Clues from a missense mutation of the adenosine A_1 receptor gene associated with early-onset Parkinson's disease

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Parkinson's disease (PD) is a complex neurodegenerative disorder for which rare and common genetic variants contribute to disease risk, onset, and progression. The genetic contribution to PD can be classified mainly in, first, rare DNA variants that are highly penetrant and therefore causal, which are typically associated with monogenic PD; and second, more common risk polymorphic variants, which individually exert a small increase in the risk of the disease, which are usually identified in the most prevalent and apparently sporadic PD (Blauwendraat et al., 2020).

The terms monogenic, familiar, and early-onset PD (EOPD) are often used indistinctly. More specifically, PD is defined as familial or sporadic, according to the presence or absence of a clear family history. Approximately 5–10% can then be classified as familial, but monogenetic PD is rare and only accounts for about 30% of familial cases and 3% to 5% of sporadic cases, while in most cases PD is due to a complex interplay between genetics and the environment (Blauwendraat et al., 2020; Guadagnolo et al., 2021). EOPD is commonly defined as an age of onset below 45 years. Monogenic forms of PD are more frequent in EOPD patients, being more than 10% of cases with onset before 45 years and more than 40% in those with onset before 30 years (Blauwendraat et al., 2020; Guadagnolo et al., 2021). Furthermore, the term monogenic, meaning complete dependence on a mutated gene, is an oversimplification, since even for some highly penetrant rare variants, the presentation of PD is dependent on other genetic and non-genetic factors. Thus, the disease might not manifest itself in some carriers of highly penetrant variants. Furthermore, when manifested, the age of onset or the degree or progression of the disease may differ between carriers in the same family (Blauwendraat et al., 2020).

There are several well-established genes in which mutations cause monogenic PD or constitute risk-conferring variants, with autosomal dominant inheritance (such as SNCA and LRRK2) and autosomal recessive inheritance (such as PRKN, PINK1, and DJ1). Collectively, rare variants in more than 20 genes have been identified so far, but the relevance of most of them is still a matter of debate, and more replication and functional validation studies are needed (Blauwendraat et al., 2020; Guadagnolo et al., 2021). Dominant mutations in SNCA, the α-synuclein gene, were identified 20 years ago as the first monogenic cause of PD, which is consistent with the critical pathogenetic role of α-synuclein. Therefore, neuronal loss in the substantia nigra pars compacta, which causes striatal dopamine deficiency, and intracellular inclusions that contain aggregates of α synuclein, which constitute the classical Lewy bodies, are the neuropathological hallmarks of PD. Pathogenic variants in the LRRK2 gene, which encodes leucine-rich repeat kinase 2, are the most common causes of autosomal dominant PD, accounting for 5% of familial and 1% of sporadic cases. Among autosomal recessive monogenic PD, pathogenic variants in the PRKN, PINK1, and DJ-1 genes account for more than 10% of cases of EOPD. Importantly, Lewy bodies are not detected in most PRKN mutations, indicating a difference in the pathogenic processes that lead to this EOPD and sporadic PD (Blauwendraat et al., 2020; Guadagnolo et al., 2021).

Jaberi et al. (2016) reported a genetic study in a family with autosomal recessive EOPD with cognitive decline, identifying two affected siblings with homozygous mutations in the adenosine A_1 receptor (A_1R) gene (ADORA1; $G279^{7.44S}$) and in the peptidyl-tRNA hydrolase domain containing 1 gene (PTRHD1) that segregated with the disease. Based on circumstantial evidence of a significant role for adenosine and A_1R in neuroprotection and neurodegeneration (Cunha, 2016), they suggested the ADORA1 mutation is the strongest candidate causative mutation. However, several months after the publication by Jaberi et al. (2016), another mutation of PTRHD1 (p.His53Tyr) was found as a possible cause of autosomal recessive intellectual disability and EOPD. This was followed by two more recent separate studies from Oman and South African families reporting the association of a 28-nucleotide frameshift deletion in the PTRHD1 coding region with EOPD and intellectual disability (reviewed in Al-Kasbi et al., 2021). Therefore, the PTRHD1 mutation might have been the main cause of EOPD simultaneously associated with the ADORA1 mutation described by Jaberi et al. (2016).

