Genetics of the P2X7 receptor and human disease

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Abstract The *P2RX7* gene is highly polymorphic, and many single nucleotide polymorphisms (SNPs) underlie the wide variation observed in P2X7 receptor responses. We review the discovery of those non-synonymous SNPs that affect receptor function and compare their frequencies in different ethnic populations. Analysis of pairwise linkage disequilibrium (LD) predicts a limited range of haplotypes. The strong LD between certain functional SNPs provides insight into published studies of the association between SNPs and human disease.

Keywords P2X7 receptor · Genetics · Single nucleotide polymorphisms · Linkage disequilibrium

Introduction

Major advances have been made in our understanding of the role the P2X7 receptor (P2X7R) has to play in human disease. Publication of the human genome sequence in 2004 [1] and the completion of the International HapMap Project in 2005 [2] has been followed by large genome-wide association studies that have found linkage for single nucleotide polymorphisms (SNPs) to human disease. It is generally assumed that SNPs identified through genetic association studies are excellent candidates for disease-causing variants. However, firm evidence for the significance of these candidates requires demonstration of their functional effect on the target protein. A potential complication of this approach comes from the existence of linkage disequilibrium within the genome such that the candidate

gene may be co-inherited with an adjoining polymorphism that is the actual disease-causing variant. It is therefore timely to examine the pattern of inheritance of combinations of SNPs within the *P2RX7* gene to define linkage disequilibrium between SNPs that have functional effects and to determine haplotype block structure of those SNPs that interact to modulate P2X7R function.

Recent advances in genetics

SNPs are the most common genetic variation in the human genome and there are currently over 14 million documented in dbSNP (www.ncbi.nlm.nih.gov/SNP; build 129), the public repository for genetic variation. Successful identification of disease-causing variations in genes before the completion of the Human Genome Project involved the use of linkage studies of large families with multiple affected members. These studies have been successful in identifying genes responsible for a number of diseases including *BRCA1* and *BRCA2* predisposing to breast cancer [3] and *APC* for familial adenomatous polyposis [4] although it has not been possible to identify susceptibility genes in the majority of complex diseases. It is hoped that with the availability of dense genetic maps of SNPs it will be possible to identify genes that contribute to susceptibility to complex common diseases using large association studies, and this approach has defined valuable loci for further study in bipolar disorder, rheumatoid arthritis, coronary artery disease, Crohn's disease, and types 1 and 2 diabetes mellitus [5].

SNPs in *P2RX7*

The *P2RX7* gene spans 53 kb with 13 exons and has been mapped to the 12q24.31 chromosomal region [6]. The

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P2RX7 gene is highly polymorphic and there are 32 non-synonymous, amino acid-altering SNPs currently documented in dbSNP (www.ncbi.nlm.nih.gov/SNP). The first to be functionally described was 1513A>C that changes glutamic acid to alanine at residue 496 (Glu⁴⁹⁶ to Ala) in the carboxyl terminus of the receptor and has an allele frequency of 0.14–0.18 in normal Caucasian populations [7]. The Glu⁴⁹⁶ to Ala polymorphism confers an unusual property upon the receptor in native or transfected cells since adenosine triphosphate (ATP)-induced ethidium uptake is abolished, but the immediate ATP-induced opening of the cation-selective channel is unaffected [8].

The second SNP to be described was 1729T>A that changes isoleucine to asparagine at residue 568 (Ile⁵⁶⁸ to Asn) which lies in a trafficking motif within the carboxyl tail of the receptor [9, 10]. This amino acid substitution prevents normal trafficking and cell surface expression of the receptor and transfection experiments have shown that P2X7R carrying the Ile⁵⁶⁸ to Asn mutation is non-functional. The 1729T>A SNP is rare in Caucasian populations with an allele frequency of 0.026 (Table 1). A third SNP, 946G>A, changes arginine to glutamine at residue 307 (Arg³⁰⁷ to Gln) in the ATP-binding pocket of the extracellular domain, and transfection experiments have shown that P2X7R carrying this mutation lacks channel and pore function although there is normal surface expression of the receptor [11]. A splice site mutation (g>t) at position +1 of the first intron causes a null allele in close to 1% of Caucasian subjects [12] due to nonsense-mediated mRNA decay. A fifth polymorphism (1096C>G) changes serine to threonine at amino acid 357 (Ser³⁵⁷ to Thr) and has an allele frequency of 0.075 in Caucasians. The Ser³⁵⁷ to Thr

