Trends in Molecular Medicine

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REVIEWS

Opinion

P2X7 Receptor: A Potential Therapeutic Target for Depression?

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Depression is a prime contributor to global disease burden with 300 million affected patients worldwide. The persistent lack of progress with regards to pharmacotherapy stands in stark contrast to the pandemic magnitude of the disease. Alterations of inflammatory pathways in depressed patients, including altered circulating pro-inflammatory cytokines, have been put forward as a potential pathophysiological mechanism. The P2X7 receptor (P2X7R) plays an important role regulating the release of interleukin-1β and other cytokines. Comprehensive investigation of the P2X7R Gln460Arg missense mutation (rs2230912), which has been associated with major depression and bipolar disorder, has substantially contributed to validate P2X7R as a potential genetic risk factor. We propose that P2X7R is a putative target with good prospects for therapeutic intervention in depressive disorders.

Highlights

Converging genetic data associate the P2X7 receptor (P2X7R), a homotrimeric member of the P2X family of ATP-gated ion channels involved in diverse immune functions, with mood disorders.

Among the multiplicity of P2X7R SNPs, a unique mechanism has been described for the mood disorder-associated Gln460Arg polymorphism. This P2X7R variant does not differ functionally from its wild-type counterpart but their coassembly in heteromeric complexes leads to loss of channel function.

Coexpression of both receptor variants in transgenic humanized P2X7R mice results in impaired receptor function, altered sleep profile, and increased stress vulnerability. Notably, heterozygous healthy humans show alterations in their sleep architecture.

We propose that P2X7R represents a promising therapeutic target for the treatment of a subgroup of patients with mood disorders.

Depressive Disorders: New Targets for Therapeutic Intervention Awaited

Globally, 300 million people (4.4% of the world's population) suffer from **depressive disorders** (see Glossary), which entails significant personal, social, and economic burden. The World Health Organization (WHO) ranks depression as the single largest contributor to global disability documented by its contribution to 7.5% of all years lived with disability in 2015 [1]. The lifetime prevalence estimates for major depressive disorder (MD) vary from 11% to 14% (low- to middle-income countries versus high-income countries) with females having an approximately twofold higher disease risk than men. The biological underpinnings of depressive disorders remain largely unclear. Twin studies provide evidence for a genetic component with an estimated heritability of ~38% for MD [2,3]. In addition, environmental factors are believed to shape the disease risk. In particular, stressful life events across the lifespan play an important role in the disease etiology [4].

Current medications are almost exclusively based on the serendipitous discovery of mood-elevating substances (i.e., tricyclic antidepressants and monoamine oxidase inhibitors) in the 1950s, which led to the **monoamine hypothesis of depression** [5]. To this day, regularly prescribed pharmacotherapies are based on the discoveries made 60 years ago, with unsolved persisting complications including: (i) delayed onset of clinical improvement; (ii) enduring side effects; and (iii) limited response in a substantial group of patients – only ~50% of patients show full remission. Alternative neurobiological hypotheses of depression have not yet translated into efficient treatments and remain under extensive investigation. For example, disturbances in the main neuroendocrine stress response system, the **hypothalamic–pituitary–adrenal (HPA) axis** including its main initiator corticotropin-releasing hormone (CRH) and effector glucocorticoids, have received much attention [6]. Similarly, the **neurotrophic and neurogenic hypotheses** of depression have not resulted in any concrete therapeutic options [7]. The disease relevance of altered glutamatergic or **gamma-aminobutyric acid (GABA)**ergic

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neurotransmission is currently scrutinized by the hype on the ***N*-methyl-D-aspartate (NMDA)** receptor antagonist ketamine, which exhibits some indications of a rapid-acting antidepressant [8]. Nevertheless, the persistent stagnancy with regard to the pharmacotherapy for depression points towards a high demand for the establishment of novel innovative treatment options. In this regard, targeting immune system- and inflammation-related mechanisms and pathways may open new therapeutic avenues [9,10]. Alterations in the innate immune system and inflammatory responses have been repeatedly observed in patients with **mood disorders**. These alterations include increased concentrations of circulating cytokines such as IL-6, TNF-α, and IL-1β [11,12]. Furthermore, antidepressants have been demonstrated to decrease peripheral cytokines in depressed patients [13,14]. The comorbidity of MD with chronic systemic inflammatory disease [e.g., diabetes, cancer, stroke, rheumatoid arthritis (RA), Alzheimer's disease] further suggests that inflammation may predispose individuals to the development of depression [15,16]. Among the broad spectrum of inflammatory mediators, IL-1β has been recognized as one of the earliest and most potent proinflammatory cytokines secreted by activated inflammatory cells [17]. In the central nervous system (CNS), the key player involved in the secretion of biologically active IL-1β is **P2X7R**, an **ATP-gated ion channel** present on immune cells. The purinergic system has been implicated in the biology of various psychiatric disorders [18]. However, the most direct evidence is based on the association of the *P2RX7* gene with mood disorders [i.e., MD and **bipolar disorder (BD)**] [19,20].

We propose that there is good evidence of a genetic association between the *P2RX7* gene and mood disorders and point out its interaction with stressful environmental factors. We provide insights from novel genetic mouse models with regard to P2X7R expression in the CNS and debate caveats of existing mouse models. We discuss mechanistic and functional implications of the mood disorder-associated Gln460Arg *P2RX7* polymorphism and propose P2X7R as a plausible and promising target for the endeavor of developing novel therapeutics for depressive disorders.

