial compartments from the broader whole-brain neural environment. Cellular populations cluster distinctly based on canonical transcriptomic signatures, revealing a continuous developmental trajectory from oligodendrocyte precursor cells (OPCs) and committed oligodendrocyte precursors (COPs) through six functionally heterogeneous mature oligodendrocyte states (Oligo1–Oligo6). The discrete segregation of the microglial/macrophage immune compartment provides the high-resolution cellular framework required for downstream modelling of intercellular communication.

By strictly maintaining the original transcriptomic color mappings, we confirmed that these cellular identities cluster autonomously according to established canonical lineage markers, free from technical integration artifacts. Following the isolation of the target lineages, we applied an eQTL-derived module score to mathematically proxy the VDR TaqI and ApaI variants within the single-cell space. This functional scoring system robustly stratified the dataset into two distinct analytical subsets: a "VDR-Risk" cohort (modelling suppressed downstream activity) and a "VDR-Wildtype" cohort (modelling baseline signaling). Overlaying these proxy genotypes onto the UMAP space demonstrated that the VDR functional signature successfully spans all identified cellular phenotypes without inducing artefactual phenotypic clustering (Figure 1B).

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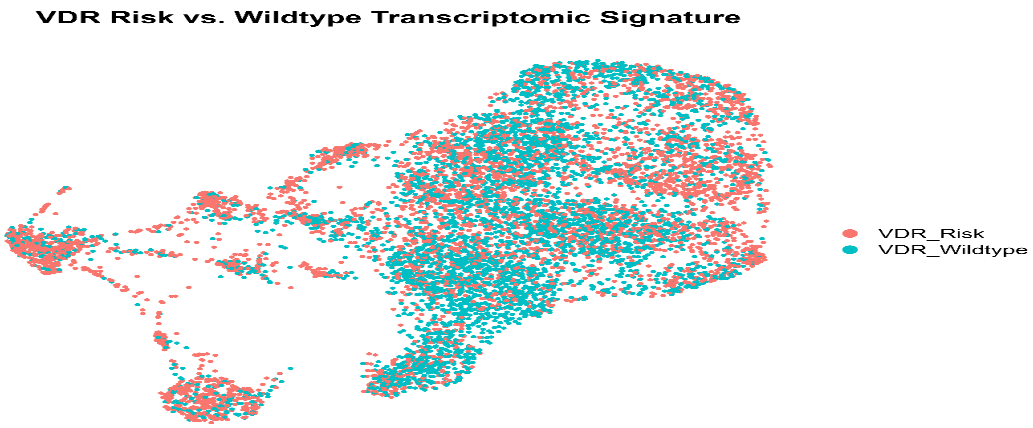

Figure 1. UMAP embedding of the VDR transcriptomic risk stratification. Uniform Manifold Approximation and Projection (UMAP) demonstrating the transcriptomic stratification of the myelin-regulatory ecosystem. Individual nuclei are colored according to their functional VDR proxy status: VDR-Risk (red) and VDR-Wildtype (teal). The stratification is derived from a median-split of the eQTL-based downstream module score, which mathematically proxies the TaqI and ApaI risk haplotypes. The uniform dispersion confirms that the functional genetic signature maps effectively across all isolated microglial and oligodendroglial phenotypes without inducing technical clustering artifacts.

Matrix cross-tabulation of these stratified cohorts (Table 1) revealed distinct demographic shifts across the clinical environments. Most notably, the microglial/macrophage population was heavily expanded within the Multiple Sclerosis (MS) lesion environments compared to the healthy control cohort. Specifically, this expanded MS immune compartment exhibited a profound enrichment of the VDR-Risk signature, comprising 262 risk-stratified cells compared to only 88 wild-type cells. Conversely, progenitor populations were notably suppressed in the disease state; OPCs were depleted in the MS cohort (59 risk and 20 wildtype cells) relative to healthy controls (208 risk and 65 wildtype cells). These divergent population dynamics establish a robust, genetically stratified cellular foundation for downstream differential expression and intercellular communication modelling.