polymorphism produces a partial loss of P2X7R function which affects both channel and pore functions [13]. A sixth polymorphism (489C>T) changes amino acid 155 from histidine to tyrosine (His¹⁵⁵ to Tyr) in the ectodomain [14] and has an allele frequency of 0.455 in Caucasians (Table 1). The His¹⁵⁵ to Tyr polymorphism produces a gain-of-function effect on P2X7R pore function and calcium influx [14]. Finally, a seventh polymorphism has been described at 474G>A which reduces receptor function in native cells [15] although confirmatory transfection experiments are awaited to verify these data.

Re-sequencing of genes for the International HapMap Project (<http://www.hapmap.org>) has shown divergence in the polymorphic structure of the *P2RX7* gene in different population samples. The HapMap data include nine non-synonymous SNPs in the *P2RX7* gene: rs17525809 (253 bp), rs208294 (489 bp), rs7958311 (835 bp), rs7958316 (853 bp), rs1718119 (1068 bp), rs2230911 (1096 bp), rs2230912 (1405 bp), rs3751143 (1513 bp), and rs1653624 (1729 bp). These SNPs are all represented in CEPH (Utah Residents with Northern and Western European Ancestry) but of note is that rs7958311 (853 bp), rs2230912 (1405 bp), and rs1653624 (1729 bp) were not found in Japanese in Tokyo, Japan or Han Chinese in Beijing, China populations. Similarly, rs7958311 (853 bp) and rs1653624 (1729 bp) were not found in the Yoruba in Ibadan, Nigeria population. In addition, rs17525809 (253 bp) was not found in YRI and rs2230912 (1405 bp) minor allele frequency was very low (0.017) compared with the CEU population (Table 2). These differences in populations may reflect positive or negative selection pressures over long time periods by environmental and disease factors.

Table 1 Minor allele frequencies of ten single nucleotide polymorphisms (SNPs) in the *P2RX7* gene in an Australian Caucasian cohort

SNP	SNP (nucleotide number)	Minor allele frequency
rs35933842	151+1g>t	0.008
rs28360447	474G>A	0.015
rs208294	489C>T	0.455
rs7958311	853G>A	0.023
rs7958316	946G>A	0.016
rs1718119	1068G>A	0.394
rs2230911	1096C>G	0.075
rs2230912	1405A>G	0.174
rs3751143	1513A>C	0.185
rs1653624	1729T>A	0.026

Genomic DNA was extracted from the peripheral blood of 1,755 normal Australian Caucasian subjects. Genotyping was performed using a Sequenom MassArray automated detection system at the Australian Genome Research Facility (Brisbane, Australia)

P2X7R and human disease

Genetic association studies of human disease with a complex pattern of inheritance have yielded valuable insights into the involvement of unsuspected genes. Several genetic association case–control studies using synonymous and non-synonymous SNPs have revealed a role for *P2RX7* as a susceptibility gene in mood disorders and in susceptibility to infections with intracellular pathogens such as tuberculosis. Three large studies in a total of 2,500 patients with either bipolar disorder or major depressive disorder [16–18] have found a significant disease association of a non-synonymous SNP (rs2230912) within the coding region of the *P2RX7* gene. This SNP codes for Gln⁴⁶⁰ to Arg in the carboxyl terminus of the receptor protein, but its functional effect on receptor function is not fully defined. P2X7R has also been shown to be important in the killing of phagocytosed *Mycobacterium tuberculosis* by extracel-

