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Abstract

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Keywords

P2RX7, purinergic receptor, splice isoform, single nucleotide polymorphism, extracellular ATP, patent, CMMB

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Significance of P2X7 Receptor Variants to Human Health and Disease

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Abstract: The human P2X7 receptor is a trimeric ligand-gated cation channel coded by the *P2RX7* gene located at chromosome position 12q24. P2X7 is expressed in a wide variety of normal and disease-associated cell types. Activation of this receptor by extracellular adenosine 5'-triphosphate results in numerous downstream events including the release of pro-inflammatory mediators, cell proliferation or death, and killing of intracellular pathogens. As a result, P2X7 plays important roles in inflammation, immunity, bone homeostasis, neurological function and neoplasia. The *P2RX7* gene encodes a P2X7 subunit 595 amino acids in length, however splice isoforms that can alter receptor expression and function, and modify the signaling properties downstream of receptor activation also exist. Moreover, the relative amount of P2X7 function varies between human individuals due to numerous single nucleotide polymorphisms resulting in either loss- or gain-of-function. Combinations of these polymorphisms give rise to various haplotypes that can also modify P2X7 function. Collectively, P2X7, and its splice and polymorphic variants are attracting considerable interest in relation to human health and disease, including the development and publication of a number of patents.

INTRODUCTION

Extracellular nucleotides and nucleosides operate through a complex network of purinergic signaling cascades involving various membrane receptors and ectoenzymes [1, 2]. One member of this network is the P2X7 receptor, a trimeric ligand-gated cation channel encoded by the *P2RX7* gene (purinergic receptor P2X, ligand-gated ion channel 7) and belonging to the P2X receptor family [3, 4]. Activation of P2X7 by extracellular adenosine 5'-triphosphate (ATP) causes the movement of Ca^{2+}, Na^{+} and K^{+} across the plasma membrane, as well as the uptake of organic cations including fluorescent dyes [3, 4]. P2X7 activation induces an array of downstream events, in a cell specific manner, including the release of pro-inflammatory mediators [5], cell proliferation or death [6], and killing of intracellular pathogens [7]. P2X7 is predominately expressed on cells of the hematopoietic, epithelial, mesenchymal and neural lineages [8]. Subsequently P2X7 plays important roles in inflammation, immunity, bone homeostasis, neurological function (including pain) and neoplasia. Moreover, the relative amount of P2X7 expression and/or function varies between human individuals, which can be explained in part by splice and polymorphic variants. Of note, a number of these variants have been associated with diseases. This review will summarize the biology of P2X7 including splice and polymorphic P2X7 variants, and detail recent patents relating to DNA and gene sequences of *P2RX7*. Recent patents relating to P2X7 antagonists and their relevance to human health and disease have been discussed elsewhere [9, 10].

THE P2X7 RECEPTOR

The P2X7 receptor is a trimeric ATP-gated cation channel comprised of homomeric subunits 595 amino acids in length [11]. Each subunit comprises intracellular amino and carboxyl termini with two hydrophobic transmembrane domains, separated by a long glycosylated

extracellular loop containing ATP-binding sites [12, 13]. These observations are supported by atomic computing modeling of the human P2X7 receptor [14], based on a crystal structure of the zebrafish P2X4 receptor [15]. P2X7 subunits may also combine with P2X4 subunits to form heteromeric receptors [16], however homomeric P2X7 receptors appear to be the dominant subtype in most cell types [11, 17].

P2X7 was originally thought to be restricted to cells of the hematopoietic lineages, however it is now evident that P2X7 is also present in other cell lineages. In regards to hematopoietic lineages in humans, P2X7 is present (in decreasing amounts) on macrophages, dendritic cells, monocytes, natural killer cells, B-lymphocytes, T-lymphocytes and erythrocytes [18-21]. P2X7 is also present on human mast cells [22], microglia [23], osteoclasts [24], and eosinophils [25]. The relative amounts of this receptor, however, in these latter cell types in relation to other human hematopoietic cell types remain unknown. Despite the presence of P2X7 on eosinophils, human neutrophils appear to be devoid of P2X7 [26, 27]. P2X7 is also present on other human cell types including osteoblasts [28], fibroblasts [29], endothelial cells [30], and epithelial cells residing in various tissues including the skin, cervix, pancreas and colon (see [31, 32] and references therein). Moreover, P2X7 is present on various neural cell types including astrocytes, oligodendrocytes, Schwann cells and neurons, however its precise localization and expression in the latter remains controversial (see [33, 34] and references therein). Finally, P2X7 is present in various neoplastic cells particularly those arising from hematopoietic and epithelial lineages (see [35, 36] and references therein).

P2X7 activation induces a number of well-established downstream signaling events in various human cell types (Fig. **1**), many of which were originally established or have been supported by studies in animal models. The best described signaling event downstream of P2X7 activation is the activation of the NALP3 inflammasome resulting in the functional maturation and release of the pro-inflammatory cytokines, interleukin (IL)-1β and IL-18 from human myeloid cells

(reviewed by [37]). Depending on the cell type, environmental conditions and concentration of extracellular ATP, P2X7 activation can also promote cell proliferation or induce cell death via a number of different intracellular pathways (reviewed by [6]). Moreover, P2X7 activation can induce phagosome-lysosome fusion resulting in the killing of intracellular mycobacteria, chlamydia and toxoplasma residing with human macrophages (reviewed by [7]). P2X7 activation can also induce the release of CD21 (complement component receptor 2), CD23 (low affinity IgE receptor), CD62L (L-selectin), matrix metalloproteinase 9 (gelatinase B) and cathepsins from lymphoid and myeloid [18, 20, 38-43]; however the mechanisms linking P2X7 to these processes remain poorly defined. Finally, P2X7 activation can induce other downstream signaling events in cells of various lineages including rapid and reversible phosphatidylserine exposure, membrane blebbing, release of microparticles and exosomes, and multinucleated cell formation (reviewed by [44]), as well as the formation of reactive oxygen and nitrogen species (reviewed by [45]).

THE HUMAN *P2RX7* GENE

The human *P2RX7* gene comprises 13 exons and is located at chromosome position 12q24 [46], a region associated with inflammatory and psychiatric disorders [47, 48]. Initially, a putative 1.8-kb promoter region upstream of exon 1 was proposed [49], however experimental evidence supporting this has been lacking. More recently, the transcription initiation site and an active promoter region have been located 91 nucleotides, and 249 to 59 nucleotides respectively upstream of exon 1 [50]. Furthermore, target sites for the microRNAs, miR-150 and miR-186 have been identified within the *P2RX7* 3'-untranslated region, which can negatively regulate P2X7 mRNA translation or P2X7 expression [51]. Putative microRNA binding sites within the *P2RX7* 3'-untranslated region have also been found for miR-625, miR-1275, miR-491-5p and miR-1302 [52], but direct evidence in relation to P2X7 expression is lacking. *P2RX7* gene expression may also be

modulated by other mechanisms including *cis*-enhancement and hypermethylation as reviewed elsewhere [32].

P2X7 SPLICE ISOFORMS

A number of splice isoforms of the human P2X7 receptor have been identified (Fig. **2**). Initially, seven variants of human P2X7, resulting from alternative splicing, were identified [53]. These were designated as P2X7B to P2X7H by the investigators, with the original, full-length receptor encoding 595 amino acids [12] defined as P2X7A. P2X7B includes the intron between exons 10 and 11 leading to a new stop codon and a truncated protein lacking the last 171 amino acids of the full-length protein, but containing an alternate 18 amino acids following the second transmembrane domain. P2X7C to P2X7F lack various exons coding the parts of the extracellular domain (exons 4, 5, 7 and 8, and 4 and 8 respectively); P2X7C and P2X7E also contain the same intron between exons 10 and 11 as P2X7B. P2X7G and P2X7H contain an additional exon (termed exon N3) in the intron between exons 2 and 3, which introduces a new start codon potentially leading to translation of a P2X7 protein lacking the first transmembrane domain. P2X7G also contains the same intron between exons 10 and 11 as P2X7B, P2X7C and P2X7E. Thus, four of the seven splice isoforms of P2X7 have an alternate short carboxyl terminus, while two potentially lack the first transmembrane domain.

Of the above P2X7 variants, two (P2X7B and P2X7H) have been studied in greater detail. P2X7B shares a similar tissue distribution to the full-length receptor (P2X7A), with P2X7B mRNA expressed at comparable or greater amounts than P2X7A mRNA [53, 54]. The relative distribution and amounts of these P2X7 isoforms however, are yet to be compared at the protein level. Functional comparisons to P2X7A, demonstrated that P2X7B can form functional channels but not pores, and is a poor inducer of caspase-3 and -7 activation [53, 54]. Co-expression of P2X7B with

P2X7A however, results in heteromeric receptors that increase the intracellular ATP content and endoplasmic reticulum Ca^{2+} content to activate NFATc1 and stimulate cell growth [54]. Collectively, these studies suggest that P2X7A and P2X7B have growth promoting activities, and that P2X7A has additional roles in inflammation and cell death [54]. In contrast to P2X7A and P2X7B, P2X7H is present at low amounts in tissues, and appears to be non-functional [53]. Despite this progress, the specific relevance of these P2X7 splice isoforms to human health and disease remains to be defined.

Three additional P2X7 variants have also been described. A ninth P2X7 variant was named P2X7I [55], and results from a rare point mutation (rs35933842; g151+1>t) in the intron between exons 1 and 2, leading to a null allele [56]. The significance of this null allele to human health and disease is unknown. However, ATP-induced killing of mycobacteria is completely ablated in macrophages from subjects heterozygous for both rs35933842 and a second loss-of-function *P2RX7* allele (rs3751143; Glu496Ala) supporting a possible role for P2X7I in increased susceptibility to tuberculosis. A tenth variant, P2X7J, has been described [55]. This P2X7 variant lacks exon 8 resulting in a truncated protein comprising 258 amino acids including an alternate 10 amino acids at the C-terminus. P2X7J is non-functional, and when co-expressed with P2X7A results in the formation of non-functional P2X7A/P2X7J heteromeric receptors. P2X7J is present in both normal and malignant cervical tissue, with higher expression in the latter and leading to the suggestion that this variant may protect malignant cells from ATP-induced apoptosis. Finally, an eleventh splice variant, P2X7K, has been found in rodents resulting from an additional exon (termed exon 1') in the intron between exons 1 and 2, and leading to a receptor with an alternate N-terminus and first transmembrane domain, with increased agonist sensitivity and propensity to form P2X7 pores [57]. However, to our knowledge this variant is yet to be described in humans.

