P2X7 receptors and multiple sclerosis: A potential biomarker and therapeutic target?

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Multiple sclerosis (MS) is a chronic, autoimmune and neuroinflammatory disease of the central nervous system (CNS) with a neurodegenerative component, characterized by demyelination and degeneration of nerve fibers. It affects mainly young adults (aged 20 to 45 years) and its causes are still unknown, but it is thought that external factors such as viruses and environmental factors trigger the disease in people with a genetic susceptibility. Patients are classified into four main categories based on the clinical course of the disease: relapsing remitting (RR)-MS, the most common form, characterized by relapses and periods of remission; secondary progressive (SP)-MS, which may develop secondarily in RR-MS patients, characterized by continuous worsening with or without periods of remission; primary progressive (PP)-MS in which symptoms continue to worsen from the onset of the disease. In particular, the PP-MS form is more resistant to the currently available pharmacological treatments used to treat the other forms, and progressive relapsing-MS, a rare form characterized by continuous disease progression from the onset.

P2X7 receptors: P2X7 receptors (P2X7Rs) belong to the P2X family of receptors, ligand-gated ion channels activated by high concentrations of adenosine triphosphate (ATP); among P2X receptors, P2X7Rs are the most studied for their relevance in inflammation and immune responses. They are homotrimers with low affinity for ATP and have a dual gating state: brief exposure to ATP at micromolar concentrations causes P2X7Rs to open as an ion-selective channel, allowing cellular Na^+ and Ca^+ influx and K^+ efflux, leading to changes in cellular ion homeostasis; at ATP concentrations above 100 nm and after prolonged exposure, P2X7R forms a non-selective membrane pore permeable to hydrophilic molecules up to 900 Da in size, leading to cytotoxicity and apoptosis (membrane blebbing, cytokine release, and cell death). Consequently, channel opening leads to cell proliferation and survival, whereas forming large pores is detrimental leading to inflammasome activation, cytokine release, and pyroptosis (Di Virgilio et al., 2019). P2X7Rs are predominantly expressed in hematopoietic and immune cells such as mast cells, monocytes, and macrophages, but are also present in glial cells of the CNS such as astrocytes, oligodendrocytes, and microglia, and of the peripheral nervous system such as satellite glial cells and Schwann cells. For example, astrocyte-neuron signaling is mainly mediated by ATP released from neurons, but P2X7Rs are also involved in communication between glia and other glial cell types (Tozaki-Saitoh et al., 2011). Thus, P2X7Rs provide a communication bridge between the immune and nervous systems and are involved in processes such as neuroinflammation and neurodegeneration. Importantly, P2XR7 are strongly implicated in the pathogenesis of autoimmune diseases, including MS.

P2X7Rs in immunity: P2X7 receptors are involved in various mechanisms of both innate and adaptative immunity. In innate immunity cells, ATP released as a result of activation of pattern recognition receptors by pathogen-associated molecular patterns or damage-associated molecular patterns, stimulates P2X7Rs. The influx of Ca^{2+} and efflux of K^+ ions induced by channel opening leads to the assembly of the nucleotide-binding domain, leucine-rich-containing family and pyrin domain-containing 3 (NLRP3) inflammasome. Activation of NLRP3 leads to cleavage of pro-caspase-1 to active caspase-1, which then activates the pro-inflammatory cytokines interleukin (IL)-1β and IL-18, which are released into the extracellular environment. IL-1β activates nuclear factor kappa B, which induces the expression of genes that maintain inflammation and IL-18 promotes IFN-γ production (Kaneko et al., 2019). Regarding adaptative immunity, P2X7Rs are expressed on both T and B cells. Activation of P2X7Rs in B cells is associated with an active pro-inflammatory phenotype, activation of matrix metalloproteinases, with the modulation of antibody production, and with the modulation of B cell migratory behavior. P2X7Rs are also expressed in $CD4^+$ and $CD8^+$ T cells, where they regulate their development, function and memory formation. For example, T regulatory (Treg) cells, which are involved in maintaining self-tolerance and play a key role in the development of autoimmune diseases, express high levels of P2X7Rs. Their activation by ATP can inhibit their suppressive potential, probably by limiting Foxp3 expression. It has been shown that P2X7R inhibition or deletion ameliorates inflammation in inflamed tissues by preserving the functions of Tregs; instead, P2X7R activation promotes the conversion of Tregs into T helper 17. Additionally, impaired P2X7R activity could lead to the overactivation of effector T cells, with a beneficial effect on cancer but a detrimental effect on autoimmunity (Grassi and Salina, 2023).