However, although the bacterial homolog (pth1) is well characterized, human PTRHD1 does not seem to function as peptidyl-tRNA hydrolase and, currently, its function remains unclear (Al-Kasbi et al., 2021). On the other hand, A_1R is known to play a very significant role in mediating the central effects of adenosine, both during physiological and pathological conditions (Cunha, 2016). Therefore, the putative role of mutated A_1R^{G279S} in the development of EOPD with cognitive decline described by Jaberi et al. (2016) could not be ruled out. Therefore, it became important to study whether the $G279^{7.44S}$ mutation has functional consequences. $G279^{7.44}$ is located in the middle of the transmembrane domain (TM) 7 of A_1R, facing the lipid bilayer, thus not being part of the orthosteric binding site. But it is also located near the conserved $NP^{7.50}xxY$ motif, which is essential to form the active conformation of the receptor (Nasrollahi-Shirazi et al., 2020; Sarasola et al., 2022). The $G279^{7.44S}$ mutation would then be foreseeable to lead to differences in agonist-induced activation without changes in agonist binding properties. Furthermore, since mutations in the TMs can affect the ability of G protein-coupled receptors to undergo folding in the endoplasmic reticulum, functional differences could be related to different densities at the plasma membrane. Finally, another possibility could be the differential ability of A_1R^{G279S} to form functional heteromers with other G protein-coupled receptors, more importantly with the dopamine D_1 receptor (D_1R) or with the adenosine A_{2A} receptor ($A_{2A}R$). Thus, functional A_1R-$A_{2A}R$ and A_1R-D_1R heteromers exert a very significant role in adenosine-mediated presynaptic and postsynaptic modulation of striatal glutamatergic transmission, respectively (Ferré et al., 2022).

Three different studies have been conducted in mammalian transfected cells (HEK-293 and HEK-293T cells) to address these questions. Using immunohistochemical and co-immunoprecipitation techniques in co-transfected cells, Jaberi et al. (2016) found no differences in the density of A_1R^{G279S} in the plasma membrane and its molecular interactions with the dopamine D_1R, compared to wild-type A_1R (i.e., A_1R^{WT}). This was confirmed by Nasrollahi-Shirazi et al. (2020) using radioligand binding, flow cytometric analysis, and bioluminescent resonance energy transfer (BRET). With radioligand binding experiments, the same authors found no differences in the binding properties of A_1R ligands, while signaling experiments implied that the $G279^{7.44S}$ mutation increases the constitutive activity and agonist-induced efficacy of A_1R. This was attributed to an enhanced conformational flexibility due to a reduced kinetic stability of A_1R^{G279S} *versus* A_1R^{WT}, as shown by analyzing the time-dependent loss of radiolabeled antagonist binding at different temperatures, and as supported by molecular dynamic simulations (Nasrollahi-Shirazi et al., 2020). In our recent study, using immunofluorescence, biotinylation, and NanoBRET techniques, we also found that A_1R^{G279S} stably expressed in HEK-293T cells shows an equivalent subcellular distribution and cell surface density as A_1R^{WT} (Sarasola et al., 2022). Furthermore, NanoBRET experiments also showed that a fluorescent selective A_1R ligand had the same affinity for A_1R^{G279S} as for A_1R^{WT}. However, in contradiction to the results obtained by Nasrollahi-Shirazi et al. (2020), using NanoBiT technology in transiently transfected HEK-293T cells, we did not observe a significant difference in the functional response of A_1R agonists, in their ability to couple with transducer proteins (Gαi, Gαq, Gα12/13, Gαs, β-arrestin2, or GRK2) (Sarasola et al., 2022).

After the report by Jaberi et al. (2016), we postulated that a possible mechanistic explanation of the pathogenetic link of the $G279^{7.44S}$ mutation with EOPD could be a loss of function of the A_1R in its interactions with the $A_{2A}R$ in the cortico-striatal glutamatergic terminals (Fernández-Dueñas et al., 2017). As we recently reviewed, adenosine plays a very significant role in local striatal modulation of cortico-striatal glutamate release and, secondarily, of acetylcholine and dopamine release (Ferré et al., 2022). This modulation is largely mediated by A_1R-$A_{2A}R$ and $A_{2A}R$-cannabinoid CB_1 receptor (CB_1R) heteromers localized in cortico-striatal glutamatergic terminals (**Figure 1**; Ferré et al., 2022). Predominant activation of A_1Rs or $A_{2A}Rs$ in the cortico-striatal terminal results in inhibition or facilitation of glutamate release, respectively, depending on the degree of constitutive activity of $A_{2A}R$, on the extracellular level of adenosine, and on the level of endocannabinoids. We have previously demonstrated that the constitutive activity of $A_{2A}R$ disappears in the A_1R-$A_{2A}R$ heteromer, but not in the $A_{2A}R$-CB_1R heteromer (Köfalvi et al., 2020). Then, the ability of endocannabinoids and other CB_1R agonists to inhibit glutamate release depends on their ability to counteract the constitutive $A_{2A}R$-mediated adenylyl cyclase activation in the $A_{2A}R$-CB_1R heteromer (**Figure 1**; Köfalvi et al., 2020).