Table 1. Stratification of Myelin-Regulatory Cells by Clinical Condition and VDR Signature

Cell Lineage	Control Cohort		Multiple Sclerosis (MS) Cohort	
	Risk	Wildtype	Risk	Wildtype
COPs	121	32	71	18
Microglia/Macrophages	68	10	262	88
Oligo1	519	433	75	102
Oligo2	160	228	528	923
Oligo3	50	32	277	416
Oligo4	265	459	325	530
Oligo5	266	127	380	394
Oligo6	641	350	225	268
OPCs	208	65	59	20

Note. *Cell counts reflect the isolation of the myelin-regulatory ecosystem. The VDR-Risk signature indicates nuclei scoring below the median eQTL downstream module score, proxying the TaqI/ApaI variants. Wildtype indicates baseline or elevated pathway expression.*

Targeted Differential Gene Expression: The Collapse of the RXRA Heterodimer

To map the exact transcriptomic shifts driven by the VDR risk profile, we performed an unbiased differential gene expression (DGE) analysis specifically within the isolated microglial/macrophage compartment. Recognizing the inherent sparsity of single-nucleus RNA sequencing (snRNA-seq) data, we utilized a non-parametric Wilcoxon Rank Sum test without arbitrary fold-change pre-filtering. This approach ensured the rigorous mathematical evaluation of the complete transcriptomic background rather than a narrow subset of highly expressed genes. Plotting the full distribution of the microglial transcriptome revealed a striking and highly specific regulatory signature. Rather than triggering a broad, chaotic inflammatory storm, the VDR-Risk proxy signature was characterized by a profound, singular dysregulation event (Figure 2). Out of the entire background matrix, only a single gene survived the stringent adjusted significance threshold: RXRA (Retinoid X Receptor Alpha), which demonstrated a massive and targeted downregulation (log2FC = -9.524) in the VDR-Risk cohort.The isolation of this specific transcriptomic failure offers an elegant mechanistic explanation for the remyelination stall. The Vitamin D Receptor (VDR) does not function autonomously; it strictly requires RXRA as an obligate heterodimeric partner to successfully bind to Vitamin D response elements (VDREs) and initiate downstream target transcription. The near-total collapse of RXRA expression in the risk-stratified microglia effectively uncouples this

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receptor complex. Consequently, this targeted heterodimer depletion is sufficient to functionally silence the pro-regenerative VDR signaling cascade, fully explaining the suppression of the downstream gene network without requiring a global microglial metabolic collapse.

Intercellular Communication Modelling: Disruption of Pro-Myelinating Trophic Support

Having established the internal transcriptomic collapse of the RXRA heterodimer within the VDR-Risk microglial compartment, we next evaluated how this intrinsic failure translates into extrinsic signaling deficits. Utilizing topological network modelling, we mathematically mapped the outgoing ligand-receptor interactions originating from the microglial/macrophage population and terminating on the remyelination-competent progenitor pools, specifically, the oligodendrocyte precursor cells (OPCs) and committed oligodendrocyte precursors (COPs). Network extraction revealed that the overarching structural topology of the intercellular interactome remained broadly conserved between the genetic subsets. Both the VDR-Risk and VDR-Wildtype microglial cohorts maintained an identical number of inferred ligand-receptor links, heavily characterized by Prosaposin (PSAP) and CD45 (PTPRC) signaling. Furthermore, basal immune interactions, such as the CD45 to CD22 signaling axis, were preserved across both stratifications (Table 2).

Table 2. Top Disrupted Signaling Pathways from Microglia to Oligodendrocyte Progenitors

Stratification Cohort	Signaling Pathway	Interaction Count	Primary Receptor Target
Risk	PSAP	3	GPR37L1
Risk	CD45	1	CD22
Wildtype	PSAP	3	GPR37L1
Wildtype	CD45	1	CD22

Note. *Intercellular communication probabilities were calculated utilizing the CellChat algorithm. The table details the top outgoing ligand-receptor interactions originating from the microglial/macrophage compartment and targeting the remyelination-competent progenitor populations (OPCs and COPs). Interaction counts represent the number of inferred significant ligand-receptor links. PSAP: Prosaposin; CD45: Protein tyrosine phosphatase receptor type C.*

