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# In Silico Analysis of Single Nucleotide Polymorphisms (SNPs) in Human *HLA-A* and *HLA-B* Genes Responsible for Renal Transplantation Rejection

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**Abstract:**

***Background*** *Chronic renal Disease (CRD) is becoming a real major public health problem worldwide, renal transplantation provides the best long-term treatment for chronic renal failure. Single-nucleotide polymorphisms (SNPs) play a major role in the understanding of the genetic basis of many complex human diseases. Also, the genetics of human phenotype variation could be understood by knowing the functions of these SNPs. It is still a major challenge to identify the functional SNPs in a disease-related gene.*

***Result*** *In this work, we explore whether SNPs mutations in HLA complex genes affect renal transplantation rejection, we have analyzed the genetic variation that can alter the expression and the function of HLA-A and HLA-B genes using computational methods. HLA-A gene contained a total of 1211 SNPs, of which 66 were nonsynonymous SNPs and 18 were in non-coding 3' untranslated region(UTR), likewise HLA-B gene contain total of 1234 SNPs of which 28 were nsSNPs and 35 were in 3'UTR region. It was found that, five of the total nsSNPs were predicted to be damaging by both the SIFT and PolyPhen servers, and these nsSNPs located within HLA-A gene, in like manner, 18 functional SNPs were predicted in 3'UTR region of HLA-B gene, through the 18 SNPs, 13 allele disrupts a conserved of 42 miRNA site, nine derived allele creates a new site of 23 miRNA (ancestral allele with support >2)*

***Conclusion*** *we propose that the main target mutation for the kidney transplantation rejection caused by coding- nonsynonymous region of HLA-A gene and non-coding 3'UTR region of HLA-B gene.*

**Key words**: Single-nucleotide, polymorphisms, renal transplantation rejection, computational methods

## Background

Chronic renal disease (CRD) can be define as slow and gradually loss of kidney function and that oftentimes lead to end stage of renal disease (ESRD) (NKF, 2013). More than 10% (>=20 million) of people age 20 years or older and about 31 million from the total population have CRD in United States in 2010. (CDC and AKF 2010) In like manner 12.5% of Canadian adult have CRD during the period 2007-2009 (Aroraetal, 2013). Finally CRD mostly common in male than female (Krol and Yee, 2011 & Elsharif and Elsharif, 2011).

Where in End Stage Renal Disease (ESRD) situation kidneys stop working quite enough to keep the body healthy and live without dialysis or transplants (AKF 2010).

The prevalence and incidence of ESRD records shown frequently increased worldwide (levey et al, 2003), where in 2001 the prevalence per million population shown 2000 in Japan, 1500 in US, 800 in European Union, and vary in developing country as below 100 in sub-Saharan Africa and India, 400 in Latin American, 600 in KSA (Barsoum, 2006), and finally 106 in Sudan 2009 (Elamin et al, 2010). Also in US 2010 nearly 117.000 people began treatment for ESRD (USRDS, 2012). Furthermore around the world 2002 there are over 1.1 million patients estimated to have ESRD with addition of 7% annually yearly (Lysaght, 2002). In conclusion we can say that chronic renal disease (CRD) is becoming a real major public health problem worldwide (Barsoum, 2006).

## Renal transplantation rejection:

Transplantation in general can be define as a process of transferring cell, tissue or organ from one individual to a second individual, where in kidney transplantation transfer kidney organ from donor to recipient (Gorczynski and Stanley, 1999). Organ shortage is a major problem for raising the numbers in renal transplantation. In addition poor and basically unchanged long-term outcomes in graft survival cause a high number of patients to return to the waiting list and dialysis treatment (Jevnikar and Mannon, 2008). Over the past decades, the acute rejection rate of kidney transplants has fallen dramatically and the 1-year graft survival rate has increased to 90% in transplantations with deceased donors and 95% with living related donors (Yates and Nicholson, 2006). The median rate of acute rejection was 18% in the recent multicenter study done by (Israni et al 2010). Renal transplantation provides the best long-term treatment for chronic renal failure (Arnutti et al, 2002).To have a well match

donor we need to do HLA typing.A well match donor is important to the success of our transplants (Butt et al, 2009).

## Human leukocyte antigens (HLA):

The human leukocyte antigen (HLA) system is the name of the major histocompatibility complex (MHC) in humans, which generally inherited from parents as a one set name haplotype, furthermore human leukocyte antigen (HLA) locus, located on chromosome:6p (short arm) in the distal portion of the 21.3 band, cover zone of about 4000 kilobase (kb) (4 X106nucleotides) (Mehra, 2001), which it the most polymorphic and gene-dense region of the human genome (Ayele et al, 2012).