P2X7 SINGLE NUCLEOTIDE POLYMORPHISMS

A recent survey of the NCBI single nucleotide polymorphism (SNP) database (http://www.ncbi.nlm.nih.gov/sites/entrez) has revealed that over 686 SNPs reside within the human *P2RX7* gene [58]. The majority of these SNPs however are intronic or synonymous. There are 28 non-synonymous SNPs, of which only 16 have been characterized for phenotype (Table **1**; Fig. **3**). Of these 16 SNPs, the loss-of-function *P2RX7* SNP, rs3751143 (Glu496Ala) [59], has been the most extensively studied (Table **2**). As seen in Table **2**, this SNP impairs a number of P2X7-mediated processes in a variety of cell types. Although direct evidence is limited, it is likely that similar functional defects may also occur for other loss-of-function *P2RX7* SNPs. In this regard, rs35933842 (g151+1>t), rs28360457 (Arg307Gln), rs2230911 (Thr357Ser) and rs1653624 (Ile568Asn) also impair ATP-induced apoptosis of macrophages and/or killing of intracellular mycobacteria or toxoplasma [60-62]. Conversely, gain-of-function SNPs, rs208294 (His155Tyr), rs7958311 (His270Arg) and rs1718119 (Ala348Thr), have been associated with increased ATP-induced IL-1β release from monocytes [63]. In contrast to most SNPs however, rs3751143 appears unique in that the amino acid substitution impairs P2X7-mediated organic cation uptake (pore function), but not P2X7-mediated currents (channel function) [64]. This observation has been recently challenged [14], however we have argued [65] that the weight of experimental evidence still favors the earlier observation [64].

Despite the phenotypic characterization of a number of *P2RX7* SNPs, the mechanisms by which the amino acid substitutions lead to altered function remain poorly understood. Amino acid substitutions in the extracellular loop are proposed to alter ATP binding [14, 66, 67]. Consistent with this hypothesis, rs28360457 codes for an amino acid substitution (Arg307Gln) [67] at a site homologous to the putative ATP-binding sites in other P2X receptors [68]. Amino acid substitutions in the transmembrane and C-terminus regions are thought to alter the gating properties

of the channel and/or to disrupt channel or pore formation [59, 62, 63], although some evidence exists that changes in the C-terminus can also alter ATP sensitivity [14]. In contrast, rs1653624 codes for an amino acid substitution (Ile568Asn) that prevents trafficking of P2X7 to the plasma membrane and leads to a complete loss-of-function when present in homozygous dosage [69].

P2X7 SINGLE NUCLEOTIDE POLYMORPHISMS & DISEASE

P2RX7 SNPs have been associated with various diseases (Table **3**). Of these, the association of rs3751143 with increased susceptibility to tuberculosis is the most robust. Of the 10 cohorts reported, 6 of these (from various racial groups) have found an increased frequency of the mutant allele in this disease (see Table **3**). Moreover, this association of rs3751143 with tuberculosis is supported by meta-analysis [70]. The increased susceptibility of patients coding rs3751143 to tuberculosis is most likely due to impaired P2X7-mediated killing of intracellular mycobacteria within macrophages [60, 71, 72], however other mechanisms such as impaired IL-1β release [73] or adaptive immune responses [74] cannot be excluded. The SNP rs3751143 has also been studied in multiple cohorts comprising patients with chronic lymphocytic leukemia (CLL) following initial observations that an increased frequency of rs3751143 may be associated with increased susceptibility to or prolonged survival in CLL [75, 76]. Neither association however was supported by additional studies [77-80] or by a meta-analysis of the combined studies [78]. Finally, an increased frequency of the mutant rs2230912 allele has been associated with mood disorders (bipolar or unipolar disorders) [81-85], however these findings have not been supported by other studies [86-88]. The rs2230912 SNP has only a minor effect on P2X7 function [14, 63] and it is currently difficult to reconcile how this SNP may be of physiological consequence in mood disorders. We have recently demonstrated however, that this SNP is in linkage disequilibrium with

three gain-of-function *P2RX7* SNPs in a haplotype potentially leading to increased amounts of circulating IL-1β and altering mood states [63].

The association of *P2RX7* SNPs with other diseases has been examined in a variety of other studies with many failing to find a significant association (Table **3**). The majority of these studies have small sample sizes and thus lack sufficient statistical power to detect if a significant association exists. Future studies examining the association of *P2RX7* SNPs with disease will benefit from the development of high-through genotyping, and the collection of large disease cohorts and appropriately matched controls. Moreover, future studies will need to consider the combined effect of multiple *P2RX7* SNPs or haplotypes. Nevertheless further studies of P2X7 in relation to human health and disease are warranted based on observations in rodent models of disease, as well as with clinical samples obtained from humans. For example, P2X7 and its variants may play an important role in inflammatory and immune disorders such as rheumatoid arthritis. ATP induces significantly higher amounts of IL-1β release in blood samples obtained from rheumatoid arthritis patients compared to controls [89], while P2X7-deficient mice (Pfizer) have reduced severity and incidence of anti-collagen antibody-induced arthritis [90]. Additionally, an important role for P2X7 and its variants is suggested in relation to bone disorders. P2X7 is present on both human osteoclasts and osteoblasts [24, 28], while P2X7-deficient mice (Pfizer) display reduced bone formation and content [91]. A final example of the potential role of P2X7 and its variants is indicated in neurodegenerative disorders such as multiple sclerosis. P2X7 expression is expressed at higher amounts on spinal cord microglia from multiple sclerosis patients compared to controls [92], while P2X7-deficient mice (Glaxo-Smith-Kline) have reduced incidence of experimental autoimmune encephalomyelitis, a model of multiple sclerosis [93]. The role of P2X7 in multiple sclerosis however, is confounded by an earlier study showing that experimental autoimmune encephalomyelitis is exacerbated in P2X7-deficient mice (Pfizer) [94].

PATENTS REGARDING THE *P2RX7* GENE & P2X7 VARIANTS

A number of patents have been filed relating to either splice or polymorphic variants of the P2X7 receptor (Table **4**). The majority of these patents outline claims for the detection of *P2RX7* variants in various disorders. Other patents mostly outline claims relating to *P2RX7* gene expression.

Several patents have been filed in relation to *P2RX7* SNPs. Patent US7767398 [95] claims a method for diagnosing a mood (affective) disorder or susceptibility to a mood disorder in an individual encoding rs2230912 or a deletion of that allele. This patent follows an earlier patent (WO2004092384; [96]) of the same name undertaken by the same inventors. The claims of this earlier patent are broader including regions in the 5' untranslated region, and exons 5 and 8 of *P2RX7*, as well as a number of other intronic and exonic *P2XR7* SNPs. The association of rs2230912 with mood disorders was originally shown by the inventors [81, 83] and subsequently validated by others [84, 85], however as mentioned above others have failed to find an association between this SNP and mood disorders [86-88]. Nonetheless, our recent data indicates that rs2230912 lies in linkage disequilibrium with a gain-of-function haplotype [63] which may account for the discordance between the various genetic studies of *P2RX7* and mood disorders [65]. In patent WO2010061246 [97], the inventors claim an *in vitro* method used to assess the sensitivity of subjects to chemotherapeutic or radiotherapeutic treatment of cancer. This assay relies on the detection of loss-of-function SNPs resulting in reduced function of the P2X7-elicited NALP3 inflammasome pathway. This patent includes the loss-of-function *P2RX7* SNPs, rs28360457, rs1653624, rs3751143 and rs2230911. This patent is based on recent data demonstrating a role for tumor-derived ATP and P2X7-mediated IL-1β release from dendritic cells in anti-tumor immune responses during chemotherapy. Treatment of human and murine cancer cells with a variety of chemotherapeutic agents or γ-irradiation induces the release of ATP into the extracellular

environment [74, 98, 99]. Moreover, *in vitro* and *in vivo* murine studies have shown that this ATP can activate P2X7 on dendritic cells to stimulate the NALP3 inflammasome and the subsequent release of biologically active IL-1β to generate interferon-γ producing anti-tumor $CD8^+$ T-cells and effective anti-tumor adaptive immunity [74]. Of clinical significance, the *P2RX7* SNP, rs3751143 (Glu496Ala), which prevents ATP-induced IL-1β release [73], is associated with reduced metastatic disease-free survival rates in breast cancer patients treated with anthracyclines [74]. Patent US20090253585 [100] claims a genotyping method of predicting the susceptibility of a subject to develop a somatosensory disorder based on the identification of at least one haplotype or SNP from a very broad range of genes including *P2RX7*. This patent includes 16 *P2RX7* SNPs (rs684201, rs685019, rs208288, rs17525809, rs208294, rs16950860, rs7958311, rs1718136, rs1718119, rs6489795, rs2230912, rs3751143, rs2230913, rs3751142, rs1621388 and rs1653625). Although a number of animal studies support a role for P2X7 in tactile sensation, nociception and pain [101-104], evidence for a role for P2X7 in somatosensory disorders in humans including the association of loss- or gain-of-function *P2XR7* SNPs is lacking. Patent US7205146 [105] claims a 12q23-qter nucleotide sequence (and an assay for its detection) which contains the *P2RX7* SNP, rs7958311. Based on case-control association studies of North American and British cohorts [105], the inventors report a significant over-representation of the adenine-containing variant of rs7958311 (coding for His at residue 270) in subjects with asthma, bronchial hyper-responsiveness or higher IgE amounts. The frequency of other *P2RX7* SNPs in this study were not significantly different between controls and cases, and to the best of our knowledge this association study has not been published in a peer-reviewed journal. Patent WO2006136791 [106] claims a method to identify a *P2XR7* haplotype containing rs169631 (intronic SNP; c>g) and rs3751143 in patients who are at risk of atherosclerosis and atherosclerosis-mediated diseases or conditions such as myocardial infarction. This genetic association is based on a cohort comprising 387 Swedish patients hospitalized due to myocardial infarction [106], however to the best of our knowledge this

association study has not been published in a peer-reviewed journal. Patent EP1741722 [107] claims materials and methods for the identification of numerous *P2RX7* SNPs including most of those listed in Table **1**. This patent is broad in its application with no apparent claims to any specific disorders.

