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Selection of human p75NTR tag SNPs and its biological significance for clinical association studies

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Abstract. To select tag single nucleotide polymorphisms (SNPs) within and around human p75 neurotrophin receptor (p75NTR) gene in Chinese Han population, the sequence involving p75NTR gene as well as the upstream and downstream of the gene was identified according to the data from National Center for Biotechnology Information (NCBI) GenBank database, and the SNP genotype data involving 63 SNPs in the regions were obtained from Chinese Han Beijing (CHB) population of HapMap database. Then, Haploview (version 4.2) was used to calculate linkage disequilibrium (LD) statistics for the selected 32 common SNPs with a minor allele frequence (MAF) more than 0.05. Haplotype blocks were constructed throughout the p75NTR gene according to the upper and the lower 95% confidence bound of D' value, and the tag SNPs were selected based on the r^2 and LOD values between SNPs as well as the results of bioinformatics analysis. The results indicated that five haplotype blocks were constructed within and around p75NTR gene and 12 tag SNPs including rs2537710, rs603769, rs614455, rs2537706, rs534561, rs2072445, rs2072446, rs7219709, rs734194, rs741071, rs741073 and rs2671641 were selected to represent the other 51 SNPs in p75NTR gene. Therefore, the 12 selected SNPs may act as tag SNPs for the entire p75NTR gene in Chinese Han population, which will provide an effective way to select tag SNPs in a whole gene, and its biological significance is to further guide the clinical association studies between the candidate gene and disease susceptibility.

Keywords: p75NTR, haplotype block, tag SNP, haplotype, bioinformatics analysis

1. Introduction

As the first cloned low-affinity nerve growth factor receptor (NGFR), p75NTR can bind to all neurotrophic factors, mediating neuronal survival, growth and apoptosis, as well as regulation of synaptic plasticity. Besides, as a co-receptor for Nogo-66 receptor (Nogo-66 receptor, NgR), p75NTR is involved in the inhibition of neurite growth in the injured central nervous system (CNS) [1,2]. Recent studies showed that the expression of p75NTR in prefrontal cortex and hippocampus in suicide sub-

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jects with depression or other mental disorders is significantly higher than that in the normal subjects [3]. The results suggest that p75NTR plays an important role in the pathogenesis of mental disorders such as depression, posttraumatic stress disorder (PTSD) and Alzheimer's disease (AD), and may be a novel therapeutic target for neuropsychiatric diseases [1–3]. Growing evidences indicate that p75NTR may be involved in the occurrence of depression and its comorbid, such as schizophrenia and PTSD [4–8]. Therefore, p75NTR can be used as an important candidate gene for the study of genetic susceptibility to neuropsychiatric diseases. Recently, many clinical association studies between p75NTR gene polymorphism and susceptibility of mental disorders, including depression and suicidal tendency have been carried out. However, only a few p75NTR SNPs have been involved in previous studies [9–11], and little is known about the global biological significance of the SNPs within the entire p75NTR gene. To comprehensively assess the potential biologic significance of all known genetic variants within the entire p75NTR gene, a SNP haplotype tagging approach as well as bioinformatics analysis is used to select representative tag SNPs. It is thus possible that the study may provide an effective way to investigate the biological significance of all the SNPs within a whole gene, and further guide the clinical association studies between the candidate gene and disease susceptibility.

2. Materials and methods

2.1. Selection of SNPs within and around p75NTR gene

The sequence within and around human p75NTR gene was identified according to the data from NCBI (www.ncbi.nlm.nih.gov) GenBank database (updated on Jun. 3, 2014). Then, all the SNPs within the whole p75NTR gene in 139 healthy CHB population were downloaded from the latest version of HapMap (www.hapmap.org) SNP database (HapMap Data Rel 28 phase II+III, August10, on NCBI B36 assembly, dbSNP b126, NGFR chr17: 44917666..44957360).