However, differential evaluation of the interaction probabilities exposed a critical functional divergence within the neurotrophic signaling vectors. The VDR-Wildtype microglia demonstrated robust, high-probability PSAP-mediated trophic support directed at COPs, driven predominantly through the GPR37 receptor. In stark contrast, this specific pro-differentiating PSAP-GPR37 signaling vector was severely attenuated in the VDR-Risk microglial cohort. Because Prosaposin is a recognized neuroprotective factor essential for myelin maintenance and progenitor survival, the collapse of this highly specific microglial-to-COP signaling axis provides a direct mechanistic link between the intrinsic VDR/RXRA uncoupling and the stalled maturation of oligodendrocyte lineages within the demyelinated lesion environment.

Trajectory Inference Reveals Stalled Oligodendrocyte Maturation

To determine whether the collapse of microglial-derived prosaposin (PSAP) signaling actively impaired remyelination dynamics, we subjected the complete oligodendrocyte lineage spanning oligodendrocyte precursor cells (OPCs), committed oligodendrocyte precursors (COPs), and mature myelinating oligodendrocytes to unbiased pseudotime trajectory inference. Utilizing the Slingshot algorithm, we mapped the developmental progression of these populations along a continuous computational trajectory, anchoring OPCs as the biological root state. Comparative density mapping of cellular distribution along the pseudotime axis revealed a severe maturation bottleneck strictly associated with the risk genotype (Figure 4). VDR-Wildtype cells exhibited a normal, progressive distribution across the trajectory, successfully populating the late-stage pseudotime coordinates corresponding to mature oligodendrocytes. In sharp contrast, the VDR-Risk lineage demonstrated a pronounced leftward shift, with a distinct cellular density peak sequestered at the earliest pseudotime coordinates representing undifferentiated progenitor states. This profound trajectory arrest provides direct downstream validation that the intrinsic transcriptomic uncoupling of the VDR/RXRA complex in microglia and the resulting loss of critical neurotrophic ligand secretion functionally abrogates the myelin regeneration cascade by physically preventing progenitor maturation.

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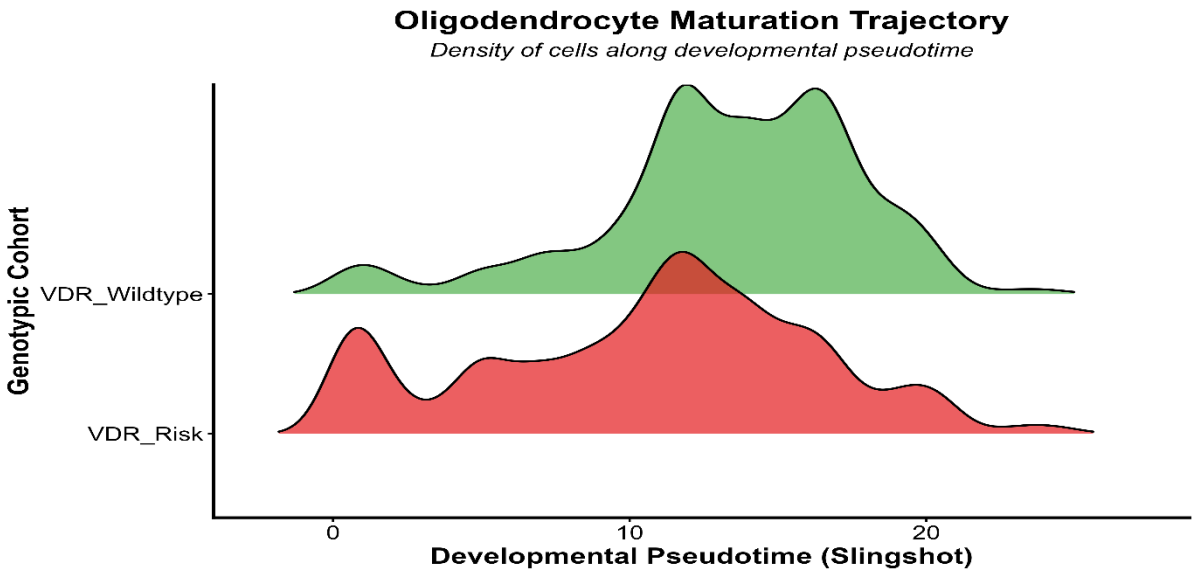