Given the potential significance of various *P2RX7* SNPs in various disorders it has also been of value to develop methods that can potentially identify subjects coding loss- or gain-of-function SNPs or haplotypes based on a phenotypic parameter such as P2X7-mediated pore formation [108, 109]. Patents US7560243 [110] and US2009053204 [111] claim a flow cytometric-based assay to measure the relative P2X7 function (pore formation) in peripheral blood monocytes. This method has been extensively described [109, 112], and the measured pore formation correlates with the capacity of monocytes to release altered ratios of tumor necrosis factor-α and IL-10 [113], and with specific *P2RX7* SNPs or haplotypes [114]. The potential clinical utility of this assay has been used to identify subjects at risk of virus-induced loss of asthma control [115]. This study however, did not report the *P2RX7* genotype of the cohort. Thus, a direct correlation between clinical status or pore formation to *P2RX7* genotype could not be ascertained.

As an alternative to the detection of P2X7-mediated pore formation, the inventors of a series of patents WO2010000041 [116], WO2009033233 [117], WO2008043145 [118], WO2008043146 [119], US7326415 [120] and WO2002057306 [121] claim the development of polyclonal and monoclonal antibodies (and related reagents) capable of binding to non-functional P2X7. Non-functional receptors may result from non-synonymous SNPs in *P2RX7* [122], however it is yet to be determined if the developed antibodies recognize all or only a sub-group of these receptors. The original antibody targeted an epitope within a loop adjacent to the extracellular ATP-binding site (residues 193-214) [123], and was found to bind non-functional P2X7 (including those arising from subjects carrying the rs3751143 mutant allele) but not to functional P2X7 [122]. Subsequently, it was shown that this antibody could bind to cells in malignant but not normal tissue, and allowed for

the differentiation of these two tissue states in melanoma, prostate cancer and breast cancer [124-126].

Other patents relate to P2X7 isoforms. The inventors of patent US7767789 [127] claim materials and methods to detect P2X7J mRNA and protein in normal and malignant tissue. This claim relates to the inventors observations that P2X7J is over-expressed in certain epithelial malignancies and promotes an anti-apoptotic phenotype [55]. The inventors of patent WO2004090097 [128] claim a P2X7 isoform, HBMYP2X7v, containing a sequence of 1521 nucleotides and encoding a polypeptide of 506 amino acids. In addition, the inventors claim methods relating to the detection of this variant in the diagnosis or treatment of a broad group of neurological and neurodegenerative disorders. However, it remains to be determined if this isoform leads to a product of functional significance or is of clinical relevance.

Finally, a number of patents relate to the expression of the *P2RX7* gene product. The inventors of patents US20090232893 [129], US20090227533 [130], US20090175827 [131] and US2009163434 [132] claim that miR-143, miR-34, miR-16 and miR-20 respectively modulate the expression of a large number of gene products, including P2X7, in a very broad group of disorders involving most of the systems within the body. Patent WO2008115387 [133] is more specific. The inventors of this patent claim the use of miR-150 and miR-186 as potential biomarkers in the management of uterine malignancies. This claim relates to data demonstrating that over-expression of these microRNAs in malignant uterine tissue down-regulates P2X7 and subsequently reduces ATP-induced apoptosis [51]. Conversely, patent WO2006024880 [134] claims the use of short inhibitory RNA (siRNA) to down-regulate P2X7 in patients from a very broad group of disorders including those of the neuronal, cardiovascular, inflammatory, cutaneous and renal systems. The potential utility of this application has been demonstrated by others using rats, where *in vivo* administration of siRNA to P2X7 impairs spinal nociceptive responses [135]. Finally, the inventors of WO2008142194 [136] claim a molecular-based method for measuring the *P2RX7* gene product

in the diagnosis and prognosis of Huntington's disease. This claim relates to data outlining evidence for increased P2X7 expression and function (Ca^{2+} flux and cell death) in neurons of mouse models of Huntington's disease [137]. Evidence however for a role for P2X7 in Huntington's disease in humans is lacking.

CURRENT & FUTURE DEVELOPMENTS

Splice and polymorphic variants of P2X7 are attracting considerable attention in human health and disease resulting in a number of related patents. Due to the prior publication of *P2RX7* and its isoforms and SNPs, the majority of patents relating to P2X7 claim the detection of *P2RX7* variants in specific or broadly specified disorders, rather than nucleotide sequences of *P2RX7*. This prior identification and publication of *P2RX7* isoforms and SNPs independently of patents has greatly assisted and most likely expedited our understanding of P2X7 in human physiology and pathophysiology. Nevertheless the scientific merit and utility of current and future patents relating to the *P2RX7* gene will need to be validated by larger sample sizes and examination by other investigators. It will also need to be determined if the relevance of such patents is restricted to specific racial groups or whether they have broader or universal application within the human population. In this regard, it still remains to be determined if many of the currently known *P2RX7* isoforms and SNPs exist in less-well studied human groups. Conversely, it remains to be determined if novel *P2RX7* variants exist in such groups. Moreover, given the highly polymorphic nature of the *P2XR7* gene and its receptor, future genetic association studies will need to include the analysis of *P2RX7* haplotypes and the detailed phenotypic characterization of the encoded receptors. Included in this, is the further understanding of the mechanisms by which *P2RX7* SNPs alter receptor expression and function including events downstream of P2X7 activation, and whether these SNPs have similar effects on *P2RX7* splice isoforms particularly P2X7B. It will also

be of importance to determine the relative effectiveness of P2X7 therapeutics, currently under development or in clinical trials [9, 10], in relation to P2X7 isoforms and polymorphic variants. Finally, given that much of our understanding of P2X7 is inferred from rodent models of inflammatory, immune, bone, and neurological disorders, as well as pain, it will be important to directly establish a role for P2X7 in these and other human diseases to help develop and validate the claims of current and future patents.

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CONFLICT IN INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Burnstock G. Purine and pyrimidine receptors. Cell Mol Life Sci 2007; 64: 1471-83.
[2] Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. Biochim Biophys Acta 2008; 1783: 673-94.

[3] Jarvis MF, Khakh BS. ATP-gated P2X cation channels. Neuropharmacology 2009; 56: 208-15.
[4] Surprenant A, North RA. Signaling at purinergic P2X receptors. Annu Rev Physiol 2009; 71: 333-59.
[5] Hughes JP, Hatcher JP, Chessell IP. The role of $P2X_7$ in pain and inflammation. Purinergic Signal 2007; 3: 163-9.
[6] Di Virgilio F, Ferrari D, Adinolfi E. $P2X_7$: a growth-promoting receptor-implications for cancer. Purinergic Signal 2009; 5: 251-6.
[7] Coutinho-Silva R, Correa G, Sater AA, Ojcius DM. The $P2X_7$ receptor and intracellular pathogens: a continuing struggle. Purinergic Signal 2009; 5: 197-204.
[8] Burnstock G, Knight GE. Cellular distribution and functions of P2 receptor subtypes in different systems. Int Rev Cytol 2004; 240: 31-304.
[9] Friedle SA, Curet MA, Watters JJ. Recent patents on novel P2X7 receptor antagonists and their potential for reducing central nervous system inflammation. Recent Pat CNS Drug Discov 2010; 5: 35-45.
[10] Guile SD, Alcaraz L, Birkinshaw TN, Bowers KC, Ebden MR, Furber M*, et al.* Antagonists of the P2X7 receptor. From lead identification to drug development. J Med Chem 2009; 52: 3123-41.
[11] Nicke A. Homotrimeric complexes are the dominant assembly state of native P2X7 subunits. Biochem Biophys Res Commun 2008; 377: 803-8.
[12] Rassendren F, Buell GN, Virginio C, Collo G, North RA, Surprenant A. The permeabilizing ATP receptor, P2X7. Cloning and expression of a human cDNA. J Biol Chem 1997; 272: 5482-6.
[13] Surprenant A, Rassendren F, Kawashima E, North RA, Buell G. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). Science 1996; 272: 735-8.
[14] Roger S, Mei ZZ, Baldwin JM, Dong L, Bradley H, Baldwin SA*, et al.* Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X7 receptor functions. J Psychiatr Res 2010; 44: 347-55.
[15] Kawate T, Michel JC, Birdsong WT, Gouaux E. Crystal structure of the ATP-gated P2X4 ion channel in the closed state. Nature 2009; 460: 592-8.
[16] Guo C, Masin M, Qureshi OS, Murrell-Lagnado RD. Evidence for functional P2X4/P2X7 heteromeric receptors. Mol Pharmacol 2007; 72: 1447-56.
[17] Torres GE, Egan TM, Voigt MM. Hetero-oligomeric assembly of P2X receptor subunits. J Biol Chem 1999; 274: 6653-9.
[18] Georgiou JD, Skarratt KK, Fuller SJ, Martin CJ, Christopherson RI, Wiley JS*, et al.* Human epidermal and monocyte-derived Langerhans cells express functional $P2X_7$ receptors. J Invest Dermatol 2005; 125: 482-90.
[19] Gu BJ, Zhang WY, Bendall LJ, Chessell IP, Buell GN, Wiley JS. Expression of $P2X_7$ purinoceptors on human lymphocytes and monocytes: evidence for nonfunctional $P2X_7$ receptors. Am J Physiol Cell Physiol 2000; 279: C1189-C97.
[20] Sluyter R, Wiley JS. Extracellular adenosine 5'-triphosphate induces a loss of CD23 from human dendritic cells via activation of $P2X_7$ receptors. Int Immunol 2002; 14: 1415-21.
[21] Stevenson RO, Taylor RM, Wiley JS, Sluyter R. The $P2X_7$ receptor mediates the uptake of organic cations in canine erythrocytes and mononuclear leukocytes: comparison to equivalent human cell types. Purinergic Signal 2009; 5: 385-94.
[22] Wareham K, Vial C, Wykes RC, Bradding P, Seward EP. Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells. Br J Pharmacol 2009; 157: 1215-24.