2.2. Construction of haplotype blocks

To select tag SNPs from the genetic variation data for the entire p75NTR gene, the common SNPs (MAF>0.05) were first selected, and haplotype blocks were constructed throughout the whole p75NTR gene, using Haploview, version 4.2 (Broad Institute of MIT and Harvard, Cambridge, Mass), a software package that provides computation of linkage disequilibrium (LD) statistics and population haplotype patterns from genotype data. Haplotype blocks represent regions with little evidence of historical recombination between common SNPs. The history of recombination between a pair of SNPs can be estimated with the use of the normalized measure of allelic association D' (value of D prime between the two loci) [12]. The criterion for SNPs selected to construct a haplotype block is that all SNPs in one region must be in strong LD with D' greater than 0.98 for the upper 95% confidence bound (C_U) and greater than 0.7 for the lower bound (C_L) [13].

2.3. Selection of tag SNPs

A maximally informative haplotype tag SNPs (htSNPs) was then selected from each block by using software Tagger program (http://www.broad.mit.edu/mpg/haploview, updated on Sep. 15, 2009) [14]. This algorithm selects a subset of variants that capture all known common genetic variations in a gene, based on an LD threshold of $r^2 \geq 0.8$ [15]. The inverse of r^2 represents the ratio of sample size needed

to detect an indirect association with an unassayed SNP to direct association at the same power. Apart from htSNPs, some SNPs out of the blocks also should be selected based on an LD threshold of $r^2 \geq 0.8$, and others out of the blocks must be selected because they could not represent each other.

To further select the more representative tag SNPs with possible functional significance, whether the above selected tag SNPs are located in the repeat sequence of p75NTR gene was analyzed using online software (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker, updated on Jan. 31, 2014), and the SNPs from the 5'-flanking region and 3'UTR of the p75NTR gene were also analyzed using online software (http://www.genome.jp/tools/motif/, updated on Aug. 10, 1998) to inspect potential transcription factor binding sites. The SNPs in the repeat sequence should not be selected, while the SNPs influencing the transcription factor sites must be selected.

Table 1

Distribution of the common p75NTR gene SNPs in Chinese Han population

SNP ID	Accession number	Location on the gene	Alleles (A:B)*	MAF#	Region
4	rs585916	-8907	C:T	0.1	5'-flanking
5	rs1035050	-8768	C:T	0.198	5'-flanking
7	rs2671687	-7942	C:T	0.209	5'-flanking
9	rs2671686	-7535	T:G	0.057	5'-flanking
13	rs2537710	-6194	A:C	0.105	5'-flanking
17	rs603769	-2695	A:G	0.209	5'-flanking
18	rs2584665	-2598	A:C	0.14	5'-flanking
20	rs575791	1976	A:G	0. 2	Intron 1
21	rs9908234	4569	A:G	0.267	Intron 1
22	rs600120	4705	A:G	0.07	Intron 1
23	rs565042	5060	A:G	0.07	Intron 1
24	rs3785931	5339	C:T	0.476	Intron 1
25	rs614455	5659	T:C	0.07	Intron 1
26	rs2072444	5956	T:C	0.453	Intron 1
27	rs657770	8371	C:A	0.07	Intron 2
29	rs2537706	13409	G:A	0.122	Intron 3
30	rs534561	13433	C:G	0.344	Intron 3
31	rs11466148	13451	C:T	0.134	Intron 3
33	rs3785930	14143	G:A	0.07	Intron 3
36	rs2072445	14933	G:T	0.07	Intron 3
37	rs2072446	15040	C:T	0.148	Exon 4
42	rs11466162	18116	G:A	0.159	3'UTR
43	rs7219709	18422	C:T	0.136	3'UTR
44	rs1804011	18491	C:A	0.116	3'UTR
46	rs7224806	18685	T:C	0.198	3'UTR
47	rs734194	18830	T:G	0.198	3'UTR
48	rs741071	18911	C:T	0.453	3'UTR
49	rs741072	18924	C:T	0.43	3'UTR
50	rs741073	19107	G:A	0.238	3'UTR
58	rs2671641	24948	G:C	0.155	3'UTR
59	rs2671642	25037	A:G	0.163	3'UTR
60	rs10491195	25517	A:G	0.105	3'UTR

Note: *: A, wild type allele; B, mutant type allele; #: MAF, minor allele frequence.