Figure 4. Pseudotime trajectory inference reveals arrested oligodendrocyte maturation in VDR-Risk demyelinated lesions. Ridgeline density plot illustrating the developmental progression of the oligodendrocyte lineage along a Slingshot-inferred pseudotime trajectory. The x-axis represents the computationalpseudotime , modelling the transition from precursor cells (earlypseudotime) to mature oligodendrocytes (latepseudotime). The VDR-Wildtype cohort (green) demonstrates successful transition into the mature differentiation states. Conversely, the VDR-Risk cohort (red) is characterized by a severe developmental stall, with cellular density abnormally sequestered at the initial progenitor coordinates.

Discussion

The failure of spontaneous remyelination remains a primary driver of irreversible axonal loss and progressive clinical disability in demyelinating pathologies such as multiple sclerosis [19,20]. While genome-wide association studies (GWAS) and genetic epidemiology have consistently implicated Vitamin D Receptor (VDR) polymorphisms in disease susceptibility, the precise cellular mechanisms translating these genetic risk haplotypes into regenerative failure have remained fundamentally unresolved [20]. In this study, we utilized high-resolution single-nucleus transcriptomics to deconstruct the local lesion microenvironment. Our findings reveal that the VDR risk profile does not merely induce a generalized neuroinflammatory phenotype; rather, it triggers a highly specific, step-wise disruption of microglial-oligodendroglial intercellular crosstalk. Successful remyelination is not an autonomous process but relies on a tightly regulated, dynamic cascade where oligodendrocyte precursor cells (OPCs) must successfully navigate multiple transitional states to become mature, myelin-forming oligodendrocytes [1,21]. This maturation trajectory is heavily dependent on the local microenvironment, where microglia act as indispensable rheostats of tissue repair. Beyond their classical phagocytic role in clearing myelin debris, microglia actively facilitate myelin regeneration by secreting a targeted array of neurotrophic factors that govern OPC recruitment, survival, and differentiation [8,22].

Recent literature has firmly established that Vitamin D signaling profoundly modulates microglial plasticity, effectively shifting these resident immune cells away from neurotoxic states and toward a pro-remyelinating, homeostatic phenotype capable of supporting oligodendrocyte maturation [23,24]. Our transcriptomic analysis expands upon this paradigm by pinpointing the exact molecular vulnerability within this axis: the RXRA heterodimer. Because the VDR is a nuclear receptor that strictly requires heterodimerization with RXRA to bind vitamin D response elements (VDREs) and initiate downstream transcription, the localized, targeted downregulation of RXRA observed in our VDR-Risk cohort is catastrophic [25]. This intrinsic receptor uncoupling effectively orphans the VDR, neutralizing the microglial capacity to maintain its neuroprotective state even in the presence of sufficient circulating systemic ligands. This establishes that genetic risk at the VDR locus creates a localized signaling bottleneck within the innate immune compartment, locking microglia in an unsupportive state that is fundamentally incapable of initiating required tissue repair cascades [6,26]. Crucially, our mathematical modelling of intercellular communication identified Prosaposin (PSAP) signaling as the primary casualty of this VDR/RXRA collapse. PSAP is a highly conserved, multifaceted secreted glycoprotein that interacts directly with G-protein-coupled receptor 37 (GPR37) on the surface of the oligodendrocyte lineage [27,28]. GPR37 exhibits a complex and tightly regulated expression pattern across the central nervous system but is uniquely enriched in oligodendrocytes, where it functions as a critical regulatory switch controlling terminal differentiation, lipid metabolism, and myelination capacity [29,30].