P2X7Rs in autoimmunity and multiple sclerosis: P2X7R has been repeatedly reported to be involved in several autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease systemic sclerosis, MS and many others, but its contribution remains controversial. Certainly, P2X7R-mediated secretion of pro-inflammatory cytokines and chemokines fuels autoimmune responses. In MS, activation of P2X7Rs on microglia and astrocytes contributes to the pathological processes, leading to the formation of the typical inflammatory plaques and damage to myelin and oligodendrocytes in the white and grey matter, with consequent neuronal loss. Increased expression of P2X7Rs has been found on several CNS cell subtypes including microglia, astrocytes and oligodendrocytes of post-mortem MS patients (Territo et al., 2021). Additional evidence for P2X7R involvement in MS pathology includes: the presence of higher density of P2X7Rs in post-mortem spinal cord samples from MS patients; the resistance of $P2X7R^{-/-}$ mice to experimental autoimmune encephalomyelitis (the experimental animal model of MS); and the lethal effect of prolonged P2X7R activation on differentiated oligodendrocytes in culture and on mature oligodendrocytes of isolated optic nerves *in vitro* and *in vivo* (Matute, 2008). Additionally, among the various genetic loci of susceptibility to MS, several variants of the *P2X7R* gene have been implicated. In particular, the non-synonymous gain-of-function single nucleotide polymorphisms rs17525809 and rs208294 in *P2X7R* have been associated with MS risk in the Spanish population (Oyanguren-Desez O et al., 2011). The gain-of-function single nucleotide polymorphisms rs1718119 and rs22390912 have been associated with a worse disease severity in Italian RR-MS patients (Guerini et al., 2022). Conversely, the rare "loss of function" single nucleotide polymorphism rs28360457, which is related to the absence of pore formation, has been suggested to confer protection against MS risk (Gu et al., 2015). For all these reasons, P2X7Rs have been identified as a potential and promising therapeutic target in order to ameliorate disease symptoms.

P2X7Rs as a biomarker: The hypothesis of using P2X7R as a biomarker of inflammation for diagnostic and therapeutic purposes is very intriguing given the role that these proteins play in NLRP3 activation. Regarding the use of P2X7R as a pharmacological target, enormous efforts have been made over the last decade and several clinical trials have been initiated by leading pharmaceutical companies to evaluate different molecules that inhibit its activation (antagonists) as potential new anti-inflammatory drugs. These trials have reached phases I and II, but have so far produced unsatisfactory results and none have yet reached the market. Of particular interest are two brain-permeable oral P2X7R antagonists (JNJ-54175446 and JNJ-55308942, Janssen), which are in phase II for major depressive disorder treatment. Furthermore, several studies have been carried out on the use of P2X7R as a diagnostic biomarker, but much remains to be done. Giuliani et al. (2019) measured P2X7R levels by ELISA in both plasma and serum samples from a small number of healthy controls (HC) and patients with various diseases (infectious diseases, cancer, ischaemic heart or brain disease, trauma, autoimmune diseases). They found that both in plasma and serum P2X7R levels were lower in HC than in patients. Additionally, P2X7R levels correlated well with C-reactive protein measurements (Giuliani et al., 2019). More recently, P2X7R levels have been measured in the peripheral blood of patients suffering from various CNS pathologies. For example, plasmatic P2X7R was found to be higher in Alzheimer's disease than in HC (Aivar et al., 2023). However, some aspects need to be further investigated: to date, there are no reliable reference values for circulating P2X7R levels in HC, and laboratory methods are far from being standardized. Further studies are needed to define what "normal" levels are and how they may vary with e.g., gender and age and also to understand in which form P2X7R is present in the circulation (monomeric/trimeric, free/associated with extracellular vesicles…) to identify the most appropriate antibodies to use.

Several radiotracers have been synthesized to image and quantify P2X7Rs in the CNS using positron emission tomography. In relation to MS, the P2X7R radiotracer [^{11}C]-SMW139 has been shown to accumulate at sites of inflammatory lesions in patients with RR-MS (Hagens et al., 2020). Also in the context of MS, *P2X7R* gene expression has been studied, at the research level, in peripheral blood mononuclear cells from patients compared to healthy controls. *P2X7R* was found to be upregulated in MS patients, particularly in women suggesting a sex-specific activation (Rump et al., 2023). Recently, P2X7R levels were also measured by our group in oligodendrocyte-derived extracellular vesicles (ODEVs) enriched from serum samples of MS patients (both PP-MS and RR-MS) and sex/age-matched HC. Extracellular vesicles (EVs) are nano-sized particles released into the extracellular space by all cell types to "communicate" with distant cells through the molecules they transport. They carry a cargo of molecules (lipids, nucleic acids, proteins…) that reflect the physiological/pathological status of the originating cell. This characteristic makes them a potential source of easily accessible biomarkers as EVs can be isolated from peripheral fluids such as blood, urine, tears, etc., including EVs produced by CNS cells, as they can cross the blood–brain barrier, due to their small size. We observed that P2X7R levels in ODEVs were higher in MS patients compared to HC, especially in PP-MS compared to both RR-MS and HC. P2X7R levels in ODEVs were also positively correlated with disease severity as measured by the Expanded Disability Status Scale (Agliardi et al., 2024). These results obtained from ODEVs are interesting because they may reflect the specific level of inflammation at the site of interest in MS, i.e. the active lesions, rather than the general level of inflammation that would be measured at the blood level instead. Additionally,

