GPCRomics of Homeostatic and Disease-Associated Human Microglia

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G-protein-coupled receptors (GPCRs) are critical sensors affecting the state of eukaryotic cells. To get systematic insight into the GPCRome of microglia, we analyzed publicly available RNA-sequencing data of bulk and single cells obtained from human and mouse brains. We identified 17 rhodopsin and adhesion family GPCRs robustly expressed in microglia from human brains, including the homeostasis-associated genes CX3CR1, GPR34, GPR183, P2RY12, P2RY13, and ADGRG1. Expression of these microglial core genes was lost upon culture of isolated cells *ex vivo* but could be acquired by human induced pluripotent stem cell (iPSC)-derived microglial precursors transplanted into mouse brains. CXCR4 and PTGER4 were higher expressed in subcortical white matter compared to cortical grey matter microglia, and ADGRG1 was downregulated in microglia obtained from normal-appearing white and grey matter tissue of multiple sclerosis (MS) brains. Single-cell RNA sequencing of microglia from active lesions, obtained early during MS, revealed downregulation of homeostasis-associated GPCR genes and upregulation of CXCR4 expression in a small subset of MS-associated lesional microglia. Functional presence of low levels of CXCR4 on human microglia was confirmed using flow cytometry and transwell migration towards SDF-1. Microglia abundantly expressed the GPCR down-stream signaling mediator genes GNAI2 (α_{i2}), GNAS (α_s), and GNA13 (α_{13}), the latter particularly in white matter. Drugs against several microglia GPCRs are available to target microglia in brain diseases. In conclusion, transcriptome profiling allowed us to identify expression of GPCRs that may contribute to brain (patho)physiology and have diagnostic and therapeutic potential in human microglia.

Keywords: brain, microglia, GPCRs, G proteins, system biology, multiple sclerosis, CXCR4

INTRODUCTION

Microglia are brain-resident phagocytic cells that contribute to brain homeostasis as well as disease (1, 2). Populating the central nervous system (CNS) during embryonic development, microglia persist for the rest of life through local self-renewal. As a consequence, they possess a unique transcriptional signature that emerged only recently from RNA sequencing (RNAseq) of purified primary cells (3). Notably, G protein-coupled receptors (GPCRs) figure prominently in the microglia transcriptome, as exemplified by the characteristic surface expression of CX3CR1, GPR34, GPR183, P2Y$_{12}$, P2Y$_{13}$, and GPR56 (3, 4). GPCRs are the senses of our cells, comprising the largest and most diverse superfamily of membrane proteins in eukaryotic cells. Of particular interest is their widespread cellular distribution and the fact that ~30% of all currently approved pharmaceuticals target them (5). GPCRs control cell and tissue physiology by regulating signaling pathways *via* heterotrimeric G proteins, which modulate cellular levels of second messengers and, in turn, a wide array of functional activities in all types of cells (6). Microglial GPCRs have been implicated in control of axon outgrowth and cortical laminar positioning during development as well as in support of survival of neurons (CX3CR1), in plasticity (P2Y$_{12}$) and complement-mediated pruning (C3AR1 and C5AR1) of synapses, in microglial brain colonization (CXCR4) and chemotaxis of microglia to injury (P2Y$_{12}$), and in neuropathic pain response (P2Y$_{12}$) (7). Exploring bulk and single cell RNAseq studies of microglia from mice and human, we here describe the expression of GPCR and G protein genes in relation to microglia homeostasis, location, health, and disease.

METHODS

RNAseq and Microarray Datasets

Genome-wide gene expression data of microglia, macrophages, and non-phagocytic cells were derived from various publicly available RNAseq data sets (**Table 1**). Numbers indicating gene expression are provided either as absolute counts, presenting fragments per kilobase of transcript per million mapped reads (FPKM) or transcripts per kilobase million (TPM), or as relative counts, related to arbitrary chosen, fixed thresholds of all genes in the gene set (e.g., the top 50% = 0.50 percentile). Heatmaps show gene expression intensity, based on the average of all genes, with white indicating low expression and red indicating high expression. *t*-distributed stochastic neighbor embedding (*t*-SNE) plots of the clusters of microglia were generated as described (15). Relative RNA expression levels for *CXCR4* and *CXCL12* from laser-dissected tissue from mixed active/inactive and inactive demyelinated lesions were obtained from a microarray dataset (18).

Flow Cytometry

Isolated human microglia from corpus callosum and subcortical white matter and occipital cortex grey matter were isolated and stained as described (4) with APC-conjugated anti-CD11b (clone ICRF44; eBioscience, San Diego, CA, USA), Alexa 700-conjugated anti-CD14 (clone MφP9; BD Biosciences, San Diego, CA, USA), PerCP-Cy5.5-conjugated anti-CD15 (clone HI98; BioLegend, San Diego, CA, USA), BB515-conjugated anti-CD45 (clone HI30; BioLegend), PE-Cy7-conjugated anti-CXCR4 (clone 12G5; BioLegend), and PE-conjugated anti-P2Y$_{12}$ (clone S16007D; BioLegend). Dead cells were visualized by fixable viability dye eFluor 780 (eBioscience). Background staining was determined using fluorescence minus one control. Membrane protein expression was measured on a FACSCanto II (BD Biosciences), and median fluorescence intensity was determined with FlowJo software version 10.1 (Ashland, OR, USA).

Transwell Migration

Human brain microglia and paired peripheral blood monocytes were separately isolated by CD11b and CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) as described (19). 2 x 10^5 cells were loaded in a volume of 100 μl RPMI1640 medium containing 0.3% bovine serum albumin on transwell filters with a pore size of 5 μm (Corning, Corning, NY, USA).

TABLE 1 | RNAseq datasets analyzed in this study.