Table 2 Minor allele frequencies of nine non-synonymous single nucleotide polymorphisms (SNPs) in *P2RX7* from four populations described by the International HapMap Project (www.hapmap.org)

CEU CEPH Utah Residents with Northern and Western European Ancestry, CHB Han Chinese in Beijing, China, JPT Japanese in Tokyo, Japan, YRI Yoruba in Ibadan, Nigeria

SNP	Base pair <i>P2RX7</i>	CEU (<i>n</i> =120)	CHB (<i>n</i> =90)	JPT (<i>n</i> =90)	YRI (<i>n</i> =120)
rs17525809	253	0.067	0.044	0.133	–
rs208294	489	0.458	0.633	0.567	0.333
rs7958311	835	0.183	0.400	0.344	0.233
rs7958316	853	0.008	–	–	–
rs1718119	1068	0.492	0.122	0.211	0.517
rs2230911	1096	0.100	0.244	0.133	0.192
rs2230912	1405	0.217	–	–	0.017
rs3751143	1513	0.136	0.205	0.239	0.067
rs1653624	1729	0.017	–	–	–

lular ATP. Extensive in vitro data have shown that mycobacteria can survive following uptake by macrophages, but that a bactericidal pathway activated by extracellular ATP via the P2X7 receptor leads both to death of the mycobacteria and subsequent apoptosis of the infected macrophage [19–23]. Epidemiological studies have confirmed the role of P2X7R in the control of tuberculosis. Thus, inheritance of the 1513A>C loss-of-function polymorphic variant of *P2RX7* confers a 3.5-fold increased lifetime risk of reactivating latent (primary) tuberculosis in two cohorts of south-east Asian migrants to Australia [22], while a Mexican study has confirmed 1513A>C as a susceptibility factor for reactivating tuberculosis [24]. A role for P2X7R has also been shown in resistance to infection with *Chlamydia trachomatis* [25, 26] although

there have been no large association studies to date studying Chlamydia infection and SNPs in *P2RX7*.

P2X7R may also play a role in bone disease and recent studies have suggested its function as a mechano-transducer in osteocytes [27] and thus inheritance of the loss-of-function P2X7R variants Glu⁴⁹⁶ to Ala and Ile⁵⁶⁸ to Asn is associated with increased fracture risk in post-menopausal females [28].

Defining haplotype structure in *P2RX7* using pairwise linkage disequilibrium

Defining linkage disequilibrium (LD) patterns in *P2RX7* for use in association studies is essential for selecting a

Fig. 1 Haploview analysis of pairwise linkage disequilibrium in the *P2RX7* gene using ten marker SNPs that change receptor function. The colours represent the relative D'/LOD score where bright red is D'=1; LOD ≥ 2 and blue D'=1; LOD < 2, shades of pink D' < 1; LOD ≥ 2, white D' < 1; LOD < 2. Numbers represent D' scores for pair-wise linkage disequilibrium

Table 3 Five haplotypes within the *P2RX7* genomic block which make up 99.9% of Australian Caucasian subjects

1068	1096	1405	1513	Frequency
G	C	A	A	0.341
A	C	A	A	0.226
G	C	A	C	0.185
A	C	G	A	0.173
G	G	A	A	0.075

minimum set of SNPs for genotyping and to show the extent of a region to be examined for functional variants. These data are also helpful to explain failure of previous association studies to detect the involvement of *P2RX7* if the chosen marker SNPs are not in LD with functional variants. We investigated pairwise LD using ten non-synonymous SNPs to define haplotype blocks in an Australian Caucasian population sample. The program Haploview (<http://www.broad.mit.edu/mpg/haploview/index.php>) was used to test for conformance with Hardy–Weinberg equilibrium, calculate D' and r^2 measures of LD between SNP pairs and to identify haplotype blocks of strong LD. In addition, we compared these data with patterns of variation for *P2RX7* found in the International HapMap Project (<http://www.hapmap.org>) database.