3. Results

3.1. Selection of SNPs within and around p75NTR gene

The full sequence of p75NTR gene with 39.728 kb in length included 10 kb upstream of the transcription start site, a total of 6 exons, 5 introns and 10 kb downstream of the stop codon, which was located on chromosome 17, position 49485293 to 49525020 (GenBank: NC000017). Total 63 SNPs within the whole p75NTR gene were obtained from the HapMap project for 139 healthy CHB population. Analysis of the genetic variation data revealed that there were 32 common SNPs (MAF>0.05), mainly distributed in 5'-flanking, intron, exon and 3' untranslated region (UTR) (see Table 1).

3.2. Construction of haplotype blocks

Fifteen of the selected SNPs constructed five blocks according to the criterion to construct a haplotype block (see Table 1 and Figure 1): block 1: from -2695 to 1976, included 3 SNPs (rs603769, rs2584665 and rs575791), located in p75NTR gene from 5'-flanking to Intron 1; block 2: from 4705 to 8371 included 6 SNPs (rs600120, rs565042, rs3785931, rs614455, rs2072444 and rs657770), located in Intron 1; block 3: from 18685 to 18830, included 2 SNPs (rs7224806 and rs734194), located in 3'UTR; block 4: from 18911 to 18924, included 2 SNPs (rs741071 and rs741072), located in 3'UTR and block 5: from 24948 to 25037, included two SNPs (rs2671641 and rs2671642), also located in 3'UTR. The total length of five blocks is 8588 bp, 21.62% of the length of the total studied sequence, containing a total of 15 SNPs, which is 46.88% of the selected 32 SNPs.

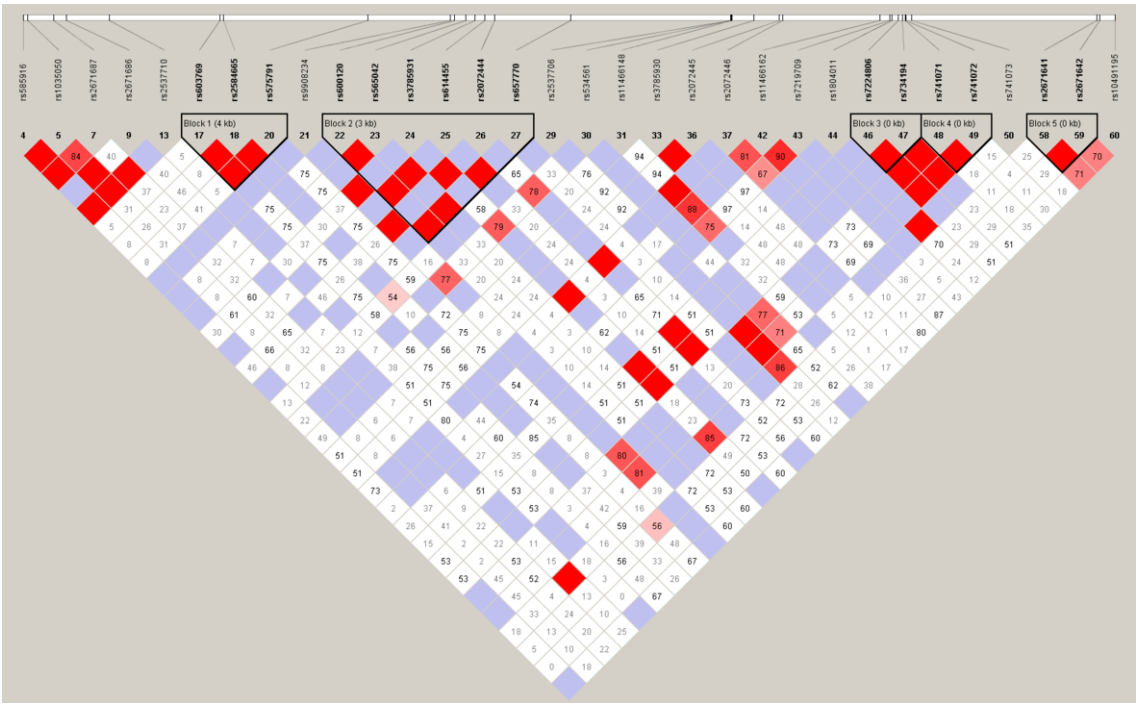

Fig. 1. Gene map and LD plot of common p75NTR gene SNPs in Chinese Han population: LD strength between SNPs, as indicated by the color scheme, was measured using a combination of the statistic D' and the LOD score (dark shade, D'=1 and LOD score ≥3; lighter shades, D'< 1 and LOD score <3). Numbers in squares are D' values.