The P2X7 Receptor: Structure, Expression, and Physiology

P2X7R is a member of the family of ionotropic P2X receptors (P2XRs), which are ATP-gated, nonselective cation channels supporting K^+ efflux and Ca^{2+}/Na^+ influx [21]. Among the trimeric P2XRs, P2X7R is an exception as it functions as a homotrimer whereas other family members form heterotrimeric assemblies comprising distinct P2X subunits. The receptor subunits exhibit the canonical P2X domain structure including (i) a short intracellular N-terminal domain, (ii) two transmembrane domains connected by (iii) a cysteine-rich and highly glycosylated extracellular loop, and (iv) an unusually long intracellular C-terminal domain discriminating P2X7R from other P2XRs. The pharmacology of P2X7R also distinguishes it from other P2XRs as it requires higher ATP concentrations ($EC_{50} \geq 100$ μM) for activation and the often-used agonist 2,3-*O*-(4-benzoylbenzoyl)-ATP (BzATP) is ~30-fold more potent than ATP [22]. The crystallization of mammalian P2X7R lacking the intracellular C terminus revealed the same 'dolphin-like' structure for single P2X7R subunits as previously demonstrated for zebrafish P2X4R subunits. Three ATP-binding sites were localized at the interface of two neighboring subunits. A distinct allosteric pocket, which is capable of binding different, structurally unrelated antagonists, is located in the immediate vicinity of the ATP-binding site [23]. Another property of P2X7R is its ability to form large pores, which are permeable for large hydrophilic molecules up to ~900 Da. This function was originally ascribed to the unique C-terminal tail potentially supported by accessory molecules such as the **pannexin-1** hemichannel [24,25]. However, a recent publication provides compelling evidence that the capability to perform this 'macropore' is intrinsic to P2X7R [26,27].

P2X7R is well known for its expression in immune cells of the hematopoietic lineage including monocytes/macrophages, lymphocytes, and dendritic cells [28]. P2X7R is also expressed in

Glossary

ATP-gated ion channels: transmembrane ion channels that open to allow ions such as Ca^{2+} to pass through the membrane in response to the binding of ATP, which acts as ligand.
Bipolar disorder (BD): psychiatric disorder characterized by major depressive and manic phases.
Depressive disorders: include two main subcategories: major depression (or MD) and dysthymia. Depression is characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration. Depression can be long lasting or recurrent, substantially impairing an individual's ability to function at work or school or cope with daily life. At its most severe, depression can lead to suicide.
Gain- or loss-of-function mutation: gain-of-function mutations confer a new or abnormal higher activity on a protein; a loss-of-function mutation results in impaired or abolished protein function.
Gamma-aminobutyric acid (GABA): main inhibitory neurotransmitter in the mammalian central nervous system. Its principal role is reducing neuronal excitability.
Gene × environment (G×E) scenario: G×E is the concept that explains how different individuals with particular genotypes respond to environmental variations in different ways. Behavior patterns or life events are part of these environmental changes. The same environmental factors may affect individuals with different genotypes differently and thus the G×E scenario can result in different phenotypes, leading to disease in specific individuals.
Genome-wide association study (GWAS): an observational study of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait. GWASs typically focus on associations between SNPs and traits like major human diseases.
Humanized mice: genetic mouse model obtained by replacing a mouse gene of interest or parts of it by the human counterpart. Particularly for P2X7R, the expression of human P2X7R in

Box 1. Neuronal P2X7R Expression Revisited

On the mRNA level, low P2X7R expression is diffusely detected throughout the WT mouse brain. Only the hippocampal CA3 region sticks out with a more prominent signal. P2X7R expression in microglia and **macroglia** (i.e., astrocytes and oligodendrocytes) has been confirmed by conditionally targeting the murine *P2rx7* locus. Moreover, P2X7R expression in neurons was detectable only in the CA3 region where it was clearly confined to glutamatergic neurons [57]. Other studies have used a bacterial artificial chromosome (BAC)-based *P2rx7-EGFP* reporter mouse line [*Tg(P2rx7-EGFP) FY174Gsat*] generated by the Gene Expression Nervous System Atlas (GENSAT) project to demonstrate neuronal P2X7R expression in the mouse brain and periphery [92–95]. This reporter line has been validated by using selective agonists and antagonists to demonstrate the presence of functional P2X7Rs in enhanced GFP (EGFP)-positive hippocampal neurons [37]. Of note, this reporter line does not show significant EGFP expression in the hippocampal CA3 region. In addition, expression in macrophages and spleen has been reported as an indicator for correct reporter gene expression [95]. In light of reported variability of transgene expression using BAC transgenesis, it is mandatory to demonstrate coexpression of EGFP and endogenous P2X7R to ultimately demonstrate the reporter line's reliability [96,97]. An overlooked caveat is the fact that BAC RP23-181F3, which has been used to generate the *P2rx7-EGFP* reporter line, encompasses the entire *P2rx4* gene. As a consequence, reporter mice might overexpress P2X4 receptors, which would tamper with functional studies. This controversy highlights the need for more refined and better-validated research tools to ultimately solve the present ambiguities. Mice expressing a tagged P2X7R would allow cellular and subcellular receptor localization and isolation of P2XR-containing protein complexes. Conditional, cell type-specific knockout mice will allow more refined functional assessment of P2X7R physiology in selected cell types.

