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Rare Intronic Variations in *TP73* Gene Found in Patients with Alzheimer's Disease

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ABSTRACT *TP73* gene encodes p73 transcription factor, crucial for neurogenesis and neuronal health maintenance. In aging brain, p73 haploinsufficiency increases deposition of tau aggregates, hallmark pathology of Alzheimer's disease. Thus, *TP73* gene can be an important candidate for studying Alzheimer's disease susceptibility. To explore the role of nucleotide variations in regulatory region of *TP73* on Alzheimer's disease, the region encompassing exon 2 of 80 Alzheimer's patients and 123 age-matched controls was sequenced. Prediction of functional impact of found variations were done by software like 'SpliceAid', '*mutation* t@sting' and 'RegRNA2.0'.Two rare variations rs5031052 (NG_017035.2: g.34771C>T) and rs141679680 (NG_017035.2:g.34875G>A) were found in Alzheimer's patients in only heterozygous condition with minor allelic frequencies 0.01875 and 0.0125 respectively, significantly higher than global MAF count (p value of z test 0.04 and 0.01 respectively), but totally absent in the control group. In silico analysis reveals the importance of these variations in splicing and microRNA binding. These variations not only introduce intronic spicing enhancer motif but also modulate splicing factor recruitment. Moreover, they regulate microRNA binding by creating or destroying miRNA binding site. Thus, the researchers report, for the first time that these two rare variations may involve in manifestation of Alzheimer's disease in our cohort.

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INTRODUCTION

Alzheimer's disease (AD) is characterized by irreparable progressive degeneration of neurons (Berchtold and Cotman 1998). *TP73*gene is a significant susceptible genetic cause of AD playing leading role in cell proliferation, differentiation, apoptosis, renewal of stem cells and cell fate commitment (Vousden and Lane 2007). This gene, on chromosomal region 1p36.33, was first described by Kaghad et al. (1997). It encodes a p53 family transcription factor called p73. The shared structural resemblance between p73 and

p53 includes an NH_2- terminal transactivation domain, a central DNA-binding domain, and a COOH-terminal oligomerization domain (Yoon et al. 2015). A complicated m-RNA expression is an important feature of this gene. Transcription from two alternative promoters give rise to functionally distinct isoforms retaining or missing the N-terminal transactivation domain known as pro-apoptotic TAp73 and anti-apoptotic δNp73 (Wang et al. 2007). In addition, alternative C terminal splicing of both transcript resulting up to seven possible variants like α, β, and so forth (Murray-Zmijewski et al. 2006).Tissue-specific monoallelic or biased allelic expression of p73 mRNA indicates the presence of functional genetic polymorphism in the regulatory sequence of this gene (Yan et al. 2002). Regulating the survival (Talos et al. 2010), proliferation (Fujitani et al. 2010) and differentiation (Agostini et al. 2010) of neural stem cell, p73 is essential for the maintenance of cognitive reserve in brain. Disorganization of Hippocampal neurons, degeneration of sympathetic neurons and atrophied cerebrum are evident in p73 knockout mice (Pozniak et al. 2002).Though it is an established fact, that this protein plays a key role in central nervous system, the number of studies exploring its association with neurodegenerative disorders are very few (Li et al. 2004; Grespi and Melino 2012) compared to that of cancers (Hamajima et al. 2002; Hishida et al. 2004; Niwa et al. 2005; Jun et al. 2007; Li at al. 2008; Gallì et al. 2009; Misra et al. 2009; Wu et al. 2017). Modulating tau phosphorylation p73 plays a role in AD pathogenicity (Cancino et al. 2013). It is also responsible for mitochondrial dysfunction (Rufini et al. 2012) a common phenomenon in AD (Hauptmann et al. 2006).

Single nucleotide variations in exon 2 of this gene were reported to accelerate the age of onset of AD in Italian population (Scacchi et al. 2009). However; no such study has so far been reported for Indian AD population. This pilot study with 80 AD subjects and 123 control individuals, investigates whether nucleotide variation in the region encompassing exon 2 of *TP73* could modulate the occurrence of AD in Indian subcontinent.

MATERIAL AND METHODS

Samples

80 patients (mean age 64.86 ± 11.46 years with 71.25 percent male and 28.75 percent female) were diagnosed with probable Alzheimer's disease using DSM -IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) criteria and NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria by the physicians. Patients with other degenerative dementia like vascular dementia, fronto-temporal dementia, dementia with lewy body etc. were not incorporated in this study. Individuals with history of cancer, head injury, brain tumour, untreated thyroid disease, vitamin B12 deficiency, HIV or other blood infections were also excluded.123 healthy age matched control individuals devoid of any identifiable neurodegenerative disease participated for this study. The study was designed and performed following the declaration of Helsinki and approved by the institutional ethics committee. All case and control subjects were chiefly Bengali-speaking and were recruited from same geographical locality to maintain similarity in ethnicity and demography between them.

Mutation Analysis

Blood samples were collected from all cases and controls after getting prior consent. Qiagen QIAamp DNA Blood Midi Kit (Catalogue No. 51185) was used to extract DNA from blood samples. Oligonucleotide primers (Forward: 5' CCACGGATGGGTCTGATCC 3' and Reverse: 5' TTAGCCCAGCGAAGGTGG 3') encompassing exon 2 regions of *TP73* gene (NCBI Reference Sequence NG_017035.2) are chosen from previously published works (Li et al. 2004). A Polymerase Chain Reaction (PCR) was standardized with a final volume of 10µl containing 20 ng of template DNA, 0.5µg of each primer, 1.5 mmol/L $MgCl_2$, dNTPs 0.1mM of each, 0.5 units of Taq DNA Polymerase (Roche, Catalogue No 11435094001). The amplification conditions were as follows, an initial denaturation at 95°C for 10 minutes, 35 cycles of 95°C for 1 min, 62°C for 45 seconds, and 72°C for 1 min, and a final extension step at 72°C for 7 min. ABI PRISM 3700 DNA Analyzer platform was used for sequencing the 428 bp amplicon.

Functional Prediction of Detected Variation

The probable damaging effects of mutant alleles at splicing and protein expression level were assessed by online software 'SpliceAid'

(http://www.introni.it/splicing.html) and 'mutation t@sting' (http://www. mutationtaster. org/). online 'RegRNA2.0' (http://regrna2. mbc. nctu. edu. tw/index.html) software was used to predict any effect of the mutant alleles in detection of functional RNA motifs.