3.3. Selection of tag SNPs

Five SNPs, including rs603769, rs614455, rs734194, rs741071 and rs2671641, were separately selected as htSNPs from the 5 blocks, based on the analysis of tagging threshold of r^2 of SNPs in each block (see Table 2). Some SNPs out of the blocks, such as rs2537710, rs2072445, rs2072446 and rs7219709, are also selected for tag SNPs according to the LD threshold of $r^2 \geq 0.8$, other 9 SNPs out of the blocks, such as rs1035050, rs2671687, rs2671686, rs9908234, rs2537706, rs534561, rs1804011, rs741073 and rs10491195, are selected for tag SNPs because they could not represent other SNPs. However, the results of bioinformatics analysis indicated that the SNPs, such as rs1035050, rs2671687 and rs2671686 from the 5'-flanking region as well as rs1804011 and rs10491195 in 3'UTR, have no effects on the transcription factor sites. In addition, rs9908234 is located in the repeat sequence of p75NTR gene. As a result, among the 9 SNPs mentioned above, only rs2537706, rs534561 and rs741073 may have functional significance. Taken together, a total of 12 tag SNPs selected in present study can capture most of SNPs within the whole p75NTR gene and might represent the possible biological significance of the p75NTR genetic variation (see Table 3).

Table 2

The linkage disequilibrium, r^2 value and LOD value between SNPs in the blocks of p75NTR gene

Block	SNP	SNP	D'	C_L*	C_U#	r^2	LOD	Average r^2	Average LOD
1	rs603769	rs2584665	1	0.74	1	0.613	6.81	0.807	10.575
		rs575791	1	0.89	1	1	14.34		
	rs2584665	rs575791	1	0.74	1	0.613	6.81	0.613	6.81
	rs575791							0.807	10.575
2	rs600120	rs565042	1	0.75	1	1	7.64	0.635	4.946
		rs3785931	1	0.13	0.99	0.085	0.84		
		rs614455	1	0.75	1	1	7.64		
		rs2072444	1	0.15	0.99	0.09	0.97		
		rs657770	1	0.75	1	1	7.64		
	rs565042	rs3785931	1	0.13	0.99	0.085	0.84	0.635	4.946
		rs614455	1	0.75	1	1	7.64		
		rs2072444	1	0.15	0.99	0.09	0.97		
		rs657770	1	0.75	1	1	7.64		
	rs3785931	rs614455	1	0.13	0.99	0.085	0.84	0.259	3.92
		rs2072444	1	0.9	1	0.953	16.24		
		rs657770	1	0.13	0.99	0.085	0.84		
	rs614455	rs2072444	1	0.15	0.99	0.09	0.97	0.635	4.946
		rs657770	1	0.75	1	1	7.64		
	rs2072444	rs657770	1	0.15	0.99	0.09	0.97	0.263	4.024
	rs657770							0.635	4.946
3	rs7224806	rs734194	1	0.89	1	1	14.05	1	14.05
	rs734194							1	14.05
4	rs741071	rs741072	1	0.89	1	0.91	15.78	0.91	15.78
	rs741072							0.91	15.78
5	rs2671641	rs2671642	1	0.82	1	0.915	10.54	0.915	10.54
	rs2671642							0.915	10.54

Note: *: C_L, the lower 95% confidence bound of the D' value; #: C_U, the upper 95% confidence bound of the D' value.