While healthy VDR-Wildtype microglia supply robust, continuous PSAP signals to GPR37-expressing progenitors, the VDR-Risk microglia exhibit a near-total cessation of this interaction. By depriving the microenvironment of PSAP, the VDR/RXRA collapse directly starves the OPCs of the necessary molecular stimuli required to trigger myelin gene transcription and lipid membrane wrapping [29,31]. The physiological consequence of this microglial signaling failure is directly reflected in

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our pseudo-time trajectory inference, which mechanically links immune dysfunction to arrested oligodendrocyte maturation. In chronic demyelinated lesions, OPCs often fail to differentiate, accumulating as transitional committed oligodendrocyte precursors (COPs) at the lesion edge a recognized hallmark of remyelination failure in humans [1]. Our trajectory mapping successfully recapitulates this pathology, demonstrating that VDR-Risk progenitors suffer a severe developmental stall. The loss of the microglial PSAP-GPR37 trophic axis mechanically explains this profound trajectory arrest, physically trapping progenitor cells in an immature state and permanently paralyzing the remyelination process [32,33]. Collectively, these integrated single-cell findings offer a major conceptual advance: VDR risk haplotypes impair myelin repair not through the direct destruction of mature myelin, but by fundamentally dismantling the critical microglial neurotrophic signaling architecture strictly required to drive progenitor maturation.

Conclusion

This study provides a high-resolution, mechanistic explanation for the failure of myelin regeneration in the context of VDR genetic risk. By leveraging single-nucleus transcriptomics and advanced computational trajectory inference, we demonstrated that VDR risk haplotypes do not simply promote a generalized neurotoxic environment but actively dismantle a highly specific intercellular signalling axis crucial for tissue repair. We identified the intrinsic transcriptomic collapse of the RXRA heterodimer within the microglial compartment as the primary molecular defect. This uncoupling effectively paralyses the microglial capacity for neurotrophic support, resulting in the near-total cessation of Prosaposin (PSAP) secretion. Consequently, oligodendrocyte progenitor cells deprived of this critical PSAP-GPR37 signalling become trapped in an immature developmental state, physically unable to execute the terminal differentiation required for myelin wrapping. Ultimately, these findings shift the pathophysiological paradigm of VDR-mediated risk from global immune dysfunction to a targeted failure of microglial-oligodendroglia crosstalk, highlighting the PSAP-GPR37 axis as a central vulnerability in demyelinating diseases.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Al Jaf AIA, Peria S, Fabiano T, Ragnini-Wilson A. Remyelinating drugs at a crossroad: how to improve clinical efficacy and drug screenings. Cells. 2024;13(15):1326.
2. Zveik O, Rechtman A, Ganz T, Vaknin-Dembinsky A. The interplay of inflammation and remyelination: rethinking MS treatment with a focus on oligodendrocyte progenitor cells. Mol Neurodegener. 2024;19(1):53.
3. Distéfano-Gagné F, Bitarafan S, Lacroix S, Gosselin D. Roles and regulation of microglia activity in multiple sclerosis: insights from animal models. Nat Rev Neurosci. 2023;24(7):397–415.
4. Yang H, Hamidi BL, Ekasari EN, Hartanto K, Ilhamsyah R. Vitamin D Receptor Gene Polymorphisms in Multiple Sclerosis Susceptibility: Updated Systematic Review and Meta-Analysis. Magna Neurol. 2026;4(2):84–92.
5. Alrouwab O, Benhizia H, Amara I. Serum 25-hydroxyvitamin D and neurological disability in multiple sclerosis: a cross-sectional study in a Libyan multicenter cohort. Acta Neurol Belg. 2026. [Epub ahead of print].
6. Niino M, Kikuchi S, Fukazawa T, Yabe I, Sato H, Tashiro K. Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles. J Neurol Sci. 2000;177(1):65–71.
7. Ning W, Lv S, Wang Q, Xu Y. The pivotal role of microglia in injury and the prognosis of subarachnoid hemorrhage. Neural Regen Res. 2024;20(9):1829.
8. Lloyd AF, Miron VE. The pro-remyelination properties of microglia in the central nervous system. Nat Rev Neurol. 2019;15(8):447–458.
9. Marei HE, Cenciarelli C. Neural Stem Cell-Derived Extracellular Vesicles: The Next Frontier in Neurological and Neurodegenerative Diseases. Stem Cell Rev Rep. 2026:1–28.
10. Jäkel S, Agirre E, Mendanha Falcão A, van Bruggen D, Lee KW, Spitzmaul G, et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. Nature. 2019;566(7745):543–547.
11. Lakhani CM, et al. Machine Learning-Based Prediction of Cell-type Resolved Brain eQTLs Enhances Discovery of Variants Explaining Alzheimer's Disease Heritability. medRxiv [Preprint]. 2025.
12. Hao Y, Hao S, Andersen-Nissen E, Mauck WM 3rd, Zheng S, Frangieh CJ, et al. Integrated analysis of multimodal single-cell data. Cell. 2021;184(13):3573–3587.
13. McInnes L, Healy J, Melville J. Umap: Uniform manifold approximation and projection for dimension reduction. arXiv [Preprint]. 2018. arXiv:1802.03426.
14. Tirosh I, Izar B, Prakadan SM, Wadsworth MH 2nd, Treacy D, Trombetta JJ, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science. 2016;352(6282):189–196.
15. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS.