Additionally, significant allosteric interactions take place between ligands that bind to orthosteric

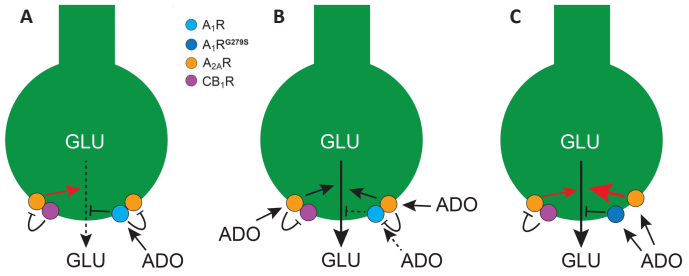

Figure 1 | **Schematic representation of cortico-striatal glutamatergic terminals and their modulatory A_1 receptor (A_1R)-A_{2A} receptor ($A_{2A}R$) and $A_{2A}R$-CB_1 receptor (CB_1R) heteromers.**
Arrows represent receptor activation or facilitation of glutamate (GLU) release. Red arrows represent constitutive activation of the $A_{2A}R$. Lines with perpendicular ending segments represent inhibitory allosteric modulation or inhibition of glutamate release. The lower and higher degrees of activation, facilitation, or inhibition are represented by broken and thicker arrows and lines, respectively. Predominant activation of A_1R or $A_{2A}R$ promotes inhibition or facilitation of GLU release, respectively. (A) Low degree of GLU release under physiological conditions, with low extracellular concentrations of adenosine (ADO), which promotes a predominant activation of A_1R in the A_1R-$A_{2A}R$ heteromer; the constitutive activation of $A_{2A}R$ in the $A_{2A}R$-CB1R heteromer depends on the degree of inhibitory control by CB_1R. (B) $A_{2A}R$ loses its constitutive activity in the A_1R-$A_{2A}R$ heteromer and plays a role with pathologically high concentrations of ADO, which facilitates the release of GLU. (C) The absence of heteromerization of A_1R^{G279S} with $A_{2A}R$ reveals a non-inhibited constitutive and agonist-induced activation of $A_{2A}R$ and a facilitatory effect on the release of GLU. Although adenosine receptors and receptor heteromers are proposed to be predominantly dimeric and tetrameric, respectively, they are represented as monomers and dimers for the sake of simplicity. Created using Illustrator 27.1.1.

sites of the A_1R-$A_{2A}R$ heteromer. On the one hand, the binding of an A_1R agonist decreases the potency and efficacy of an $A_{2A}R$ agonist (Sarasola et al., 2022). Since adenosine has higher affinity for A_1R than for $A_{2A}R$, this allosteric interaction ensures that, under physiological variations of extracellular adenosine, the functional effect of A_1R activation in the cortico-striatal terminal predominates over the effect of $A_{2A}R$ activation (**Figure 1A**). On the other hand, higher pathological levels of adenosine can overcome the allosteric interaction imposed by activated A_1R and promote an opposite effect, through a reciprocal antagonistic interaction, by which binding of an $A_{2A}R$ agonist decreases A_1R function (**Figure 1B**; Ferré et al., 2022). The same type of allosteric interaction, but in the $A_{2A}R$-CB_1R heteromer, can promote the counteraction of the antagonistic effect of CB_1R agonists on $A_{2A}R$-mediated signaling in the $A_{2A}R$-CB_1R heteromer (Ferré et al., 2022), altogether maximizing $A_{2A}R$-mediated glutamate release (**Figure 1B**).

In fact, we were able to demonstrate, using NanoBiT technology, that A_1R^{G279S} does not form heteromers with $A_{2A}R$. Molecular dynamic simulations allowed us to propose an indirect mechanism by which the $G279^{7.44S}$ mutation in TM 7 of A_1R weakens the TM 5/6 interface of the A_1R-$A_{2A}R$ heteromer. As expected, the lack of A_1R-$A_{2A}R$ heteromerization was associated with the disappearance of the A_1R agonist-induced allosteric modulation of $A_{2A}R$ signaling and the restoration of the constitutive activity of the $A_{2A}R$ (Sarasola et al., 2022). Therefore, this could confer an increased sensitivity of cortico-striatal glutamatergic terminals (**Figure 1C**), which could enhance the well-established striatal glutamatergic hyperactivity of PD (Blandini et al., 1996; Campanelli et al., 2022). This hyperglutamatergic state involves pre- and postsynaptic mechanisms and has been suggested to be a critical mechanism underlying different striatal alterations associated with PD in the early and advanced symptomatic stages of the disease (Campanelli et al., 2022).

Although we do not yet know the pathogenetic contribution of the $G279^{7.44S}$ mutation of A_1R in EOPD described by Jaberi et al. (2016), to our knowledge, this is the first example of a single missense mutation that specifically results in the impairment of G protein-coupled receptor heteromerization, which probably results in pathological implications. It would then be important to look for $G279^{7.44S}$ or other functionally similar mutations of A_1R in other clinical conditions where alterations in the function of A_1R-$A_{2A}R$ heteromers in cortico-striatal terminals have been proposed, such as restless legs syndrome (Ferré et al., 2022) or other neuropsychiatric disorders associated with alterations in cortico-striatal transmission.

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