[23] Chakfe Y, Seguin R, Antel JP, Morissette C, Malo D, Henderson D*, et al.* ADP and AMP induce interleukin-1beta release from microglial cells through activation of ATP-primed P2X7 receptor channels. J Neurosci 2002; 22: 3061-9.
[24] Hoebertz A, Townsend-Nicholson A, Glass R, Burnstock G, Arnett TR. Expression of P2 receptors in bone and cultured bone cells. Bone 2000; 27: 503-10.
[25] Ferrari D, Idzko M, Dichmann S, Purlis D, Virchow C, Norgauer J*, et al.* P2 purinergic receptors of human eosinophils: characterization and coupling to oxygen radical production. FEBS Letters 2000; 486: 217-24.
[26] Martel-Gallegos G, Rosales-Saavedra MT, Reyes JP, Casas-Pruneda G, Toro-Castillo C, Pérez-Cornejo P*, et al.* Human neutrophils do not express purinergic P2X7 receptors. Purinergic Signal 2010; 6: 297-306.
[27] Vaughan KR, Stokes L, Prince LR, Marriott HM, Meis S, Kassack MU*, et al.* Inhibition of neutrophil apoptosis by ATP is mediated by the P2Y11 receptor. J Immunol. 2007; 179: 8544-53.
[28] Gartland A, Hipskind RA, Gallagher JA, Bowler WB. Expression of a P2X7 receptor by a subpopulation of human osteoblasts. J Bone Miner Res 2001; 16: 846–56.
[29] Solini A, Chiozzi P, Morelli A, Fellin R, Di Virgilio F. Human primary fibroblasts in vitro express a purinergic P2X7 receptor coupled to ion fluxes, microvesicle formation and IL-6 release. J Cell Sci 1999; 112: 297-305.
[30] Wilson HL, Varcoe RW, Stokes L, Holland KL, Francis SE, Dower SK*, et al.* P2X receptor characterization and IL-1/IL-1Ra release from human endothelial cells. Br J Pharmacol 2007; 151: 115-27.
[31] Garcia-Marcos M, Pochet S, Marino A, JP. D. P2X7 and phospholipid signalling: the search of the "missing link" in epithelial cells. Cell Signal 2006; 18: 2098-104.
[32] Gorodeski GI. P2X7-mediated chemoprevention of epithelial cancers. Expert Opin Ther Targets 2009; 13: 1313-32.
[33] Anderson CM, Nedergaard M. Emerging challenges of assigning P2X7 receptor function and immunoreactivity in neurons. Trends Neurosci 2006; 29: 257-62.
[34] Duan S, Neary JT. P2X7 receptors: properties and relevance to CNS function. Glia 2006; 54: 738-46.
[35] Deli T, Csernoch L. Extracellular ATP and cancer - an overview with special reference to P2 purinergic receptors. Pathol Oncol Res 2008; 14: 219-31.
[36] White N, Burnstock G. P2 receptors and cancer. Trends Pharmacol Sci 2006; 27: 211-7.
[37] Di Virgilio F. Liaisons dangereuses: $P2X_7$ and the inflammasome. Trends Pharmacol Sci 2007; 28: 465-72.
[38] Farrell AW, Gadeock S, Pupovac A, Wang B, Jalilian I, Ranson M*, et al.* P2X7 receptor activation induces cell death and CD23 shedding in human RPMI 8226 multiple myeloma cells. Biochim Biophys Acta 2010; 1800: 1173-82.
[39] Gu B, Bendall LJ, Wiley JS. Adenosine triphosphate-induced shedding of CD23 and L-selectin (CD62L) from lymphocytes is mediated by the same receptor but different metalloproteases. Blood 1998; 92: 946-51.
[40] Gu BJ, Wiley JS. Rapid ATP-induced release of matrix metalloproteinase 9 is mediated by the P2X7 receptor. Blood 2006; 107: 4946-53.
[41] Jamieson GP, Snook MB, Thurlow PJ, Wiley JS. Extracellular ATP causes of loss of L-selectin from human lymphocytes via occupancy of P2Z purinocepters. J Cell Physiol 1996; 166: 637-42.
[42] Lopez-Castejon G, Theaker J, Pelegrin P, Clifton AD, Braddock M, Surprenant A. $P2X_7$ receptor-mediated release of cathepsins from macrophages is a cytokine-independent mechanism potentially involved in joint diseases. J Immunol 2010; 185: 2611-9.

[43] Sengstake S, Boneberg EM, Illges H. CD21 and CD62L shedding are both inducible via P2X7Rs. Int Immunol 2006; 18: 1171-8.
[44] Qu Y, Dubyak GR. P2X7 receptors regulate multiple types of membrane trafficking responses and non-classical secretion pathways. Purinergic Signal 2009; 5: 163 - 73.
[45] Hewinson J, Mackenzie AB. P2X7 receptor-mediated reactive oxygen and nitrogen species formation: from receptor to generators. Biochem Soc Trans 2007; 35: 1168-70.
[46] Buell GN, Talabot F, Gos A, Lorenz J, Lai E, Morris MA*, et al.* Gene structure and chromosomal localization of the human $P2X_7$ receptor. Receptors Channels 1998; 5: 347-54.
[47] Kato T. Molecular genetics of bipolar disorder and depression. Psychiatry Clin Neurosci 2007; 61: 3-19.
[48] Shen N, Tsao BP. Current advances in the human lupus genetics. Curr Rheumatol Rep 2004; 6: 391-8.
[49] Li CM, Campbell SJ, Kumararatne DS, Bellamy R, Ruwende C, McAdam KPWJ*, et al.* Association of a polymorphism in the P2X7 gene with tuberculosis in a Gambian population. J Infect Dis 2002; 186: 1458-62.
[50] Zhou L, Luo L, Qi X, Li X, Gorodeski GI. Regulation of P2X7 gene transcription. Purinergic Signal 2009; 5: 409-26.
[51] Zhou L, Qi X, Potashkin JA, Abdul-Karim FW, Gorodeski GI. MicroRNAs miR-186 and miR-150 down-regulate expression of the pro-apoptotic purinergic P2X7 receptor by activation of instability sites at the 3'-untranslated region of the gene that decrease steady-state levels of the transcript. J Biol Chem 2008; 283: 28274-86.
[52] Rahman OA, Sasvari-Szekely M, Szekely A, Faludi G, Guttman A, Nemoda Z. Analysis of a polymorphic microRNA target site in the purinergic receptor P2RX7 gene. Electrophoresis 2010; 31: 1790-5.
[53] Cheewatrakoolpong B, Gilchrest H, Anthes JC, Greenfeder S. Identification and characterization of splice variants of the human $P2X_7$ ATP channel. Biochem Biophys Res Commun 2005; 332: 17-27.
[54] Adinolfi E, Cirillo M, Woltersdorf R, Falzoni S, Chiozzi P, Pellegatti P*, et al.* Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor. FASEB J 2010; 24: 3393-404.
[55] Feng Y-H, Li X, Wang L, Zhou L, Gorodeski GI. A Truncated P2X7 Receptor Variant (P2X7-j) Endogenously Expressed in Cervical Cancer Cells Antagonizes the Full-length P2X7 Receptor through Hetero-oligomerization. J Biol Chem 2006; 281: 17228-37.
[56] Skarratt KK, Fuller SJ, Sluyter R, Dao-Ung LP, Gu BJ, Wiley JS. A 5' intronic splice site polymorphism leads to a null allele of the P2X7 gene in 1% to 2% of the Caucasian population. FEBS Lett 2005; 579: 2675-8.
[57] Nicke A, Kuan YH, Masin M, Rettinger J, Marquez-Klaka B, Bender O*, et al.* A functional P2X7 splice variant with an alternative transmembrane domain 1 escapes gene inactivation in P2X7 knock-out mice. J Biol Chem 2009; 284: 25813-22.
[58] Sun C, Chu J, Singh S, Salter R. Identification and characterization of a novel variant of the human P2X7 receptor resulting in gain of function. Purinergic Signal 2010; 6: 31-45.
[59] Gu BJ, Zhang WY, Worthington RA, Sluyter R, Dao-Ung P, Petrou S*, et al.* A Glu-496 to Ala polymorphism leads to loss of function of the human $P2X_7$ receptor. J Biol Chem 2001; 276: 11135-42.
[60] Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Wiley JS, Britton WJ. Gene dosage determines the negative effects of polymorphic alleles of the P2X7 receptor on adenosine triphosphate-mediated killing of mycobacteria by human macrophages. J Infect Dis 2005; 192: 149-55.

[61] Lees MP, Fuller SJ, McLeod R, Boulter NR, Miller CM, Zakrzewski AM*, et al.* P2X7 receptor-mediated killing of an intracellular parasite, Toxoplasma gondii, by human and murine macrophages. J Immunol 2010; 184: 7040-6.

[62] Shemon AN, Sluyter R, Fernando SL, Clarke AL, Dao-Ung LP, Skarratt KK*, et al.* A Thr357 to Ser polymorphism in homozygous and compound heterozygous subjects causes absent or reduced $P2X_7$ function and function impairs ATP-induced mycobacterial killing by macrophages. J Biol Chem 2006; 281: 2079-86.

[63] Stokes L, Fuller SJ, Sluyter R, Skarratt KK, Gu BJ, Wiley JS. Two haplotypes of the P2X7 receptor containing the Ala-348 to Thr polymorphism exhibit gain-of-function effect and enhanced interleukin-1β secretion. FASEB J 2010; 24: 2916-27.

[64] Boldt W, Klapperstuck M, Buttner C, Sadtler S, Schmalzing G, Markwardt F. $Glu^{496}Ala$ polymorphism of human $P2X_7$ receptor does not affect its electrophysiological phenotype. Am J Physiol Cell Physiol 2003; 284: C749-C56.

[65] Sluyter R, Stokes L, Fuller SJ, Skarratt KK, Gu BJ, Wiley JS. Functional significance of P2RX7 polymorphisms associated with affective mood disorders. J Psychiatr Res 2010; 44: 1116-7.

[66] Cabrini G, Falzoni S, Forchap SL, Pellegatti P, Balboni A, Agostini P*, et al.* A His-155 to Tyr polymorphism confers gain-of-function to the human P2X7 receptor of human leukemic lymphocytes. J Immunol 2005; 175: 82-9.

[67] Gu BJ, Sluyter R, Skarratt KK, Shemon AN, Dao-Ung LP, Fuller SJ*, et al.* An Arg^{307} to Gln polymorphism within the ATP binding site causes loss of function of the human $P2X_7$ receptor. J Biol Chem 2004; 279: 31287-95.

[68] Roberts JA, Vial C, Digby HR, Agboh KC, Wen H, Atterbury-Thomas A*, et al.* Molecular properties of P2X receptors. Pflugers Arch - Eur J Physiol 2006; 452: 486-500.

[69] Wiley JS, Dao-Ung LP, Li C, Shemon AN, Gu BJ, Smart ML*, et al.* An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human $P2X_7$ receptor. J Biol Chem 2003; 278: 17108-13.

[70] Xiao J, Sun L, Yan H, Jiao W, Miao Q, Feng W*, et al.* Metaanalysis of P2X7 gene polymorphisms and tuberculosis susceptibility. FEMS Immunol Med Microbiol 2010; 60: 165-70.

[71] Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Goldberg H, Marks GB*, et al.* A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. Am J Respir Crit Care Med 2007; 175: 360-6.

[72] Saunders BM, Fernando SL, Sluyter R, Britton WJ, Wiley JS. A loss-of-function polymorphism in the human $P2X_7$ receptor abolishes ATP mediated killing of mycobacteria. J Immunol 2003; 171: 5442-6.

