ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 12(A), pp. 29827-29831 December, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

FUNCTIONAL AND GENOTYPIC STUDY OF ADD1 GENE VARIANT ASSOCIATED WITH HYPERTENSIVE POPULATION IN PUNJAB

Harsimran Kaur* Satbir Kaur and Praveen P.Balgir

Department of Human Genetics, PUP

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0912.2939

ARTICLE INFO

Article History:

Received 4th September, 2018
Received in revised form 25th October, 2018
Accepted 23rd November, 2018
Published online 28th December, 2018

Key Words:

ADD1, Hypertension, Insilico analysis, PCR-RFLP, Diuretics, Metabolic disorders

ABSTRACT

Background: An inappropriate increase in peripheral vascular resistance relative to the cardiac output is a cause of essential hypertension. Numerous genetic markers have been identified in the regulation of blood pressure and essential hypertension. Alpha adducin (ADD1) is one such marker. ADD1 protein, found in the renal tubule, is involved in cellular signal transduction and interacts with other membrane skeleton proteins that affect ion transport across the cell membrane. Mutated ADD1 may affect the regulation of some factors in the Na transport system in the luminal part of the cell. So, ADD1 can be considered as a 'renal hypertensive gene' that affects the capacity of the tubular epithelial cell to transport Na and hence, affects blood pressure. Diuretic agents are drugs that increase renal excretion of water and solutes (mainly sodium salt).The Gly460Trp variant of the alpha-adducin gene influences the constitutive capacity of the kidney to reabsorb sodium, thus implying a modulation of the Blood pressure responsiveness to a diuretic ,which inhibits such renal mechanism. Thus, yielding diuretics a more beneficial therapeutic effect.

Aims and objective: This study is to carried out for survey of SNPGly460Trp prevalent among hypertensive patients from Punjab and role of ADD1 gene variant rs4961 in causation of any other metabolic disorder.

Materials and methods: Genotyping of SNPs predicted by Insilco analysis of this gene sequences will be done through RFLP-PCR analysis.

Results: In silico analysis reports that the SNP, rs4961 expressing the amino acid variant (G460W) has significant damaging effect and can be considered to be functionally important. The allelic distribution of ADD1 rs4961 showed, this locus was found to be polymorphic in Punjabi population. it was found significantly associated with hypertension in Punjabi population. Mutant allele of rs4961 is not responsible for causation of any other metabolic disorder in hypertensive patients. but muatant allele T shows significant results with causation of other metabolic disorders in patients under diuretic therapy.(p=0.035)

Conclusion: This study can be a way to "measure" the overall clinical impact of the ADD1 Trp allele and then to estimate the size of the population that may be affected by this genetic mechanism is to apply a very selective pharmacologic tool that is able to interfere with the sequence of events that are triggered by this allele.

INTRODUCTION

Hypertension is defined as blood pressure measurements of 140/90 mm Hg or greater. Essential hypertension is a highly prevalent, complex, multifactorial disorder that arises from the genetic predisposition and environmental risk factors. Numerous genetic markers have been identified in the regulation of blood pressure and essential hypertension One such marker that has drawn substantial attention is adducin (*ADD1)* gene.ADD1 is one such gene that dis-plays larger blood pressure changes with body sodium variation, causing hypertension. Alpha adducin has been mapped by positional cloning to human chromosome 4p16.3. In human a adducing gene contains 16 exons and spans about 85 kb of genomic DNA (Lin B. *et al.,* 1995).ADD1protein, found in the renal tubule, is involved in cellularsignal transduction and interacts with other membraneskeletonproteins that affect ion transport across the cellmembrane. Mutated ADD1 may induce an alteration in actin spectrin based membrane skeleton (Karrie Mei-Yee_Kiang *et al*., 2018) that may affect the regulation of other factors in the Na transport system, such as anion exchanger, epithelial Na channels (Cantiello, H. F. *et al*.,1995;

Corresponding author:* **Harsimran Kaur
Department of Human Genetics, PUP

Berdiev., B. K. *et al* 1996)and Na-K-Cl cotransport in the luminal part of the cell (Wu *et al*., 1994)