The *HLA* complex genes and their protein products have been divided into three classes on the basis of their tissue distribution, structure, and function, where Class I MHC antigens are divided to (major or classical) encoded by gens *HLA*(*A*,*B* or *C* ) and (minor) encoded by the HLA-E, -F, -G, -H loci (Garc´ıa et al, 2012 & Mahdi, 2013). By the same token Class II MHC antigens encoded by gens *HLA*(*DP*, *DM*, *DOA*, *DOB*, *DQ*, and *DR*) loci, also *HLA-A*, *B*, *C* and *D* region products are involved in list of the immunoglobulin supergene family (Bodmer, 1987), finally MHC antigens Class III contains loci responsible for complement, hormones, intracellular peptide processing and other developmental characteristics Thus the Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the *HLA* genes (shankarkumar, 2004) expression of MHC gene products is greatly enhanced by cytokines, especially interferon, which stimulates transcription of MHC genes ( Zabriskie, 2009), lastly

2558 alleles of *HLA* class I and II have been known up to 2010 ( Marsh et al, 2010).

## *HLA-A* and *HLA-B* Genes (major histocompatibility complex, class I, A/B):

*HLA-A* and *HLA-B* are protein-coding genes which belongs to the HLA class I heavy chain, their structure is a heterodimer and composed of a heavychain prove in the membrane and a light chain (beta-2 microglobulin) of 12 kDa molecular weight (Mehra, 2001). Class I molecules play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen, the heavy chain is about 45 kDa (kilo Dalton) and its gene contains 8 exons, exon 1 encodes the commander peptide, exons 2 and 3 encode the alpha1 and alpha2 domains and both of them bind the peptide, beside exon 4 encodes the alpha3 domain and exon 5 encodes the trans membrane region, lastly exons 6 and 7 encode the cytoplasmic tail, polymorphisms within exon 2 and exon 3 are responsible for the peptide binding specificity of each class one molecule, typing for these polymorphisms is routinely done for bone marrow and kidney transplantation, now a day hundreds of *HLA-A/B* alleles have been described (NCBI, 2008). Function of both (HLA-A/B) are Involved in the presentation of foreign antigens to the immune system (UniProt, 2013). *HLA-A/B* genes are associated with many disease (MalaCards, 2013), furthermore related super-pathways such as, Allograft rejection pathway, beside this genes include MHC class I receptor activity and receptor binding, lastly *HLA-F* is an important paralog of this genes (GeneCards, 2013).

## Methods Used for HLA Detecting:

Recently, more precise DNA-based HLA typing methods using molecular techniques, such as, sequence-specific priming (SSP), sequence-specific oligonucleotide probing (SSOP), Sequence based typing (SBT) and reference strand-based conformation analysis (RSCA). The resolution of the HLA typing defines the level of HLA mismatch, low-resolution serologic typing can identify antigen mismatch (HLA-A2 vs. -A11), where a high-resolution typing can identify an allele or allelic subtype-level mismatch (HLA-A*0201 vs. -A*0205), that why DNA based method had more sensitivity, accuracy, specificity, and resolving power than serologic typing methods (Mahdi, 2013)

## Single nucleotide polymorphisms (SNPs):

Most human genetic variation is represented by single nucleotide polymorphisms (SNPs), and many human SNPs are believed to cause phenotypic differences between individuals, these variations are considered as being the cause of diseases, differences in response to treatment, susceptibility to diseases or being neutral having no impact at all. ( Drazen et al., 1999 and Drazen et al., 1999) SNPs are DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome is altered. They are found throughout the genome in exons, introns intergenic regions, promoters, enhancers, SNP in a promoter can influence gene expression ( Drazen et al., 1999 and Drazen et al., 1999),a new functional polymorphisms called miRSNPs/polymorphism located at microRNA binding sites of functional gene can influence gene expression by interfering with microRNA function of those SNPs within microRNAs (miRNAs), their targets have been shown to alter miRNA function in some genetic diseases (Sethupathy& Collins, 2008). Micro RNAs are small non-coding RNAs that are

approximately 20–25 nucleotides in length (Stark et al, 2005). They regulate the gene expression in multiple target genes through sequence-specific hybridization (Stark et al, 2005) presently the identification of SNPs responsible for specific phenotypes seems to be a problem, since requiring multiple testing of hundreds or thousands of SNPs in candidate genes (Krawczak and Fenton, 2000) hence computational tools was used here.