[73] Sluyter R, Shemon AN, Wiley JS. Glu^{496} to Ala polymorphism in the $P2X_7$ receptor impairs ATP-induced IL-1β release from human monocytes. J Immunol 2004; 172: 3399-405.

[74] Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C*, et al.* Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β-dependent adaptive immunity against tumors. Nature Med 2009; 15: 1170-8.

[75] Thunberg U, Tobin G, Johnson A, Soderberg O, Padyukov L, Hultdin M*, et al.* Polymorphism in the P2X7 receptor gene and survival in chronic lymphocytic leukaemia. Lancet 2002; 360: 1935-9.

[76] Wiley JS, Dao-Ung LP, Gu BJ, Sluyter R, Shemon AN, Li C*, et al.* A loss-of-function polymorphic mutation in the cytolytic *P2X7* receptor gene and chronic lymphocytic leukaemia: a molecular study. Lancet 2002; 359: 1114-9.

[77] Nuckel H, Frey UH, Durig J, Duhrsen U, Siffert W. 1513A/C polymorphism in the P2X7 receptor gene in chronic lymphocytic leukemia: Absence of correlation with clinical outcome. Eur J Haematol 2004; 72: 259-63.

[78] Sellick GS, Rudd M, Eve P, Allinson R, Matutes E, Catovsky D*, et al.* The P2X7 receptor gene A1513C polymorphism does not contribute to risk of familial or sporadic chronic lymphocytic leukemia. Cancer Epidemiol Biomarkers Prev 2004; 13: 1065-7.

[79] Starczynski J, Pepper C, Pratt G, Hooper L, Thomas A, Hoy T*, et al.* The P2X7 receptor gene polymorphism 1513 A>C has no effect on clinical prognostic markers, in vitro sensitivity to fludarabine, Bcl-2 family protein expression or survival in B-cell chronic lymphocytic leukaemia. Br J Haematol 2003; 123: 66-71.

[80] Zhang LY, Ibbotson RE, Orchard JA, Gardiner AC, Seear RV, Chase AJ*, et al.* P2X7 polymorphism and chronic lymphocytic leukaemia: Lack of correlation with incidence, survival and abnormalities of chromosome 12. Leukemia 2003; 17: 2097-100.

[81] Barden N, Harvey M, Gagne B, Shink E, Tremblay M, Raymond C*, et al.* Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. Am J Med Genet B Neuropsychiatr Genet 2006; 141: 374-82.

[82] Hejjas K, Szekely A, Domotor E, Halmai Z, Balogh G, Schilling B*, et al.* Association between depression and the Gln460Arg polymorphism of P2RX7 gene: A dimensional approach. Am J Med Genet B Neuropsychiatr Genet 2009; 150: 295-9.

[83] Lucae S, Salyakina D, Barden N, Harvey M, Gagne B, Labbe M*, et al.* P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. Hum Mol Genet 2006; 15: 2438-45.

[84] McQuillin A, Bass NJ, Choudhury K, Puri V, Kosmin M, Lawrence J*, et al.* Case-control studies show that a non-conservative amino-acid change from a glutamine to arginine in the P2RX7 purinergic receptor protein is associated with both bipolar- and unipolar-affective disorders. Mol Psychiatry 2009; 14: 614-20.

[85] Nagy G, Ronai Z, Somogyi A, Sasvari-Szekely M, Rahman OA, Mate A*, et al.* P2RX7 Gln460Arg polymorphism is associated with depression among diabetic patients. Prog Neuropsychopharmacol Biol Psychiatry 2008; 32: 1884-8.

[86] Green EK, Grozeva D, Raybould R, Elvidge G, Macgregor S, Craig I*, et al.* P2RX7: A bipolar and unipolar disorder candidate susceptibility gene? Am J Med Genet B Neuropsychiatr Genet 2009; 150: 1063-9.

[87] Grigoroiu-Serbanescu M, Herms S, Muhleisen TW, Georgi A, Diaconu CC, Strohmaier J*, et al.* Variation in P2RX7 candidate gene (rs2230912) is not associated with bipolar I disorder and unipolar major depression in four European samples. Am J Med Genet B Neuropsychiatr Genet 2009; 150: 1017-21.

[88] Lavebratt C, Aberg E, Sjoholm LK, Forsell Y. Variations in FKBP5 and BDNF genes are suggestively associated with depression in a Swedish population-based cohort. J Affect Disord 2010; 125: 249-55.

[89] Al-Shukaili A, Al-Kaabi J, Hassan B. A comparative study of interleukin-1beta production and p2x7 expression after ATP stimulation by peripheral blood mononuclear cells isolated from rheumatoid arthritis patients and normal healthy controls. Inflammation 2008; 31: 84-90.

[90] Labasi JM, Petrushova N, Donovan C, McCurdy S, Lira P, Payette MM*, et al.* Absence of the P2X7 receptor alters leukcoyte function and attenuates an inflammatory response. J Immunol 2002; 168: 6436-45.

[91] Ke HZ, Qi H, Weidema AF, Zhang Q, Panupinthu N, Crawford DT*, et al.* Deletion of the $P2X_7$ nucleotide receptor reveals its regulatory roles in bone formation and resorption. Mol Endocrinol. 2003; 17: 1356-67.

[92] Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C*, et al.* COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. BMC Neurol 2006; 6: 12.

[93] Sharp AJ, Polak PE, Simonini V, Lin SX, Richardson JC, Bongarzone ER*, et al.* P2x7 deficiency suppresses development of experimental autoimmune encephalomyelitis. J Neuroinflammation 2008; 5: 33.
[94] Chen L, Brosnan CF. Exacerbation of experimental autoimmune encephalomyelitis in P2X7R-/- mice: evidence for loss of apoptotic activity in lymphocytes. J Immunol 2006; 176: 3115-26.
[95] Barden N, Sillaber I, Paez-Pereda M. Means and methods for diagnosing and treating affective disorders. US7767398, 2010.
[96] Barden N, Sillaber I, Paez-Pereda M. Means and methods for diagnosing and treating affective disorders. WO2004092384, 2004.
[97] Apetoh L, Tesniere A, Ghiringhelli F, Zitvogel L, Kroemer G. Use of P2X7 pathway for assessing the sensitivity of a subject to a cancer treatment. WO2010061246, 2010.
[98] Martins I, Tesniere A, Kepp O, Michaud M, Schlemmer F, Senovilla L*, et al.* Chemotherapy induces ATP release from tumor cells. Cell Cycle 2009; 8: 3723-8.
[99] Ohshima Y, Tsukimoto M, Takenouchi T, Harada H, Suzuki A, Sato M*, et al.* γ-Irradiation induces $P2X_7$ receptor-dependent ATP release from B16 melanoma cells. Biochim Biophys Acta 2010; 1800: 40-6.
[100] Diatchenko L, Maixner W. Identification of genetic polymorphic variants associated with somatosensory disorders and methods of using the same. US20090253585, 2009.
[101] Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P*, et al.* Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 2005; 114: 386-96.
[102] Donnelly-Roberts DL, Jarvis MF. Discovery of P2X7 receptor-selective antagonists offers new insights into P2X7 receptor function and indicates a role in chronic pain states. Br J Pharmacol 2007; 151: 571-9.
[103] Honore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, Mikusa JP*, et al.* A-740003 [N-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat. J Pharmacol Exp Ther 2006; 319: 1376-85.
[104] Nelson DW, Gregg RJ, Kort ME, Perez-Medrano A, Voight EA, Wang Y*, et al.* Structure-activity relationship studies on a series of novel, substituted 1-benzyl-5-phenyltetrazole P2X7 antagonists. J Med Chem 2006; 49: 3659-66.
[105] Keith T, Little RD, Van Eerdewegh P, Dupuis J, Del Mastro RG, Simon J*, et al.* Nucleotide and amino acid sequences relating to respiratory diseases and obesity. US7205146, 2007.
[106] Olsson PG, Hulthe J, McPheat W, Eriksson POG. Polymorphisms and haplotypes in P2X7 gene and their use in determining susceptibility for atherosclerosis-mediated diseases. WO2006136791, 2006.
[107] Morten JE. Polymorphisms in the human P2X7 gene. EP1741722, 2007.
[108] Jursik C, Sluyter R, Georgiou JG, Fuller SJ, Wiley JS, Gu BJ. A quantitative method for routine measurement of cell surface $P2X_7$ receptor function in leucocyte subsets by two-colour time-resolved flow cytometry. J Immunol Methods 2007; 325: 67 – 77.
[109] Korpi-Steiner NL, Sheerar D, Puffer EB, Urben C, Boyd J, Guadarrama A*, et al.* Standardized method to minimize variability in a functional P2X7 flow cytometric assay for a multi-center clinical trial. Cytometry B Clin Cytom 2008; 74: 319-29.
[110] Denlinger LC, Hogan KJ, Bertics PJ, Schell K. White blood cell functional assay. US7560243, 2009.
[111] Denlinger LC, Hogan KJ, Bertics PJ, Schell K. Functional genomic pore assay for mixed cell populations. US2009053204, 2009.