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these results suggest a potential diagnostic power of P2X7R measurement in ODEVs to help differentiate PP-MS from RR-MS. Again, further studies in larger cohorts are needed, as well as a standardized method for enrichment and analysis of extracellular vesicles which is currently far from being applied in clinical diagnostics.

In the future, several methods could be used for the P2X7R assay, some of which are summarized in **Figure 1**, taking into account the invasiveness, costs, and specificity of the procedures (**Figure 1**).

Future challenges and opportunities: In conclusion, because of its crucial role in the inflammatory cascade and the autoimmune process, P2X7R represents both a promising therapeutic target for MS and a potential biomarker for diagnostic procedures. The results obtained in ODEVs enriched from peripheral blood of different MS subtypes deserve further investigation to understand whether P2X7R involvement could be specific for the PP-MS form as an example. If confirmed, this could lead to earlier and more tailored pharmacological and rehabilitation treatments for patients. P2X7R may be useful in identifying MS patients who are most likely to benefit from a pharmacological treatment that can disable/block P2X7R activation. Future studies should aim to investigate the differential expression in different CNS cells, including by studying specific EVs. To date, both large pharmaceutical companies and small biotech companies are still interested in finding compounds that could efficiently antagonize P2X7R activation. Indeed, a large number of preclinical compounds have shown excellent activity at the human P2X7 receptor but lack potency in rodents, limiting further *in vivo* studies. Additionally, more effort is needed to understand the properties a P2X7R antagonist must have to act in the CNS in terms of solubility, lipophilicity, and half-life. A better understanding of P2X7R biology in the near future would help to overcome these limitations and bridge the translation gap that still exists for the promising use of P2X7R as a disease biomarker and therapeutic target.

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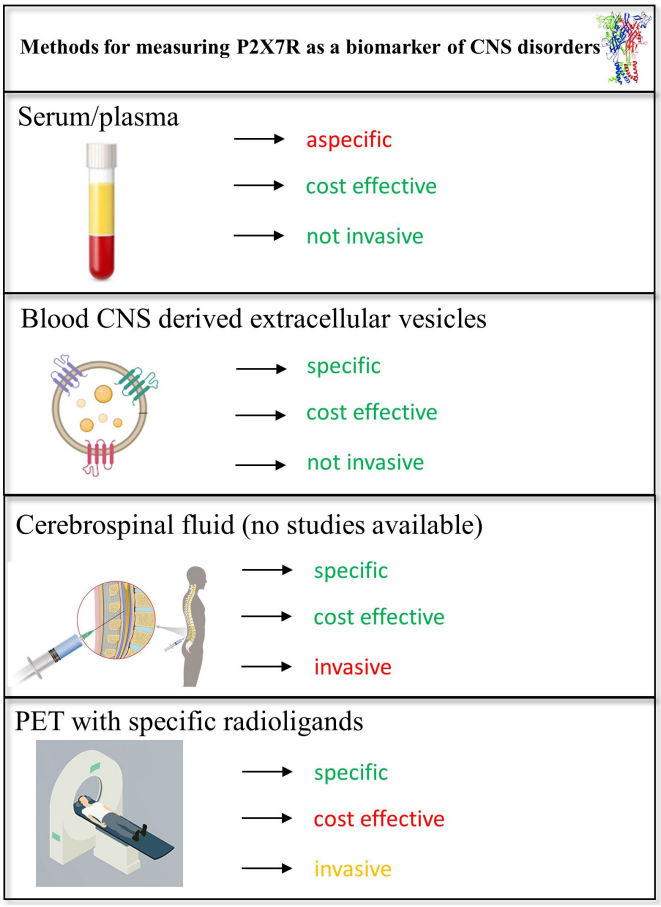

Figure 1 | **Schematic representation of the use of P2X7R as a biomarker for CNS disorders.**
The figure shows some possible ways to measure P2X7R and their advantages in terms of specificity, invasiveness, and cost/benefit ratio. CNS: Central nervous system; PET: positron emission tomography.

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