Towards the convergent therapeutic potential of GPCRs in autism spectrum disorders

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Abstract

Autism spectrum disorders (ASD) are diagnosed in 1/100 childbirth worldwide, based on two core symptoms, deficits in social interaction and communication and stereotyped behaviours. G protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors that mediate the transfer of extracellular signals to convergent intracellular signalling and downstream cellular responses that are dysregulated in ASD. Despite hundreds of GPCRs are expressed in the brain, only 23 GPCRs are genetically associated to ASD according to the Simons Foundation Autism Research Initiative (SFARI) gene database: oxytocin OTR, vasopressin V1A, V1B, metabotropic glutamate mGlu5, mGlu7, GABAB, dopamine D1, D2, D3, serotoninergic 5-HT1B, β2-adrenoceptor, cholinergic M3, adenosine A2A, A3, angiotensin AT2, cannabinoid CB1, chemokine CX3CR1, orphan GPR37, GPR85 and olfactory OR1C1, OR2M4, OR2T10, OR52M1. Here, we review the therapeutical potential of these 23 GPCRs, in addition to 5-HT2A, 5-HT6 and 5-HT7 for their relevance to ASD. We discuss their genetic association with ASD, the effects of their genetic and pharmacological manipulation in animal models and humans, their existing pharmacopeia towards core symptoms of ASD and rank them based on these evidences. Among these 23 GPCRs, we highlight that OTR, V1A, mGlu5, D2, 5-HT2A, CB1, and GPR37 are the best therapeutic targets. We conclude that the dysregulation of GPCRs and their signalling is a convergent pathological mechanism of ASD and their therapeutic potential has only begun as multiple GPCRs could mitigate ASD.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder diagnosed around the age of 3 with a worldwide prevalence of around 1/100 child births (Zeidan et al., 2022). Core clinical symptoms are defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V): social interaction and communication deficits and stereotyped, restrained or compulsive behaviours. ASD often associates with comorbid symptoms, such as anxiety, epilepsy, sleep disturbances, motor coordination impairment, gastrointestinal disorders or intellectual disability. Its aetiology remains partially resolved, as 70% of cases remain sporadic, highlighting the polygenic and environmental complexity of the disease. To date, no pharmacological treatment exists for the core social symptoms of autism. Clinical trials failed because of an important placebo effect, lack of efficacy and the large diversity of patients. New advances need to overcome these challenges. First, subtypes of patients determined on their genetic or neuropathological mechanism profile should be tested rather than patients from the whole spectrum. Second, the identification of robust therapeutic targets and the development of potent drugs should thwart the placebo effect. More than a thousand of candidate genes are listed in the Simons Foundation Autism Research Initiative (SFARI) database (*https://gene.sfari.org*), and rather than a single mutation, accumulation of deleterious alleles and copy number variants may underlie the pathological process among affected individuals (Manoli and State, 2021). Recent genome wide association studies of large ASD cohorts robustly identified hundreds of candidate genes that fall in two main convergent neurobiological mechanisms, namely 'gene expression regulation' or

'neuronal communication, signalling or plasticity' (De Rubeis et al., 2014; Satterstrom et al., 2020; Pintacuda et al., 2023). G protein-coupled receptors (GPCRs) are master regulators of these convergent mechanisms. In addition, their fine-tuned pharmacology and their diversity could represent the greatest therapeutic options for ASD to lead to successful clinical trials. In this review, we demonstrate why this receptor family meets all criteria of convergent therapeutic targets for ASD.

GPCRs and their signalling are dysregulated in ASD

Canonical GPCRs display an extracellular domain composed of the N-terminus and three extracellular loops (EL1-3) that connect seven transmembrane (TM) helices. Occupancy of the ligand binding pocket leads to conformational changes of the helices that transmit activation to the intracellular domain, composed of three intracellular loops (IL1-3) and a C-terminus region with an 8^{th} helix parallel to the plasma membrane (**Figure 1**). This intracellular domain is involved in the recruitment and activation of direct transducers that activate downstream intracellular signalling. GPCRs are translated inside the membrane of the endoplasmic reticulum (ER), thus actively exported to the plasma membrane. Due to their molecular complexity, they are often prone to misfolding or lack of ER export, which may result in cell toxicity (Beerepoot et al., 2017). Most GPCRs and their transducers are expressed in the Central Nervous System (CNS) (Regard et al., 2008; Marti-Solano et al., 2020), with over two hundred variants in GPCR genes associated with ASD (SFARI). Furthermore, transcriptomic data and meta-analysis from prefrontal cortex tissue showed that GPCRs are the most frequently dysregulated genes in ASD and revealed around 200 GPCRs potentially linked to ASD (Hormozdiari et al., 2015; Monfared et al., 2021). Among these, serotonin *HTR2A* , adenosine *ADORA1* and adrenergic *ADRA1D* are the most dysregulated.

GPCRs and their downstream signalling pathways, are also affected in ASD. Upon activation, GPCRs couple to several heterotrimeric G protein (α, β and γ subunits) and recruit β-arrestins that impact on the kinetics of intracellular signalling pathways such as extracellular signal-regulated kinases (ERK) or promote receptor endocytosis (**Figure 1A**). $G\alpha_{s/olf}$ protein activates adenylyl cyclases that hydrolyse ATP into cAMP, which is degraded by phosphodiesterases into AMP. cAMP activates exchange protein activated by cAMP (EPAC), calcium ion channels and protein kinase A (PKA). Conversely, $G\alpha_{i/o}$ inhibits adenylyl cyclases and cAMP production. $G\alpha_{q/11}$ proteins activate phospholipase Cβ, which hydrolyses phosphatidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). Released IP_3 binds to ryanodine receptors on the endoplasmic reticulum, which leads to calcium release from this subcellular location. Both calcium and DAG activate protein kinase C (PKC) and its effectors, such as Akt (or protein kinase B) or ERK. $G\alpha_{12/13}$ activates Rho guanine nucleotide exchange Factor (GEF) and RhoA, which acts on the cytoskeleton to promote neurite formation. G$\beta\gamma$ proteins also participate in downstream signalling through activation of G protein-coupled inwardly-rectifying potassium channels (GIRK) or other channels. The most affected transducers in ASD are $G\alpha_i$ and $G\alpha_{12/13}$ proteins (Monfared et al., 2021). Following activation, GPCRs are internalized in intracellular compartments (e.g., endosomes) together with their transducers and participate in the signalling cascades (Vilardaga et al., 2022). Downstream intracellular signalling network leads to integrated cellular processes, translation of specific mRNAs (Musnier et al., 2012; León et al., 2014; Tréfier et al., 2018) and gene transcription via the transcription factor cAMP-responsive element binding protein (CREB), among many others. Globally, GPCRs are key master upstream regulators of Wnt/β-catenin, ERK, PKC, Pi3K/Akt, CREB, PTEN and mTOR intracellular pathways that are convergently dysregulated in ASD (O'Roak et al., 2012; Gazestani et al., 2019; Pintacuda et al., 2023). In an attempt to estimate their potential as master regulators of genes involved in ASD, we confronted 'Kyoto Encyclopedia of Genes and Genomes' (KEGG) pathways, 'Reactome' pathways, 'Gene Ontology' (GO) terms and our knowledge (**supporting information**) to the 1045 ASD candidate genes from the SFARI list. We identified 23 GPCRs and 129 genes linked to GPCRs (**Figure 2** , **Table S1**) in this list, accounting for at least 15% of the candidate genes. Interestingly, 2 orphan GPR37 and GPR85 and 4 olfactory OR1C1, OR2M4, OR2T10, OR52M1 receptors with relatively unknown functions in the brain belong to this list. This proportion might increase with future gain of knowledge on signalling and cellular processes under the control of GPCRs, especially their effect on translation and transcription.