Statistical Analysis

z test was performed (www.princeton.edu/~otorres/Excel/proportions.xls) to compare the proportions of mutant alleles in Indian AD patients and in the global population. The frequencies of minor alleles in global population were taken from dbSNP report of NCBI (http://www.ncbi.nlm.nih.gov/). The reported respective MAF or Minor Allele Count for these two variations are T = 0.0060/30 and A = 0.0024/12 (http://www.ncbi.nlm.nih.gov/).

RESULTS

Two rare variations rs5031052 (NG_017035.2:g.34771C>T) and rs141679680 (NG_017035.2:g.34875G>A), (Fig. 1) located on 3682335th and3682439th nucleotide positions respectively on chromosome 1(GRCh38 Assembly) were found. rs5031052 resides in the 3rd nucleotide

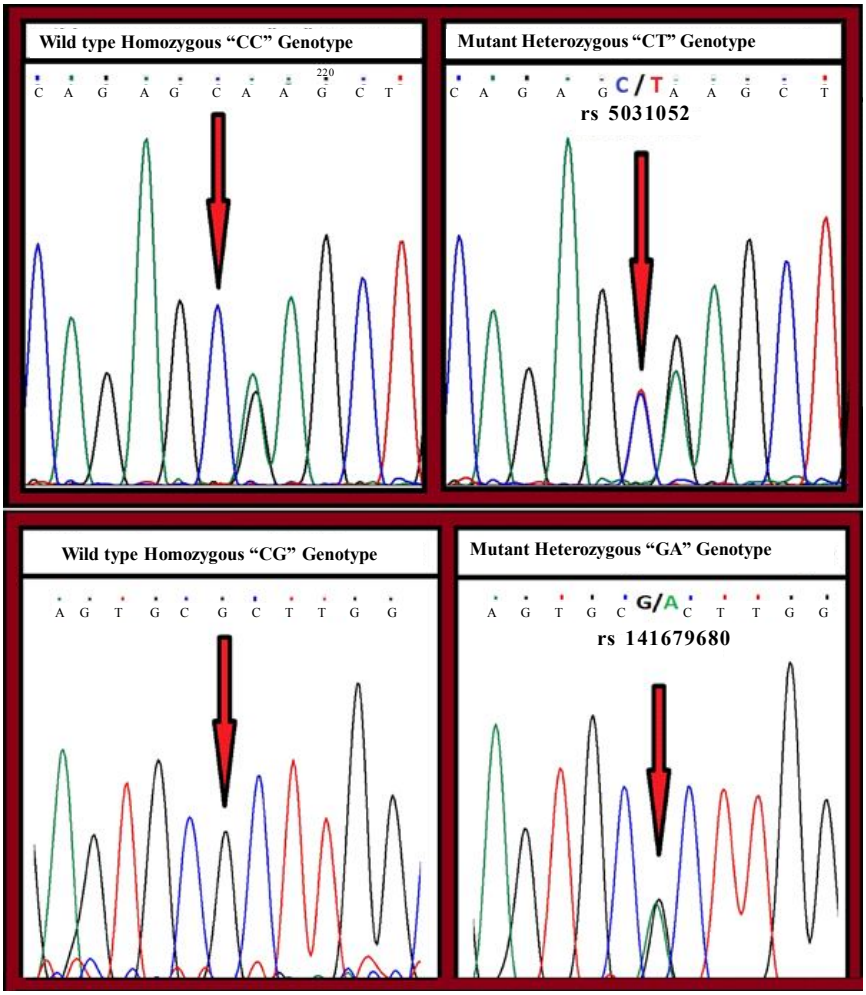

Fig. 1. Chromatograms showing the wild type and mutant genotypes of the detected variations

position of exon 2, that is, in 5' untranslated region or upstream of translational initiation site whereas, rs141679680 is the 9th nucleotide downstream of exon 2 of *TP73* gene.

Genotypic and Allele Frequency

Three and two out of 80 AD patients carried the heterozygous variation rs5031052 and rs141679680 respectively. Thus, for rs5031052, the CT genotypic and T allele frequencies are 0.0375 and 0.01875 respectively. For rs141679680 the GA genotypic frequency is 0.025 and A allelic frequency is 0.0125 in AD affected individuals. Both variations occur in heterozygous condition and totally absent in age matched control group. Table 1 shows that both mutant alleles are in significantly higher frequencies in AD patients than the total global population (p value of z test 0.04 and 0.01 for "T" of rs5031052 and "A" of rs141679680 respectively).

In-silico Analysis

Mutation Taster Result

This software predicts that rs141679680 is a polymorphic variation, and rs5031052 is a disease-causing variation. Both are anticipated to change splice site. Many protein features might be changed. The analysis reveals that transactivation domain, DNA binding domain, oligomerization domain, sterile alpha motif (SAM domain), regions involved in interaction with Homeodomain-interacting protein kinase 2 (HIPK2), WW domain-containing oxidoreductase (WWOX), Abelson murine leukemia viral oncogene homolog 1 (ABL1), polo-like kinase 1 (PLK1), tyrosine kinase like SRC and HCK, residues for Zinc binding, WW binding, sumoylation etc might get lost as they are situated downstream of altered splice site (Table 2).

Splice Aid Analysis for rs5031052

According to this software, mutant T allele of this variation introduce a binding site for a serine/ arginine rich splicing factor SC35 involved in splicing complex formation. The ATP dependent interaction between pre-m-RNA and snRNPs like U1 and U2 are also regulated by this splicing factor (Fig. 2).

Splice Aid Analysis for rs141679680

The result shows that the mutant A allele of this variation abolishes a binding site for Muscleblind-like protein1 (MBNL1) protein which is involved in the regulation of alternative splicing of pre-mRNA. Splicing of specific pre-mRNA targets are either activated or repressed after binding of this protein (Fig. 2).