MATERIAL AND METHOD

Functional analysis of snps of ADD1 gene

ADD1can be considered as a 'renal hypertensive gene'. Abnormalities in renal sodium reabsorption may be involved in the development and maintenance of experimental and clinical hypertension (P. Manuta 1999). Bioinformatics tools used to retrieve data about SNPs based on gene of interest have been documented (Clinford *et al*., 2004). A total of 1,113 SNPs associated withADD1gene were retrieved from the single-nucleotide polymorphism data-base (dbSNP) (Wheeler., D.L. 2008). We used functional single-nucleotide polymorphism (F-SNP) database for selecting nsSNPsofADD1 gene. Out of 1,113 SNPs associated with ADD1gene, We employed SIFT, PolyPhen, PANTHER, I-MUTANT tools on 9 SNPs to obtain the functional impact of nsSNPs, resulting in amino acid changes.I-Mutant2.0 is a Support Vector Machine -based web server for the automatic prediction of protein stability changes upon single-site mutations.I-Mutant2.0 correctly predicts whether the protein mutation stabilises or destabilises the protein.I-Mutant2.0 can predict the direction of the free energy change and its value. The DDG value is calculated from the unfolding Gibbs free energy value of the mutated protein minus the unfolding Gibbs free energy value of the wild type (Kcal/mol).

Genotyping of ADD1 gene: Patients with EH (n=400) and normotensive controls (n=200) were consecutively selected from different hypertension outpatient clinic and medical center, respectively, of Punjab. Genomic DNA was extracted from peripheral blood using salting out method. The genotyping of G614T polymorphism of ADD1 was done by PCR-RFLP technique.DNA fragments were amplified in a total volume of 20 µl PCR reaction Amplified products were digested with Sau96I enzyme (NBE Inc., US) at 65 °C for 20 mins. All products were loaded onto 3 percent agarose and electrophoresed. The length of PCR amplification product with G614T was 147 bp. The Sau 96I restriction enzymes were used to distinguish 614G/T, resulting in 122 bpand 25 bp fragments in the presence of the G allele.

RESULTS AND DISCUSSION

Our in silico analysis reports that the SNP, rs4961 expressing the amino acid variant (G460W) has significant.damaging effect and can be considered to be functionally important. It has been reported that G460W genotype for rs4961 of the ADD1gene is associated with erythrocyte sodium transport (Katsua T., 2003). Manunta *et al*. have reported that the tryptophan (Trp) adducin variants are characterized by reduced fractional excretions of lithium and uric acid, which suggests increased proximal sodium reabsorption, thereby causing the risk for hypertension.

Table Functional effect of nsSNPs obtained from various tools

s.no	SNP ID	SNP Type	Allele change	FS score	Amino acid change	SIFTscore	Polyphen score	Panther	I-Mutant DDG Score and stability
1.	rs4971	Non-synonmous	T/A	0.635	Tyr270Asn	0.00(Dam)	2.922	Sub PSEC -5.15755 P- Deleterious 0.89637	-1.37 Decrease
2.	rs4972	Non-synonmous	A/T	0.867	Glu376Asp	0.07(Dam)	1.668	Sub PSEC -4.58712 P- Deleterious 0.90802	0.02 Increase
3.	rs4962	Non-synonmous	A/T	0.677	Asn510Ile	0.02(Dam)	2.463	Sub PSEC -5.49861 P- Deleterious 0.92404	1.58 Increase
4.	rs4963	Non-synonmous	C/G	0.849	Ser586Cys	0.05(Dam)	1.397	Sub PSEC -2.83418 P- Deleterious 0.45864	-0.71 Decrease
5.	rs11792	Non-synonmous	G/T	0.5	Glu661Asp	1.00(tol)	1.184	Sub PSEC -1.57927 P- Deleterious 0.19455	-0.51 Decrease
6.	rs4690006	Non-synonmous	C/A	1	Leu387Met	0.18(Dam)	0.132	Sub PSEC -3.97579 P- Deleterious 0.72627	NA Decrease
7.	rs13306092	Non-synonmous	G/A	0.1	Arg570His	0.09(Dam)	0.198	Sub PSEC -2.61526 P- Deleterious 0.40499	-0.51 Decrease
8	rs13306093	Non-synonmous	C/T	0.195	Pro624Leu	0.09(Dam)	1.195	Sub PSEC -0.45575 P- Deleterious 0.07281	-0.71 Decrease
9.	rs4961	Non-synonmous	G/T	0.625	Gly460Trp	0.05(Dam)	1.746	Sub PSEC -3.12257 P- Deleterious 0.5306	-0.28 Decrease