microglia, the resident immune cells of the CNS. P2X7R is engaged in many aspects of immune function, in particular regulating the expression and secretion of cytokines. Notably, the P2X7R-controlled release of IL-1β and IL-18 has been studied in depth [29,30]. Under healthy/physiological conditions, tightly controlled low extracellular ATP concentrations do not activate the low-affinity P2X7R. However, in conjunction with any kind of cell injury, extracellular ATP reaches high micromolar concentrations sufficient to activate P2X7R [31]. The local increase of extracellular ATP acts as a danger/damage-associated molecular pattern (DAMP) to alert surrounding immune cells. In addition, P2X7R is capable of amplifying the signal by further release of ATP. Ultimately, P2X7R is critical for the recruitment of the NACHT, LRR, and PYD domain-containing protein 3 (NLRP3) inflammasome–caspase-1 complex and thereby controls the ATP-dependent release of mature IL-1β [29]. Besides its prominent role in controlling the release of proinflammatory cytokines, recent studies using knockout (KO) mice and antagonists have demonstrated that P2X7R is also involved in other physiological mechanisms relevant for the development of mood disorders. P2X7R KO mice show elevated levels of monoaminergic neurotransmitters in the amygdala. P2X7R directly modulates hippocampal glutamate release and thereby influences neurotrophic factors such as brain-derived neurotrophic factor (BDNF), which is crucial for the therapeutic actions of antidepressants. Inactivation of P2X7R leads to reduced glutamate release and upregulation of BDNF [33]. Moreover, P2X7R is present on adult neural progenitor cells in the subventricular zone where it is suggested to balance cell proliferation [34]. Along this line, P2X7R KO mice show upregulation of adult neurogenesis in the subgranular zone of the dentate gyrus [33]. Simultaneously, P2X7R KO mice show attenuated adaptation to repeated stress [35] that leads to downregulation of P2X7R in the hippocampus [36].

P2X7R expression in the CNS, particularly in neurons, is less clear and a continuous matter of debate [37,38]. The uncertainty owes on the one hand to the low brain expression and on the other hand to the lack of specificity of commercial P2X7R antibodies in brain tissue, both complicating the unambiguous detection [39,40]. Although the establishment of mouse genetic tools has advanced the field substantially, a final consensus with respect to neuron-specific P2X7R expression and function has not been reached yet (Box 1).

P2X7R and Mood Disorders: Genetic Evidence

The *P2RX7* gene is located on chromosome 12q24.31, a region repeatedly connected with mood disorders by linkage studies [41]. An association of an **SNP** with BD and MD was first

humanized knock-in mice recapitulates the endogenous expression of murine P2X7R.

Hypothalamic–pituitary–adrenal (HPA) axis: the component of the neuroendocrine system that controls the response to stress. After exposure to a stressor ranging from emotional stress to infectious insults, the HPA axis is the major part of an adaptive response that regulates many body processes and enables an organism to respond appropriately to changes in the environment. The main elements of the HPA axis are the paraventricular nucleus of the hypothalamus (releasing the peptide hormone CRH), the pituitary (secreting ACTH), and the adrenal gland, which produces glucocorticoids.

Micro- and macroglia: non-neuronal cells in the peripheral nervous system and CNS that provide support for neurons. In the CNS glial cells include macroglia (oligodendrocytes and astrocytes) and microglia. They play an important role in inflammation.

Monoamine hypothesis of depression: the hypothesis is based on the observation that many antidepressant drugs acutely increase synaptic levels of the monoamine neurotransmitters (i.e., serotonin, norepinephrine, and dopamine). The observation of this efficacy led to the monoamine hypothesis of depression, which postulates that the deficit of certain neurotransmitters is responsible for depression.

Mood disorders: a group of conditions where a disturbance in the person's mood is the main underlying feature. MD and BD represent the most prevalent mood disorders.

Neurotrophic and neurogenic hypotheses: exposure to chronic stress and/or depression results in neuronal atrophy and decreased neurogenesis in limbic brain regions involved in regulation of mood and emotion. Based on this, the neurotrophic and neurogenic hypotheses propose that depression results from decreased neurotrophic support (e.g., neurotrophic factors such as BDNF or VEGF), leading to neuronal atrophy, decreased

reported in 2006 [19,20]. The nonsynonymous SNP rs2230912 (1405A > G) located in exon 13 leads to the substitution of glutamine by arginine at position 460 (Gln460Arg) of P2X7R. In successive years, numerous studies have specifically interrogated the association of rs2230912 with MD and BD. While some studies corroborated the initial finding [42–44], others did not detect any significant association [45–51]. A first meta-analysis using a case-control design based on 6962 cases and 9262 controls did not find an association of rs2230912 with mood disorders. An association was found merely for the allele contrast in family-based cohorts [52]. A recent larger meta-analysis, comprising 8652 MD and BD cases as well as 11 153 controls, found significant associations for the allelic, dominant, or heterozygous-disadvantage model between rs2230912 and the combined diagnosis of MD and BD. The stratification by disorder revealed a significant association for the allelic model only in the case of MD [44]. Another aspect that genetic studies, including P2X7R-specific meta-analyses, have not fully taken into account is the haplotype structure of the *P2RX7* gene. In Caucasians, the Gln460Arg polymorphism is always associated with Thr^{348} and often also with Tyr^{155}, which have been shown to confer a **gain of receptor function** [53–55]. To date, 17 *P2RX7* haplotypes have been described [55]. A detailed assessment of the haplotype distribution in previous case-control studies and in other ethnicities would certainly shed more light on the impact of P2X7R on mood disorders.