Table 3

Tag SNPs of p75NTR gene

Block	Tag SNP	Captured SNPs	Location on the gene	Variation (A/B)*	MAF#	Region
	rs2537710	rs585916, rs2537710	-6194	A/C	0.105	5'-flanking
1	rs603769	rs603769, rs2584665, rs575791	-2695	A/G	0.209	5'-flanking
2	rs614455	rs614455, rs565042, rs600120, rs2072444, rs657770	5659	T/C	0.07	Intron 1
	rs2537706	rs2537706	13409	G/A	0.122	Intron 3
	rs534561	rs534561	13433	C/ G	0.344	Intron 3
	rs2072445	rs2072445, rs3785930	14933	G/T	0.07	Intron 3
	rs2072446	rs11466148, rs2072446	15040	C/T	0.148	Exon 4
	rs7219709	rs11466162, rs7219709	18422	C/T	0.136	3'UTR
3	rs734194	rs734194, rs7224806	18830	T/G	0.198	3'UTR
4	rs741071	rs741071, rs741072	18911	C/T	0.453	3'UTR
	rs741073	rs741073	19107	G/A	0.238	3'UTR
5	rs2671641	rs2671642, rs2671641	24948	G/C	0.155	3'UTR

Note: *: A, wild type allele; B, mutant type allele; #: MAF, minor allele frequence.

4. Discussion

Compared with Trk receptor, p75NTR lacks enzyme activity and thus could not trigger a series of phosphorylation cascades by tyrosine kinase. However, as a low affinity neurotrophin receptor, p75NTR can bind to all neurotrophic factors, mediate neuronal survival and growth, induce neuronal apoptosis, and regulate synaptic plasticity. In addition, as a co-receptor for NgR, it is involved in the inhibition of neurite growth in the injured CNS [1,2]. Studies showed that the expression of p75NTR in prefrontal cortex and hippocampus in suicide subjects with depression or other mental disorders is significantly up-regulated [3]. The results suggest that p75NTR plays a pivotal role in the pathogenesis of mental disorders such as depression, PTSD and AD, and may be a novel therapeutic target for neuropsychiatric diseases [1–3]. Indeed it has been shown that p75NTR, with neuronal cell apoptotic effects and regulation of synaptic plasticity, is involved in the occurrence of depression and its comorbid, such as schizophrenia and PTSD [4–8]. As a result, p75NTR can be used as an important candidate gene for the study of genetic susceptibility to neuropsychiatric diseases. Recently, many clinical association studies between p75NTR gene polymorphism and susceptibility of mental disorders, including depression and suicidal tendency, have been carried out. However, only a few p75NTR SNPs, such as rs575791, rs2537706, rs2072446, rs11466155 and rs734194, have been involved in previous studies [9–11], and little is known about the global biological significance of the genetic variants within the whole p75NTR gene.

To address this problem, tag SNPs should be selected to represent the other SNPs in entire p75NTR gene. For this purpose, the sequence within and around human p75NTR gene is identified according to the data from NCBI GenBank database, and the SNP genotype data involving 63 SNPs of p75NTR gene are obtained from CHB population of HapMap database. Then, Haploview (version 4.2) is used to calculate LD statistics for the selected 32 common SNPs (MAF>0.05). Haplotype blocks are constructed throughout the p75NTR gene according to C_U and C_L, and the tag SNPs are selected based on the r^2 and LOD values between SNPs as well as the results of bioinformatics analysis. The results indicate that five haplotype blocks are constructed and a total of 12 tag SNPs including rs2537710,

rs603769, rs614455, rs2537706, rs534561, rs2072445, rs2072446, rs7219709, rs734194, rs741071, rs741073 and rs2671641 are selected to capture most of the genetic variation of the entire p75NTR gene and might represent the possible biological significance of the p75NTR genetic variation. Therefore, future research will be conducted to investigate the clinical association studies between the selected tag SNPs of p75NTR gene and the susceptibility of mental disorders such as depression, PTSD and AD. Taken together, the method to select tag SNPs in this study may provide an effective way to select tag SNPs in a whole gene, and its biological significance is to further guide the clinical association studies between the candidate gene and disease susceptibility.