The structural analysis of ADD1 protein shows G460W to be in the coiled and disordered region and hence, this polymorphism causing a change from aliphatic to aromatic amino acid may alter the function of that region of the protein and affect its stability. This polymorphism causing a change from aliphatic to aromatic amino acid found in the coiled and disordered region might alter the function of that region of the protein and affect its stability. F-SNP assesses the deleterious effect of SNPs by calculating a specific functional significance (FS) score for each nsSNP. The deleterious SNP has a FS score value between Seven nsSNPs, rs4971, rs4972, rs4962, rs4963, rs11792, rs4690006 and rs4961 are found to have deleterious FS scores in the range of 0.5-1. They are found to be deleterious by having changes in the protein-coding region except for one nsSNP, rs11792. SNPs with minor allele frequency (MAF)[0.05 are examined for disease association (Tsunoda *et al*.,2004). For rs4961, MAF value is found to be[0.05 in various populations such as AFR (Africa), EUR (Europe), EAS (East Asia), GIH (Gujrat Indians in Houston, Texas), MEX (Mexican), AFA (African American), EUA (European American) and HCH (Han Chinese). Significant change in protein-coding region is found for rs4961 that replaces glycine460 (G460) in ADD1 protein with tryptophan (W) because of the nucleotide change at 2,038th position where GGG is replaced with TGG i.e., G (guanine) to T (thymine). This indicates a change from alkyl group to an aromatic group in the side chains of the amino acids (the base represented in bold caption is the nsSNP.

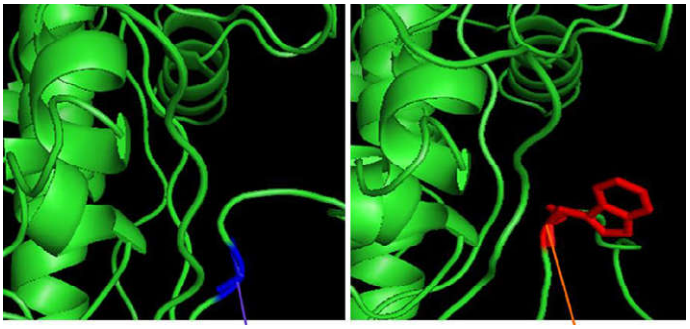

Figure 1 The native protein glycine (460) and mutant protein structure with tryptophan (460) for SNP rs4961

The change in amino acids from Glycine (G) to tryphophan (W) at 460 position is observed to be in the loop region of the modeled protein as shown in Figure 1 . a Helices and b sheets are the majority of secondary structures found in proteins which are interspersed with regions of irregular structure referred to as coil. They can possess structural significance, and can be the location of the functional portion or active site of the protein (Scheeff, E. D. *et al*., 2003). The direction of loop regions for both native and mutant proteins is also found to be altered as depicted polymorphism occurring in this region can alter the structure and function of the protein.

In the insilico analysis it was found that the SNP, rs4961 expressing the amino acid variant (G460W) has significant damaging effect and can be considered to be functionally important. It has been reported that G460W genotype for rs4961 of the ADD1 gene is associated with erythrocyte sodium transport (Katsua *et al*., 2003)

Genetic variability among ADD1 gene

ADD1 gene rs4961 variability in hypertensive Punjabi population

For rs4961, the amplified product of 149 bp was obtained, which was subjected to restriction digestion with Sau96I enzyme. The representative agarose gels of PCR and restriction digestion yielded 142 bp and 25 bp products are shown in Figure 2

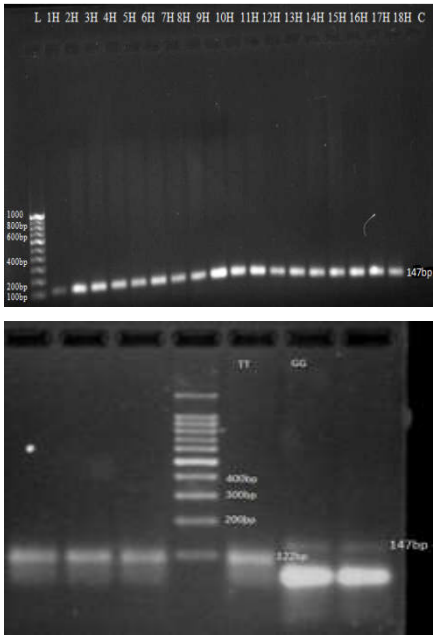

Figure 2 Representative 2% Agarose gel showing (a) 147 bp PCR product ADD1 gene rs4961.100bp DNA ladder (b) Restriction fragment digestion showing GG genotype (147 bp, and TT 122 bp)

The frequencies of GG (69.5%), GT (21%) and TT (9.5%) in hypertensive subjects were compared with respective genotypes in normotensive subjects (80.5%, 21% and 9.5%).