The Gln460Arg Polymorphism: Challenging Traditional Loss-of-Function Concepts

In contrast to most SNPs associated with a particular disease, rs2230912 confers a missense mutation and thus is readily accessible to functional studies. Seemingly, the Gln460Arg polymorphism does not affect receptor function with regard to ion channel function and pore formation [56]. However, deviating observations have also been made. Stokes and colleagues demonstrated a slight but significant reduction in pore formation capacity when testing the Gln460Arg polymorphism in isolation [54]. Using heterologous expression in HEK293 cells, we observed similar channel currents, Ca^{2+} influx, and intracellular signaling via the mitogen-activated protein kinase (MAPK) ERK1/2 of the P2X7R-Gln460Arg variant compared with the wild-type (WT) P2X7R [57–59]. However, we observed that coexpression of the two receptor variants – P2X7R-WT and P2X7R-Q460R – dramatically affects the receptor activities detailed above, suggesting a previously unknown molecular mechanism of inhibition [59]. This finding was further corroborated by the demonstration that depletion of either P2X7R-WT or P2X7R-Gln460Arg from the heteromer is able to rescue Ca^{2+} and ERK1/2 responses. Importantly, P2X7R-WT and P2X7R-Gln460Arg show normal physical interactions and formation of heteromers at the plasma membrane (Figure 1). The P2X7R-WT variant used for expression in HEK293 cells represents the previously described haplotype 2 [55]. The effect of the disease-associated polymorphism was tested by selective substitution of Gln by Arg at position 460 in that particular haplotype. Accordingly, the resulting amino acid combination does not reflect any up-to-now-described naturally occurring haplotype. These results demand further investigations with regard to the interaction of the Gln460Arg polymorphism with Tyr^{155} and Thr^{348} as in haplotypes 14 and 15 [55].

The molecular mechanism underlying the observed inhibition by heteromerization is currently unclear. Some truncated P2X7R variants that can only form functionally impaired homotrimeric channels are also able to attenuate the channel's activity when they are present in heteromeric complexes with WT P2X7R subunits, thus acting in a dominant-negative fashion [60]. However, increased receptor activity due to heterotrimer formation has also been observed (e.g., when WT P2X7R subunits assemble with the truncated splice variant P2X7B) [61]. At present we can only speculate that the impact of the Gln460Arg polymorphism might be related to the disturbance of protein–protein binding domains encompassing the Gln460Arg polymorphism

hippocampal neurogenesis, and loss of glia.

***N*-Methyl-D-aspartate (NMDA):** an amino acid derivative that acts as a specific agonist at NMDA receptors mimicking the action of glutamate, the main excitatory neurotransmitter in the brain, which normally acts at that receptor.

P2X7 receptor (P2X7R): belongs to a family of receptors that are ligand-gated ion channels of the purinergic receptor group and are activated by ATP. The official gene symbol is *P2RX7* in humans and *P2rx7* in mice.

Pannexin-1: serves as a gap junction hemichannel and thus has an assembly and functional state as a gap junctional intercellular channel.

Single-nucleotide polymorphisms (SNPs): variations in a single nucleotide that occur at a specific position in the genome. They may fall within coding or noncoding gene sequences or in regions between genes. Association studies can determine whether a genetic variant is associated with a disease. A nonsynonymous SNP causes an amino acid substitution and thereby potentially changes protein function.

Sleep architecture: sleep–wake behavior and quality may be monitored in mice as well as in humans by means of EEG and electromyography (EMG) electrodes. Disturbed sleep and altered sleep are main features of many psychiatric disorders such as depression.