Species	Cells	Dataset	Reference
various	microglia	bulk RNAseq of isolated cells from cortex (GM) (human) or whole brains (mouse)	Geirsdottir et al. (8)
human	brain lysate, microglia	bulk RNAseq of isolated cells from cortex (GM)	Galatro et al. (9)
human	microglia	bulk RNAseq of isolated cells from cortex (GM)	Gosselin et al. (10)
human	microglia	bulk RNAseq of isolated cells from corpus callosum (WM) and occipital cortex (GM) of control and MS donors	Van der Poel et al. (4)
human	microglia	bulk RNAseq of isolated cells from corpus callosum (WM) and occipital cortex (GM) of control donors	Mizee et al. (unpublished data)
mouse	various	bulk RNAseq of (isolated cells from) whole brains (neurons, oligodendrocytes, astrocytes, and microglia)	Zhang et al. (11)
mouse	microglia, macrophages	bulk RNAseq of isolated cells from whole organs	Van Hove et al. (12)
human	iPSC-derived iMPs	bulk RNAseq of isolated cells transplanted into the brain of neonatal mice in vivo or cultured in vitro	Svoboda et al. (13)
mouse	microglia	single cell RNAseq of cells from brains of 5XFAD mice	Keren-Shaul et al. (14)
human	microglia	single cell RNAseq of cells from cortical (GM) biopsies of active MS lesions	Masuda et al. (15)
human	microglia	single cell RNAseq of cells from cortical (GM) surgically resected brain tissue	Olah et al. (16)
human	microglia	bulk RNAseq of isolated cells from cerebral cortex (GM)	Olah et al. (17)

WM, white matter; GM, grey matter.

100 ng/ml SDF-1 (stromal cell-derived factor-1, CXCL12) was added as chemoattractant to the lower compartment. After 4 h incubation at 37°C, cells in the lower compartment were harvested and quantified by flow cytometry at a fixed high speed for 120 sec.

Statistics

All analyses were performed in GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). When data were not normally distributed, non-parametric tests, either Wilcoxon or Mann-Whitney U, were performed.

RESULTS

Species and Cell Type Specificity of GPCR and GPCR Signaling Molecule Expression in Microglia

To explore the presence of GPCRs in microglia, we utilized the list of GPCRs not involved in olfaction, taste, light perception, and pheromone signaling as provided by the International Union of Basic and Clinical Pharmacology (IUPHAR)/British Pharmacological Society (BPS) Guide to Pharmacology (https://www.guidetopharmacology.org) (20). According to the GRAFS classification (21), the 384 receptors comprise 303 rhodopsin, 33 adhesion, 22 glutamate, 15 secretin, and 11 frizzled family members.

To identify GPCR genes that are reliably expressed in human microglia, we first tested bulk RNAseq expression data of homeostatic microglia from different vertebrate species we recently published (8). We found 83 GPCR genes in the top 50% (0.5 percentile) of all genes in the human and/or mouse gene sets (**Figure 1A**). 42 of these genes were lowly expressed (0.5–0.67 percentile), 12 genes were medium expressed (0.68–0.85 percentile), and 29 genes belonged to the highly expressed genes (0.86–1.0 percentile). While the homeostatic microglia marker genes CX3CR1, GPR34, GPR183, P2RY12, P2RY13, and ADGRG1 (encoding GPR56) were highly expressed in both species, expression of other GPCR genes was medium, low, or even absent in either human or mouse. For example, transcription of ADGRE1, encoding F4/80 in mouse, was lacking in human microglia, in line with its exclusive expression in human eosinophils (22). Other genes with a restricted, high expression in either mouse or human microglia were CCR6, GPR84, GPR146, and FPR1, respectively. Notably, ADGRE5 (encoding CD97), which is abundantly expressed by all types of bone marrow-derived leukocytes (23) was lowly expressed in mouse and human microglia, in line with previous findings (24). The previously disputed gene ADGRB1 (encoding BAI1) (25) was undetectable in mouse and human microglia.

We confirmed the list of well-expressed human microglia GPCR genes in four other bulk RNAseq studies of primary human microglia [(4, 9, 10) and Mizee et al, unpublished data]. We found 15 genes abundantly expressed across the different data sets, which formed, together with two genes with a medium expression in grey matter microglia but high expression in white matter microglia (see below), the core microglia GPCR gene set

for our further analyses (for all other genes, see the supplementary information). This gene set comprises rhodopsin (e.g., adenosine, chemokine, complement peptide, lysophospholipid, purinergic, and orphan receptors) as well as adhesion, but not secretin, glutamate, or frizzled family members. Among the 17 selected genes were the homeostatic microglia marker genes CX3CR1, GPR34, GPR183, P2RY12, P2RY13, and ADGRG1.