The frequencies of wild (0.8) and mutant allele (0.2) were compared with respective allelic frequencies in controls (0.88 and 0.12) was also found to be statistically significant ($p<0.0005$). However, the allelic frequency of mutant allele T was observed to be higher in hypertensive subjects as compared to frequency of that allele in normotensive subjects and relative risk was found to be 1.20 folds. The Gly460Trp polymorphism was found significantly associated with hypertension at allelic level (P<0.01; OR=0.85; 95%CI=0.75-0.96) in Han Chinese population (Pan-Pan Liu *et al*., 2015).

Table 4.16 Genotypic and allelic disrtibution of ADD1 rs4961 between and normotensive and hypertensive subjects

Sample Group	Genotypes			Alleles		$\chi2$ (df=1)	p-value (S/NS)	Relative risk	Odds Ratio
	GG	GT	TT	G Count (Freq.)	T Count (Freq.)				
Normotensive subjects n=200 (%)	161 (80.5%)	30 (15%)	9 (4.5%)	352(0.88)	48 (0.12)	11.91	0.0005 (S)	1.192 (1.085 to1.294)	1.833 (1.305 to 2.579)
Hypertensive subjects n=400 (%)	278 (69.5%)	84 (21%)	38 (9.5%)	640(0.8)	160(0.2)				

ADD1 gene polymorphism rs4961 was also found significantaly associated with hypertension in Madeira island population.(odds ratio 2.484, P = .01) (Ana Célia Sousa *et al*., 2017). A significant association was found between *ADD1* gene G614T polymorphism and EH in Chinese patients Lifang Wang. The rs4961 polymorphism of the ADD1 gene is associated with essential hypertension. We found a significant association between the Gly460Trp gene polymorphism of the alpha adducin and hypertension in a North Indian population (PDF) Study of Alpha Adducin Gene Polymorphism in Young Essential Hypertensive North Indians.

The allelic distribution of ADD1 rs4961 showed, this locus was found to be polymorphic in Punjabi population The allelic frequency of G and T allele was found to be 0.8 and 0.2 respectively in Punjabi population. In comparison with europian frequency of G and T allele was similar as shown in Table

Population table				
ID	Field1	Field2	Field3	Field4
3	SNP ID	Population	Allelic Frequency	
4	rs4961		G	T
5		EAS(East Asia)	0.5475	0.4524
6		EUR(EUROPEAN)	0.7951	0.2047
7		AFR(Africa)	0.9508	0.0492
8		AMR(America)	0.8255	0.1744
9		SAS(South Africa)	0.79996	0.2003
15		Hypertensive Punjabi population(Present study)	0.7862	0.2138
16		HAPMAP-CEU(Europian)	0.7964	0.2035
17		HAPMAP-HCB(Asian)	0.5357	0.4642
18		HAPMAP-JPT(Asian)	0.4244	0.5755
19		HAPMAP-YRI(Sub-Saharan African)	0.9601	0.0398
20		HAPMAP-ASW(Southwest USA)	0.9591	0.0408
21		HAPMAP-CHB(Asian)	0.4268	0.5731
22		HAPMAP-CH(Chinese)	0.4882	0.5117
23		HAPMAP-GIH(Gujrati Indian in Texas)	0.8352	0.1647
24		HAPMAP-LWK(Kenya)	0.9444	0.0555
25		HAPMAP-MKK(Maasai in Kinyawa	0.8846	0.1153
26		HAPMAP-TSI(Toscans in Italy)	0.8068	0.1931

Source: https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs4961
***Allelic frequency similar to the present study are highlighted in blue color**

be statistically non-significant (p=0.50) . Both alleles do not appear to be associated with causation of MD in hypertensive patients.