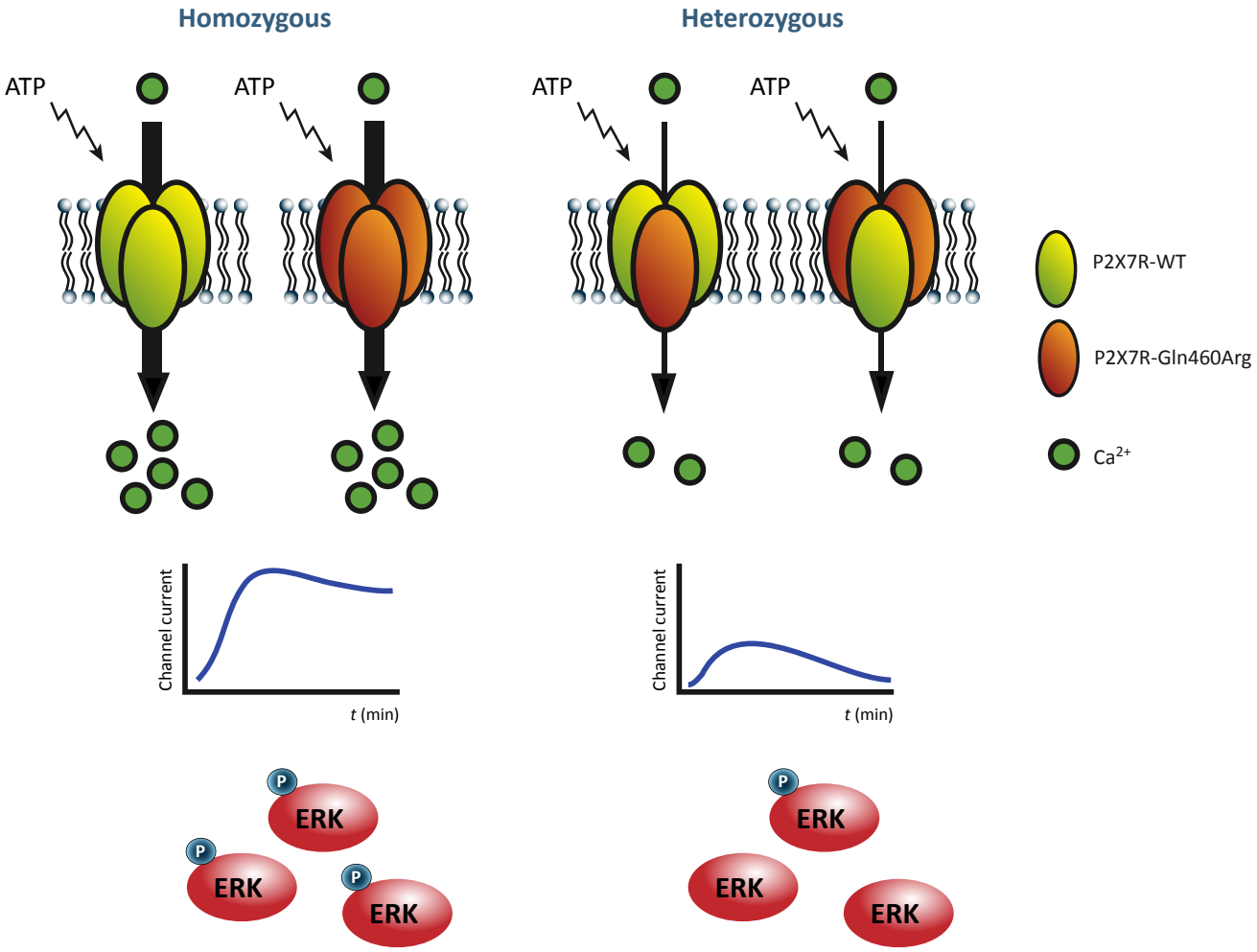

Figure 1. The Gln460Arg Polymorphism Diminishes P2X7 Receptor (P2X7R) Function in Heterozygosis. The P2X7R-Gln460Arg variant shows no functional differences to the P2X7R-wild type (WT) when both forms are expressed alone as homotrimers (homozygosis). However, coexpression of the two forms (heterozygosis) diminishes calcium intake, channel currents, and intracellular signaling (ERK1/2 phosphorylation). Coimmunoprecipitation and fluorescence resonance energy transfer (FRET) studies proved that the P2X7R-Gln460Arg variant physically interacts with P2X7R-WT and localizes the trimer to the cell membrane. Silencing of either of the variants rescues the loss-of-function phenotype.

such as a Src homology 3 domain (residues 441–460) [62]. Alternatively, the interaction of WT and mutant P2X7R subunits might entail conformational changes of the channel that ultimately result in reduced P2X7R activity. Unfortunately, the recently crystallized P2X7R excluded the long C-terminal domain, which could have provided valuable structural insights [23]. The mode of action of the Gln460Arg polymorphism (i.e., through heteromerization with WT subunits of the channel) may represent a general mechanism of action of human mutations. This mechanism is in line with the heterozygote disadvantage association found in the initial human genetic study and in the latest meta-analysis [19,44].

Humanized P2X7R Mice: Interrogating the Gln460Arg Polymorphism *In Vivo*

Genetic mouse models are invaluable tools to address gene function in an *in vivo* context [63]. Three independent constitutive P2X7R KO mouse lines have been generated by

GlaxoSmithKline, Pfizer, and Lexicon Genetics [64–66]. Of note, neither the Glaxo nor the Pfizer mice are full KOs as some splice variants have been reported to evade the inactivation [67,68]. Only P2X7R KO mice by Pfizer and Lexicon Genetics have been tested with regard to endophenotypes related to mood disorders. P2X7R KO mice consistently show signs of antidepressant-like behavior in the tail suspension test (i.e., reduced immobility) [32,66]. This phenotype has been recapitulated by the application of the P2X7R antagonist Brilliant Blue G (BGG) to WT mice but not when the antagonist was applied to P2X7R KO mice. A similar phenotype has been observed in the forced swim test, in particular following repeated testing [32,35,66]. In addition, P2X7R KO mice react with reduced hyperlocomotion in response to amphetamine, a phenotype that again can be phenocopied by BBG treatment [32]. Increased anxiety-related behavior was observed in one study but not in the others [35]. However, the translation of a constitutive KO to the human situation is inaccurate considering that the genetic association is not a null allele but an SNP leading to an amino acid substitution. Therefore, we generated **humanized mice** substituting exon 2–13 of the murine P2X7R by the human WT and Gln460Arg variants, respectively [57,58]. These mouse lines express human P2X7Rs in a spatial and temporal manner, which is similar to the mouse P2X7R. Moreover, the pore formation assay confirmed the expected approximately tenfold higher affinity for the agonist BzATP measured in humanized mice compared with WT animals. Therefore, we believe that these mice represent an invaluable *in vivo* model to evaluate any compound directed against the human P2X7R.