We next tested the expression of microglial GPCRs in other cell types of the CNS. The three most highly expressed genes, ADGRG1, P2RY12, and CX3CR1, also appeared top-abundant in whole brain tissue (10) (**Figure 1B**). All selected genes were more abundantly expressed in pure microglia as compared to the whole human cortex, which was further corroborated by data on gene expression in the major cell types of the mouse CNS (11) (**Figure 1C** and **Supplementary Table 1**). Except for Cxcr4, expression in microglia was higher as compared to neurons, astrocytes, and oligodendrocytes. Interestingly, high expression in microglia regularly correlated with gene activity in oligodendrocytes, albeit at a lower level.
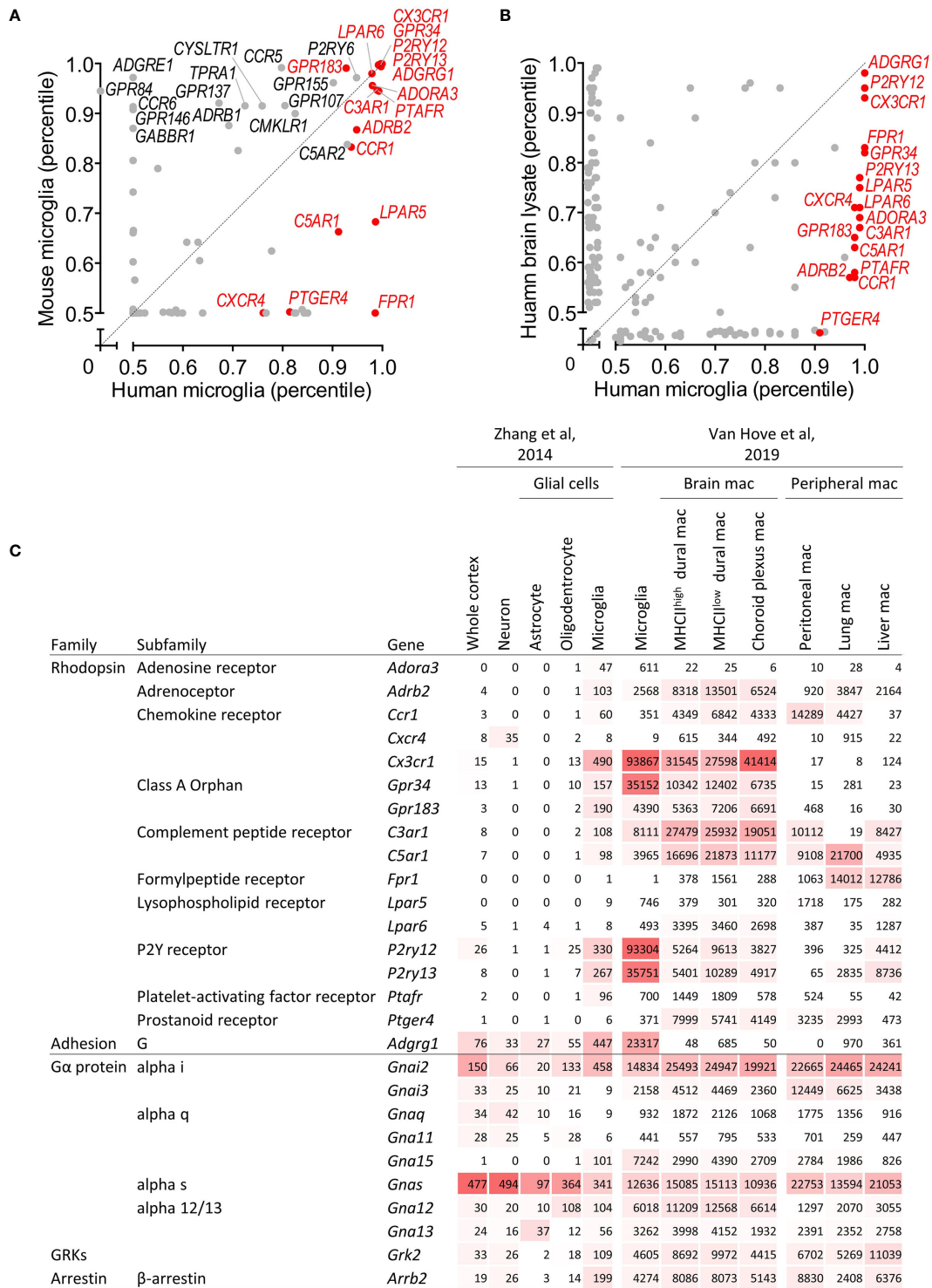
Next to parenchymal microglia, the CNS harbors border-associated macrophages with distinct transcriptional signatures, residing in the dura mater, subdural meninges, and choroid plexus (12). Most microglia GPCRs were expressed also in non-parenchymal macrophages of the CNS, at comparable, higher, or lower level (**Figure 1C** and **Supplementary Table 1**). Inclusion of tissue-resident macrophages from peritoneum, lung, and liver unveiled similar patterns (12). The signature genes Cx3cr1, Gpr34, P2ry12, P2ry13, and Adgrg1 were particularly expressed in microglia.

Upon ligation, GPCRs diversify downstream signaling through four main classes of Gα subunit – Gα$_s$, Gα$_{i/o}$, Gα$_{q/11}$, and Gα$_{12/13}$ (6). Moreover, G protein-coupled receptor kinases (GRKs) phosphorylate intracellular domains of GPCRs and function together with β-arrestin to regulate the GPCR desensitization (6). Human and mouse microglia expressed GNAI2 (Gα$_{i2}$), GNAS (Gα$_s$), GNA13 (Gα$_{13}$), GRK2, and ARRB2. In addition, mouse microglia also expressed Gna15 (Gα$_{15}$) (**Figure 1C** and **Supplementary Table 1**). Gnai2, Gna15, Grk2, and Arrb2 were higher expressed in microglia as compared to neurons, astrocytes, and oligodendrocytes, while Gnas and Gna12 were expressed in different cell types. G protein, GRK, and β-arrestin gene expression levels in macrophages in- and out-side the CNS were mostly comparable.

Regional and Disease Specificity of GPCR and GPCR Signaling Molecule Expression in Human Microglia

Dissected from their natural microenvironment, microglia change their transcriptional program and alter morphological and functional characteristics (10, 19). Indeed, expression of ADORA3, ADRB2, CXCR4, CX3CR1, GPR34, LPAR5, LPAR6, P2RY12, P2RY13, and ADGRG1 was strongly downregulated or even lost in microglia cultured ex vivo for 1 or 7 days (**Figure 2** and **Supplementary Table 2**).

Human induced pluripotent stem cells (iPSCs) can be differentiated into induced microglial precursors cells (iMPs)