Splicing Regulatory Motif Analysis via RegRNA 2.0 for rs5031052

The mutant T allele creates a 6 bases long intron splicing enhancer (ISE) motif namely, ighg2

Table 1: Comparisons of mutant allelic frequencies ("T" of rs5031052 and "A" of rs141679680) between AD patients in Indian cohort and Global population

Comparing proportions of found mutant allele in Indian AD patients with global population (z-test)

		rs5031052					
Proportions of Mutant	*"T" allele*	*Number of chromosomes*	*p-hat*	*q-hat*	*std. error*	*z-value*	*p-value*
AD subject	0.01875	160	0.006395	0.993605	0.006402	1.991551	0.04813**
Global population	0.006	5000					
		rs141679680					
Proportions of Mutant	*"T" allele*	*Number of chromosomes*	*p-hat*	*q-hat*	*std. error*	*z-value*	*p-value*
AD subject	0.0125	160	0.002713	0.997287	0.004178	2.417644	0.01675**
Global population	0.0024	5000					

** Proportions significantly different at 95%

Table 2: In-silico analysis of found variations in TP 73 by Mutation Taster software programme

Inferred changes inTP-73 expression	*Amino acid residue(s) spliced out*	*Protein feature*	*Function(s)*	*Probable damaging effects of spicing*
Both of the variations rs141679680 (a polymorphic variation), and rs5031052 (a disease causing variation) are responsible for splice site change. They alter many downstream protein features by splicing mechanism.	1-46	Transactivation domain	It interacts with the basal transcriptional machinery needed for transactivation.	Transactivation property of p73 may be altered.
	27	Interaction with PLK1	It abolishes transcriptional activity of p73, reduce its pro apoptotic function.	Pro apoptotic function may be modulated.
	28	Interaction with		
	99	SRC and HCK	Hinders transcriptional activity and apoptosis.	Apoptosis may be hampered.
		Interaction with ABL1	It increases stability and function of p73.	Destabilization of p73.
	131-310	DNA binding domain	Needed for binding with specific DNA sequence on target gene.	Interaction with p73 target gene may be abolished.
	194,197			
	258,262	Zinc binding	Required for stabilization of p73 protein.	Destabilization of p73.
	345-380	Interaction with HIPK2	It modulates cell cycle and apoptosis.	Apoptotic process may be hampered.
	345-386	Oligomerization domain	Needed for formation of p73 oligomer. The oligomer is required for high-affinity DNA binding and transcriptional activation.	DNA target binding and transcription may be disturbed.
	483-487	WW binding motif	Responsible for the proteo-some base degradation of p73.	Degradation of p73 may be altered.
	485-551	SAM domain	It regulates transcription of target genes as well as the proteome based degradation of p73.	Transactivation and degradation of p73 may be disrupted.
	487	WWOX binding	It increases the proapo-ptotic activity of p73.	Pro apoptotic function may be modulated.
	627	Sumoylation	Needed for apoptosis, stress response, cell cycle, nuclear-cytoplasmic transport and protein stability.	All of these processes may be impeded due to destabilization of p73.

cgamma2 (immunoglobulin heavy chain subclass g2 - cgamma2 gene) - intron 1 ("GTGAGC") which is absent in wild type.

Micro RNA Target Site Prediction via RegRNA 2.0 for rs5031052

Mutant T allele creates a 23-nucleotide binding motif for micro RNA hsa-miR-3616-3p "UCCUGCAGAGUGAGCUGCCCUCG" which is absent in wild type (Fig. 3).

Micro RNA Target Site Prediction via RegRNA 2.0 for rs141679680

Analysis reveals that there is a 27 neocleotide long hsa-miR-4435 micro RNA binding motif"UCUCUGUGAGUGCGCUUGGCUGGCCAG" in wild type. Whether the mutant A allele abolishes this motif (Fig. 3).

DISCUSSION

Eukaryotic genome employs differential or alternative splicing as a prospective mechanism which ensures increased structural and functional diversity of proteins (Black 2003). Data suggest that above 90 percent of the estimated 20,000 to 25,000 protein coding genes in human are post transcriptionally modified by differential splicing (Wang et al. 2008). Apart from its evolutionary significance (Irimia et al. 2007), alternative splicing also contributes to the pathology of several diseases (Ward and Cooper

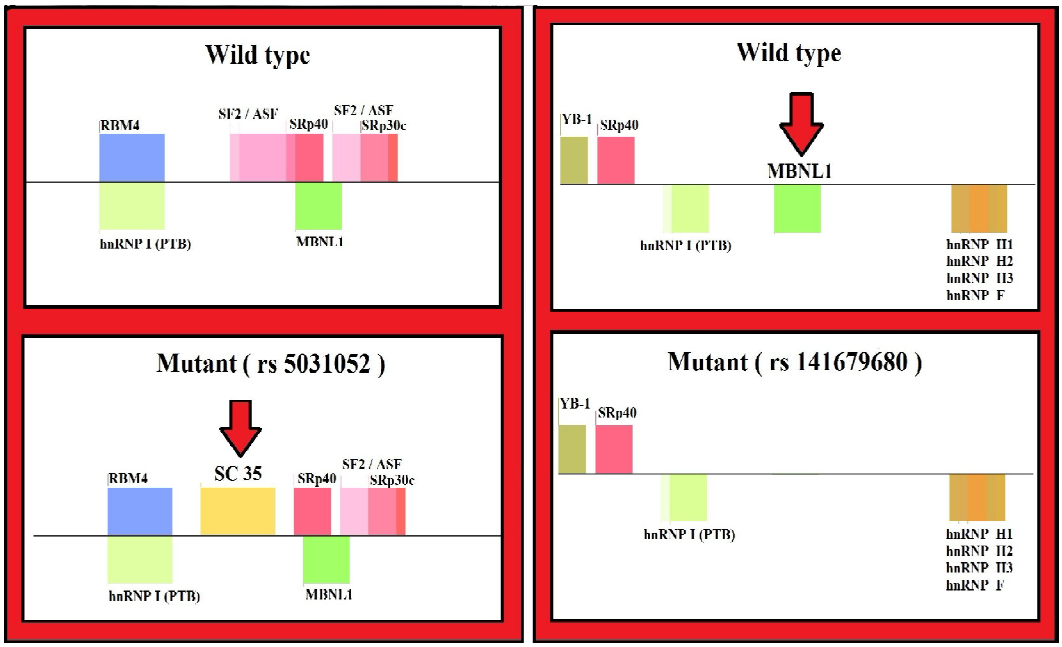

Fig. 2. Results showing the splicing factor binding site prediction for the detected variations using Splice Aid software