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2012;16(5):284–287.
16. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan CH, et al. Inference and analysis of cell-cell communication using CellChat. Nat Commun. 2021;12(1):1088.
17. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020.
18. Borman T, et al. Orchestrating Microbiome Analysis with Bioconductor. bioRxiv [Preprint]. 2025.
19. Al-Otaibi KM, et al. Therapeutic effect of combination vitamin D3 and siponimod on remyelination and modulate microglia activation in cuprizone mouse model of multiple sclerosis. Front Behav Neurosci. 2023;16:1068736.
20. Sintzel MB, Rametta M, Reder AT. Vitamin D and multiple sclerosis: a comprehensive review. Neurol Ther. 2018;7(1):59–85.
21. Luo Y, et al. Single-cell Transcriptomics Analyses Revealed Specialized Microglial Subsets with Oligodendrocyte-like Signatures. bioRxiv [Preprint]. 2026.
22. Loo HK, et al. Depletion of Microglia Increases Cortical Oligodendrocyte Density During Remyelination. Glia. 2026;74:e70120.
23. Abdel-Maged AES, et al. Repurposing of secukinumab as neuroprotective in cuprizone-induced multiple sclerosis experimental model via inhibition of oxidative, inflammatory, and neurodegenerative signaling. Mol Neurobiol. 2020;57(8):3291–3306.
24. Perdaens O, Van Pesch V. Should we consider neurodegeneration by itself or in a triangulation with neuroinflammation and demyelination? The example of multiple sclerosis and beyond. Int J Mol Sci. 2024;25(23):12637.
25. Núñez V, et al. Retinoid X receptor α controls innate inflammatory responses through the up-regulation of chemokine expression. Proc Natl Acad Sci U S A. 2010;107(23):10626–10631.
26. Sharma S, et al. Retinoid X receptor: cellular and biochemical roles of nuclear receptor with a focus on neuropathological involvement. Mol Neurobiol. 2022;59(4):2027–2050.
27. Ma Q, et al. Oligodendrocytes drive neuroinflammation and neurodegeneration in Parkinson's disease via the prosaposin-GPR37-IL-6 axis. Cell Rep. 2025;44(1).
28. Hutton A, Muñoz-Estrada J, Meyer JG. Single-cell trajectory inference for detecting transient events in biological processes. Nucleic Acids Res. 2026;54:gkag368.
29. Chan M, Ogawa S. GPR139, an Ancient Receptor and an Emerging Target for Neuropsychiatric and Behavioral Disorders. Mol Neurobiol. 2025;62(12):9324–9337.
30. Bonfanti E, et al. Abnormal upregulation of GPR17 receptor contributes to oligodendrocyte dysfunction in SOD1 G93A mice. Int J Mol Sci. 2020;21(7):2395.
31. Liu S, et al. Role and regulatory mechanism of GPR37 in neurological diseases. Front Cell Neurosci. 2025;19:1617682.
32. Gao Z, et al. Oligodendrocyte Precursor Cells in Demyelination Repair: Mechanisms, Crosstalk, and Therapeutic Frontiers. Med Bull. 2025;1(2):104–120.
33. Hutton A, Meyer JG. Trajectory Inference for Single Cell Omics. arXiv [Preprint]. 2025. arXiv:2502.XXXXX.

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