Family	Subfamily	Gene	Whole cortex	Neuron	Astrocyte	Oligodentrocyte	Microglia	Microglia	MHCII[high] dural mac	MHCII[low] dural mac	Choroid plexus mac	Peritoneal mac	Lung mac	Liver mac
			Zhang et al, 2014 — Glial cells					Van Hove et al, 2019 — Brain mac				Peripheral mac		
Rhodopsin	Adenosine receptor	Adora3	0	0	0	1	47	611	22	25	6	10	28	4
	Adrenoceptor	Adrb2	4	0	0	1	103	2568	8318	13501	6524	920	3847	2164
	Chemokine receptor	Ccr1	3	0	0	1	60	351	4349	6842	4333	14289	4427	37
		Cxcr4	8	35	0	2	8	9	615	344	492	10	915	22
		Cx3cr1	15	1	0	13	490	93867	31545	27598	41414	17	8	124
	Class A Orphan	Gpr34	13	1	0	10	157	35152	10342	12402	6735	15	281	23
		Gpr183	3	0	0	2	190	4390	5363	7206	6691	468	16	30
	Complement peptide receptor	C3ar1	8	0	0	2	108	8111	27479	25932	19051	10112	19	8427
		C5ar1	7	0	0	1	98	3965	16696	21873	11177	9108	21700	4935
	Formylpeptide receptor	Fpr1	0	0	0	0	1	1	378	1561	288	1063	14012	12786
	Lysophospholipid receptor	Lpar5	0	0	0	0	9	746	379	301	320	1718	175	282
		Lpar6	5	1	4	1	8	493	3395	3460	2698	387	35	1287
	P2Y receptor	P2ry12	26	1	1	25	330	93304	5264	9613	3827	396	325	4412
		P2ry13	8	0	1	7	267	35751	5401	10289	4917	65	2835	8736
	Platelet-activating factor receptor	Ptafr	2	0	0	1	96	700	1449	1809	578	524	55	42
	Prostanoid receptor	Ptger4	1	0	1	0	6	371	7999	5741	4149	3235	2993	473
Adhesion	G	Adgrg1	76	33	27	55	447	23317	48	685	50	0	970	361
Gα protein	alpha i	Gnai2	150	66	20	133	458	14834	25493	24947	19921	22665	24465	24241
		Gnai3	33	25	10	21	9	2158	4512	4469	2360	12449	6625	3438
	alpha q	Gnaq	34	42	10	16	9	932	1872	2126	1068	1775	1356	916
		Gna11	28	25	5	28	6	441	557	795	533	701	259	447
		Gna15	1	0	0	1	101	7242	2990	4390	2709	2784	1986	826
	alpha s	Gnas	477	494	97	364	341	12636	15085	15113	10936	22753	13594	21053
	alpha 12/13	Gna12	30	20	10	108	104	6018	11209	12568	6614	1297	2070	3055
		Gna13	24	16	37	12	56	3262	3998	4152	1932	2391	2352	2758
GRKs		Grk2	33	26	2	18	109	4605	8692	9972	4415	6702	5269	11039
Arrestin	β-arrestin	Arrb2	19	26	3	14	199	4274	8086	8073	5143	8830	2408	6376

FIGURE 1 | Identification of GPCR and GPCR signaling molecule genes expressed in microglia. **(A)** Scatter plot providing GPCR gene expression in microglia isolated from human and mice as percentile of all human/mouse genes (8). **(B)** Scatter plot providing GPCR gene expression in brain and microglia of human as percentile of all genes (10). **(C)** Expression of GPCR and GPCR signaling molecule genes in mouse cortical tissue, neurons, astrocytes, oligodendrocytes, and microglia (11), and in mouse microglia, non-parenchymal brain and peripheral tissue macrophages (12). Expression is provided as FPKM (11) and gene count (12), respectively. mac, macrophages.

showing the characteristic morphology and gene expression of primary human microglia (13). Notably, iMPs expressed the GPCRs typically found in microglia. When transplanted into the brains of NOD scid gamma (NSG) mice, carrying the human transgenes encoding IL-3, SCF, GM-CSF, and CSF1, expression of *ADRB2*, *CX3CR1*, *GPR183*, *FPR1*, *LPAR5*, *LPAR6*, *P2RY12*, *P2RY13*, and *ADGRG1* was further enhanced at day 10 and/or 60 (**Figure 2** and **Supplementary Table 2**). This induction was not seen in iMPs cultured for the same period *in vitro*, which rather resulted in a downregulation of the expression of several GPCRs.

Various studies have established regional differences in microglia gene expression (2). Using data from our laboratory, we compared GPCR expression between human microglia obtained from subcortical white and cortical grey matter (4) (**Figure 2** and **Supplementary Table 2**). We found seven genes ≥2-fold higher expressed in either white or grey matter, respectively. In a second, independent dataset, we could confirm a higher gene expression in white matter microglia for *CXCR4* and *PTGER4* (Mizee et al, unpublished data). Single cell RNAseq has facilitated the investigation of microglial heterogeneity within brain regions. A study addressing this question showed,

with the exception of *C3AR1*, a similar activity of GPCR genes in cortical microglia subsets (16) (**Supplementary Figure 1**).

Changes in gene expression in mouse models for Alzheimer's disease and amyotrophic lateral sclerosis (ALS) have led to the description of a phenotype referred to as disease-associated microglia, associated with significantly reduced expression of several signature GPCR genes (14). When comparing gene expression in pure microglia from normal-appearing, non-lesional tissue of deceased multiple sclerosis (MS) autopsy cases and tissue of non-pathological brains (**Figure 2** and **Supplementary Table 2**), we found downregulation of *ADGRG1* in both, white and grey matter, while expression of other GPCR genes was not changed.

Single cell RNAseq data of (models of) Alzheimer's disease, ALS, and MS revealed that pathological reprogramming only occurs in small subsets of disease-associated microglia that coexist with large subsets of homeostatic microglia and small subsets of infiltrating monocytes (14–16). Notably, disease-associated microglia in lesion biopsies of patients with histologically confirmed early active MS pathology showed reduced expression of the core signature genes *CX3CR1*, *GPR34*, *GPR183*, *P2RY12*, *P2RY13*, and *ADGRG1* (15, 26) (**Figures 3A, B**).