In a contrary study, the ADD1 polymorphism predicted the high risk of mortality in type 2 diabetic patients and increased mean common carotid IMT in relation to hypertension. (Yazdanpanah M *et al*., 2005) Furthermore, this polymorphism was also found to be associated with atherosclerosis, stroke and MI .Similarly, 460Trp allele was associated with stroke in Dutch women and the risk was elevated in the presence of systolic hypertension (Zafarmand MH.,2008)In contrast, it was not associated with MI in Dutch men (Pahor M *et al*.,2000)

Disrtibution of ADD1 rs4961 alleles among patients on diuretics therapy, with and without MD

Out of 400 hypetensive subjects 118 (29.5%) of the hypertensive subjects were taking Diuretics with other drug combinations. Of them 34.7% hypertensive subjects were suffering from other MD.

Comparison of allelic disrtibution of rs 4961 was statistically Significant (p=0.03) among hypertensive subjects with MD and without MD.

Mutant allele T was is associated with causation of other MD in hypertensive taking Diuretics.

Another similar study was conducted which shows that α-adducin polymorphism associated with increased risk of adverse cardiovascular outcomes after the use of diuretics (Tobias Gerhard *et al*.,2008). In another study in carriers of the adducin variant, diuretic therapy was associated with a lower risk of combined MI or stroke than other antihypertensive therapies (Bruce M. Psaty *et al*., 2002)

CONCLUSION

We have determined the non-synonymous sin-gle-nucleotide polymorphisms (nsSNPs) of a adducin1 (ADD1) gene and its variations in different populations to understand its role in hypertension.

Table Genotypic and allelic disrtibution of ADD1 rs4961 between hypertensive patients with MD and without MD

Sample Group	Genotypes GG	GT	TT	Alleles G	T	χ2 (df=1)	p-value (S/NS)	Relative Risk	Odds Ratio
HT patients with MD n (%) 114 (28.5)	78 (68.4)	23 (20.17)	13 (11.4)	179 (78.5)	49 (21.4)	0.44	0.50 (NS)	0.9133 (0.7078 to 1.198)	0.8796 (0.6044 to 1.275)
HT patients without MD n (%) 286 (71.5)	200 (69.9)	61 (21.3)	25 (8.7)	461 (80.59)	111 (19.4)				

Sample Group	Diuretic therapy 118 (29.5%) Genotypes GG 88 (74.5)	GT 19 (16.1)	TT 11 (9.4)	Alleles G	T	χ2 (df=1)	p-value (S/NS)	Relative Risk	Odds Ratio
HT patients with MD n (%) 42 (34.71)	32 (76.1)	8 (19.0)	02 (4.7)	72 (85.7)	12 (14.28)	0.86	0.035 (S)	1.262 (0.793 to 2.158)	1.415 (0.6968 to 2.904)
HT patients without MD n (%) 76 (65.29)	56 (73.6)	11 (14.4)	09 (11.8)	123 (80.9)	29 (19.07)				

28.5% patients taking antihypertensive medications were having other MD. Allelic disrtibution of rs4961 between hypertensive patients with MD and without MD was found to

The amino acid change found for rs4961 is from glycine to tryptophan, i.e., from analkyl amino acid to an aromatic amino acid. This residual change is observed in the coiled region of

the protein and is also predicted to be disordered by computational algorithm. Protein disorder plays an important role in structural and functional genomics. Genotypic and allelic frequency of rs4961 revealed statistically significant difference between hypertensive and normotensive individuals pointing to their role in susceptibility to the development of hypertension. Genotypic and allelic distribution of gene variants amongst the patients at SNP rs4961 revealed a significant association with respect to development of metabolic disorders. on comparison of allelic frequencies of hypertensive patients taking Diuretics therapy in combination with other drugs, rs4961 revealed statistical significant propensity to develop MD. Although an abundant amount of research has already been carried out in the field of anti-hypertensive pharmacogenetics, the data is still scanty. Research in the area will continue to extend from both technological advances in genotyping and more information of human genome. The pharmacogenetics research demands that future work be characterized by creative innovational collaboration, close coordination and the establishment of consortia among research groups.