GPCRs are the most druggable targets for ASD

GPCRs respond to diverse natural signals ranging from photons, amino acids, peptides up to large glycosylated proteins. This receptor family comprises more than 800 GPCRs, subdivided into five classes according to the International Union of Basic & Clinical Pharmacology (IUPHAR) nomenclature and classification (Alexander et al., 2021): the largest rhodopsin-like class A, the secretin/class B, the glutamate/class C, the Frizzled class and the adhesion class. Sensory GPCRs (olfactory, vision, taste and pheromone receptors), which account for most GPCR genes, are included mostly in class A and a few in class C. Although hundreds of GPCRs remain without any identified ligand, 24% of these so-called "orphan receptors" (including olfactory receptors) are dysregulated in ASD. In addition to natural ligands, drugs can modulate GPCR activity, inducing diverse pharmacological profiles (**Figure 1B**). They can either be chemical compounds, peptides, large autoantibodies and more recently, antibody fragments (Mujić-Delić et al., 2014). Orthosteric agonists, inverse agonists and antagonists occupy the natural ligand binding pocket and activate, inactivate the receptor and/or prevent the binding of the endogenous ligand respectively. In contrast, by binding to allosteric sites, positive (PAM) or negative (NAM) allosteric modulators enhance or decrease GPCR activity only in the presence of agonists. While their intrinsic instability has been a major issue for resolving their 3 dimensional-structure, many GPCR structures in inactive, intermediate and active conformations became available with the development of cryo-electron microscopy. These recent advances facilitate *in silico* design of more selective drug. Finally, GPCRs are modulated by interacting partners. They can form cell-specific homo- or hetero-oligomers depending on the GPCR composition and subcellular localisation in a particular cell type. Each GPCR in the oligomer or expressed in the same cell may influence the signalling network of other GPCRs, opening a new area of GPCR pharmacology. Their activity is also modulated through reciprocal functional interactions with scaffolding protein partners (*e.g.,* Shank1-3), ion channels and tyrosine kinase receptors. Different ligands may favour one coupling or protein partner interaction, leading to what is called 'signalling bias' (**Figure 1A**). This pharmacological property of GPCRs is of great interest for therapeutic applications, as one biased drug can induce one signalling cascade over the others, hence possibly avoiding side effects. Considering their unique characteristics, GPCRs offer many levels of leveraging as therapeutic targets for neurological disorders.

More than 30% of the drugs approved on the global market target a GPCR in various disorders, including neurological conditions for 20% of them (Hauser et al., 2017; Alexander et al., 2021). This proportion will increase as nearly half of GPCRs in the CNS remain orphan. So far, no pharmaceutical agent has reached the market to improve primary symptoms of ASD. The few GPCRs tested in clinical trials are $mGlu_5$, $GABA_B$, V_{1A} and CB_1 and are all listed in the SFARI database (**Table S1**). Therefore, the therapeutic potential of GPCRs has only begun. In this review, we explored the therapeutical values of these 23 GPCRs identified in the SFARI list, in addition to 5-HT_{2A}, 5-HT_6 and 5-HT_7receptors for their relevance to ASD (**supporting information**). We studied the potential deleterious effect of the different variants associated with ASD, their dysregulation in ASD post-mortem tissues and their pharmacogenomic to conclude on their potential involvement in the aetiology of ASD (**Table S2**). We addressed the behavioural consequences of their genetic and pharmacological manipulation in animal models (**Table S3**) and whether these models recapitulate the validity criteria defined for psychiatric diseases applied to ASD (Chadman et al., 2019). We analysed their pharmacological landscape (*e.g.,* specific drugs on the market or in clinical trials), the availability of GPCR structures, and when available, the results of clinical trials (**Table S4**). We also reported their known downstream signalling and their interacting partners (**Table S5**). Finally, we reviewed their cellular localisation and levels of expression in the CNS (**Figures 2-3, Table S6**). Based on these evidences, we conclude on the therapeutic potential of these 26 GPCRs (**Figure 4**).

Oxytocin and vasopressin receptors

Oxytocin (OT) was first described in the 1960s for its effects on reproduction and maternal behaviours (Froemke and Young, 2021). Extremely conserved in mammals, OT and its paralog arginine vasopressin (AVP) in the CNS modulate social recognition and memory, defensive behaviours, trust, empathy and maternal attachment (Macdonald and Macdonald, 2010; Rae et al., 2022). Mice lacking OT peptide (*Oxt*KO

mice) may display impairment in social memory and aggressive and anxious-like behaviours (**Table S3**), but these phenotypes are inconsistent across different laboratories and parental genotypes. Administration of OT in the lateral ventricles or in the medial amygdala of *Oxt* KO mice normalizes anxious-like behaviour and restores social recognition (Ferguson et al., 2001; Mantella et al., 2003). Lastly, Brattleboro rats, which carry a frameshift deletion in the*Avp* gene leading to the lack of AVP, display defective social preference (Surget and Belzung, 2008) whereas *Avp* KO mice are lethal, in the absence of peripheral AVP administration (Zelena, 2017).

Several studies have associated the OT-AVP family with autism spectrum disorders, because they regulate social behaviours and OT plasma levels are lower in ASD children (Cataldo et al., 2018; John and Jaeggi, 2021; Rae et al., 2022). Logically, many studies have investigated the therapeutic potential of OT or AVP for ASD. Intranasal administration of OT at low dose improves emotion recognition in young men with autistic condition (Guastella et al., 2010a). Furthermore, OT inhalation increased trust and interactions in adults with ASD (Andari et al., 2010) and reduced severe repetitive behaviours (Hollander et al., 2003). Unexpectedly, administration of OT failed to improve social abilities over placebo in phase 2 clinical trials and induced frequent side effects in several studies (Leppanen et al., 2018; Sikich et al., 2021; Witte et al., 2022). In fact, OT administration may not be effective in all patients, but rather only in subtypes of patients with OT deficiency. Interestingly, administration of AVP in humans improves the recognition of happy and angry social faces compared to neutral faces (Guastella et al., 2010b). A phase 2 clinical trial showed that four weeks of intranasal administration of AVP in 30 ASD children improved their social skills and reduced anxiety and repetitive behaviours, with minimal side effects (Parker et al., 2019). Whereas AVP remains to be tested in a larger cohort of patients, the first results indicate that it might be more efficient than OT to provide pro-social effects. Thus, despite mitigated results, the OT-AVP family remains of interest for ASD. Actually, OT and AVP might not be idealistic treatments as both bind and activate with nanomolar affinity the four highly conserved oxytocin receptor (OTR), vasopressin V_{1A} and V_{1B} receptors in the CNS and V_2 receptor in the periphery. Therefore, in the following section, we review the therapeutic potential of OTR, V_{1A} and V_{1B} for treatment in ASD.