Family	Subfamily	Gene	Gosselin et al, 2017 Microglia 0 d	1 d	7 d	Svoboda et al, 2019 iMPs	in vivo 10 d	60 d	in vitro 10 d	60 d	Van der Poel et al, 2019 WM CON	MS	GM CON	MS
Rhodopsin	Adenosine receptor	ADORA3	162	11	46	22265	4864	23998	30578	20433	4359	3640	4780	4124
	Adrenoceptor	ADRB2	86	2	2	2274	1381	8628	1495	673	5246	4813	2837	2975
	Chemokine receptor	CCR1	125	92	188	11746	4952	14665	14118	7546	2840	2712	5234	3877
		CXCR4	111	21	8	2141	90	293	8542	1841	15831	17984	1449	5984
		CX3CR1	920	4	2	5008	74358	346492	146	2243	9175	12011	19974	22293
	Class A Orphan	GPR34	542	26	57	44970	32383	106441	47660	37619	5318	5089	6863	6563
		GPR183	139	44	84	3199	5383	19006	1018	1841	14883	13521	2913	4397
	Complement peptide receptor	C3AR1	222	86	242	15484	9734	18585	24888	25134	6653	5580	6517	6158
		C5AR1	108	346	360	3273	483	1840	5131	7335	7527	8141	2089	2427
	Formylpeptide receptor	FPR1	394	65	323	8478	2127	27248	7967	2325	3730	2117	8204	2937
	Lysophospholipid receptor	LPAR5	202	21	42	5096	5311	21826	4454	3400	2662	2591	4430	3798
		LPAR6	232	38	66	11368	9222	19940	3599	3276	9451	8748	15106	18231
	P2Y receptor	P2RY12	1050	1	3	4949	22464	219754	704	184	8274	9272	23102	20329
		P2RY13	253	1	5	2487	8282	40696	735	756	5488	4518	5786	6253
	Platelet-activating factor receptor	PTAFR	116	116	37	14233	11427	49899	20366	11692	4132	4410	4404	4745
	Prostanoid receptor	PTGER4	34	39	37	311	121	276	1583	1144	6769	7984	783	1628
Adhesion	G	ADGRG1	477	7	10	9485	11692	43760	4	17	12277	5267	20512	10089
Gα protein	alpha i	GNAI2	285	410	423	39251	23304	29388	31371	18922	7050	7402	6785	7064
		GNAI3	21	22	25	8743	5210	5650	6868	6046	1969	2081	1419	1404
	alpha q	GNAQ	32	21	18	1142	253	599	573	452	2680	2808	2937	2992
		GNA11	7	7	13	126	47	90	137	117	479	407	381	615
		GNA15	36	63	52	3761	2210	3504	3203	3741	1385	1691	915	776
	alpha s	GNAS	426	413	472	6137	2921	5578	6861	5762	13587	15412	11851	14254
	alpha 12/13	GNA12	18	81	97	248	114	193	638	1062	1494	2388	751	1124
		GNA13	64	41	37	3689	1738	3542	3777	2180	12252	20970	3007	3684
GRKs		GRK2	183	88	61	1020	464	981	549	522	6982	6244	7575	7469
Arrestin	β-arrestin	ARRB2	324	255	197	7238	2364	4697	5784	4465	4696	5024	5987	5873

FIGURE 2 | Gene expression of selected GPCRs and GPCR signaling molecules in human microglia. Expression of GPCR and GPCR signaling molecule genes in microglia cultured *ex vivo* for 1 and 7 days (10), in iPSC-derived iMPs transplanted neonatal mouse brains or cultured *in vitro* for 10 and 60 days (13), and in white matter and grey matter microglia from control and MS brains (4). Expression is provided as TPM (10) and gene count (4, 13), respectively. CON, control; WM, white matter; GM, grey matter.

In contrast, both disease-associated microglia and infiltrating monocytes had upregulated expression of *CXCR4*. In disease-associated microglia in surgically resected material of Alzheimer's disease patients, GPCR expression was not altered (16).
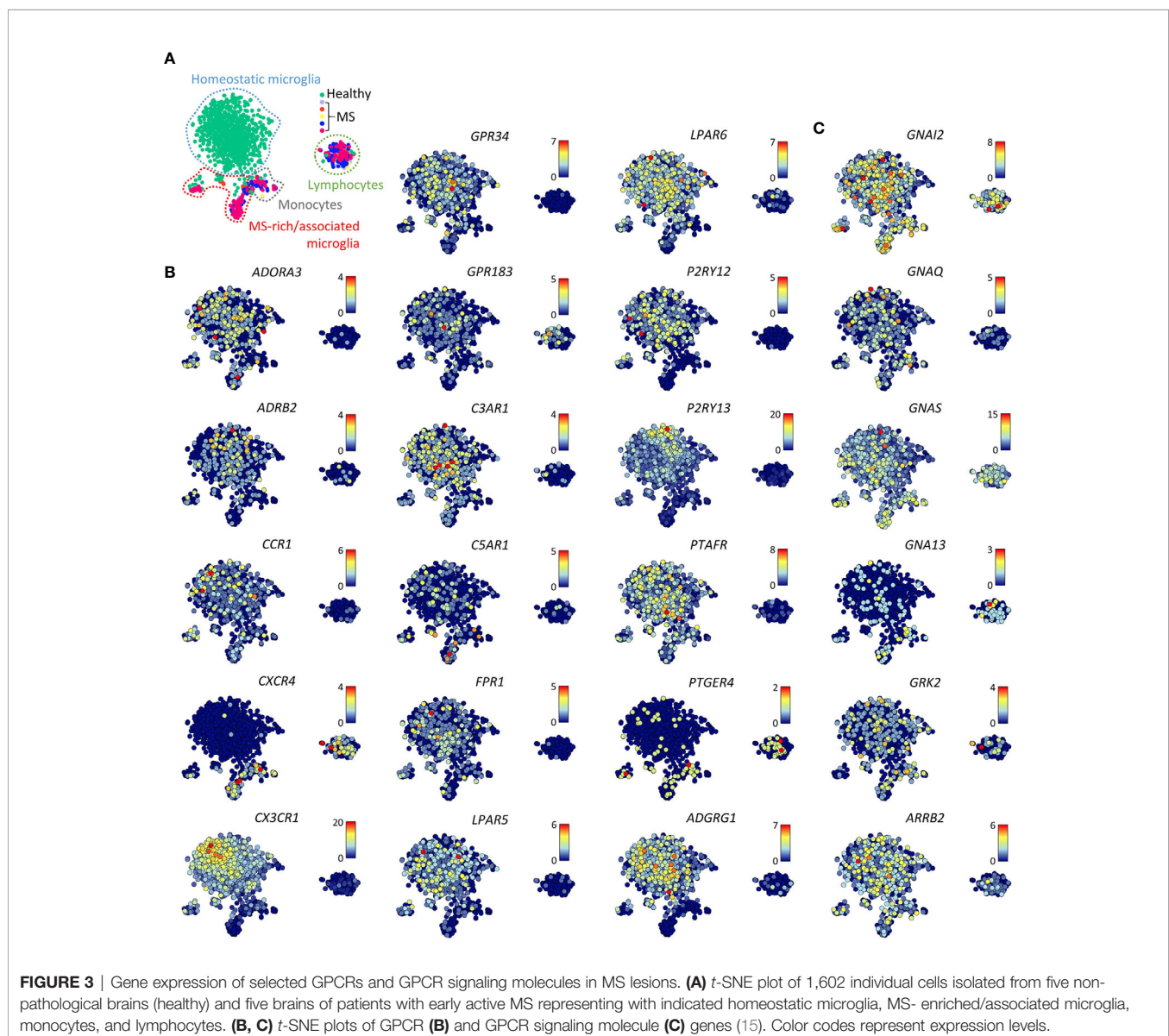
The late onset of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and ALS, has triggered interest in the effect of aging on microglia gene expression. A study of aged microglia found a lower expression of the microglia signature genes *GPR183*, *P2RY12*, and *P2RY13* (17).