Distributions of genotype frequencies of the ten SNPs in the Australian Caucasians were all in Hardy–Weinberg equilibrium, and minor allele frequencies are summarized in Table 1. A haplotype block was found to include base positions 1068, 1096, 1405, and 1513 (Fig. 1) and this block overlaps that previously published [16, 17]. It is likely that the base position 1729 lies within this block, but the low frequency of 1729A minor allele precluded its inclusion. Analysis of the HapMap International Project (<http://www.hapmap.org>) data confirmed significant pairwise LD between base positions 1068 and 1096, 1405, and

1513 (not shown). For these four SNPs 99.9% of our Australian Caucasian subjects had one of five haplotypes: GCAA, ACAA, GCAC, ACGA, and GGAA (Table 3). The 489 base position was not part of this haplotype block but was in high LD with 1405 ($D'=0.81$) and 1729 ($D'=0.94$). LD between 489 and 1405 was confirmed in the CEU population using the HapMap (<http://www.hapmap.org>) data. Analysis of 1,166 subjects that were genotyped at both 489 and 1405 and 1,170 subjects genotyped at both 489 and 1513 confirmed that 1405G and 1513C minor alleles were associated with the 489T minor allele (Table 4).

Table 4 Genotype of Australian Caucasian subjects who have been typed at (a) both 489 and 1405 bp of the gene ($n=1,166$) and (b) both 489 and 1513 bp of the gene ($n=1,170$)

		CC	489 CT	TT
1405	AA	305	358	100
	AG	21	240	108
	GG	0	4	30
1513	AA	280	392	107
	AC	46	192	102
	CC	0	22	29

Fig. 2 ATP-induced ethidium uptake into HEK293 cells transfected with P2X7 constructs mutated at polymorphic positions that alter function. **a** Gain-of-function mutation His¹⁵⁵ to Tyr is shown either on a wild-type background or combined with the Gln⁴⁶⁰ to Arg (Q460R) variation (H155Y Q460R). **b** Loss-of-function mutation Glu⁴⁹⁶ to Ala is shown either on a wild-type background (E496A) or combined with the His¹⁵⁵ to Tyr (H155Y) variation (H155Y E496A)

We have investigated these particular strong pairwise LD relationships by transfecting HEK293 cells with constructs carrying a combination of His¹⁵⁵ to Tyr and Gln⁴⁶⁰ to Arg mutations and a combination of His¹⁵⁵ to Tyr and Glu⁴⁹⁶ to Ala mutations. This approach allows an assessment of whether a gain-of-function polymorphism can modulate the functional effect of a second co-inherited polymorphism. Figure 2 shows that the gain-of-function Tyr¹⁵⁵ mutation increases ATP-induced ethidium uptake by approximately 50% over a wild-type receptor, while the Arg⁴⁶⁰ mutation shows reduced ethidium uptake. The combination shows that the Tyr¹⁵⁵ rescued the function of Arg⁴⁶⁰ (Fig. 2a), whereas the Tyr¹⁵⁵ mutation could not rescue the loss-of-function seen with the Ala⁴⁹⁶ mutation (Fig. 2b).

In summary, strong LD exists between four non-synonymous *P2RX7* SNPs that form five haplotypes in 99.9% of Caucasian subjects. Significantly, SNPs within this block are in high LD with the 489 base position (His¹⁵⁵ to Tyr) functional variant. Analysis of 1405A>G and 1513A>C revealed both minor alleles to have originally occurred on a 489T background allele. Individual *P2RX7* SNPs should not be considered in isolation and interaction of combinations of functional variants within P2X7R must be considered when analyzing P2X7R studies. These data are important for use in interpreting results of association studies, as SNPs that alter P2X7R channel and pore function are candidates for disease-associated variants. In addition, genotyping costs of future association studies using Caucasian subjects can be reduced by using tag SNPs that identify each of the five haplotypes with LD between four SNPs.

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