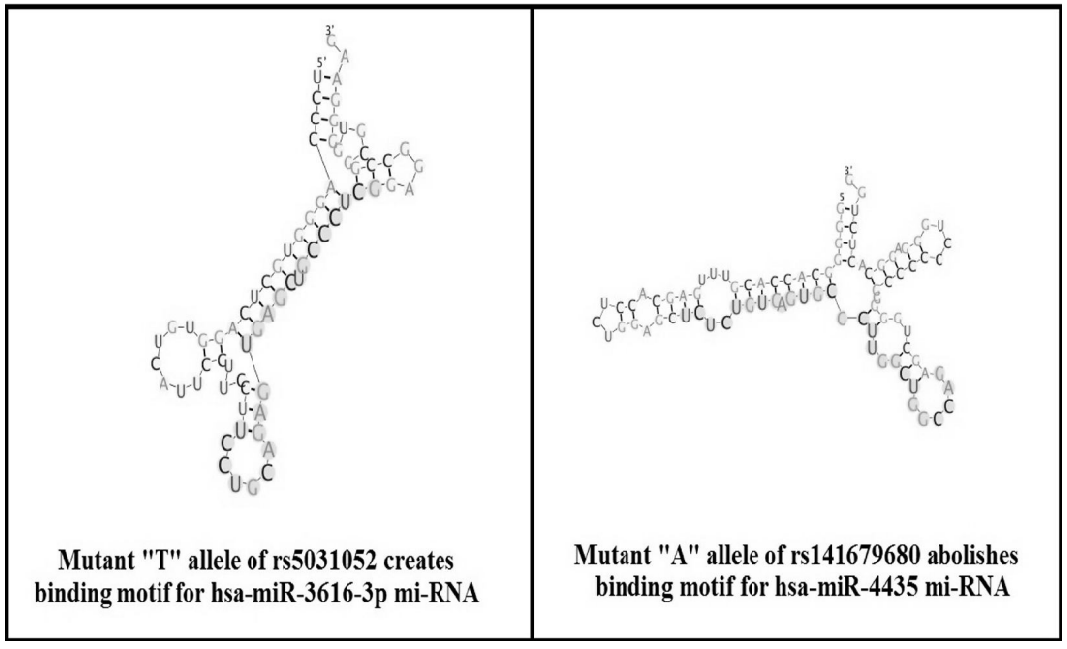

Fig. 3. Results showing the Micro RNA target site prediction for the detected variations using RegRNA 2.0 software

2010). *TP73* gene encodes p73 protein, involved in cell growth suppression and induction of apoptosis (Zhu et al. 1998). *TP73* is a prominent example of alternatively spliced gene, producing diverse isoforms of p73 protein (α,β, γ, δ, ε, η and ζ) with discrete biological functions (Ueda et al. 1999).

Two rare variations rs5031052 and rs141679680 in the region encompassing exon 2 of *TP73* in AD patients were found, and in-silico analysis indicates their role in splicing. Several researches exhibit the differential transactivation property of p73 isoforms. Isoform β is the strongest (Yang et al. 2002), α, β and η are weaker transactivators, where ζ and ε are inert (Ishimoto et al. 2002) and ζ is yet uncharacterized (Nozell et al. 2003). These variations may exert a role in the transactivation by splicing out the TA domain. The researchers' in-silico analysis also indicates that the aminoacid region for potential DNA binding (131 - 310) and oligomerization (345 - 386) may get lost due to this splicing event. Moreover, the structural analysis of p73 reveals that the α isoform possess a C terminal sterile alpha motif (SAM) which regulate transcription of target genes (Nozell et al. 2003) as well as the proteome based degradation of p73 (Peschiaroli et al. 2009). This important SAM domain may be trimmed off by splicing.

Researches disclose that a coordinated cross talking between p73 and several other proteins is needed for its transcriptional and biological activities (Watanabe et al. 2002). The results of this study show that these variations can splice out the amino acid residues 345 to 380, the region previously reported to interacts with a conserved serine/threonine nuclear kinase called Homeodomain-interacting protein kinase 2 (HIPK2) to modulate cell cycle and apoptosis (Kim et al. 2002). Studies also revealed that a diminished transcriptional activity of p73 is ensued when it interacts with a tumor suppressor called WW domain–containing oxidoreductase (WWOX) and redistributed from nucleus to cytoplasm (Aqeilan et al. 2005). Researchers suggest that cytoplasmic p73, through its WW domain-binding PY motif interacts with WWOX and increase its proapoptotic activity in a transcription independent manner (Fabbri et al. 2005). Moreover, Itch, a HECT-type E3 ubiquitin protein ligase binds to PY motif of p73 through its WW domain and operates proteosome base degradation of p73 (Rossi et al. 2005), whereas, YAP1 (Yes-associated protein 1) displaces Itch, binds to PY motif and stabilizes p73 (Levy et al. 2007). These interactions may be hampered as these variations can splice out the WW binding motif in 483-487 amino acid region.

The result also shows that these two variations impair sumoylation, a post transcriptional modification engaged in several cellular activities like apoptosis, stress response, cell cycle, nuclear-cytoplasmic transport and protein stability etc (Hay et al., 2005). The lysine - 627 residue at COOH- terminal of p73 α, binds to a small ubiquitin-like modifier1 (SUMO-1) protein and become covalently modified (Minty at al. 2000). The lysine-627 may get lost by splicing.

Various studies reveal that phosphorylation of Thr-27 residue by polo like kinase 1(PLK1) abolish transcriptional activity of p73 and hence, reduce its pro apoptotic function (Koida et al. 2008; Soond et al. 2008). Whereas, Tyr -99 phosphorylation by c Abl is evident for increase stability and function of p73 (Tsai et al. 2003). HCK and SRC phosphorylate p73 at Tyr -28 and impede transcriptional activity and apoptosis (Paliwal et al. 2007). Our analysis has explored that all of these phosphorylation events may be inhibited due to these variations.

Puca et al. in 2011, shows that the DNA binding domain of p53 is stabilized by zinc atom. Zinc plays a potent role in rescuing p53 from its mutant to functional conformation in several cancerous cell lines and hence hindering tumor progression and revitalizing drug sensitivity (Puca et al. 2011). Interestingly, this zinc mediated conformational stability is stronger in p73 than p53 as chelation of zinc by EDTA damages the DNA binding capacity of p73β more severely than p53 (Lokshin et al. 2007). Splicing out the aminoacids residues involved in Zn –binding, these variations may abrogate the stabilization of p73 protein.