Expression of GPCR signaling molecule genes in the data sets studied here was quite stable. Separation of microglia from their CNS microenvironment or transfer of iMPs into NSG mouse brains only moderately affected signaling molecule gene expression (**Figure 2**). However, white matter microglia more abundantly expressed *GNA13* as compared to grey mater microglia. GPCR signaling molecule genes expression was not altered in normal-appearing or lesional MS microglia from either white or grey matter (**Figure 3C**).

Expression and MS-Associated Upregulation of CXCR4 by Human White Matter Microglia

The presence and MS-associated upregulation of *CXCR4* expression in microglia is of particular interest since Werner et al. recently showed that CXCR4 distinguishes monocytes from microglia in mice (27). To test whether human microglia express CXCR4, we analyzed freshly isolated cells by flow cytometry, shown here for three donors with MS (**Figure 4A**). Expression was detectable, albeit levels were moderate on white matter microglia and low on grey matter microglia (**Figures 4A, B**). On lesional microglia, we noticed a slightly higher expression of CXCR4 as well as lower expression of P2Y$_{12}$ compared to

FIGURE 3 | Gene expression of selected GPCRs and GPCR signaling molecules in MS lesions. **(A)** *t*-SNE plot of 1,602 individual cells isolated from five non-pathological brains (healthy) and five brains of patients with early active MS representing with indicated homeostatic microglia, MS- enriched/associated microglia, monocytes, and lymphocytes. **(B, C)** *t*-SNE plots of GPCR **(B)** and GPCR signaling molecule **(C)** genes (15). Color codes represent expression levels.

microglia from subcortical normal-appearing white matter, the latter in line with Zrzavy et al. (26) (**Figures 4A, C**). Whole tissue gene expression microarray analysis of laser-dissected control white matter and white matter MS lesions (18) further confirmed increased expression of *CXCR4* in the rim of mixed active/inactive but not inactive lesions (**Figure 4D**).

To test whether expression of CXCR4 on microglia is functional, we studied transwell migration in response to SDF-1 (CXCL12) (14). SDF-1 binds to CXCR4 and CXCR7, the latter however is not expressed by microglia (**Supplementary Table 2**). Of note SDF-1 stimulated chemotaxis of monocytes, white matter and grey matter microglia at levels corresponding with the presence of CXCR4 (**Figure 4E**). We conclude that CXCR4 expression on human microglia is functional.

DISCUSSION

GPCRs constitute an important share of the sensome of eukaryotic cells. By analyzing various RNAseq datasets, we here provide a comprehensive overview of their presence in microglia. We identified 17 GPCR genes that are robustly transcribed in adult human microglia, including the homeostatic core genes *CX3CR1*, *GPR34*, *GPR183*, *P2RY12*, and *P2RY13*, and *ADGRG1*, but also *ADORA3*, *ADRB2, CCR1, C3AR1, C5AR1, LPAR5, LPAR6*, and *PTAFR*. GPCRs genes well expressed in human microglia but hardly found in mouse microglia were *CXCR4*, *FPR1*, and *PTGER4*.