Oxytocin receptor

The oxytocin receptor gene (*OXTR*) spans over 4 exons and encodes 5 splicing transcript variants that differ in their 5' untranslated region (UTR) leading to only one receptor, OTR. Decades of research identified several agonists of OTR (**Table S4**), such as the potent peptide agonist Thr^4Gly^7-OT (TGOT) (Elands et al., 1988), the $G\alpha_q$-biased agonist carbetocin (Passoni et al., 2016) and the first chemical agonist LIT001 (Frantz et al., 2018). However, all these ligands also bind vasopressin receptors. OTR expression is found in CNS regions critical for the regulation of social behaviour and emotion (**Figure 3**) and might be sexually dimorphic depending on the brain region and species (Dumais et al., 2013). In humans, *OXTR* transcript levels peak after birth, during all infancy and reduce in adolescents and adults (Kang et al., 2011). This corresponds to oxytocinergic neuron development in the same critical period as observed in mice (Soumier et al., 2022). OTR is involved in complex social behaviours, like maternal care, social recognition, aggression, mating but also in pair bonding, empathy and could exert anxiolytic effects (Jurek and Neumann, 2018). *Oxtr* KO mice have an autism-like phenotype, with both social deficits and stereotyped behaviours whereas heterozygous mice express only social deficits. *Oxtr* KO also display deficits in social memory and pup vocalisations following maternal separation and aggressive behaviour (**Table S3**). Whereas increased self-grooming, anxious-like behaviours and cognitive inflexibility have been observed, results are inconsistent through laboratories or mouse lines. However, *Oxtr*KO in monogamous prairie voles leads to deficits in social novelty and increased repetitive behaviours, but no impairment in social interactions, vocalisations or maternal behaviour (Horie et al., 2019; Berendzen et al., 2022). Interestingly, intraventricular administration of OT or AVP restores the social deficits in *Oxtr* KO mice via V_{1A} receptors (Sala et al., 2011). This finding highlights the crosstalk within this GPCR family. More than twenty variants in the *OXTR* gene have been associated with ASD (**Table S2**). Interestingly, variants are mostly located outside the receptor coding region, leading to potential receptor expression dysregulation.

Vasopressin V_{1A} and V_{1B}receptors

AVP, well known as the antidiuretic hormone via the activation of V_2 receptors, binds V_{1A} and V_{1B} receptors in the CNS. *AVPR1A* and*AVPR1B* genes encode each, only one transcript variant and the V_{1A} and $V1_B$ receptors respectively.

V_{1A} receptor is involved in maternal care, social recognition, affiliative behaviour and pair bonding (Koshimizu et al., 2012). Administration of the V_{1A} antagonist d(CH2)5Tyr(Me)AVP into the medial amygdala of rats affects maternal memory (Nephew and Bridges, 2008). Furthermore, *Avpr1a* KO mice and hamsters display defective social memory, interaction and communication, reduced anxious-like behaviours and inconsistent levels of aggressive behaviour across species (**Table S3**). Several studies have associated the length of the promoter and the 5'UTR of the*AVPR1A* gene, which regulate V_{1A} expression levels, with important social deficits. Indeed, most variants associated with ASD risk are identified in these regions (**Table S2**), which either influence human relationships and altruism (Walum et al., 2008; Meyer-Lindenberg et al., 2009), personality in primates (Hopkins et al., 2012) or social behaviour in rodents (Hammock et al., 2005). Recently, administration of the selective V_{1A} antagonists RG7713 or balovaptan improved socialisation and communication in men with ASD (Umbricht et al., 2017; Bolognani et al., 2019; Schnider et al., 2020). Despite these promising results in phase 2 clinical trials, balovaptan failed to improve social abilities over placebo in phase 3 (Jacob et al., 2022). Further investigations are still required to understand the therapeutic potential of $V_{1A.}$as it is not yet clear whether it should be activated or inhibited to improve social skills.

V_{1B} receptor deletion in mice (*Avpr1b*KO) leads to increased dominance, decreased aggressive behaviour and vocalisations and impaired motivation and social memory (**Table S3**). Three independent studies have identified variants in the*AVPR1B* gene linked to ASD (**Table S2**), mood disorders and aggressive behaviour. Accordingly, administration of the antagonist nelivaptan (**Table S4**) normalizes aggressive, chasing and anxious-like behaviours in rodents (Blanchard et al., 2005; Salomé et al., 2006). Oral administration of nelivaptan is currently in clinical trials for anxiety and depression.

In conclusion, data in animals and humans support that OTR and V_{1A} receptors may be involved in the aetiology of autism and are major therapeutic targets for ASD, whileV_{1B}might be of interest for aggressive and anxious-like behaviours. Nevertheless, so far, clinical trials failed to bypass the placebo effect observed in patients. Regarding their conservation, their crosstalk and the existence of homo- and hetero-oligomers of these three receptors (Terrillon et al., 2003; Dekan et al., 2021), further investigations are needed to identify the most suitable targets (*e.g.* , which receptor or oligomer, which signalling pathway) and respective ligands of this family.

Metabotropic glutamate and GABA receptors

Glutamate and γ-aminobutyric acid (GABA) are the two major neurotransmitters in the CNS. They bind their cognate class C GPCRs, metabotropic glutamate mGluRs and $GABA_B$ receptors respectively. In contrast to class A GPCRs, glutamate and GABA bind to the large extracellular N-terminal domain called the Venus Fly Trap, which closes upon activation. In addition, they form constitutive oligomers, which lead to specific rearrangements of subunits during activation. They are mainly expressed in pre- and postsynaptic compartments in the brain (**Figures 2-3**) and participates in the excitatory and inhibitory balance in the CNS (Nelson and Valakh, 2015), which is hypothesised to be dysregulated in ASD.

$mGlu_5$

The *GRM5* gene encodes two splice variants of $mGlu_5$ ($mGlu_{5a}$ and $mGlu_{5b}$), with the $mGlu_{5b}$ receptor expressed predominantly during the adult stage (**Table S6**). Activation of $mGlu_5$ induces synaptic plasticity, which requires *de novo* mRNA translation through phosphorylation of eIF2α (Di Prisco et al., 2014). *Grm5* KO mice display ASD-related core symptoms (**Table S3**), deficits in social interaction, increased stereotyped and compulsive behaviours. Furthermore, they show hyperactivity, reduced anxious-like behaviours and sensorimotor gating deficits (Brody et al., 2004; Xu et al., 2021). Five independent studies have identified over twenty rare variants in the *GRM5* gene of ASD patients (**Table S2**), highlighting *GRM5* as one of the

most susceptible genes in ASD (Nisar et al., 2022). Alterations in $mGlu_5$ receptor signalling or expression affect synaptic and neuronal development, trademarks of ASD and intellectual disability (D'Antoni et al., 2014). Higher $mGlu_5$ protein expression was reported in different brain regions including cerebellar vermis region and superior frontal cortex in children with ASD (Fatemi et al., 2011) and in prefrontal cortex of patients with monogenic Fragile X syndrome (FXS) (Lohith et al., 2013). In contrast, lower $mGlu_5$ mRNA and protein expression was reported in the dorsolateral prefrontal cortex of ASD patients (Chana et al., 2015). Thus, administration of the selective antagonist mavoglurant and the NAM basimglurant in the FXS mouse model (*Fmr1* KO) (**Table S4**) improved its broad range of phenotypes (Scharf et al., 2015). However, administration of these compounds failed to provide similar therapeutic benefits in FXS patients in phase 2b/3 clinical trials (Jacquemont et al., 2011, 2014; Lozano et al., 2015). These compounds are still in clinical trials for dyskinesia, obsessive–compulsive disorders and depression. Altogether, data favour the role of $mGlu_5$ in ASD pathogenesis and explain why it is one of the first GPCRs targeted for ASD. However, targeting $mGlu_5$ even with a selective negative allosteric modulator did not pass the placebo effect in patients. This might be attributed to differences in receptor expression.