## Material and methods

### Bioinformatics processing and data analysis

In this study the decision of choosing the right set of SNPs to be screened was a critical one. One possible way to overcome this problem was be to prioritize SNPs according to their structural and functional significance using different bioinformatics prediction tools.

### Datasets

The SNPs and their related proteins sequence for both *HLA-A* and *HLA-B* genes were obtained from the SNPs database (dbSNPs) for our computational analysis, besides other two database (http://www.ncbi.nlm.nih.gov/SNP/) for SNPs and http://www.uniprot.org for SNPs related protein sequence, then SNPs identification was performed using a SNPs-database server (dSNPs)

### Predicting damaging amino acid substitutions using SIFTv5.1 (Sorting Intolerant from Tolerant).

An online computational program to detect a harmful nonsynonymous single-base nucleotide polymorphism (nsSNP), it performs an alignments between an order sequence with a large number of homologous sequences to predict if an amino

acid substitution affects protein function (Ng and Henikoff, 2001). The score result of each residual ranges from zero to one, where the amino acid substitution is predicted damaging when the score is below or equal to 0.05, and tolerated if the score is greater than 0.05 (Ng and Henikoff, 2003). SIFT version 5.1 is available at http://sift.bii.a-star.edu.sg/index.html.

**Prediction of functional modification of coding nsSNPs using Polyphen-2 (Polymorphism Phenotyping v2):-**

Its software tool to predicts possible impact of an amino acid substitution on the structure and function of a human protein by analysis of multiple sequence alignment and protein 3D structure, in addition that calculates position-specific independent count scores (PSIC) for each of two variants, and then computes the PSIC scores difference between two variants. The higher a PSIC score difference, the higher the functional impact a particular amino acid substitution is likely to have (Ramensky et al, 2002). Prediction outcome can be one of probably damaging, possibly damaging, or benign and also can be indicated by a vertical black marker inside a color gradient bar, where green is benign and red is damaging (Adzhubei et al., 2013). PolyPhen version 2.2.2r398 is available at http://genetics.bwh.harvard.edu/pph2/index.shtml

**I-Mutant Suite (Predictor of effects of single point protein mutation) :-**

Protein stability change disturbs both protein structure and protein function (Daggett and Fersht, 2003). I-Mutant is a suite of Support Vector Machine based predictors integrated in an unique web server, which used to predict the protein stability changes at single-site mutations starting from the protein structural or sequence information (Capriotti et al, 2005). I-

Mutant 3.0 is available at http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I Mutant3.0.cgi

**Protein 3D structural Modeling:-**

Modeling SNPs on the 3D structure of the proteins is a very helpful action in order to predict the effect that SNPs may cause on the structural level. Therefore we used CPH models 3.2 server to predict the 3D structure for those proteins with an unknown 3D structure model. It is in fact a protein homology modeling server, where the template recognition is based on profile-to-profile alignment, guided by secondary structure and exposure predictions (Nielsen et al 2010).

**Modeling amino acid substitution:-**

UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data. Chimera (version 1.8) software was used to scan the 3D (three-dimensional) structure of specific protein, and hence modifies the original amino acid with the mutated one to see the impact that can be produced. The outcome is then a graphic model depicting the mutation. Chimera (version 1.8) currently available within the Chimera package and available from the Chimera web site http://www.cgl.ucsf.edu/chimera/

**PolymiRTS data base 3.0 to Polymorphism in microRNA Target Site**

PolymiRTS database was designed specifically for the analysis of the 3'UTR region, it aims to identify single-nucleotide polymorphisms (SNPs) that affect miRNA targeting in human and mouse. we used this server at this stage to determine SNPs

that may alter miRNA target sites, all SNPs located within the 3′UTRs of *HLA* complex genes (*HLA-A/B*) were selected separately and submitted to the program,

## Results and discussion

The *HLA-A* and *HLA-B* genes investigated in this work were retrieved from the dbSNP database (National Center for Biotechnology Information). *HLA-A* gene was containing a total of 1211 SNPs, of which 66 were non-synonymous SNPs and 18 were in non-coding 3 untranslated region, likewise *HLA-B* gene was containing total of 1234 SNPs of which 28 were nsSNPs and 35 were in 3′UTR region, total SNPs contain SNPs of 3′UTR/5′UTR near to gene, non-coding 3′UTR/5′UTR, intron, coding synonymous and coding non-synonymous regions. Initially non-synonymous coding SNPs and 3′UTR SNPs were selected for our investigation. The distribution of SNPs located in both coding non-synonymous and 3′UTR for both genes is shown in **(Fig. 1.),** it can be seen that 3.8% of the total SNPs are nsSNPs, and 2.1% of the total SNPs are located in 3′UTR.