According to the results of this study, mutant T allele of rs5031052 variation incorporates a SC 35 binding site. SC 35 (spliceosome component of 35 kDa) is a non-snRNP splicing factor (Fu and Maniatis, 1990), encoded by SRSF2 (Serine/arginine-rich splicing factor 2) gene. This SR family protein is needed to initiate the assembly of ATP- dependent spliceosome complex for consecutive and alternative pre-mRNA splicing (Fu and Maniatis 1992). It also regulates transcriptional elongation (Lin et al. 2008). Post translational modification like phosphory-

lation and acetylation of SC 35 is crucial for cellular apoptosis (Edmond et al. 2011), cell proliferation and genomic stability during organogenetic events (Xiao et al. 2007). Again, it was found that the mutant A allele of rs141679680 abolishes MBNL1 binding site. Muscleblind-like protein 1, encoded by MBNL1 gene (Ishikawa et al. 1997), is a splicing factor of Muscleblind (Mbl) family which is critical for cell differentiation (Schiano et al.2017) and mammalian organogenesis. The zinc finger domain of MBNL1 binds to intronic sequence and either repress or activate splicing(Fernandez-Costa et al. 2011). MBNL1 is also play key role in central nervous system (CNS) disorder. Perturbed splicing regulation by MBNL1 was reported in myotonic dystrophy (DM) associated abnormality of CNS (Charizanis et al. 2012). Tau aggregation is resulted from loss of function of MBNL1 in DM (Dhaenens et al. 2008).

Several research works documented that Micro RNA (miRNA) represents a class of non-protein coding, 22 nucleotide long RNA molecules modulating the expression of over 60 percent of human protein coding genes (Friedman et al. 2009),involved in myriad biological process like cell proliferation, differentiation, apoptosis and metabolism (Bartel 2009).The complementary target site of miRNA lies usually in 3' UTR, but can also exist in 5'UTR or in coding region of the mRNAs (Dzikiewicz-Krawczyk 2014). This miRNA-mRNA interaction negatively influences gene expression by translational repression, cleavage or destabilization of mRNA (Bushati and Cohen 2007). Enormous number of evidences show that polymorphisms in miRNA target site impair binding of miRNA (Sethupathy and Collins 2008; Bhattacharya et al. 2014) and alters gene expression, which interns influence the risk of various diseases like colorectal cancers (Naccarati et al. 2012) gastric cancer (Chen et al. 2015), non-Hodgkin's lymphoma (Diao et al. 2014), osteosarcoma (Lv et al. 2014), hepatocellular carcinoma (Tan et al. 2015),Chronic lymphocytic leukemia (Rodríguez-Vicente et al. 2015), cervical cancer (Mi et al. 2014), prion disease(Saba et al. 2014), myocardial infarction (Nossent et al. 2011), metabolic syndrome (Ye et al. 2013), HBV-associated liver diseases (Zhang et al. 2015), Alzheimer's disease (Mallick and Ghosh 2011; Delay et al. 2014), anxiety and depressive disorders(Jensen et al. 2014), hypertension (Sethupathy et al. 2007), and cognitive performance (Yang et al. 2014).Thus, to predict whether our variations affect mi-RNA binding, in-silico analysis was performed and interesting results was found. The rs5031052 variation introduces a hsa-miR-3616-3p binding site, and rs141679680 eliminates a hsa-miR-4435 binding site. Thus we can anticipate that creation or deletion of mi-RNA binding sites in the 5'UTR of TP73 gene probably lead to modification in translational level and subsequently change its expression, which in turn, may leads to neurodegeneration.

CONCLUSION

Thus, taking these findings into consideration, it can be interpreted that these two SNPs may perform critically in splicing of *TP73* both by introducing intronic splicing enhancer motif and modulating recruitment of splicing factors. These variations thus demonstrate that *TP73* gene is playing a cardinal role in AD development. To the best of the researchers' knowledge, this is the first report indicating association of these SNPs with Alzheimer's disease. To conclude, the significantly increased frequencies of mutant alleles from reported MAF count (0.01875 vs. 0.006 p value of z test = 0.04 and 0.0125 vs. 0.0024 p value of z test = 0.01 for rs5031052 T allele and rs141679680 A allele respectively) and its complete absence in control individuals, pointed towards the fact that these variations can work as prognostic biomarkers for AD. But this can only be confirmed after screening of bigger sample size. However, replicated studies in other ethnically varied populations can only affirm the apparent association of these polymorphisms with AD. Although, detail experiments are required for the functional validation of the in-silico analysis, we must weigh the likelihood that these nucleotide variations may enact in the pathology of AD in our population.

RECOMMENDATIONS

Comprehensive investigations at molecular level are needed for proclamation of biological roles of these two polymorphisms in AD pathogenesis.

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REFERENCES

Agostini M, Tucci P, Chen H, Knight RA, Bano D, Nicotera P, Mc Keon F, Melino G 2010. p73 regulates maintenance of neural stem cell. *Biochem Biophys Res Commun*, 403: 13-17.

Aqeilan RI, Donati V, Palamarchuk A, Trapasso F, Kaou M, Pekarsky Y, Sudol M, Croce CM 2005. WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulateits transcriptional function.*Cancer Res,* 65: 6764-6772.

Bartel DP 2009. MicroRNAs: Target recognition and regulatory functions. *Cell*, 136: 215-233.

Berchtold NC, Cotman CW 1998. Evolution in the conceptualization of dementia and Alzheimer's disease: Greco-Roman period to the 1960s. *Neurobiol Aging,* 19: 173-189.

Bhattacharya A, Ziebarth JD, Cui Y 2014. PolymiRTS Database 3.0: Linking polymorphisms in microRNAs and their target sites with human diseases and biological pathways. *Nucleic Acids Res*, 42: D86-91.

Black DL 2003. Mechanisms of alternative pre-messenger RNA splicing. *Annu Rev Biochem,* 72: 291-336.

Bushati N, Cohen SM 2007. microRNA functions. *Annu Rev Cell Dev Bio,* 23: 175-205.

Cancino GI, Miller FD, Kaplan DR 2013. p73 haploin sufficiency causes tau hyperphosphorylation and tau kinase dysregulation in mouse models of aging and Alzheimer's disease. *Neurobiol Aging* , 34: 387-399.