$mGlu_7$

The *GRM7* gene encodes two isoforms ($mGluR_{7a}$, $mGluR_{7b}$) that differ in their C-terminus, potentially leading to different protein-protein interactions and receptor coupling. $mGlu_7$ is expressed during the critical neurodevelopmental period, when it augments synapse formation and stabilisation (Song et al., 2021). Compared to other mGluRs, $mGlu_7$ has less affinity for glutamate, hence is considered as an "emergency brake". $mGlu_7$ are predominantly localised at presynaptic sites that regulate neurotransmitters release of glutamate or GABA. Interestingly, the non-selective $mGlu_{4,6,7}$ agonist L-AP4 negatively regulates glutamate or GABA release whereas the selective PAM AMN082 positively affects the extracellular glutamate levels and negatively the GABA levels (Manahan-Vaughan and Reymann, 1995; Mitsukawa et al., 2005; Li et al., 2008). *Grm7* KO mice display intact social interaction, but social memory deficits (**Table S3**), which might be explained by their global learning defects. Overall, *Grm7* KO mice and mice carrying the Ile154Thr $mGlu_7$ mutation identified in ASD patients recapitulate comorbid symptoms, such as anxious-like behaviours, motor coordination impairment and seizures (Fisher et al., 2020, 2021). 21 SNPs and CNVs in the *GRM7* gene have been associated with ASD (**Table S2**). In particular, the Ile154Thr, Arg658Trp and Thr675Lys mutants lead to reduced $mGlu_7$surface expression and/or degradation. Dysregulated levels of $mGlu_7$ results in lack of axonal growth due to altered cAMP-PKA-ERK signalling and reduced number of synapses in primary neuronal cultures, which is rescued by the PAM AMN082 (Song et al., 2021). This is in line with reduced expression of $mGlu_7$in post-mortem motor cortex samples from patients with Rett syndrome (RTT) and in a mouse model of RTT (*Mecp2* KO) (Bedogni et al., 2016; Gogliotti et al., 2017). In conclusion, $mGlu_7$ is a promising target as it could contribute to ASD pathogenesis. Furthermore, selective agonists or PAM (e.g., AMN082) already exist and normalise comorbid symptoms in mouse models of ASD via the regulation of glutamate and GABA release, and possibly in patients as well.

$GABA_B$ receptor

Metabotropic $GABA_B$ receptors are obligatory hetero-oligomers of $GABA_{B1}$ and $GABA_{B2}$through their C-terminus coiled-coiled domain. Presynaptic $GABA_B$ receptors suppress neurotransmitter release whereas postsynaptic receptors induce slow inhibitory postsynaptic currents, which shunt the excitatory currents (Lüscher et al., 1997). $GABA_B$ receptor deletion in mice (*Gabbr1-Gabbr2*double KO) leads to stress-induced social withdrawal, emotional behavioural disturbances and increased anxious- and anti-depressive-like behaviours (**Table S3**). The effect of *Gabbr2* deletion alone has not been reported yet. However, *Xenopus tropicalis*tadpole larvae carrying the Ala567Thr, Ser695Ile and Ile705Asn $GABA_{B2}$ mutants identified in ASD and epileptic patients, display increased seizure-like behaviour and altered swimming patterns that are partially rescued by the selective $GABA_B$agonist baclofen (Yoo et al., 2017). Of note, when expressed in heterologous HEK293 cells, these three mutants disrupt $GABA_B$ activation. Four genomic studies revealed the association of the *GABBR2* gene with ASD and RTT (**Table S2**). Administration of baclofen normalises the behaviours observed in a mouse model of FXS, in an idiopathic BTBR mouse model of ASD and in the

C58 inbred mouse strain (Henderson et al., 2012; Silverman et al., 2015). Despite its first beneficial effect and its good tolerance in FXS and ASD patients, baclofen clinical trials were discontinued after phase 2 for its lack of efficacy (Berry-Kravis et al., 2012; Veenstra-VanderWeele et al., 2017). Nevertheless, baclofen is currently tested as an adjuvant therapy to risperidone for irritability (Mahdavinasab et al., 2019). Finally, in agreement with the unbalanced GABA and glutamate transmission hypothesis in ASD, reduced expression levels of the $GABA_B$ receptor were observed in the cerebellum, in the cingulate cortex and in the fusiform gyrus of ASD patients (Fatemi et al., 2009; Oblak et al., 2010).

In conclusion, $GABA_B$ receptor remains a promising therapeutic target for ASD according to the genomic and genetic data in patients and in animal models. However, its efficacy might be greater in combination with the administration of other ligands, such as risperidone for irritability or drugs targeting mGluRs to restore the excitatory and inhibitory balance.

Biogenic amine receptors

Biogenic amine receptors are class A GPCRs that interact with endogenous aminergic ligands, such as adrenaline, noradrenaline, dopamine and serotonin (5-hydroxytryptamine, 5-HT).

Dopamine receptors

Dopaminergic D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors regulate broad functions: locomotion including voluntary movement, reward processing, learning, motivated behaviour, action selection, sleep, attention, and decision making (Mishra et al., 2018), some of which, when dysregulated, are comorbid symptoms of ASD (DiCarlo et al., 2022).

D_1 receptors are particularly enriched in D_1 striato-nigral GABAergic medium spiny neurons of the striatum. They have the lowest dopamine affinity among all the dopaminergic receptors, suggesting that they are activated by high phasic dopamine release, while D_2-like receptors might detect low tonic dopamine levels (Beaulieu and Gainetdinov, 2011). D_1 receptor may have a role, although controversial, in social behaviour (Scerbina et al., 2012; Campi et al., 2014). Rat carrying the Ile116Ser mutation in the D_1 receptor exhibited ASD-like social symptoms with reduced social interaction (sociability and social novelty) and ultrasonic vocalisations in pups while calling their mothers (**Table S3**). However, no stereotyped behaviours were observed for this rat model. This mutant has reduced expression at the cell surface and impaired G protein coupling. Administration of the D_1 receptor antagonist SCH23390 ameliorated stereotyped behaviours in mice lacking the tyrosine hydroxylase that catalyses dopamine synthesis (Chartoff et al., 2001). Furthermore, administration of the approved antipsychotic antagonist flupentixol at low doses reduced the rate of deliberate self-harm injuries in schizophrenic patients (Ruhrmann et al., 2007; Witt et al., 2021). The antagonist ecopipam is currently in phase 2 clinical trials for the treatment of Tourette's syndrome, characterised by repetitive tics (Gilbert et al., 2018). Conversely, excessive activation of the D_1 receptor induces an autistic-like phenotype in WT mice (Lee et al., 2018). Lastly, one study reported that three common SNPs located in the 5'UTR of the *DRD1* gene (**Table S2**) are associated with severe impairments in social interaction, non-verbal communication and increased motor stereotypies.

D_2 receptors encoded by the *DRD2* gene comprise two splicing isoforms, short D_{2S} and long D_{2L} differing in their IL3. D_{2S} serves as an auto-receptor regulating dopamine release and dopamine synthesis while D_{2L} is a postsynaptic receptor (Negyessy and Goldman-Rakic, 2005). These receptors are mainly expressed in neurons, with the highest levels in GABAergic indirect D_2striato-pallidal medium spiny neurons of the striatum, but also in astrocytes and oligodendrocytes (**Figure 2**). D*rd2* KO mice show great impairments in social behaviour (sociability and social novelty), impaired social olfaction and stereotyped behaviours (**Table S3**). Moreover, *Drd2* heterozygous mice exposed to early maternal separation stress also display social interaction deficits and stereotyped behaviours. This phenotype seems exclusively mediated by the dorsal striatum as specific knock-down of the D_2 receptor in this structure is sufficient to recapitulate all the behavioural impairments reported in *Drd2* KO mice (Lee et al., 2018). Conversely, D_2 receptor overexpression in the striatum and olfactory tubercle revealed impairment in sociability only in female mice and vocalisation. Among the dopamine receptors, the *DRD2* gene displays the highest number of SNPs

associated with ASD (**Table S2**). Currently, the only available approved treatments for ASD patients are antipsychotics (**Table S4**), such as aripiprazole or risperidone, which antagonise D_2 receptor in addition to other GPCRs, to treat irritability, aggressive and repetitive behaviours (McDougle et al., 2005; Varni et al., 2012). Additionally, two other D_2 antagonists, pimozide and olanzapine, are antipsychotics used in clinics for schizophrenia and Tourette's syndrome, leading to potential amelioration of speech impairment (Maguire et al., 2004; Pringsheim and Marras, 2009).