[112] Denlinger LC, Schell K, Angelini G, Green D, Guadarrama A, Prabhu U*, et al.* A novel assay to detect nucleotide receptor P2X7 genetic polymorphisms influencing numerous innate immune functions. J Endotoxin Res 2004; 10: 137-42.
[113] Denlinger LC, Angelini G, Schell K, Green DN, Guadarrama AG, Prabhu U*, et al.* Detection of human P2X7 nucleotide receptor polymorphisms by a novel monocyte pore assay predictive of alterations in lipopolysaccharide-induced cytokine production. J Immunol 2005; 174: 4424-31.
[114] Denlinger LC, Coursin DB, Schell K, Angelini G, Green DN, Guadarrama AG*, et al.* Human P2X7 pore function predicts allele linkage disequilibrium. Clin Chem 2006; 52: 995-1004.
[115] Denlinger LC, Shi L, Guadarrama A, Schell K, Green D, Morrin A*, et al.* Attenuated P2X7 pore function as a risk factor for virus-induced loss of asthma control. Am J Respir Crit Care Med 2009; 179: 265-70.
[116] Barden JA, Gidley-Baird A. Anti-P2X7 peptides and epitopes. WO2010000041, 2010.
[117] Barden JA, Gidley-Baird A. Novel P2X7 epitopes. WO2009033233, 2009.
[118] Barden JA, Gidley-Baird A. Hybridomas producing antibodies against non functional P2X7 receptor. WO2008043145, 2008.
[119] Gidley-Baird AB, J.A. Antibodies against non-functional P2X7 receptor. WO2008043146, 2008.
[120] Barden JA, Gidley-Baird A. Antibodies to non-functional P2X7 receptor. US7326415, 2008.
[121] Gidley-Baird A, Barden JA. Antibodies to non-functional P2X7 receptor diagnosis and treatment of cancers and other conditions. WO2002057306, 2002.
[122] Barden JA, Sluyter R, Gu BJ, Wiley JS. Specific detection of non-functional human $P2X_7$ receptors in HEK293 cells and B-lymphocytes. FEBS Lett 2003; 538: 159-62.
[123] Worthington RA, Smart ML, Gu BJ, Williams DA, Petrou S, Wiley JS*, et al.* Point mutations confer loss of ATP-induced human $P2X_7$ receptor function. FEBS Letters 2002; 512: 43-6.
[124] Slater M, Danieletto S, Gidley-Baird A, Teh LC, Barden JA. Early prostate cancer detected using expression of non-functional cytolytic P2X7 receptors. Histopathology 2004; 44: 206-15.
[125] Slater M, Danieletto S, Pooley M, Cheng Teh L, Gidley-Baird A, Barden JA. Differentiation between cancerous and normal hyperplastic lobules in breast lesions. Breast Cancer Res Treat 2004; 83: 1-10.
[126] Slater M, Scolyer RA, Gidley-Baird A, Thompson JF, Barden JA. Increased expression of apoptotic markers in melanoma. Melanoma Res 2003; 13: 137-45.
[127] Gorodeski G, Feng Y-H, Li X. Truncated proteins as cancer markers. US7767789, 2010.
[128] Franco DL, Ramanathan CS, Lewis MA, Feder JN. Polynucleotide encoding a novel human P2X7 splice variant, HBMYP2X7v. WO2004090097, 2004.
[129] Bader AG, Byrom M, Johnson CD, Brown D. Mir-143 regulated genes and pathways as targets for therapeutic intervention. US20090232893, 2009.
[130] Bader AG, Patrawala L, Wiggins JF, Byrom MW, Johnson CD, Brown D. Mir-34 regulated genes and pathways as targets for therapeutic intervention. US20090227533, 2009.
[131] Byrom M, Patrawala L, Johnson CD, Brown D, Bader AG. Mir-16 regulated genes and pathways as targets for therapeutic intervention. US20090175827, 2009.
[132] Bader AG, Byrom M, Johnson CD, Brown D. Mir-20 regulated genes and pathways as targets for therapeutic intervention. US20090163434, 2009.
[133] Gorodeski G, Potashkin J, Cheatham B. Screening, diagnosing, treating and prognosis of pathophysiologic status by RNA regulation. WO2008115387, 2008.

[134] Jimenez AI, Sesto A, Roman JP, Gascon I, Gonzalez De Buitrago G, Jimenez MC*, et al.* Methods and compositions to inhibit P2X7 receptor expression. WO2006024880, 2006.
[135] Chu YX, Zhang Y, Zhang YQ, Zhao ZQ. Involvement of microglial P2X7 receptors and downstream signaling pathways in long-term potentiation of spinal nociceptive responses. Brain Behav Immun 2010; 24: 1176-89.
[136] Lucas JJ, Miras-Portugal MT, Diaz-Hernandez M. Method for the in vitro diagnosis/prognosis of Huntington's chorea. WO2008142194, 2008.
[137] Diaz-Hernandez M, Diez-Zaera M, Sanchez-Nogueiro J, Gomez-Villafuertes R, Canals JM, Alberch J*, et al.* Altered P2X7-receptor level and function in mouse models of Huntington's disease and therapeutic efficacy of antagonist administration. FASEB J 2009; 23: 1893-906.
[138] Adriouch S, Bannas P, Schwarz N, Fliegert R, Guse AH, Seman M*, et al.* ADP-ribosylation at R125 gates the P2X7 ion channel by presenting a covalent ligand to its nucleotide binding site. FASEB J 2008; 22: 861-9.
[139] Fuller SJ, Stokes L, Skarratt KK, Gu BJ, Wiley JS. Genetics of the P2X7 receptor and human disease. Purinergic Signal 2009; 5: 257-62.
[140] Sluyter R, Shemon AN, Barden JA, Wiley JS. Extracellular ATP increases cation fluxes in human erythrocytes by activation of the $P2X_7$ receptor. J Biol Chem 2004; 279: 44749-55.
[141] Sluyter R, Dalitz JG, Wiley JS. $P2X_7$ receptor polymorphism impairs extracellular adenosine 5'-triphosphate-induced interleukin-18 release from human monocytes. Genes Immunity 2004; 5: 588-91.
[142] Sluyter R, Shemon AN, Wiley JS. $P2X_7$ receptor activation causes phosphatidylserine exposure in human erythrocytes. Biochem Biophys Res Commun 2007; 355: 169-73.
[143] Shemon AN, Sluyter R, Wiley JS. Rottlerin inhibits $P2X_7$ receptor stimulated phospholipase D activity in chronic lymphocytic leukaemia B-lymphocytes. Immunol Cell Biol 2007; 85: 68-72.
[144] Gartland A, Skarratt KK, Hocking LJ, Stokes L, Clifton-Bligh R, Parsons C*, et al.* Loss of function polymorphisms in the P2X7 receptor gene are associated with accelerated lumbar spine bone loss in postmenopausal women. J Bone Mineral Res 2008; 23: S296.
[145] Ohlendorff SD, Tofteng CL, Jensen JEB, Petersen S, Civitelli R, Fenger M*, et al.* Single nucleotide polymorphisms in the P2X7 gene are associated to fracture risk and to effect of estrogen treatment. Pharmacogenet Genomics 2007; 17: 555-67.
[146] Nissen N, Madsen JS, Bladbjerg EM, Beck Jensen JE, Jorgensen NR, Langdahl B*, et al.* No association between hip geometry and four common polymorphisms associated with fracture: The Danish osteoporosis prevention study. Calcif Tissue Int 2009; 84: 276-85.
[147] Mrazek F, Gallo J, Stahelova A, Petrek M. Functional variants of the P2RX7 gene, aseptic osteolysis, and revision of the total hip arthroplasty: A preliminary study. Hum Immunol 2010; 71: 201-5.
[148] Lee KH, Sung SP, Kim I, Jin HK, Eun KR, Yoon SS*, et al.* P2X7 receptor polymorphism and clinical outcomes in HLA-matched sibling allogeneic hematopoietic stem cell transplantation. Haematologica 2007; 92: 651-7.
[149] Jamieson SE, Peixoto-Rangel AL, Hargrave AC, Roubaix LAD, Mui EJ, Boulter NR*, et al.* Evidence for associations between the purinergic receptor P2X7 (P2RX7) and toxoplasmosis. Genes Immunity 2010; 11: 374-83.
[150] Sambasivan V, Murthy KJR, Reddy R, Vijayalakshimi V, Hasan Q. P2X7 gene polymorphisms and risk assessment for pulmonary tuberculosis in Asian Indians. Disease Markers 2010; 28: 43-8.
[151] Xiao J, Sun L, Jiao W, Li Z, Zhao S, Li H*, et al.* Lack of association between polymorphisms in the P2X7 gene and tuberculosis in a Chinese Han population. FEMS Immunol Med Microbiol 2009; 55: 107-11.

[152] Taype CA, Shamsuzzaman S, Accinelli RA, Espinoza JR, Shaw MA. Genetic susceptibility to different clinical forms of tuberculosis in the Peruvian population. Infect Genet Evol 2010; 10: 495-504.
[153] Nino-Moreno P, Portales-Perez D, Hernandez-Castro B, Portales-Cervantes L, Flores-Meraz V, Baranda L*, et al.* P2X7 and NRAMP1/SLC11 A1 gene polymorphisms in Mexican mestizo patients with pulmonary tuberculosis. Clin Exp Immunol 2007; 148: 469-77.
[154] Mokrousov I, Sapozhnikova N, Narvskaya O. Mycobacterium tuberculosis co-existence with humans: Making an imprint on the macrophage P2X7 receptor gene? J Med Microbiol 2008; 57: 581-4.
[155] Tekin D, Kayaalti Z, Dalgic N, Cakir E, Soylemezoglu T, Isin Kutlubay B*, et al.* Polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis in Turkish children. Pediatr Infect Dis J 2010; 29: 779-82.
[156] Sharma S, Kumar V, Khosla R, Kajal N, Sarin B, Sehajpal PK. Association of P2X7 receptor +1513 (A>C) polymorphism with tuberculosis in a Punjabi population. Int J Tuberc Lung Dis 2010; 14: 1159-63.
[157] Palomino-Doza J, Rahman TJ, Avery PJ, Mayosi BM, Farrall M, Watkins H*, et al.* Ambulatory blood pressure is associated with polymorphic variation in P2X receptor genes. Hypertension 2008; 52: 980-5.
[158] Haas SL, Ruether A, Singer MV, Schreiber S, Bocker U. Functional P2X7 receptor polymorphisms (His155Tyr, Arg307Gln, Glu496Ala) in patients with Crohn's disease. Scand J Immuno 2007; 65: 166-70.
[159] Eslick GD, Thampan BV, Nalos M, McLean AS, Sluyter R. Circulating interleukin-18 concentrations and a loss-of-function P2X7 polymorphism in heart failure. Int J Cardiol 2009; 137: 81-3.
[160] Portales-Cervantes L, Nino-Moreno P, Doni-z-Padilla L, Baranda-Candido L, Garci-a-Hernandez M, Salgado-Bustamante M*, et al.* Expression and function of the P2X7 purinergic receptor in patients with systemic lupus erythematosus and rheumatoid arthritis. Hum Immunol 2010; 71: 818-25.
[161] Lester S, Skarratt K, Gu B, Downie-Doyle S, Wiley J, Rischmueller M. Functional polymorphisms of the P2X7 gene influence susceptibility to Ro/la autoantibody-positive primary Sjogren's syndrome. Arthritis Rheumatism 2008; 58: S792.
[162] Forchap SL, Anandacoomarasamy A, Wicks J, Di Virgilio F, Baricordi OR, Rubbini M*, et al.* P2X7 gene polymorphisms do not appear to be a susceptibility gene locus in sporadic cases of systemic lupus erythematosus. Tissue Antigens 2008; 72: 487-90.
[163] Dao-Ung LP, Fuller SJ, Sluyter R, Skarratt KK, Thunberg U, Tobin G*, et al.* Association of the 1513C polymorphism in the P2X7 gene with familial forms of chronic lymphocytic leukaemia. Br J Haematol 2004; 125: 815-7.
[164] Paneesha S, Starczynski J, Pepper C, Delgado J, Hooper L, Fegan C*, et al.* The P2X7 receptor gene polymorphism 1513A>C has no effect on clinical prognostic markers and survival in multiple myeloma. Leukemia Lymphoma 2006; 47: 281-4.
[165] Jalbout M, Bouaouina N, Chouchane L. Genetic variation in the P2X7 apoptosis purinoreceptor correlated with anti-nuclear and cytoskeleton autoantibodies induction in nasopharyngeal carcinoma Cancer Therapy 2004; 2: 463-8.
[166] Dardano A, Falzoni S, Caraccio N, Polini A, Tognini S, Solini A*, et al.* 1513A>C polymorphism in the P2X7 receptor gene in patients with papillary thyroid cancer: Correlation with histological variants and clinical parameters. J Clin Endocrinol Metab 2009; 94: 695-8.
[167] Erhardt A, Lucae S, Unschuld PG, Ising M, Kern N, Salyakina D*, et al.* Association of polymorphisms in P2RX7 and CaMKKb with anxiety disorders. J Affect Disord 2007; 101: 159-68.