Charizanis K, Lee KY, Batra R, Goodwin M, Zhang C, Yuan Y, Shiue L, Cline M, Scotti M M, Xia G, Kumar A, Ashizawa T, Clark HB, Kimura T, Takahashi MP, Fujimura H, Jinnai K, Yoshikawa H, Gomes-Pereira M, Gourdon G, Sakai N, Nishino S, Foster TC, Ares M Jr, Darnell RB, Swanson MS 2012. Muscle blind-like 2-mediated alternative splicing in the developing brain and dysregulation in myotonicdystrophy. *Neuron*, 75: 437-450.

Chen Q, Qin R, Fang Y, Li H, Liu Y 2015. A functional variant at the miR-214 binding site in the methylenetetrahydrofolatereductase gene alters susceptibility to gastric cancer in a Chinese Han population. *Cell Physiol Biochem*, 36: 622-630.

Delay C, Dorval V, Fok A, Grenier-Boley B, Lambert JC, Hsiung GY, Hébert SS 2014. Micro RNAs targeting Nicastrin regulate Aβ production and are affected by target site polymorphisms. *Front Mol Neurosci*, 7: 67.

Dhaenens CM, Schraen-Maschke S, Tran H, Vingtdeux V, Ghanem D, Leroy O, Delplanque J, Vanbrussel E, Delacourte A, Vermersch P, Maurage CA, Gruffat H, Sergeant A, Mahadevan MS, Ishiura S, Buée L Cooper TA, Caillet-Boudin ML, Charlet-Berguerand N, Sablonnière B, Sergeant N 2008. Overexpression of MBNL1 fetal isoforms and modified splicing of Tau in the DM1 brain: Two individual consequences of CUG trinucleotide repeats. *Exp Neurol*, 210: 467-478.

Diao L, Su H, Wei G, Li T, Gao Y, Zhao G, Guo Z 2014. Prognostic value of microRNA 502 binding site SNP in the 3'-untranslated region of the SET8 gene in patients with non-Hodgkin's lymphoma. *Tumori*, 100: 553-558.

Dzikiewicz-Krawczyk A 2014. MicroRNA-binding site polymorphisms in hematological malignancies. *J Hematol Oncol*, 7:83.

Edmond V, Moysan E, KhochbinS, Matthias P, Brambilla C, Brambilla E, Gazzeri S, Eymin B 2010. Acetylation and phosphorylation of SRSF2 control cell fate decision in response to cisplatin. *EMBO J*, 30: 510-523.

Fabbri M, Iliopoulos D, Trapasso F, Aqeilan RI, Cimmino A, Zanesi N Yendamuri S, Han SY, Amadori D, Huebner K, Croce CM 2005. WWOX gene restoration prevents lung cancer growth in vitro and in vivo. *Proc Natl Acad Sci USA*, 102: 15611-15616.

Fernandez-Costa JM, Llamusi MB, Garcia-Lopez A, Artero R 2011. Alternative splicing regulation by muscle blind proteins: From development to disease. *Biol Rev Camb Philos Soc*, 86: 947-958.

Friedman RC, Farh KK, Burge CB, Bartel DP 2009. Most mammalian m RNAs are conserved targets of microRNAs. *Genome Res*, 19: 92-105.

Fu XD, Maniatis T 1990. Factor required for mammali an spliceosome assembly is localized to discrete regions in the nucleus. *Nature*, 343: 437-441.

Fu XD, Maniatis T 1992. Isolation of a complementary DNA that encodes the mammalian splicing factor SC35. *Science*, 256: 535-538.

Fujitani M, Cancino GI, Dugani CB, Weaver IC, Gauthier-Fisher A, Paquin A, Mak TW, Wojtowicz MJ, Miller FD, Kaplan DR 2010. Ap73 acts via the bHLH Hey2 to promote long-term maintenance of neural precursors. *Curr Biol*, 20: 2058-2065.

Gallì P, Cadoni G, Volante M, De Feo E, Amore R, Giorgio A, Arzani D, Paludetti G, Ricciardi G, Boccia SA 2009. Case-control study on the combined effects of p53 and p73 polymorphisms on head and neck cancer risk in an Italian population. *BMC Cancer*, 9: 137.

Grespi F, Melino G 2012. P73 and age-related diseases: Is there any link with Parkinson Disease? *Aging (Albany NY)*, 4: 923-931.

Hamajima N, Matsuo K, Suzuki T, Nakamura T, Matsuura A, Hatooka S, Shinoda M, Kodera Y, Yamamura Y, Hirai T, Kato T, Tajima K 2002. No associations of p73 G4C14-to-A4T14 at exon 2 and p53 Arg72Pro polymorphisms with the risk of digestive tract cancers in Japanese. *Cancer Lett*, 181: 81-85.

Hauptmann S, Keil U, Scherping I, Bonert A, Eckert A, Müller WE 2006. Mitochondrial dysfunction in sporadic and genetic Alzheimer's disease. *Exp Gerontol*, 41: 668-673.

Hay RT 2005. SUMO: A history of modification. *Mol Cell*, 18: 1-12.

Hishida A, Matsuo K, Tajima K, Ogura M, Kagami Y, Taji H, Morishima Y, Emi N, Naoe T, Hamajima N 2004. Polymorphisms of p53 Arg72Pro, p73 G4C14-to-A4T14 at exon 2 and p21 Ser31Arg and

the risk of non-Hodgkin's lymphoma in Japanese. *Leuk Lymphoma*, 45: 957-964.

Irimia M, Rukov JL, Penny D, Roy SW 2007. Functional and evolutionary analysis of alternatively spliced genes is consistent with an early eukaryotic origin of alternative splicing. *BMC Evol Biol*, 7: 188.

Ishikawa K, Nagase T, Nakajima D, Seki N, Ohira M, Miyajima N, Tanaka A, Kotani H, Nomura N, Ohara O 1997. Prediction of the coding sequences of unidentified human genes. VIII. 78 new cDNA clones from brain which code for large proteins in vitro. *DNA Res*, 4: 307-313.

Ishimoto O, Kawahara C, Enjo K, Obinata M, Nukiwa T, Ikawa S 2002. Possible oncogenic potential of DeltaNp73: A newly identified isoform of human p73.*Cancer Res*, 62: 636-641.

Jensen KP, Kranzler HR, Stein MB, Gelernter J 2014. The effects of a MAP2K5 micro RNA target site SNP on risk for anxiety and depressive disorders. *Am J Med Genet B Neuropsychiatr Genet*, 165B: 175-183.