D_3 receptor expression is conserved between humans and rodents and controls habituation to novelty (Mishra et al., 2018). *Drd3* KO mice display hyperactive and addictive behaviours, with particular vulnerability to alcohol and drug abuse (**Table S3**), but their social skills or stereotyped behaviours have not been reported yet. Three independent studies identified three SNPs in the*DRD3* gene associated with ASD (**Table S2**). The antipsychotic cariprazine, a partial agonist for D_3 that also binds D_2 receptors with lower affinity, is approved for the treatment of schizophrenia and bipolar disorder. Interestingly, administration of cariprazine improved social behaviours in a dose-dependent way in male rat models of ASD exposed to valproic acid*in utero* (Román et al., 2021), which makes this ligand a potential treatment for ASD.

In conclusion, alteration in any of these three dopaminergic receptors result in autistic-like symptoms in animal models and in genetic association with ASD. However, approved ligands targeting the D_2 receptor are already on the market to ameliorate autistic symptoms, especially repetitive behaviours, and could be tested for social symptoms, highlighting this receptor as a promising target for ASD treatment. Nonetheless, other dopamine receptors might be of interest; for example, the less known *DRD5* gene that display the highest number of distinct missense and loss of function variants in the general population (Hauser et al., 2018).

Serotonin 5-HT_{1B}

Dysregulation in 5-HT levels in different CNS structure has been observed in ASD (Pourhamzeh et al., 2022) while enhanced 5-HT release restores social deficit in several ASD mouse models (Walsh et al., 2021). The dup15q11-q13 mouse model of ASD displays reduced serotoninergic activity of the dorsal raphe nucleus, associated with low 5-HT levels in all CNS regions and impaired social interaction (Farook et al., 2012; Nakai et al., 2017). As more than 25% of the ASD patients show increased 5-HT blood levels, 5-HT is considered as a biomarker for a subgroup of patients (Gabriele et al., 2014; Muller et al., 2016). All 14 serotonin receptors encode a GPCR, except the channel receptor 5-HT_3. They modulate cognition, memory, sleep, appetite, respiration, thermo-regulation and mood (Berger et al., 2009). 5-HT_{1B} belongs to the 5-HT_1 receptor family that are encoded by 7 genes (*HTR1A-F*). It exerts a consistent effect on anxious-like behaviours, as administration of the selective full agonist CP94253 or antagonists SB 216641 and GR 127935 (**Table S4**) leads to anxiogenic or anxiolytic effect in rodents. In addition, administration of CP94253 reduced aggressive behaviour in resident male mice, whereas anpirtoline, that also targets 5-HT_3 channels, restored isolation-induced impairments, increased pain threshold and exerted anti-depressive effects in mice (Schlicker et al., 1992; Fish et al., 1999). In agreement with pharmacological studies, *Htr1B* KO mice display decreased anxious-like behaviours, exacerbated aggressive behaviour, deficits in maternal behaviour, improved cognitive flexibility and vulnerability to drug abuse (**Table S3**). So far, only two variants associated with ASD have been reported from two independent studies (**Table S2**).

Beside the *HTR1B* gene, evidence has highlighted that the*HTR2A* gene fulfils the SFARI criteria as a strong candidate and targeting 5-HT_6 or 5-HT_7 improve core symptoms in mouse models of ASD (**supporting information**). Thus, potentially the 14 serotonin receptors are of interest for ASD. Highly promising drugs targeting multiple 5-HT receptors, such as arylpiperazine derivative drugs (Lacivita et al., 2021) and the first 5-HT_7 biased agonist (El Khamlichi et al., 2022) could be tested to improve core ASD symptoms. Currently, antipsychotic (aripiprazole and risperidone) administration to treat irritability, aggressive and repetitive behaviours in ASD patients partially activates 5-HT_{1A} and inhibits 5-HT_{2A}.

β2-αδρενοςεπτορ

Noradrenaline and adrenaline activate the α_1, α_2, β_1, β_2 and β_3-adrenoceptors with different potencies. In

the CNS, they control cognition, memory, emotions and stress-induced behaviours. Only the *ADRB2* gene is present in the SFARI list and encodes the β_2-adrenoceptor. Despite its vital cardiac function,*Adrb2* KO mice are fertile and viable and display increased anxious-like behaviours and decreased depressive-like behaviours (**Table S3**). Increased adrenergic neuron activity from the locus coeruleus or increased noradrenaline plasma concentration has been associated with aberrant attention and decreased interest in ASD individuals (Bast et al., 2018; Beversdorf, 2020). Accordingly, two common SNPs associated with ASD (**Table S2**) show enhanced isoproterenol agonist-induced response. Furthermore, studies have suggested an association between prenatal exposure to β_2-adrenoceptor agonists and ASD (Gidaya et al., 2016). Interestingly, administration of the approved β_2/β_3-adrenergic antagonist propranolol improves verbal responses and social interactions and decreases anxiety in ASD patients (Hegarty et al., 2017). Therefore, the β_2-adrenoceptor is an interesting target for ASD and the approved drug propranolol (**Table S4**) may help to normalise core social symptoms. Further investigations should also address the potential interest of the other members of this family, such as*ADRA1D,* one of the most downregulated genes in the prefrontal cortex of ASD patients (Monfared et al., 2021) whose targeting with clonidine improves hyperarousal, hyperactivity and social relationships in individuals with ASD (Ming et al., 2008).

Other class A receptors

Adenosine receptors

Adenosine receptors are divided into four different subtypes, namely A_1, A_{2A}, A_{2B} and A_3, among which A_1 and A_{2A} show the highest affinity for adenosine (Alexander et al., 2021). Brain adenosine receptors have important roles in different processes, such as neuroplasticity, sleep-wake cycle, locomotion, and cognition (Wei et al., 2011).

A_{2A} receptor encoded by the *ADORA2A*gene, stimulates glutamate release at presynaptic terminals and myelination by oligodendrocytes (De Nuccio et al., 2019). Post-synaptic A_{2A} receptors are highly enriched in D_2GABAergic striato-pallidal medium spiny neurons of the striatum to modulate locomotion and anxious-like behaviours (Coelho et al., 2014). Accordingly, *Adora2a* KO mice display motor impairment and anxious-like behaviours (**Table S3**). A_{2A} might act as a regulator of other GPCRs (**Table S5**), as it forms many different hetero-oligomers with D_2, $mGlu_5$, δ opioid receptors and orphan GPR88 (Ciruela et al., 2011; Pellissier et al., 2018; Laboute et al., 2020). During brain development, GABAergic synapses, which release adenosine and ATP in addition to GABA, are the first synapses to be formed and are crucial for the construction of the neural network. Activation of A_{2A} receptors is necessary and sufficient to prune GABAergic synapses during this period (Gomez-Castro et al., 2021). In contrast, any impairment in A_{2A} signalling or expression may result in GABAergic synapse alteration and cognitive deficits in adults, as observed in animals administered with A_{2A}antagonists during development. Spontaneous stereotypies often result from unbalanced cortical glutamatergic and GABAergic afferences (glutamate hyperactivity) on the striatum and decreased activation of the efferent subthalamic nucleus, as observed in ASD patients and animal models (Li and Pozzo-Miller, 2020). Consistently, administration of the selective A_{2A} agonist CGS21680 normalises aberrant vertical repetitive behaviours in BTBR and C58 inbred mice (Amodeo et al., 2018; Lewis et al., 2019) via its action on D_2medium spiny neurons, which in turn restore the neurotransmission on efferent subthalamic nucleus. So far, only one study has associated four SNPs in the *ADORA2A* gene with autism and severe anxiety (**Table S2**).

The **A_3 receptor** promotes expression of the serotonin transporter (SERT) to the cell surface (Campbell et al., 2013). Thus, lack of A_3 signalling decreases SERT cell surface expression and leads to extracellular accumulation of serotonin, as observed in ASD patients (see section 4 on serotonin). Actually, two variants in the *ADORA3* gene are associated with ASD, (**Table S2**) with impaired adenosine binding (Campbell et al., 2013). Consequently, *Adora3* KO mice show anxious-like and despair behaviours (**Table S3**).

