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

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Significance of P2X7 Receptor Variants to Human Health and Disease

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Running title: P2X7 receptor variants

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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.

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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]

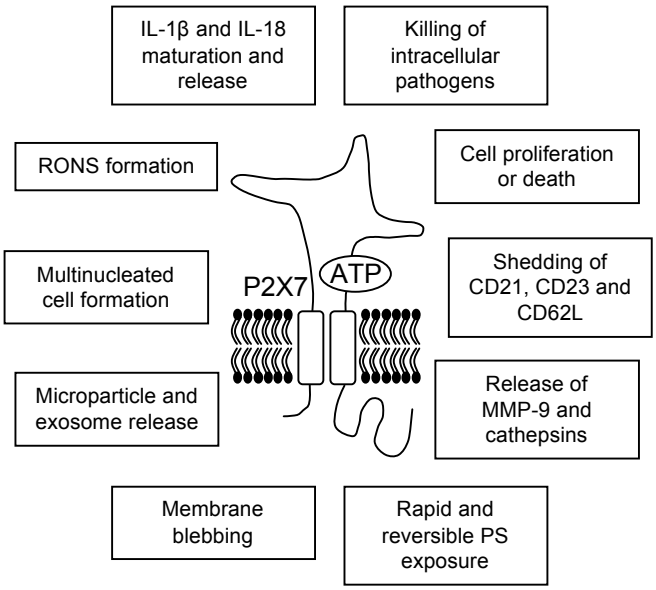
Psychiatric Disorders				
Anxiety	rs1718119	rs2230912 plus 10 other	German	[167]
Bipolar disorder	rs2230912	16 other	French Canadian	[81]
Bipolar or unipolar disorder	rs2230912	rs208293, rs208294 rs504677 rs1718119	British	[84]
Bipolar or unipolar disorder	rs2230912 (increased clinical score)		Hungarian	[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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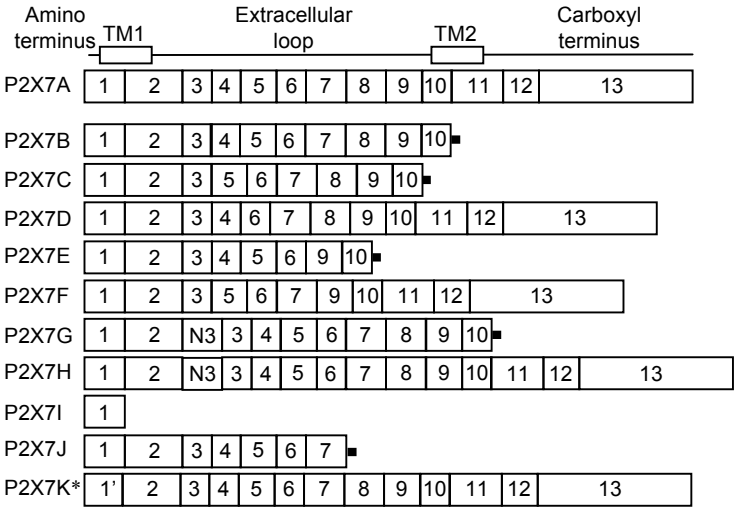
Release of MMP-9 and cathepsins
Membrane blebbing
Rapid and reversible PS exposure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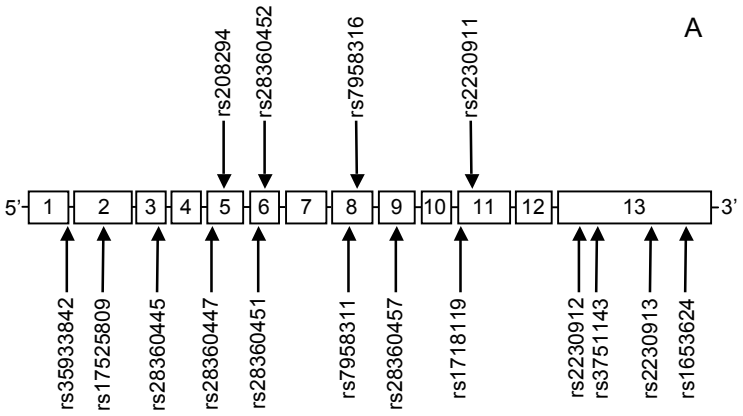
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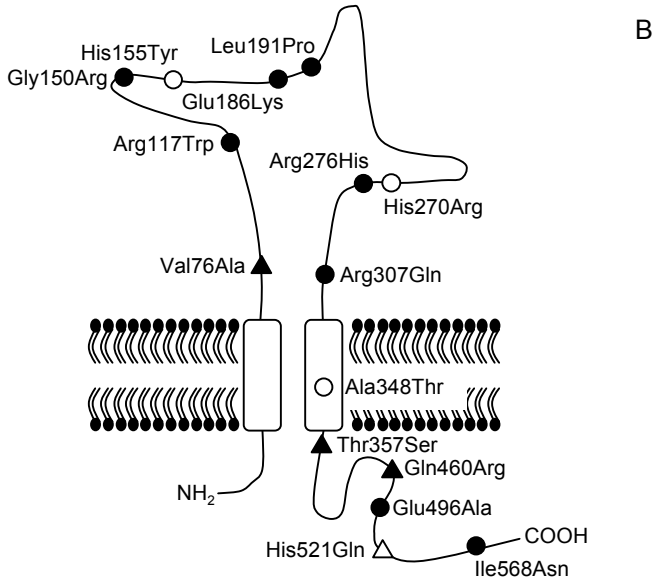
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