Figure 5 summarizes the findings of this study. As expected, isolated microglia rapidly lost expression of several GPCRs when
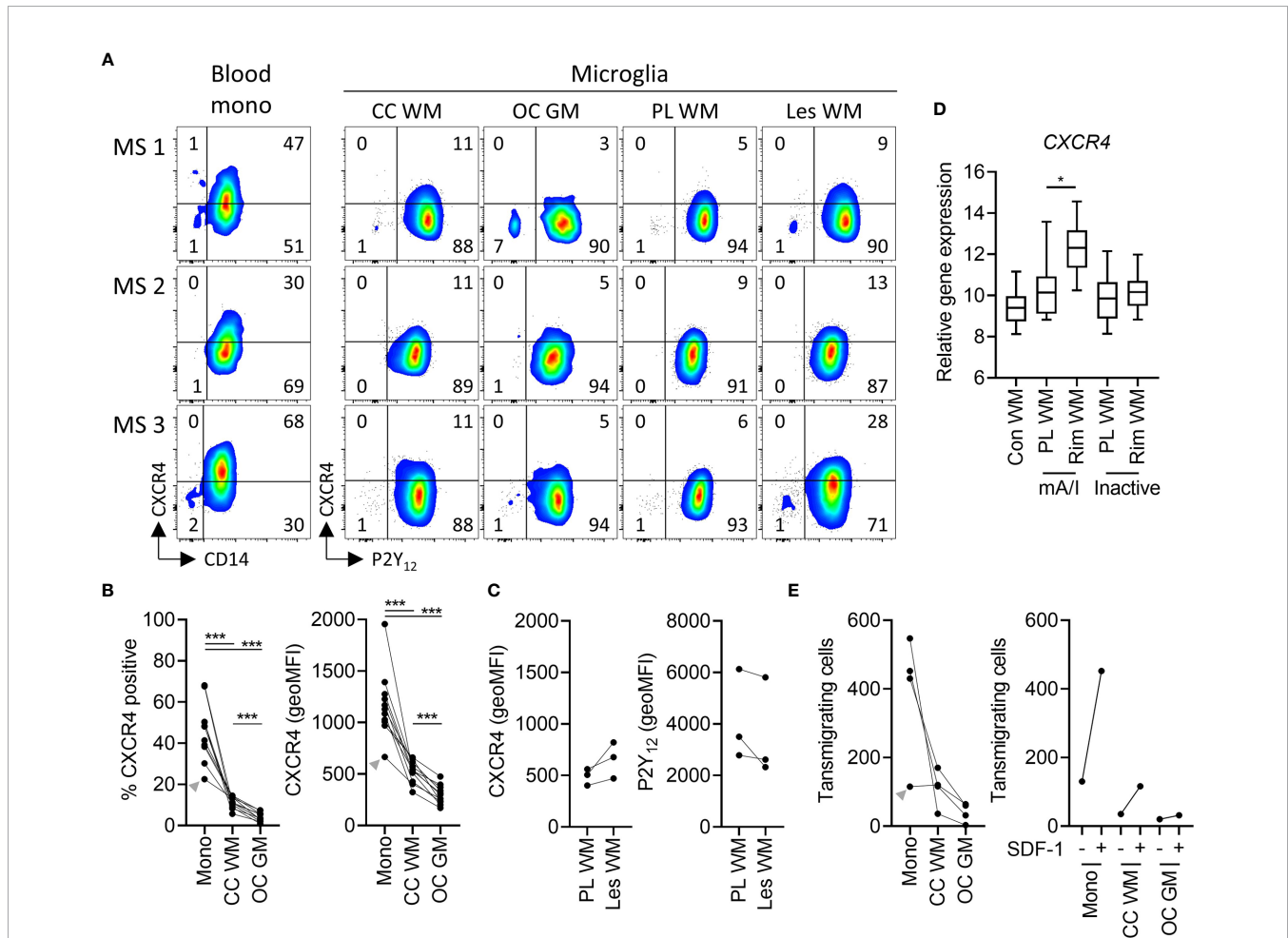
FIGURE 4 | Expression of functional CXCR4 in white and grey matter microglia in health and MS. **(A)** Representative dot plots of CXCR4 expression on paired peripheral CD14$^+$ blood monocytes and P2Y$_{12}$$^+$ microglia from corpus callosum white matter and occipital cortex grey matter, as well as P2Y$_{12}$$^+$ microglia from peri-lesional and lesional subcortical WM of three MS brain donors measured by flow cytometry. **(B)** Quantification of CXCR4 expression (percentage of positive cells and geoMFI) on monocytes, WM microglia, and GM microglia of n=11 brain donors (3 MS, 8 non-MS; Wilcoxon-signed rank test; ***p < 0.0005). **(C)** Quantification of CXCR4 and P2Y$_{12}$ expression on microglia from peri-lesional and lesional WM of three MS brain donors. **(D)** Quantification of tissue gene expression of *CXCR4* in control as well as peri-lesional and lesional WM from mixed active/inactive and inactive MS lesions (18) (Wilcoxon-signed rank test; *p < 0.05). **(E)** Quantification of transwell migration of monocytes, white matter microglia, and grey matter microglia in response to the CXCR4 ligand SDF-1 of n=4 brain donors (left panel). Of note, SDF-1-stimulated transwell migration was higher as compared to spontaneous transwell migration in all three cell types (n=1 brain donor; right panel). A donor with a relatively low expression of CXCR4 on monocytes also showed low monocyte transmigration towards SDF-1 (grey arrowhead). mono, monocytes; CC, corpus callosum; WM, white matter; OC, occipital cortex; GM, grey matter; PL, peri-lesional; Les, lesional; mA/I, mixed active/inactive; geoMFI, geometric mean fluorescence intensity.

cultured *ex vivo*, which limits the value of *in vitro*-expanded primary cells for functional studies. In contrast, iPSC-derived iMPs had a GPCR expression remarkably similar to primary microglia, which further equalized upon transfer into NSG mouse brains, ectopically providing critical human growth factors building the microglia phenotype. Regional diversity of microglia has been suggested (2), and we indeed found more abundant expression of *CXCR4* and *PTGER4* in white matter as compared to grey matter microglia in two independent studies [(4) and Mizee et al., unpublished data].

Disease-associated microglia in mouse models of AD and ALS downregulate expression of various GPCR genes, including *Cx3cr1*, *P2ry12*, *P2ry13*, and *Adgrg1* (14). The data presented here refer to MS, an inflammatory demyelinating and neurodegenerative disease. Bulk primary microglia collected post-mortem from normal-appearing tissue of MS donors showed downregulation of *ADGRG1* (4) in grey and white matter. *ADGRG1* encodes the adhesion family GPCR GPR56 (28), which is more widely expressed in the CNS and has been linked in neuronal precursors with cortical lamination and in oligodendrocyte precursors with proliferation (29). Abundant presence of GPR56 on human microglia (4) and regulation of synaptic refinement through a mouse GPR56 splicing isoform (30) have been reported only recently. Obviously, *ADGRG1* expression not only distinguishes microglia from other macrophages but also fades out in response to minor changes in the microenvironment, stressing its value as indicator of microglia homeostasis.

In biopsies of active lesions in early cases of MS, diseases-associated microglia subsets showed the expected general downregulation of GPCR genes associated with microglia homeostasis. Single-cell mass cytometry indeed confirmed the appearance of active MS lesion-enriched clusters with a down-regulated surface expression of P2Y$_{12}$, CX3CR1, and GPR56 (31).