In conclusion, A_{2A} shows the greatest promise to mitigate repetitive behaviours and anxiety in ASD. However, most of A_{2A} agonists have failed in clinical trials (Guerrero, 2018) due to severe side effects, including CNS

excitotoxicity (**Table S4**). Only few of them have been approved, such as the agonist regadenoson. An alternative strategy would be to consider other members of this family, such as the *ADORA1* gene, which is one of the most downregulated GPCR genes in patients (Monfared et al., 2021) and whose targeting in combination to A_{2A} agonists improves stereotyped behaviours (Lewis et al., 2019). A better specificity could also be achieved by targeting A_{2A}hetero-oligomers such as D_2-A_{2A}-mGluR$_5$ to avoid side effects.

Angiotensin AT_2 receptor

Angiotensin receptors are divided into AT_1 and AT_2 subtypes. They are activated by different maturation products of angiotensinogen peptides, namely angiotensin II and III. Only the AT_2 receptor is implicated in different neurological disorders such as ASD, schizophrenia, Parkinson's disease (PD) and Alzheimer's disease (Firouzabadi et al., 2016; Szczepanska-Sadowska et al., 2022). Its functions in the brain remain elusive. To date, no data from genetic or pharmacological manipulation support a role of AT_2 in social or stereotyped behaviours (**Table S3**). However, *Agtr2* KO mice show impaired reward processing and locomotion. Interestingly, administration of the AT_2 receptor selective agonist C21/M024 improves cognition in a mouse model of Alzheimer's disease (Jing et al., 2012). Finally, four independent studies have associated the *AGTR2* gene on the chromosome X with ASD and X-linked intellectual disability (**Table S2**). Therefore, together with its unknown expression and function in the CNS, further studies are required to conclude on the therapeutic potential of AT_2.

Cannabinoid CB_1 receptor

Endogenous cannabinoids regulate dopamine circuits that are crucial for reward processes linked to addiction and for synaptic transmission through neurotransmitter release modulation (Zhang et al., 2004). Cannabinoid receptors are composed of CB_1 and CB_2. CB_1 mediates the central effects of cannabis and its derivatives. *Cnr1* KO mice show deficits in social interaction and communication, two core symptoms of ASD (**Table S3**). They also exhibit anxiogenic, context-dependent social aggressive and depressive-like behaviours and improved social memory. Interestingly, administration of endocannabinoids improves social interactions via the potentiation of reward processes and inhibition of social anxiety in BTBR and *Fmr1* KO mice (Wei et al., 2017). Three independent studies have reported more than thirty variants in the *CNR1* gene associated with ASD (**Table S2**). Some states in the USA have already authorised cannabis to treat self-injurious or aggressive behaviours in ASD patients. While the first results of clinical trials with a combination of cannabidiol and delta-9-THC showed no side effects, but mitigated results (Aran et al., 2021), few case studies have shown improvement of core and comorbid symptoms in children (Carreira et al., 2022). Thus, further testing is required and will be obtained with the administration of CB_1 NAM cannabidiol or endocannabinoid mix that are currently in clinical trials for ASD (Aran et al., 2021). In conclusion, multiple evidences highlight CB_1 receptor as one of the most promising GPCR target to treat core and associated symptoms in ASD.

Chemokine CX_3CR1 receptor

Chemokine receptors are a vast family of GPCRs involved in the immune system. Both secreted and membrane-bound chemokine CX3CL1 activate the C-X3-C motif chemokine receptor 1 (CX_3CR1 or GPR13). In humans, the *CX3CR1* gene encodes 4 transcript variants and two protein isoforms that differ in their N-terminus domain (Marti-Solano et al., 2020). CX_3CR1 is expressed on microglia where it is activated by CX3CL1 release from neurons upon inflammatory response and during synaptic maturation and pruning (Jung et al., 2000; Soriano et al., 2002; Zhan et al., 2014). *Cx3cr1* KO mice display social interaction deficits and increased motor stereotypies (**Table S3**), associated with decreased functional brain connectivity from the prefrontal cortex, similarly to observations in ASD patients. Moreover, in animals exposed to social isolation, levels of *Cx3cr1*transcripts were increased in the prefrontal cortex, nucleus accumbens and hippocampus (Zhou et al., 2020). Three rare missense deleterious mutations in the *CX3CR1* gene have been associated with schizophrenia and ASD (**Table S2**).

In conclusion, CX_3CR1 plays a major role in neuron-microglia mutual interaction, highlighting the growing evidence of microglia in neurodevelopmental disorders, including ASD (Lukens and Eyo, 2022). CX_3CR1 is a

promising target to treat ASD. However, development of specific compounds will be necessary to demonstrate its beneficial effect.

Muscarinic acetylcholine M_3 receptor

In addition to ionotropic receptors, acetylcholine activates five muscarinic M_1-M_5 GPCRs. Whereas many receptor ligands, including allosteric modulators, have been reported, only few of them are selective for a receptor subtype (**Table S4**). The *CHRM3* gene is complex, spans over 550 kb and includes 7 exons, with only exon 7 encoding the M_3 receptor. It has 10 described and 21 predicted transcript variants. Like other muscarinic receptors, M_3 modulates excitatory transmission, neuronal development including cellular proliferation and survival, neuronal differentiation and controls food intake, learning and memory (Yamada et al., 2001; Poulin et al., 2010). *Chrm3* KO mice or knock-in of a mutant receptor whose IL3 cannot be phosphorylated, significantly altered hippocampus-dependent contextual fear memory formation and decreased paradoxical sleep (**Table S3**). However, no study has investigated the ASD-like symptoms in these animals nor the therapeutic potential of muscarinic ligands. Seven variants have been associated with ASD in six independent studies (**Table S2**), suggesting the potential involvement of M_3 in ASD aetiology. Interstitial deletion in the 1q43 region, which mostly affects the *CHRM3* gene, is associated with ASD, intellectual disability, seizures, microcephaly and congenital malformation (van Bever et al., 2005; Hiraki et al., 2008). Whereas reduced cholinergic enzyme activity has been observed in cortical areas of ASD patients (Perry et al., 2001), further evidence is needed to conclude on the potential interest of muscarinic receptors as therapeutic targets for ASD.

Orphan and olfactory receptors

Hundreds of orphan and olfactory GPCRs are expressed in the CNS and represent new potential therapeutic targets for neurological disorders (Khan and He, 2017) including ASD. Interestingly, orphan GPR37 and GPR85 are the top dysregulated GPCR genes in ASD tissues (Monfared et al., 2021). Except their classification by sequence homology to the class A of GPCRs, the study of orphan or olfactory receptors remains challenging due to the lack of any identified ligand or poorly known function.