Jun HJ, Park SH, Lee WK, Choi JE, Jang JS, Kim EJ, Cha SI, Kim DS, Kam S, Kim CH, Kang YM, Jung TH, Park JY 2007. Combined effects of p73 and MDM2 polymorphisms on the risk of lung cancer. *Mol Carcinog*, 46: 100-105.

Kaghad M, Bonnet H, Yang A, Creancier L, Biscan JC, Valent A, Minty A, Chalon P, Lelias JM, Dumont X, Ferrara P, McKeon F, Caput D 1997. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell*, 90: 809-819.

Kim EJ, Park JS, Um SJ 2002. Identification and characterization of HIPK2 interacting with p73 and modulating functions of the p53family in vivo. *J Biol Chem*, 277: 32020-32028.

Koida N, Ozaki T, Yamamoto H, Ono S, Koda T, Ando K, Okoshi R, Kamijo T, Omura K, Nakagawara A 2008. Inhibitory role of Plk1 in the regulation of p73-dependent apoptosis through physical interaction and phosphorylation. *J Biol Chem*, 283: 8555-8563.

Levy D, Adamovich Y, Reuven N, Shaul Y 2007. The Yes-associated protein 1 stabilizes p73 by preventing itch-mediated ubiquitination of p73.*Cell Death Differ*, 14: 743-751.

Li C, Chen K, Liu Z, Wang LE, Gershenwald JE, Lee JE, Prieto VG, Duvic M, Grimm EA, Wei Q 2008. Polymorphisms of TP53 Arg72Pro, but not p73 G4C14>A4TA4 and p21 Ser31Arg, contribute to risk of cutaneous melanoma. *J Invest Dermatol*, 128: 1585-1588.

Li G, Wang LE, Chamberlain RM, Amos CI, Spitz MR, Wei Q 2004. p73 G4C14-to-A4T14 polymorphism and risk of lung cancer. *Cancer Res*, 64: 6863-6866.

Li Q, Athan ES, Wei M, Yuan E, Rice SL, Vonsattel JP, Mayeux RP, Tycko B 2004. TP73 allelic expression in human brain and allele frequencies in Alzheimer's disease. *BMC Med Genet*, 2: 5-14.

Lin S, Coutinho-Mansfield G, Wang D, Pandit S, Fu XD 2008. The splicing factor SC35 has an active role in transcriptional elongation. *Nat Struct Mol Biol*, 15: 819-826.

Lokshin M, Li Y, Gaiddon C, Prives C 2007. p53 and p73 display common and distinct requirements for sequence specific binding to DNA. *Nucleic Acids Res*, 35: 340-352.

Lv H, Pei J, Liu H, Wang H, Liu J 2014. A polymorphism site in the pre miR 34a coding region reduces miR 34a expression and promotes osteosarcoma cell proliferation and migration. *Mol Med Rep*, 10: 2912-2916.

Mallick B, Ghosh Z 2011. A complex crosstalk between polymorphic microRNA target sites and AD prognosis. *RNA Biol*, 8: 665-673.

Mi Y, Wang L, Zong L, Pei M, Lu Q, Huang P 2014. Genetic variants in micro RNA target sites of 37 selected cancer-related genes and the risk of cervical cancer. *PLoS One*, 9: 1-9.

Minty A, Dumont X, Kaghad M, Caput D 2000. Covalent modification of p73alpha by SUMO-1. Two-hybrid screening with p73 identifies novel SUMO-1-interacting proteins and a SUMO-1 interaction motif. *J Biol Chem*, 275: 36316-36323.

Misra C, Majumder M, Bajaj S, Ghosh S, Roy B, Roychoudhury S 2009. Polymorphisms at p53, p73, and MDM2 loci modulate the risk of tobacco associated leukoplakia and oral cancer. *Mol Carcinog*, 48: 790-800.

Murray-Zmijewski F, Lane DP, Bourdon JC 2006. p53/p63/p73 isoforms: An orchestra of isof orms to harmonise cell differentiation and response tostress. *Cell Death Differ*, 13: 962-972.

Naccarati A, Pardini B, Stefano L, Landi D, Slyskova J, Novotny J, Levy M, Polakova V, Lipska L, Vodicka P 2012. Polymorphisms in miRNA-binding sites of nucleotide excision repair genes and colorectal cancer risk. *Carcinogenesis*, 33: 1346-1351.

Niwa Y, Hirose K, Matsuo K, Tajima K, Ikoma Y, Nakanishi T, Nawa A, Kuzuya K, Tamakoshi A, Hamajima N 2005. Association of p73 G4C14-to-A4T14 polymorphism at exon 2 and p53 Arg72Pro polymorphism with the risk of endometrial cancer in Japanese subjects. *Cancer Lett*, 219: 183-190.

Nossent AY, Hansen JL, Doggen C, Quax PH, Sheikh SP, Rosendaal FR 2011. SNPs in microRNA binding sites in 3'-UTRs of RAAS genes influence arterial blood pressure and risk of myocardial infarction. *Am J Hypertens*, 24: 999-1006.

Nozell S, Wu Y, Mc Naughton K, Liu G, Willis A, Paik JC, Chen X 2003. Characterization of p73 functional domains necessary for transactivation and growth suppression. *Oncogene*, 22: 4333-4347.

Paliwal P, Radha V, Swarup G 2007. Regulation of p73 by Hck through kinase-dependent and independent mechanisms. *BMC Mol Biol*, 8: 1-8.

Peschiaroli A, Scialpi F, Bernassola F, Pagano M, Melino G 2009. The F-box protein FBXO45 promotes the proteasome-dependent degradation of p73. *Oncogene*, 28: 3157-3166.

Pozniak CD, Barnabé-Heider F, Rymar VV, Lee AF, Sadikot AF, Miller FD 2002. p73 is required for survival and maintenance of CNS neurons. *J Neurosci*, 22: 9800-9809.

Puca R, Nardinocchi L, Porru M, Simon AJ, Rechavi G, Leonetti C, Givol D, D'Orazi G 2011. Restoring p53 active conf ormation by zinc increases the

resp onse of mutant p53 tumor cells toanticancer drugs. *Cell Cycle*, 10: 1679-1689.