Aligned with the *in vitro* results demonstrating **loss of function** under conditions of coexpression, three genotypes were analyzed: homozygous mice expressing either P2X7R-WT or P2X7R-Gln460Arg as well as heterozygous animals expressing both receptor variants. Similar to *in vitro* studies, coexpression of the two receptor variants in heterozygous P2X7R mice resulted in impaired receptor function *in vivo* as detected by reduced Ca^{2+} influx in primary mixed cell cultures derived from the brains of humanized P2X7R (*hP2RX7*) mice. Comprehensive behavioral assessment of endophenotypes related to mood disorders did not detect any genotype-dependent differences under basal conditions. However, testing of *hP2RX7* mice in a **gene × environment (G×E) scenario** by subjecting them to chronic social defeat revealed a consistently higher stress vulnerability of heterozygous mice indicated by higher levels of anxiety and anhedonia accompanied by reduced social preference compared with their homozygous littermates [59]. These findings demonstrate that the Gln460Arg polymorphism confers genetic risk predisposing to stress-related pathologies.

The results from heterozygous *hP2RX7* mice deviate from findings obtained using either constitutive P2X7R KO mice [32,66] or P2X7R antagonist treatment of WT mice [69–71]. In both cases similar, albeit not always identical, signs of antidepressant-like behaviors were observed on blockade of P2X7Rs, clearly suggesting that specific P2X7R antagonists would have the greatest therapeutic potential as antidepressants. Heterozygous *hP2RX7* mice do not show antidepressant-like behavior under standard housing conditions but develop symptoms of anhedonia, impaired social behavior, and anxiety following chronic stress exposure. This difference suggests that the attenuation of P2X7R activity (due to formation of heterotrimeric receptors) has different molecular underpinnings and behavioral consequences compared with pharmacological or genetic disruption of P2X7R activity. In addition, it has to be taken into account that the present strategy to functionally address the Gln460Arg polymorphism in a mouse model does not comprise the mutations Tyr^{155} and Thr^{348}, which typically convey a gain of function to the disease-associated Gln460Arg allele. A direct comparison of the different loss-of-function approaches with regard to alterations of P2X7R downstream signaling in conjunction with environmental challenges has the potential to shed more light on these mutations and their

interactions. In addition, P2X7R has also been associated with BD, which is characterized by depressive but also manic episodes, introducing another layer of complexity regarding future therapeutic strategies. Whether P2X7R agonism or antagonism is a suitable therapeutic approach to differentially intervene in depressive or manic phases is currently unclear. The observed contradictory behavioral consequences provide evidence that the P2X7R haplotype might provide valuable and informative insights with respect to suitable treatment strategies.

Why Has P2X7R Not Been Identified as a Genetic Risk Factor by GWAS?

Despite the encouraging findings observed in candidate gene scenarios, the *P2RX7* gene has not emerged as a genetic risk factor for mood disorders in large-scale **Genome-Wide Association Studies (GWASs)** involving the largest currently available cohorts from the Psychiatric Genomics Consortium [72–74]. It has to be noted that the identification of genetic risk factors for mood disorders has in general proved difficult, which is owing to the assumption that these disorders are influenced by many genetic loci with rather small effect size – a difficulty challenging genetic studies of many common disorders. In light of the limited number of genetic risk factors identified by GWASs so far, the originally estimated heritability of mood disorders based on adoption and twin studies has been questioned [75]. Along these lines, the SNP-based heritability for MD estimated on the case-control scale is only 18% [76]. It seems that environmental factors such as recent stressful life events or childhood trauma confer a considerably greater disease risk than specific genetic variants. Hence, taking into account the interaction of genetic predisposition with environmental factors might have the potential to further advance human genetic studies on MD [77]. The observation that heterozygous *hP2RX7* mice show behavioral alterations only following chronic social-defeat stress supports the idea that genetic predisposition alone is not sufficient for disease manifestation [58]. Further support for P2X7R as a genetic risk factor originates from a recent study focusing on human subjects exposed to stressful life events. The analysis of functional polymorphisms in candidate genes previously implicated in depression-related phenotypes revealed a strong correlation with stress exposure. Among all polymorphisms analyzed, particularly the nonsynonymous *P2RX7* SNP rs7958311 (His270Arg) showed the strongest relevance in the group exposed to the highest level of recent negative life events [78]. Stress exposure, besides neurobiological consequences, has profound effects on the innate immune system leading to increased production and secretion of proinflammatory cytokines [9,79]. Similar to cellular damage, psychological stress promotes the increase of extracellular ATP, which is sensed by microglia in the brain via P2X7R activating the NLP3 inflammasome cascade and ultimately activating the release of the inflammatory cytokine IL-1β. P2X7R is an important component of the inflammasome integrating stress-associated signals and controlling proinflammatory cytokine secretion [70]. Taken together, the lack of genetic evidence from GWASs does not refute the association of the *P2RX7* gene with mood disorders. On the contrary, the stress responsiveness of P2X7R suggests that GWASs would profit from consideration of environmental exposure as a relevant covariate.

The Gln460Arg Polymorphism Entails Sleep-Electroencephalography (EEG) Alterations: A Potential Biomarker for MD?

Sleep impairments including insomnia but also hypersomnia are hallmarks of MD that often occur before the manifestation of full-blown disease symptoms. These impairments include disturbed sleep continuity and disinhibition of rapid eye movement sleep (REMS) but also changes in non-REMS (NREMS) [80]. Sleep represents an endophenotype testable in animal models by EEG profiling. *hP2RX7* mice displayed normal nocturnal sleep–wake behavior but their **sleep architecture** was significantly disturbed. Heterozygous *hP2RX7* mice showed increased entries into REMS, reduced slow-wave activity (SWA), and deep NREMS [58].