GPR37

GPR37 or parkin-associated endothelin-like receptor (Pael-R) is closely related to endothelin GPCRs. Several potential natural peptides have been reported activating GPR37 (**Table S4**), but remains to be confirmed. GPR37 is characterized by a poor export from ER to plasma membrane in heterologous cell lines, which is either rescued by deletion of its long N-terminus domain, oligomerization with A_{2A}or D_2 receptors, or interaction with syntenin 1 through their PDZ domain (Dunham et al., 2009; Hertz et al., 2019). GPR37 is up-regulated during oligodendrocyte differentiation where it inhibits late-stage differentiation and myelination (Yang et al., 2016). GPR37 is also located in dopaminergic axon terminals of the substantia nigra where it controls dopamine release through a direct interaction with the dopamine transporter (Marazziti et al., 2007). *Gpr37* KO mice display obsessive compulsive behaviours, decreased locomotion, reduced colon motility and abnormal sensorimotor gating (**Table S3**). They may have increased anxious-like behaviours, but this phenotype varies depending on the tests, sex and housing conditions. Conversely, transgenic mice overexpressing *Gpr37* show increased methamphetamine-induced stereotyped behaviours, motor coordination and locomotion (Imai et al., 2007). Despite social interactions remain to be investigated, *Gpr37* mice rather display a large variety of comorbid symptoms of ASD associated with altered striatal dopamine signalling, a feature of ASD (Li and Pozzo-Miller, 2020). Interestingly, variants of the dopamine transporter gene, its direct interactor, are also associated with ASD (DiCarlo et al., 2019) and lead to similar alterations of dopamine transmission in the striatum. The *GPR37*gene has been identified in the first autism locus (AUTS1) on chromosome 7q31–33. Since then, nine variants in this gene have been associated with ASD (**Table S2**). Therefore, several pieces of evidence confirm that GPR37 might be an interesting target for ASD. However, selective ligands should be developed and tested in preclinical models to further strengthen its therapeutic potential for ASD.

GPR85

GPR85/SREB2 belongs to the super-conserved receptor expressed in the brain (SREB) family. The *GPR85* gene encodes 7 predicted and 3 transcript variants due to alternative splicing of the 3'UTR. They all encode the extremely conserved GPR85, which shares 100% homology and strong expression throughout the CNS in humans and mice (**Figure 3**). It is expressed in all types of neurons and microglia (**Figure 2**). At the molecular level, GPR85 directly interacts with SHANK3 or PSD95 scaffolding partners through its PDZ domain in its C-terminus, and indirectly with neuroligin through PSD95 (Fujita-Jimbo et al., 2015; Jin et al., 2018). In the adult hippocampus, GPR85 negatively regulates neurogenesis and dendritic morphology, thereof controlling brain size (Chen et al., 2012). *Gpr85* KO mice display increased neurogenesis associated with enlarged brain size and increased cognitive abilities in spatial tasks (**Table S3**). Conversely, mice overexpressing *Gpr85* in forebrain neurons show core symptoms of ASD, social interaction deficits and restrictive behaviours, in addition to cognitive inabilities, abnormal sensorimotor gating and reduced dendritic arborisation (Matsumoto et al., 2008; Chen et al., 2012). Two independent studies reported five variants in the human *GPR85* gene in Japanese ASD patients (**Table S2**), including one variant in the 3'UTR. Furthermore, two studies have found downregulated *GPR85* transcripts and decreased *GPR85* splicing events in the cortex of ASD patients (Voineagu et al., 2011; Monfared et al., 2021). Interestingly, increased *Gpr85* mRNA levels have been found in the striatum and prefrontal cortex of mice overexpressing Shank3 (Jin et al., 2018). Although studies on GPR85 remain sparse and no drug are available, data from mice and patients converge on its therapeutic potential to improve social interaction deficits.

Olfactory receptors

In humans, 387 genes encode olfactory receptors (OR) in addition to 462 pseudogenes. ORs, encoded by a single exon, are subdivided in aquatic ancestry class I receptors clustered on human chromosome 1 (OR1-15) and the largest terrestrial ancestry class II (OR51-56) located on different chromosomes (Olender et al., 2020). They detect odorant volatile molecules, although most of them remain orphan. Since their discovery, growing evidence have shown OR expression outside the olfactory epithelium, primarily in testis, then in most tissues, including the CNS. Their roles in development, chemotaxis, tissue injury and regeneration are starting to be deciphered. Only few studies have associated the *OR1C1* , *OR2M4* , *OR2T10* and *OR52M1* genes with ASD, with the strongest evidence for *OR1C1* (**Table S2**). Furthermore, other OR genes were also identified in association studies (Ruzzo et al., 2019), in particular with schizophrenia. Often qualified as 'ectopic' outside the olfactory epithelium, their expression is rather conserved among species (De la Cruz et al., 2009; Olender et al., 2016). OR1C1, OR2M4 and OR2T10 are present in the CNS, in contrast to OR52M1, which is conserved between humans and rodents (**Figures 2-3** , **Table S4**). OR1C1 and OR2T10, specific to apes, are both detected in the cortex, with OR1C1 also found in the pons, cerebellum, hippocampus and amygdala (**Table S6**). OR2M4 is conserved in apes, cows and pigs and is detected in neurons of most CNS areas. Their function remains to be elucidated in the CNS as no ligands nor animal models are available. In conclusion, the function of orphans and ORs only starts to be elucidated in the CNS and they could be of interest for ASD in the future with the development of selective drugs.

GENERAL CONCLUSIONS and FUTURE DIRECTIONS

In this review, we highlighted the involvement of the different GPCRs in the aetiology of ASD and as potential targets. We analysed the effect of GPCR variants on their level of expression, ligand binding, receptor folding or activation of downstream signalling pathways (**Table S2**). Variants located in introns, untranslated regions or coding regions of $mGlu_7$, 5-HT_{2A}, CB_1, GPR37 and GPR85 receptor genes are consistent with the decreased levels of transcript expression observed in patients (Monfared et al., 2021). Based on the evidences in ASD patients, their function and localisation (**Figures 2-3**), and their behavioural predictive validity in animal models (**Table S3**), we classified these GPCRs. OTR, V_{1A}, $mGlu_5$, D_2, 5-HT_{2A}, CB_1 and GPR37 receptors fulfil most, if not all, criteria. V_{1A} display a clear potential for social interaction, D_2 and GPR37 for stereotyped behaviours, and $mGlu_5$, OTR, CB_1 and 5-HT_{2A} eventually for both core symptoms. Overall, it is surprising that out of 800 GPCRs, only 23 GPCRs are included in the SFARI list, and that all these genes are classified in the second category, namely 'strong candidate gene'. We propose to move OTR, CB_1 and V_{1A} receptors to the first category 'high confidence genes', and add 5-HT_{2A} to the list. In this

review, we also suggest $GABA_{B1}$, 5-HT_6, 5-HT_7, D_4 and D_5 has potential candidates for ASD. Increasing pieces of evidence showed the functional crosstalk between GPCRs within a cell, to control a specific function, such as D_2, A_{2A} and $mGlu_5$ for the control of motor activity (Ciruela et al., 2011). In fact, independently of their physical interaction, GPCRs are not individual entities but should rather be considered as a set of GPCRs and isoforms working together in a cell to orchestrate the different signals and regulate downstream signalling network and related cellular processes. Up to hundreds of GPCRs are expressed in the same brain structures or cell types, with the highest diversity in the striatum, cortex and hypothalamus (Vassilatis et al., 2003; Marti-Solano et al., 2020). In 2018, Babu and colleagues identified hundreds of missense and CNV variants in the genes coding for V_{1B}, D_1, D_2, and D_3, 5-HT_{2A}, β_2-adrenoceptors and $GABA_B$ (Hauser et al., 2018) that might influence their ligand binding or transducer recruitment (**Table S4**). This area of research based on receptor bias is known as pharmacogenomics. Considering the major impact of GPCR signalling (**Table S5**) that are altered in ASD (De Rubeis et al., 2014; Hormozdiari et al., 2015; Gazestani et al., 2019), any slight modification in a GPCR or in a combination of GPCRs would lead to drastic signalling defects and neuronal pathogenicity. Here, we found that at least 15% of the genes listed in the SFARI database are in the signalling networks and cellular downstream processes of GPCRs. Therefore, application of pharmacogenomics to hundreds of GPCRs expressed in the CNS remains an outstanding hypothesis to fully decipher the global effect of GPCRs on pathological processes underlying ASD. Therefore, the impact of GPCRs for autism research has only begun to be highlighted, and rather than a single entity, GPCRs should be considered as one global functional unit of GPCRs expressed in a cell that control signalling networks, in order to understand their contribution to ASD aetiology.