The distribution of the chemokine receptor CXCR4 is of particular interest. CXCR4 binds CXCL12 (SDF-1), which is expressed by the brain vasculature and upregulated in MS lesions (32). Werner et al. recently reported that CXCR4 distinguishes brain-infiltrating monocytes from resident microglia in mice (27). We here demonstrate that human microglia from white and – to a lesser extent – grey matter express CXCR4. Transmigration towards SDF-1 confirmed the relevance of the presence of CXCR4 on microglia. Expression of CXCR4 by both microglia and infiltrating monocytes and upregulation by small subset of MS-associated lesional microglia (15) makes this chemokine receptor, involved in immune cell homeostasis and margination (33), a potentially interesting target for therapeutic intervention. Indeed, *Cxcr4* gene ablation reduced monocyte infiltration and response gene expression in experimental stroke in mice (27).

When testing the expression of genes encoding signaling molecules downstream of GPCRs, in particular Gα proteins, GRKs, and β-arrestin (6), we found robust expression of *GNAI2*, *GNAS*, *GNA13*, *GRK2*, and *ARRB2*. Further, mice, but not human, abundantly expressed *Gna15*. Expression of these genes did not depend on the specific microenvironment of

Family	Subfamily	Gene	In vitro culture	iMPs in vivo	GM vs WM	MS NAWM	MS NAGM	MS GM lesion	Gi/Go	Gq/G11	Gs	G12/G13	Drugs
Rhodopsin	Adenosine receptors	ADORA3	↓						●				✓
	Adrenoceptors	ADRB2	↓	↑					●		●		✓
	Chemokine receptors	CCR1							●				
		CXCR4	↓		↓	↑	↑		●				✓
		CX3CR1	↓	↑	↑			↓	●				
	Orphan receptors	GPR34	↓	↑					●				
		GPR183		↑	↓				●			●	
	Complement peptide receptors	C3AR1							●				
		C5AR1			↓				●			●	
	Formylpeptide receptors	FPR1		↑	↑	↓			●	●			
	Lysophospholipid (LPA) receptors	LPAR5	↓	↑					●	●		●	
		LPAR6	↓						●	●		●	
	P2Y receptors	P2RY12	↓	↑	↑			↓	●				✓
		P2RY13	↓	↑				↓	●				
	Platelet-activating factor receptor	PTAFR		↑					●	●			
	Prostanoid receptors	PTGER4			↓		↑		●		●		✓
Adhesion	G	ADGRG1	↓	↑		↓	↓	↓		●		●	
G protein	Alpha 12/13	GNA13			↓								

FIGURE 5 | Tabular summary of human microglia GPCR gene regulation, signaling, and drug availability. See Discussion for details. Arrows indicate the direction of regulation. Colored dots indicate prevalence of signaling of GPCRs through the respective G proteins (red = high; light red = medium) and abundance of gene expression of the G proteins in microglia (large dots = high; small dots = low). WM, white matter; GM, grey matter; NAGM, normal-appearing grey matter; NAWM, normal-appearing white matter.

microglia and was found at similar levels in other macrophages. $G\alpha_s$-coupled receptors activate adenylate cyclase, leading to cAMP accumulation. While *GNAS* transcripts were abundant in microglia, only two highly expressed GPCRs primarily couple to this $G\alpha$ subunit (*ADRB2*, *PTGER4*) suggesting rather limited augmentation of cAMP levels in microglia through GPCR signaling. $G\alpha_i$-coupled receptors inhibit the cAMP-dependent pathway by inhibiting adenylyl cyclase activity. Our data indicate that $G\alpha_i$, the preferred $G\alpha$ subunit of 12 of the 17 abundant microglia GPCRs, is well expressed in microglia, implying inhibition of cAMP-dependent protein kinase (PKA). $G\alpha_{12/13}$-coupled receptors activate the small GTPase RhoA. We found *GNA13* abundantly transcribed in white but not grey matter, which explains why initial studies of cortical microglia reported dim expression (10). Finally, $G\alpha_q$-coupled receptors activate phospholipase C to increase intracellular calcium concentration as well as activate protein kinase C, which results in Raf kinase activation of the MAPK pathway. *GNAQ* transcript levels were generally low, suggesting that LPAR5, LPAR6, and GPR56, which can engage $G\alpha_{12/13}$ as well as $G\alpha_q$, may rather control cell cytoskeleton remodeling and thus regulate microglia migration. Yet, in particular for GPR56, molecular mechanisms additional to G protein signaling may apply (28, 34).

GPCRs are known for their excellent drugability. A survey at DrugBank (https://www.drugbank.ca/) revealed approved drugs against at least five highly expressed microglia GPCRs with indications covering, amongst others, conditions of the lungs (ADORA3, ADRB2), eye (ADRB2), blood (CXCR4, P2Y$_{12}$), heart (P2Y$_{12}$), and uterus (PTGER4). Moreover, rodent models suggest efficacy of CXCR4 targeting in the treatment of stroke and glioma (35). Studying the effects of small molecules, penetrating the blood–brain barrier, on microglia *in vitro* and *in vivo* may disclose novel opportunities for the treatment of brain diseases in which microglia emerge as central players, including neurodevelopmental disorders (e.g., autism), neurodegenerative and -inflammatory conditions (e.g., Alzheimer's disease and MS), and chronic pain (1).

In summary, we here describe the GPCR repertoire of human microglia based on publicly available bulk and single-cell RNAseq data. GPCRs that belong to the core signature of microglia are abundantly expressed under homeostatic and rapidly downregulated under non-homeostatic conditions, making them interesting models for studying microenvironmental factors that shape microglia identity during brain development and disease. Datasets of microglia from brain donors with neurological diseases only lately became available and require further investigation. This in particular holds true as drugs targeting different highly expressed microglia GPCRs have been developed, implying their potential application for CNS diseases in which microglia figurate.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/ (accessible under accession code GEO: GSE52564, GSE98969, GSE99074, GSE108000, GSE111972, GSE124335, GSE128855, GSE134707, GSE139194), https://www.synapse.org/#!Synapse:syn21438358 and http://shiny.maths.usyd.edu.au/Ellis/MicrogliaPlots/.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FUNDING

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.674189/full#supplementary-material

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