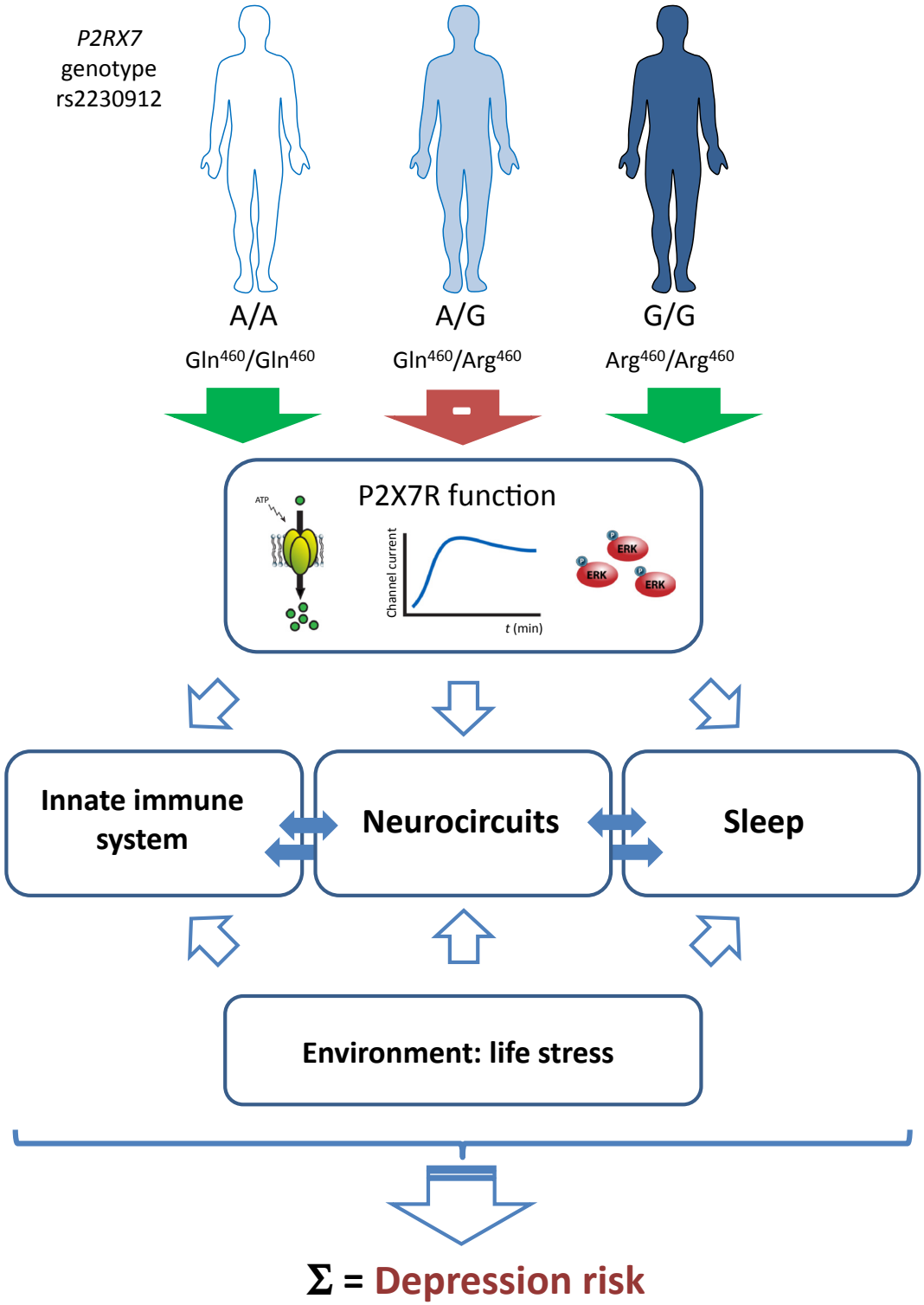

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Figure 2. Hypothesis of How the P2X7 Receptor (P2X7R) SNP rs2230912 (Gln460Arg) in Combination with Environmental Factors Determines the Risk for Depression. The SNP has a direct impact on P2X7R activity, which modulates various physiological processes differentially. Heterozygosity for this

(Figure legend continued on the bottom of the next page.)

Analogous sleep impairments have been observed in patients suffering from mood disorders [81,82]. Parallel sleep-EEG recordings in healthy human subjects genotyped for rs2230912 revealed NREMS instability and alterations in NREMS morphology in heterozygous (G/A) carriers compared with homozygous (A/A) subjects [58]. The tested subjects were healthy volunteers with no obvious signs of sleep impairments. These findings suggest that sleep disturbances in heterozygote carriers might represent early disease symptoms or signs of a prodromal disease state, which, when exposed to stressful live events, might develop into disease (Figure 2). Therefore, the Gln460Arg polymorphism in combination with sleep phenotyping might have the potential to serve as a biomarker for MD. What, then, is the role of P2X7R in regulating sleep? As already discussed, P2X7R controls the release of various cytokines including IL-1β, which are also well-known as sleep-regulatory substances playing an important role in sleep regulation during inflammation [83]. Therefore, alterations in P2X7R activity in heterozygote mice or humans might affect sleep-regulatory substances and ultimately the regulation of sleep parameters in a direct or indirect manner. In this context, it is of interest that P2X7R expression is under circadian control and upregulated following sleep deprivation in rodents and humans [50].