GPCRs meet all the criteria of therapeutic targets for ASD to bypass the placebo effect observed in clinical trials. They contribute to the polygenic ASD aetiology, pathogenic variants are recessive, they are therapeutically rescuable, and they are *per essence* membrane receptors that display a large pharmacopeia of safe and efficient drugs. We analysed the therapeutic potential of these 26 GPCRs and categorized them as 'high', 'moderate' and 'low' candidates, based on 1) drugs that are already approved or tested for a related disorder, 2) their beneficial effects in animal models of ASD or in clinical trials, 3) their pharmacogenomic profile and 4) their safety (**Table S4** ,**S6**). We classified $mGlu_5$, $GABA_B$, D_2, 5-HT_{2A}, 5-HT_7, CB_1 as 'high' candidates and OTR, V_{1A}, $mGlu_7$, D_1, 5-HT_{1B} and M_3 as 'moderate' candidates due to the lack of selective ligands. However, the high number of variants of $mGlu_5$ and 5-HT_{2A} might compromise their responsiveness to drugs (Hauser et al., 2018) and might explain why $mGlu_5$-targeted clinical trials have failed. We excluded β_2-adrenoceptors, A_{2A}, and AT_2 as toxicity or severe side effects have been reported in clinical trials. Finally, despite their strong potential in the future, orphan and olfactory receptors belong to the 'low' category as there are no natural ligands or drugs clearly identified. We also ranked $CX3CR_1$, V_{1B}, $D_3$and A_3 as 'low' candidates, as no selective ligands have been developed and the adequate pharmacological profile remains to be investigated. Finally, considering their involvement in ASD aetiology, their therapeutic potential and results of clinical trials (**Figure 4**), we conclude that D_2, 5-HT_{2A}, CB_1, OTR, $V_{1A}$and GPR37 are the most promising targets for clinical development. D_2, 5-HT_{2A} and CB_1 are already ongoing for irritability, repetitive behaviours, aggressive and self-injury behaviours, but could be tested on other core symptoms, especially CB_1 on social scales. In the near future, when specific ligands will be developed, OTR, V_{1A} and GPR37 should be tested as well. The recent development of antibody fragments targeting GPCRs (Mujić-Delić et al., 2014) and the emergence of high throughput screening by DNA-based bar-coded chemical libraries (Madsen et al., 2020) should boost the identification of new drugs to target GPCRs, including orphan and olfactory receptors. Interestingly, antibody fragments display all types of pharmacological profiles, and can also be used as chaperones, or target oligomers of GPCRs. Considering that GPCRs function as a global GPCR module in a cell, a similar approach might also be applied for future drug development. Finally, the use of several drugs or of a drug targeting multiple GPCRs might be relevant for ASD. Such drugs already exist. For example, aripiprazole, risperidone or cariprazine targets multiple dopamine, serotonin, histamine and/or adrenoceptors (**Table S4**) or arylpiperazine derivatives target multiple 5-HT receptors (Lacivita et al., 2021). Based on their fine-tune pharmacology (biased ligands, oligomerization, global GPCR entities) and their diversity, GPCRs represent the greatest therapeutic options for ASD and hold the promise to successful clinical trials.

Abbreviations

ASD autism spectrum disorders

cAMP adenosine 3',5 cyclic monophosphate

CNS central nervous system

CNV copy number variants

CREB cAMP-responsive element binding protein

DAG diacylglycerol

ER endoplasmic reticulum

ERK extracellular signal-regulated kinase

FXS fragile X syndrome

GABA γ-aminobutyric acid

GIRK G protein-coupled inwardly-rectifying potassium channels

GPCR G-protein coupled receptor

GO gene ontology

5-HT 5-hydroxytryptamine

IP_3 inositol 1,3,4- triphosphate

KEGG Kyoto encyclopaedia of genes and genomes pathway

KD knock-down

KI knock-in

KO knock-out

NAM negative allosteric modulator

OR olfactory receptor

PAM positive allosteric modulator

PD Parkinson's disease

PKA protein kinase A

PLC phospholipase CRTK receptor tyrosine kinase

RTT Rett syndrome

SNP single nucleotide polymorphism

Tg transgenic animals

UTR untranslated region

WT wild type

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FIGURES

Figure 1 GPCR signalling and pharmacology

A) GPCR are composed of seven transmembrane domains connecting the extracellular domain [N-terminus, extracellular loops (EL) 1-3] where various ligands bind the receptor (*e.g.,* natural ligands and chemi-

cals/antibodies), to the intracellular domain [intracellular loops (IL) 1-3, helix H8, C-terminus] that recruit the direct transducers, which activate a signalling network (*e.g.,* Akt, ERK) and integrated cellular processes. A biased ligand has the particular pharmacological profile to favour signalling pathways within the complex receptor signalling network. Here, a G protein-biased ligand that favours G protein coupling (black arrow) over β-arrestin recruitment (grey arrow) is shown. The radial graph represents the maximum efficacy (Emax) of this G protein-biased ligand to stimulate the indicated signalling responses. The scale is indicated as % of the efficacy of the natural ligand. **B**) GPCRs display a rich pharmacopoeia of ligands, with agonists, antagonists and inverse agonists that bind to the orthosteric binding site (e.g., the binding site of the natural ligand) to respectively activate or prevent the agonist binding and inactivate the receptor. Other ligands bind to allosteric sites that increase or decrease the efficacy or efficiency of the natural ligand, respectively called positive or negative allosteric modulators.

Figure 2 At least 15% of SFARI genes participate in GPCR activity and signalling processes

GPCR ligands are synthesized by metabolic enzymes, loaded by their transporters into synaptic vesicles that fuse with the presynaptic membrane upon increase of intracellular calcium, leading to neurotransmitter release in the synaptic cleft. These neurotransmitters or ligands are either recaptured by membrane transporters, degraded, or bind and activate their cognate GPCR. Even in the absence of ligand, GPCRs are present in preformed higher complexes with scaffolding partners, channels, cytoskeleton and signalling transducers. Upon GPCR activation, transducers activate enzyme and channel effectors to produce second messengers. These second messengers activate major kinases and guanine nucleotide exchange factor (GEF) that tune up or down downstream cellular processes, including translation and transcription. Syndromic (in red), high confidence (category 1 in dark orange), strong candidate (category 2 in light orange) and suggestive evidence (category 3 in green) genes are coloured according to SFARI gene scoring and colour code (gene.sfari.org/about-gene-scoring, **Table S1**) and additional GPCR genes are in black. GPCRs are localized at pre, post-synaptic compartment of neurons, in astrocytes or in unknown or other cell types according to their expression pattern (see text for further details).

Figure 3 GPCR localisation and expression in the human and mouse brain

Relative expression and localisation of the 25 GPCRs are presented on the murine and human brain templates from the protein atlas database (www.proteinatlas.org). After comparison to protein expression for consistency (only available for the 5-HT_{1B}, 5-HT_{2A}, 5-HT_7, A_{2A}, V_{1B}, M_3, CB_1, CX_3CR1, D_2, $GABA_{B2}$ and GPR37), relative RNA levels are represented as high (brown, over 20 normalised transcript expression values, expressed as nTPM), moderate (red, 10-20 nTPM), low (pink, 2-10 nTPM) and just detectable (light pink, 0.1-2 nTPM) expression in the CNS (expression in the other organs are indicated in **Table S4 and S6**). Brain templates are from Servier Medical Art.

Figure 4 Workflow to assess the relevance to ASD of GPCR genes extracted from the SFARI database and their therapeutic potential

Twenty-three GPCRs have been considered in this review, and for each of them, the data obtained *in vitro* , in animal models (mainly mouse) and the features of GPCR variants and expression levels encountered in ASD patients have been discussed side-by-side (see text for further details).

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