Rodríguez-Vicente AE, Quwaider D, Benito R, Misiewicz-Krzeminska I, Hernández-Sánchez M, de Coca AG, Fisac R, Alonso JM, Zato C, de Paz JF, García JL, Sarasquete ME, Hernández JÁ, Corchado JM, González M, Gutiérrez NC, Hernández-Rivas JM 2015. MicroRNA-223 is a novel negative regulator of HSP90B1 in CLL. *BMC Cancer*, 15: 238.

Rossi M, De Laurenzi V, Munarriz E, Green DR, Liu YC, Vousden KH, Cesareni G, Melino G 2005. The ubiquitin-protein ligase Itch regulates p73 stability. *EMBO J*, 24: 836-848.

Rufini A, Niklison-Chirou MV, Inoue S, Tomasini R, Harris IS, Marino A, Federici M, Dinsdale D, Knight RA, Melino G, Mak TW 2012. TAp73 depletion accelerates aging through metabolic dysregulation. *Genes Dev*, 26: 2009-2014.

Saba R, Medina SJ, Booth SA 2014. A functional SNP catalog of overlapping miRNA-binding sites in genes implicated in prion disease and other neurodegenerative disorders. *Hum Mutat*, 35: 1233-1248.

Scacchi R, Gambina G, Moretto G, Corbo RM 2009. Association study between P53 and P73 gene polymorphisms and the sporadic late-onset form of Alzheimer's disease. *J Neural Transm (Vienna)*, 116: 1179-1184.

Schiano C, Rienzo M, Casamassimi A, Soricelli A, Napoli C 2017. Splicing regulators in endothelial cell differentiation. *J Cardiovasc Med (Hagerstown),* 18(10): 742-749.

Sethupathy P, Borel C, Gagnebin M, Grant GR, Deutsch S, Elton TS, Hatzigeorgiou AG, Antonarakis SE 2007. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: A mechanism for functional single-nucleotide polymorphisms related to phenotypes. *Am J Hum Genet*, 81: 405-413.

Sethupathy P, Collins FS 2008. MicroRNA target site polymorphisms and human disease. *Trends Genet*, 24: 489-497.

Soond SM, Barry SP, Melino G, Knight RA, Latchman DS, Stephanou A 2008. p73-mediated transcriptional activity is negatively regulated by polo-like kinase 1.*Cell Cycle*, 7: 1214-1223.

Talos F, Abraham A, Vaseva AV, Holembowski L, Tsirka SE, Scheel A, Bode D, Dobbelstein M, Brück W, Moll UM 2010. p73 is an essential regulator of neural stem cell maintenance in embryonal and adult CNS neurogenesis. *Cell Death Differ*, 17: 1816-1829.

Tan C, Liu S, Tan S, Zeng X, Yu H, Li A, Bei C, Qiu X 2015. Polymorphisms in microRNA target sites of forkhead box O genes are associated with hepatocellular carcinoma. *PLoS One*, 10, e0119210: 1-10.

Tsai KK, Yuan ZM 2003. c-Abl stabilizes p73 by a phosphorylation-augmented interaction. *Cancer Res*, 63: 3418-3424.

Ueda Y, Hijikata M, Takagi S, Chiba T, Shimotohno K 1999.New p73 variants with altered C-terminal structures have varied transcriptional activities. *Oncogene*, 18: 4993-4998.

Vousden KH, Lane DP 2007. p53 in health and disease. *Nat Rev Mol Cell Biol*, 8: 275-283.

Wang J, Liu YX, Hande MP, Wong AC, Jin YJ, Yin Y 2007. TAp73 is a downstream target of p53 in controlling the cellular defense against stress. *J Biol Chem*, 282: 29152-29162.

Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB 2008. Alternative isoform regulation in human tissue transcriptomes. *Nature,* 456: 470-476.

Ward AJ, Cooper TA 2010. The pathobiology of splicing. *J Pathol*, 220: 152-163.

Watanabe K, Ozaki T, Nakagawa T, Miyazaki K, Takahashi M, Hosoda M, Hayashi S, Todo S, Nakagawara A 2002. Physical interaction of p73 with c-Myc and MM1, a c-Myc-binding protein, and modulation of the p73function. *J Biol Chem*, 277: 15113-15123.

Wu J , Xiao Y, Xia C, Yang F, Li H, Shao Z, Lin Z, Zhao X 2017. Identification of biomarkers for predicting lymph node metastasis of stomach cancer using clinical DNA methylation data. *Dis Markers,* 2017: 5745724.

Xiao R, Sun Y, Ding JH, Lin S, Rose DW, Rosenfeld MG, Fu XD, Li X 2007. Splicing regulator SC35 is essential for genomic stability and cell proliferation during mammalian organogenesis. *Mol Cell Biol*, 27: 5393-5402.

Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW 2002. Allelic variation in human gene expression. *Science*, 297: 1143.

Yang A, Walker N, Bronson R, Kaghad M, Oosterwegel M, Bonnin J, Vagner C, Bonnet H, Dikkes P, Sharpe A, McKeon F, Caput D 2000. p73 deficient mice have neurological, pherom onal and inflammatory defects but lack spontaneoustumours. *Nature*, 404: 99-103.

Yang A, Kaghad M, Caput D, McKeon F 2002. On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet*, 18: 90-95.

Yang L, Zhang R, Li M, Wu X, Wang J, Huang L, Shi X, Li Q, Su B 2014. A functional MiR-124 binding-site polymorphism in IQGAP1 affects human cognitive performance. *PLoS One*, 9, e107065.

Ye Q, Zhao X, Xu K, Li Q, Cheng J, Gao Y, Du J, Shi H, Zhou L 2013. Polymorphisms in lipid metabolism related mi RNA binding sites and risk of metabolic syndrome. *Gene*, 528: 132-138.

Yoon MK, Ha JH, Lee MS, Chi SW 2015. Structure and apoptotic function of p73. *BMB Rep*, 48: 81-90.

Zhang G, Li N, Li Z, Zhu Q, Li F, Yang C, Han Q, Lv Y, Zhou Z, Liu Z 2015. microRNA-4717 differentially interacts with its polymorphic target in the PD1 3' untranslated region: A mechanism for regulating PD-1 expression and function in HBV-associated liver diseases.*Oncotarget*, 6: 18933-18944.

Zhu J, Jiang J, Zhou W, Chen X 1998. The potential tumor suppressor p73 differentially regulates cellular p53 target genes. *Cancer Res*, 58: 5061-5065.

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