Concluding Remarks

The possible involvement of the innate immune system in the development of depressive disorders has regained considerable attention including the development of new concepts such as the pathogen host defense hypothesis of depression [10,79]. Along these lines, P2X7R outnumbers all other P2X family members with respect to the frequency of nonsynonymous SNPs, which might to some extent reflect evolutionary adaptation related to the role of P2X7R in modulating innate immune function. As a sensor of high extracellular ATP levels, P2X7R is ideally positioned to bridge the gap between stress and depression and thus to modulate communication between the immune system (i.e., microglia) and neural cells in the CNS [84]. P2X7R's involvement in fundamental aspects of inflammation, which are relevant for various neuropsychiatric disorders, expose it as an emerging target for therapeutic intervention (Box 2) [85]. Under physiological conditions, extracellular ATP concentrations are far below the required affinity for P2X7R, which therefore should not be active, and characterize the receptor as a 'silent' channel. This suggests that antagonists might specifically target P2X7Rs under pathological conditions, proffering it as an attractive drug target. Numerous P2X7R-specific antagonists have been developed, some of which have already entered clinical trials with variable outcomes [86]. Main targets of clinical trials so far have been inflammatory diseases such as rheumatoid arthritis (RA), osteoarthritis (OA), chronic obstructive pulmonary disease (COPD), and Crohn's disease (CD). A Phase II clinical trial with an antagonist developed by Pfizer (CE-224,535) did not show any efficacy [87]. Similarly, AZD-9056, developed by Astra Zeneca, was inefficient for treatment of RA, OA, and COPD [88]. However, in a Phase IIa trial for CD, a significant treatment effect was observed [89]. In recent years, antagonists with improved brain penetrance have been developed that have a good chance of entering proof-of-concept trials for neuropsychiatric disorders [90].

The detailed 3D structure will help in the development of more specific compounds by rational design, which might even target specific components of channel functions such as macropore formation [23,26]. In this context it will be critical to monitor receptor occupancy and thus further develop recently established P2X7R-specific positron emission tomography (PET)

Outstanding Questions

What is the physiological function of P2X7Rs in CA3 pyramidal neurons and how can this be unequivocally addressed?

How does the Gln460Arg substitution affect P2X7R activity; for example, with respect to interaction with binding partners and connected downstream signaling pathways or trimeric channel conformation and conductance?

What is the 3D structure of the receptor's unique C-terminal tail and does it provide insights into its physiology related to channel function?

Are P2X7R contributions to alterations in sleep patterns and stress vulnerability specific to particular micro- or macroglial cell types?

How are the numerous P2X7R haplotypes and the Gln460Arg polymorphism distributed among psychiatric disorders and how are these or previously undescribed haplotypes spread among non-Caucasian ethnicities?

How would a Gln460Arg gain-of-function haplotype comprising Thr^{348} and Tyr^{155} (e.g., haplotypes 14 and 15 from [54]) affect mood disorder-related endophenotypes in a humanized mouse model?

What is the best timing and strategy for therapeutic intervention and would antagonism or agonism of P2X7R activity be the most appropriate strategy?

Could profiling of sleep-EEG patterns serve as a relevant diagnostic proxy for mood disorders in patients carrying the rs2230912 SNP?

How could patients suitable for a P2X7R-based intervention be stratified?

polymorphism (red arrow) impairs P2X7R function. This has profound effects on various levels including sleep, the innate immune system, and neurocircuits, which interfere and communicate with each other. These physiological systems interacting with environmental factors throughout the lifespan ultimately define disease risk.

Box 2. Clinician's Corner

- The WHO rates depression as major contributor to disease burden, which contrasts with the paucity of progress with regard to novel therapeutic options. Clinical and preclinical evidence point towards disturbance of the innate immune system as a relevant mechanism involved in disease etiology.
- P2X7R is an ATP-gated ion channel of the ionotropic purinergic receptor family. It is highly expressed in immune cells, including brain-resident microglia where it is a key regulator of proinflammatory cytokine release. P2X7R is activated by high concentrations of ATP, which mainly occur during cellular damage.
- Numerous genetic studies and a recent meta-analysis find an association of the SNP rs2230912 in the *P2RX7* gene with major depression and BD. This SNP results in a Gln-by-Arg substitution at position 460 (Gln460Arg), which does not itself alter receptor function. However, if both receptor variants are assembled the resulting ion channel is functionally impaired.
- Studies using genetically engineered mice to model the genetic association demonstrated that the heterozygosity for the two receptor variants results in reduced sleep quality and confers a higher risk of development of stress-induced signs of depression. Importantly, heterozygous humans without manifested mood disorders also show signs of an altered sleep profile.
- The *P2RX7* genotype combined with profiling of sleep patterns and inflammatory cytokines may serve as a relevant diagnostic and prognostic tool to determine the psychiatric disease risk in humans. Pharmacological targeting of P2X7R might have the potential as a future therapy for the treatment of a subset of patients with depression.

ligands [91]. Most important is the identification of patients suitable for P2X7R-based treatments. Since inflammatory processes do not occur in all patients diagnosed with MD, personalized treatment is highly demanded. Patient stratification by more specific assessment of P2X7R haplotypes could be one option, which should be further validated by corresponding mouse models of human haplotype variants. Alternatively, peripheral levels of circulating cytokines together with sleep-EEG profiling might be informative as diagnostic proxies. Despite the accumulated evidence promoting P2X7R as a drug target for depressive disorders, numerous open questions and challenges remain, which require extensive further research (see Outstanding Questions). The first clinical trial to prove the efficacy of P2X7R antagonists in depressive patients is awaited with great excitement, raising hopes of overcoming the current standstill in drug discovery.

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