Acknowledgement

This study is funded by DBT-IPLS

Reference

Bakhrom K. Berdiev, Ramon Latorre, Dale J. Benos, and Iskander I. Ismailov. Actin Modifies Ca21 Block of Epithelial Na1 Channels in Planar Lipid Bilayers

Clifford, R. J., Edmonson, M. N., Titia, C. N., *et al*. (2004): Bioinformatics tools for single nucleotide polymorphism discovery and analysis. *Annals of the New York Academy of Sciences.*, 20: 101-109.

Gerhard, T., Gong, Y., Beitelshees, A, L., Mao,X., Lobmeyer, M, T., Cooper-DeHoff, R, M., Langaee, T, Y., Schork, N, J., . Shriver, M, D., Pepine, C, J., Johnson, J, A. (2008): α-adducin polymorphism associated with increased risk of adverse cardiovascular outcomes: results from invest-genes. *Am Heart J*., 156(2): 397-404.

Karrie Mei-Yee, Kiang., Gilberto, Ka-Kit, Leung. (2018): A Review on Adducin from Functional to Pathological Mechanisms: Future Direction in Cancer. *BioMed Research International*., 14.

Katsua, T., Ishikawa, K., Sugimoto, K., *et al*. (2003): Salt sensitivity of Japanese from the viewpoint of gene polymorphism. *Hypertension Research.*, 26: 521-525.

Manunta, P., Burnier, M., Amico, M. D., *et al*. (1999): Adducin polymorphism affects renal proximal tubule reabsorption in hypertension. *Hypertension.*, 33: 694-697

Mojgan ,Y., Fakhredin, A .,Albert , H., Yurii, S., Ben A. O., Bruno, H.C. S., Huibert, A.P. P., Steven ,W.J. Jacqueline, C.M. W., Joop, A.M.J.L. J., and Cornelia., M.(2018): The α-Adducin Gene Is Associated With Macrovascular Complications and Mortality in Patients With Type 2 Diabetes. *Diabetes.,* 55(10):2922-7.

Pahor, M., Psaty, BM., Alderman, MH., Applegate, WB., Williamson, JD., Furberg, CD.(2000): Therapeutic benefits of ACE inhibitors and other antihypertensive drugs in patients with type 2 diabetes. *Diabetes Care*., 23(7):888-92.

Panel, Horacio, F.CantielloAdriana G.Prat. (1996): Role of Actin Filament Organization in Ion Channel Activity and Cell Volume Regulation. *Elsevier., 43: 373-396.*

Pan-Pan, L., Qi-Long, Z., Fang Yuan., Fa-De, Z., Rui Fan., Li-Juan, F., Ling-Mei, H., Xu-Jun, Q., Shiwei Duan and Li-Na, Z., (2017): Association between ADD1 Gly460Trp Polymorphism and Essential Hypertension in Han Chinese. Journal of hypertension., 96(42): e7861.

Psaty, B, M. (2012): Conducting comparative effectiveness research on medications: the views of a practicing epidemiologist from the other Washington. *Value Health*., 15(2): 394-396.

Scheeff, E. D., & Fink, J. L. Hoboken, NJ, *et al*. (2003): Fundamentals of protein structure Structural bioinformatics. *Wiley.,*: 15-39.

Sousa,AC., Palma Dos Reis, R., Pereira, A., Borges, S., Freitas, AI., Guerra, G., Góis, T., Rodrigues, M., Henriques, E., Freitas, S., Ornelas, I., Pereira, D., Brehm, A., Mendonça, MI.(2017): Relationship between ADD1 Gly460Trp gene polymorphism and essential hypertension in Madeira Island. *Medicine*., 96(42):e7861.

Tsunoda, T., Lathrop, G. M., Sekine, A., *et al*. (2004): Variation of gene-based SNPs and linkage disequilibrium patterns in the human genome. Human Molecular Genetics., 13: 1623-1632.

Wheeler, D. L., Barrett, T., Benson, D. A., *et al*. (2008): Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research.*, 36 :13-21.

Wu M, et al. (1994): Four distinct and unusual linker proteins in a mitotically dividing nucleus are derived from a 71-kilodalton polyprotein, lack p34cdc2 sites, and contain protein kinase A sites. *Mol Cell Biol*., 14(1):10-20.

How to cite this article:

Harsimran Kaur Satbir Kaur and Praveen P.Balgir.2018, Functional and Genotypic Study of Add1 Gene Variant Associated with Hypertensive Population in Punjab. *Int J Recent Sci Res.* 9(12), pp. 29827-29831.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0912.2939

****