[168] Hansen T, Jakobsen KD, Fenger M, Nielsen J, Krane K, Fink-Jensen A*, et al.* Variation in the purinergic P2RX7 receptor gene and schizophrenia. Schizophrenia Research 2008; 104: 146-52.

FIGURE LEGENDS

Fig. (1). Signaling events downstream of human P2X7 receptor activation. P2X7 is a trimeric ligand-gated cation channel (only one P2X7 subunit is shown). Activation of P2X7 by extracellular adenosine 5'-triphosphate (ATP) induces a number of downstream signaling events, as illustrated, in a variety of cell types. IL, interleukin; MMP-9, matrix metalloproteinase 9; PS, phosphatidylserine; RONS, reactive oxygen and nitrogen species.

Fig. (2). Splice isoforms of the human P2X7 receptor. Top schematic representation shows the domains of the full-length human P2X7 receptor including the first (TM1) and second (TM2) transmembrane domains. Remaining schematic representations show the regions encoding the original, full-length human P2X7 receptor (P2X7A) and its splice isoforms (P2X7B to P2X7K). Numbered boxes and solid lines represent exons and alternate coding regions respectively. Additional exons, 1' and N3, present in the introns (not shown) between exons 1 and 2, and exons 2 and 3 respectively, are termed as originally described [55, 57]. The additional exon in P2X7G and P2X7H introduces a new start codon potentially leading to translation of a P2X7 protein lacking the first transmembrane domain. *To date P2X7K has been observed only in rodents. Schematic representations of the P2X7 isoforms are adapted from previous studies [53, 55].

Fig. (3). Characterized single nucleotide polymorphisms of the human *P2RX7* gene. Schematic representations of the human (A) *P2XR7* gene and (B) P2X7 subunit displaying single nucleotide polymorphisms. (A) Solid lines and numbered boxes represent introns (not to scale) and exons respectively. An additional exon, N3, in the intron between exons 2 and 3 is not shown. (B) Open circles, closed triangles and closed circles represent gain-, partial loss- and loss-of-function polymorphisms respectively; the open triangle represents a polymorphism with neutral effect on P2X7 function, but reduced sensitivity to inhibition by extracellular Ca^{2+}. SNP rs35933842, which

leads to a null allele, is located in the intron between exons 1 and 2, and thus is not shown in the P2X7 subunit. Schematic representation of the human P2X7 subunit is adapted from a schematic of the murine P2X7 subunit of a previous study [138].

Table 1. Characterized Single Nucleotide Polymorphisms of the Human *P2RX7* Gene.

dbSNP ID	Base change	Amino Acid change	Effect on function	Minor Allele Frequency[1]	Reference
rs35933842	gt>tt	-	Loss	0.008	[56]
rs17525809	GTG>GCG	Val76Ala	Partial loss	0.062	[14, 63]
rs28360445	CGG>TGG	Arg117Trp	Loss	nd	[14]
rs28360447	GGA>AGA	Gly150Arg	Loss	0.018	[14, 63]
rs208294	CAT>TAT	His155Tyr	Gain	0.439	[14, 58, 63, 66, 139]
rs28360451	GAA>AAA	Glu186Lys	Loss	nd	[14]
rs28360452	CTC>CCC	Leu191Pro	Loss	nd	[14]
rs7958311	CAT>CGT	His270Arg	Gain	0.255	[63]
rs7958316	CGT>CAT	Arg276His	Loss	0.020	[63]
rs28360457	CGG>CAG	Arg307Gln	Loss	0.013	[67]
rs1718119	GCT>ACT	Ala348Thr	Gain	0.400	[14, 58, 63]
rs2230911	ACT>AGT	Thr357Ser	Partial loss	0.083	[62]
rs2230912	CAG>CGG	Gln460Arg	Partial loss	0.170	[63]
rs3751143	GAG>GCG	Glu496Ala	Loss	0.175	[14, 58, 59]
rs2230913	CAC>CAG	His521Gln	Neutral[2]	0.020	[14]
rs1653624	ATC>AAC	Ile568Asn	Loss	0.029	[14, 69]

[1]Minor allele frequencies were previously described [63] except rs2230913 (http://www.ncbi.nlm.nih.gov/projects/SNP); nd, not determined.

[2]Neutral effect; however the mutant receptor displays reduced sensitivity to inhibition by extracellular Ca^{2+}.

Table 2. Functional Defects of the rs3751143 (Glu496Ala) Single Nucleotide Polymorphism

Functional Defect	Cell Types	References
Ba^{2+} (Ca^{2+}), Rb^{+} (K^{+}) flux	Monocytes, B- and T-lymphocytes, erythrocytes	[59, 73, 108, 140]
Ethidium^{+}, Yo-Pro-1^{2+} uptake	Macrophages, dendritic cells, Langerhans cells, monocytes, natural killer cells, B- and T-lymphocytes	[18, 20, 59, 60, 71-73, 76, 108, 109, 112-114, 122, 141]
CD23 shedding	Dendritic cells, Langerhans cells	[18, 20]
CD62L shedding	Monocytes, lymphocytes	[73]
IL-1β release	Monocytes	[73]
IL-18 release	Monocytes	[141]
TNF-α/IL-10 ratio	Whole blood	[113]
Matrix metalloproteinase 9 release	Monocytes, B- and T-lymphocytes	[40]
Phosphatidylserine exposure	Erythrocytes	[142]
Phospholipase D activation	B-lymphocytes	[143]
Mycobacterial killing	Macrophages	[60, 71, 72]
Toxoplasma killing	Macrophages	[61]
Cell death	Macrophages, B- and T-lymphocytes	[59, 60, 71, 72, 76]

Table 3. Association of *P2RX7* Single Nucleotide Polymorphisms with Human Disorders

Disorder	Associated Allele(s)[1]	Non-associated Allele(s)[2]	Population	References
Bone Disorders				
Accelerated Lumbar Spine Bone Loss	rs28360457	rs35933842, rs28360447 rs3751143, rs1653624	Scottish post-menopausal women	[144]
Fracture risk	rs3751143, rs1653624	rs28360457	Danish	[145]
Hip geometry		rs3751143, rs1653624	Danish	[146]
Periprosthetic osteolysis	rs28360457 (increase hip arthroplasty failure risk)	rs35933842, rs3751143, rs1653624	Czech	[147]
Infectious Diseases				
Allogeneic stem cell transplantation	rs3751143 (reduced survival due to infection)		Korean	[148]
Toxoplasmosis	rs1718119		North American, Brazilian	[149]
Tuberculosis	rs2393799 (↓)	rs3751143	Gambian	[49]
Tuberculosis	rs2393799, rs1653624 (↓)	rs3751143	Asian Indian	[150]
Tuberculosis		rs2393799, rs3751143	Chinese Han	[151]
Tuberculosis		rs3751143	Peruvian	[152]
Tuberculosis	rs3751143	rs2393799	Various[3]	[70]
Tuberculosis	rs3751143	rs2393799	Mexican Mestizo	[153]
Tuberculosis	rs3751143	rs2393799	Russian Caucasian	[154]
Tuberculosis	rs3751143		Australian Vietnamese	[71]
Tuberculosis	rs3751143		Australian Asian	[71]
Tuberculosis	rs3751143		Turkish children	[155]
Tuberculosis	rs3751143		Punjabi	[156]
Inflammatory and cardiovascular disorders				
Ambulatory blood pressure	rs591874	13 other	British	[157]
Crohn's disease		rs208294, rs28360457, rs3751143	German	[158]
Heart failure		rs3751143	Australian	[159]
Rheumatoid		rs2393799,	Mexican	[160]

arthritis		rs3751143		
Sjögren's Syndrome	rs2230912 (increased Ro/La autoantibody diversification)		Australian	[161]
Systemic lupus erythematosus		rs2393799, rs3751143	Mexican	[160]
Systemic lupus erythematosus		rs3751143	Australian, Italian	[162]
Malignancies				
Breast cancer (anthracyline-treated)	rs3751143 (reduced metastatic-free survival)		French	[74]
Chronic lymphocytic leukemia	rs3751143 (increased survival)		Swedish	[75]
Chronic lymphocytic leukemia	rs3751143		Australian	[76]
Chronic lymphocytic leukemia		rs3751143	German	[77]
Chronic lymphocytic leukemia		rs3751143	British, various[4]	[78]
Chronic lymphocytic leukemia		rs3751143	British	[79]
Chronic lymphocytic leukemia		rs3751143	British	[80]
Chronic lymphocytic leukemia (familial)	rs3751143		Australian, British	[163]
Chronic lymphocytic leukemia (familial)		rs3751143	British	[78]
Multiple myeloma		rs3751143	British	[164]
Nasopharyngeal carcinoma	rs3751143 (increased autoantibodies)		Tunisian	[165]
Papillary thyroid cancer	rs3751143 (follicular variant only)	rs208294	Italian	[166]