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References

[1] T. Fujii and H. Kunugi, p75NTR as a therapeutic target for neuropsychiatric diseases, Curr. Mol. Pharmacol. **2** (2009), 70–76.
[2] F. Zeng, J.J. Lu, X.F. Zhou and Y.J. Wang, Roles of p75NTR in the pathogenesis of Alzheimer's disease: A novel therapeutic target, Biochem. Pharmacol. **82** (2011), 1500–1509.
[3] Y. Dwivedi, H.S. Rizavi, H. Zhang, A.C. Mondal, R.C. Roberts, R.R. Conley, N. Ghanshyam and G.N. Pandey, Neurotrophin receptor activation and expression in human postmortem brain: Effect of suicide, Biol. Psychiat. **65** (2009), 319–328.
[4] X.M. Li, F. Han, D.J. Liu and Y.X. Shi, Single-prolonged stress induced mitochondrial-dependent apoptosis in hippocampus in the rat model of post-traumatic stress disorder, J. Chem. Neuroanat. **40** (2010), 248–255.
[5] B. Xiao, B. Yu, H.T. Wang, F. Han and Y.X. Shi, Single-prolonged stress induces apoptosis by activating Cytochrome C/Caspase-9 pathway in a rat model of post-traumatic stress disorder, Cell Mol. Neurobiol. **31** (2011), 37–43.
[6] J. Ding, F. Han and Y. Shi, Single-prolonged stress induces apoptosis in the amygdala in a rat model of post-traumatic stress disorder, J. Psychiatr. Res. **44** (2010), 48–55.
[7] N.H. Woo, H.K. Teng, C.J. Siao, C. Chiaruttini, P.T. Pang, T.A. Milner, B.L. Hempstead and B. Lu, Activation of p75NTR by proBDNF facilitates hippocampal long-term depression, Nat. Neurosci. **8** (2005), 1069–1077.
[8] K. Martinowich, R.J. Schloesser, Y. Lu, D.V. Jimenez, D. Paredes, J.S. Greene, N.H. Greig, H.K. Manji and B. Lu, Roles of p75NTR, Long-term depression, and cholinergic transmission in anxiety and acute stress coping, Biol. Psychiatry **71** (2012), 75–83.
[9] H. Kunugi, R. Hashimoto, M. Yoshida, M. Tatsumi and K. Kamijima, A missense polymorphism (S205L) of the low-affinity neurotrophin receptor p75NTR gene is associated with depressive disorder and attempted suicide, Am. J. Med. Genet. Part B **129B** (2004), 44–46.
[10] T. Fujii, N. Yamamoto, H. Hori, K. Hattori, D. Sasayama, T. Teraishi, M. Hashikura, M. Tatsumi, N. Okamoto, T. Higuchi and H. Kunugi, Support for association between the Ser205Leu polymorphism of p75NTR and major depressive disorder, J. Hum. Genet. **56** (2011), 806–809.
[11] Y.T.A. Gau, Y.J. Liou, Y.W.Y. Yu, T.J. Chen, M.W. Lin, S.J. Tsai and C.J. Hong, Evidence for association between genetic variants of p75 neurotrophin receptor (p75NTR) gene and antidepressant treatment response in Chinese major depressive disorder, Am. J. Med. Genet. Part B **147B** (2008), 594–599.
[12] J.C. Barrett, B. Fry, J. Maller and M.J. Daly, Haploview: Analysis and visualization of LD and haplotype maps, Bioinformatics **21** (2005), 263–265.
[13] M.J. Daly, J.D. Rioux, S.F. Schaffner, T.J. Hudson and E.S. Lander, High-resolution haplotype structure in the human genome, Nat. Genet. **29** (2001), 229–232.
[14] Z. Xu, N.L. Kaplan and J.A. Taylor, TAGster: Efficient selection of LD tag SNPs in single or multiple populations, Bioinformatics **23** (2007), 3254–3255.
[15] C.S. Carlson, M.A. Eberle, M.J. Rieder, Q. Yi, L. Kruglyak and D.A. Nickerson, Selecting a maximally informative set of single nucleotide polymorphisms for association analyses using linkage disequilibrium, Am. J. Hum. Genet. **74** (2004), 106–120.