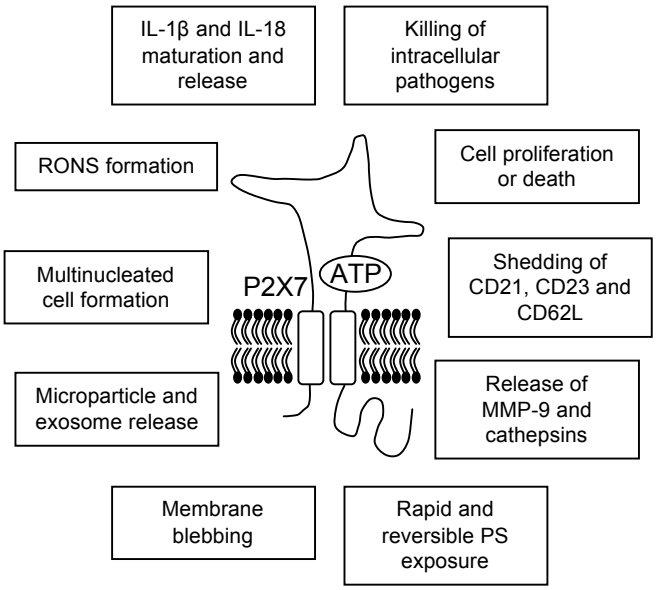
Psychiatric Disorders				
Anxiety	rs1718119	rs2230912 plus 10 other	German	[167]
Bipolar disorder	rs2230912	16 other	French Canadian	[81]
Bipolar or unipolar disorder	rs2230912	rs208293, rs208294 rs504677 rs1718119	British	[84]
Bipolar or unipolar disorder	rs2230912 (increased clinical score)		Hungarian	[82]
Bipolar or unipolar disorder	rs1653625		Hungarian	[52]
Bipolar or unipolar		rs2230912 plus 8 other	British	[86]
Bipolar or unipolar disorder		rs2230912	German, Polish, Romanian, Russian	[87]
Unipolar disorder	rs2230912	15 other	German Caucasian	[83]
Unipolar disorder	rs2230912		Hungarian diabetics	[85]
Unipolar disorder		rs2230912	Swedish	[88]
Schizophrenia		rs2230912 plus 8 other	Danish	[168]

[1]Allele frequency significantly greater in cases compared to controls unless otherwise indicated (↓); comments in parenthesis indicate that the allele frequency was not significantly different between cases and controls, but correlated with specified clinical parameters.
[2]Allele frequency not significantly different between cases and controls.
[3]Meta-analysis of multiple studies (see corresponding reference for details).
[4]Includes meta-analysis of multiple studies (see corresponding reference for details).

Table 4. Patents Related to the *P2RX7* Gene and P2X7 Variants.

Publication Number	Reference Number and Title	Inventors	Publication Date
US7767789	[127] Truncated proteins as cancer markers	Gorodeski, G., Feng, Y-H., Li, X.	2010/08/03
US7767398	[95] Means and methods for diagnosing and treating affective disorders	Barden, N., Sillaber, I., Paez-Pereda, M.	2010/08/03
WO2010061246	[97] Use of P2X7 pathway for assessing the sensitivity of a subject to a cancer treatment	Apetoh, L., Tesniere, A., Ghiringhelli, F., Zitvogel, L., Kroemer, G.	2010/06/03
WO2010000041	[116] Anti-P2X7 peptides and epitopes	Barden, J.A., Gidley-Baird, A.	2010/01/07
US20090253585	[100] Identification of genetic polymorphic variants associated with somatosensory disorders and methods of using the same	Diatchenko, L., Maixner, W.	2009/10/08
US20090232893	[129] Mir-143 regulated genes and pathways as targets for therapeutic intervention	Bader, A.G., Byrom, M., Johnson, C.D., Brown, D.	2009/09/17
US20090227533	[130] Mir-34 regulated genes and pathways as targets for therapeutic intervention	Bader, A.G., Patrawala, L., Wiggins, J.F., Byrom, M.W., Johnson, C.D., Brown, D.	2009/09/10
US7560243	[110] White blood cell functional assay	Denlinger, L.C., Hogan, K.J., Bertics, P.J., Schell, K.	2009/07/14
US20090175827	[131] Mir-16 regulated genes and pathways as targets for therapeutic intervention	Byrom, M., Patrawala, L., Johnson, C.D., Brown, D., Bader, A.G.	2009/07/09
US20090163434	[132] Mir-20 regulated genes and pathways as targets for therapeutic intervention	Bader, A.G., Byrom, M., Johnson, C.D., Brown, D.	2009/06/25
WO2009033233	[117] Novel P2X7 epitopes	Barden, J.A.,Gidley-Baird, A.	2009/03/09
US2009053204	[111] Functional genomic pore assay for mixed cell populations	Denlinger, L.C., Hogan, K.J., Bertics, P.J., Schell, K.	2009/02/26

WO2008142194	[136] Method for the in vitro diagnosis/prognosis of Huntington's chorea	Lucas, J.J., Miras-Portugal, M.T., Diaz-Hernandez, M.	2008/11/27
WO2008115387	[133] Screening, diagnosing, treating and prognosis of pathophysiologic status by RNA regulation	Gorodeski, G., Potashkin, J., Cheatham, B.	2008/09/25
WO2008043145	[118] Hybridomas producing antibodies against non functional P2X7 receptor	Barden, J.A., Gidley-Baird, A.	2008/04/17
WO2008043146	[119] Antibodies against non-functional P2X7 receptor	Gidley-Baird, A. Barden, J.A.	2008/04/17
US7326415	[120] Antibodies to non-functional P2X7 receptor	Barden, J.A., Gidley-Baird, A.	2008/02/05
EP1741722	[107] Polymorphisms in the human P2X7 gene	Morten, J.E.	2007/01/10
WO2006024880	[134] Methods and compositions to inhibit P2X7 receptor expression	Jimenez, A.I., Sesto, A., Roman, J.P., Gascon, I., Gonzalez De Buitrago, G., Jimenez, M.C., Williams, G.O.	2006/03/09
US7205146	[105] Nucleotide and amino acid sequences relating to respiratory diseases and obesity	Keith, T., Little, R.D., Van Eerdewegh, P., Dupuis, J., Del Mastro, R.G., Simon, J., Allen, K., Pandit, S.	2007/04/17
WO2006136791	[106] Polymorphisms and haplotypes in P2X7 gene and their use in determining susceptibility for atherosclerosis-mediated diseases	Olsson, P.G., Hulthe, J., McPheat, W., Eriksson, P.O.G.	2006/12/28
WO2004092384	[96] Means and methods for diagnosing and treating affective disorders	Barden, N., Sillaber, I., Paez-Pereda, M.	2004/10/28
WO2004090097	[128] Polynucleotide encoding a novel human P2X7 splice variant, HBMYP2X7v	Franco, D.L., Ramanathan, C.S., Lewis, M.A., Feder, J.N.	2004/10/21
WO2002057306	[121] Antibodies to non-functional P2X7 receptor diagnosis and treatment of cancers and other conditions	Gidley-Baird, A., Barden, J.A.	2002/07/17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Cell proliferation or death
Multinucleated cell formation
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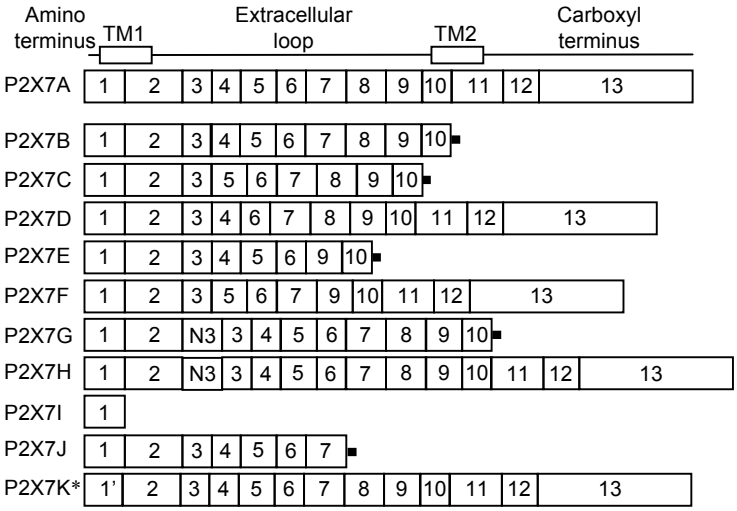
Microparticle and exosome release
Release of MMP-9 and cathepsins
Membrane blebbing
Rapid and reversible PS exposure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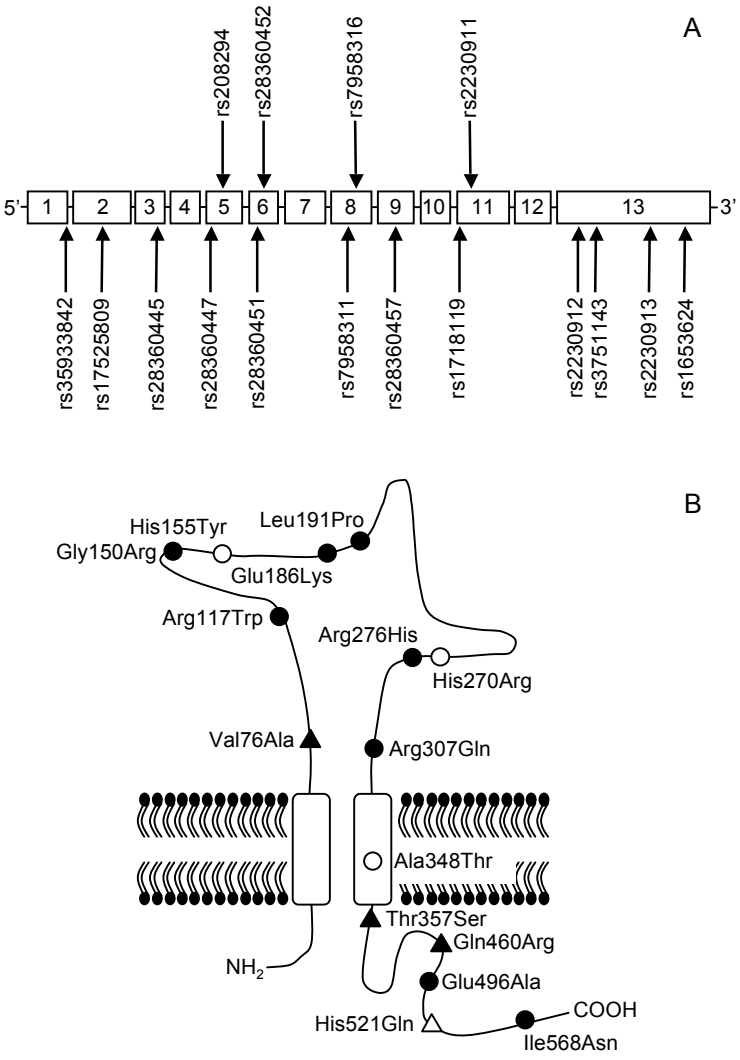
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