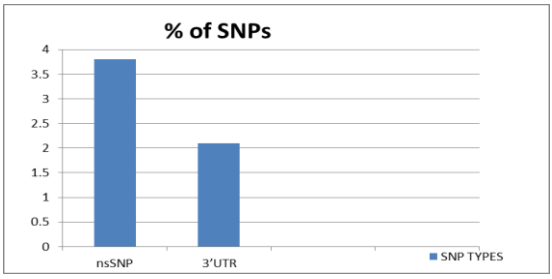



**Fig.1 Figure show the distribution of coding non-synonymous and 3UTR SNPs found in dbSNPs database.**

## Deleterious nsSNPs predicted by both SIFT and PolyPhen (*HLA-A* gene)

The rs number (reference SNPs) of 66 nsSNPs were submitted as a batch to the SIFT program and PolyPhen server. Among the 66 nsSNPs, five were predicted to be damaging by both the SIFT and PolyPhen server, which form 7.6% of the nsSNPs, furthermore damaging nsSNPs were confirmed using SIFT and PolyPhen protein sequence input option. The results are listed in **(Table 1)**

## Damaging nsSNPs predicted by both SIFT and PolyPhen (*HLA-B* gen)

In this step 28 nsSNPs were submitted as a batch to the SIFT and PolyPhen programs. Among the 28 nsSNPs, only one SNP (rs1050393) (A/G) was predicted to be damaging by a PolyPhen server, with 0.994 damaging score, beside deleterious SNP form 3.6% of the total nsSNPs. Damaging nsSNP was confirmed using protein sequence input option of SIFT and PolyPhen tools.

**Table (1):- Prediction result of SIFT and PolyPhen programs**

| Gene name | SNP ID | Chromosome location | Nucleotide change | Amino Acid Change | PolyPhen-2 result | PSIC SD | SIFT result | Tolerance Index |
|---|---|---|---|---|---|---|---|---|
| HLA-A | rs13870 4952 | chr6 29912022 29912023 | C/G | Q 248 H | PROBABLY DAMAGING | 0.96 4 | DAMA GING | 0.03 |
| | rs15002 8516 | chr6 29912041 29912042 | A/G/T | V 255 M | PROBABLY DAMAGING | 1 | DAMA GING | 0.01 |
| | rs15013 5282 | chr6 29911982 29911983 | C/T | A 235 V | PROBABLY DAMAGING | 0.99 7 | DAMA GING | 0.04 |
| | rs17185 861 | chr6 29911314 29911315 | A/C/G/T | R 205 H | POSSIBLY DAMAGING | 0.79 3 | DAMA GING | 0.05 |
| | rs41541 319 | chr6 29910540 29910541 | A/C/G/T | H 27 Q | PROBABLY DAMAGING | 0.99 7 | DAMA GING | 0 |

PolyPhen-2 result: POROBABLY DAMAGING (more confident prediction) / POSSIBLY DAMAGING (less confident prediction), PSIC SD: Position-Specific Independent Counts software Tolerance Index : Ranges from 0 to 1.

The amino acid substitution is predicted damaging is the score is <= 0.05, and tolerated if the score is > 0.05.

## Prediction of change in stability due to mutation used I-Mutant 3.0 server

We submitted independently five proteins sequence containing nsSNPs which predicted to be damaging both using SIFT and PolyPhen again to I-Mutant 3.0 server ,the output showed that three of five SNPs (rs150135282/ rs17185861/ rs41541319) were predicted to decrease protein stability, SNPs with ID (rs138704952/ rs150028516) were predicted to increase protein stability, above results demonstrated that all proteins stability were changed due to SNPs alteration (**Table 2**).

**Table(2):- Prediction result of I-Mutant software**

| Gene name | SNP ID | Amino acid position | WT | MT | SVM2 Prediction Effect | DDG Value Prediction Kcal/mol | RI |
|---|---|---|---|---|---|---|---|
| HLA-A | rs138704952 | 248 | Q | H | Increase | 0.8 | 7 |
| | rs150028516 | 255 | V | M | Increase | 0.65 | 4 |
| | rs150135282 | 235 | A | V | Decrease | -0.12 | 5 |
| | rs17185861 | 205 | R | H | Decrease | -1.13 | 8 |
| | rs41541319 | 27 | H | Q | Decrease | -1.29 | 9 |

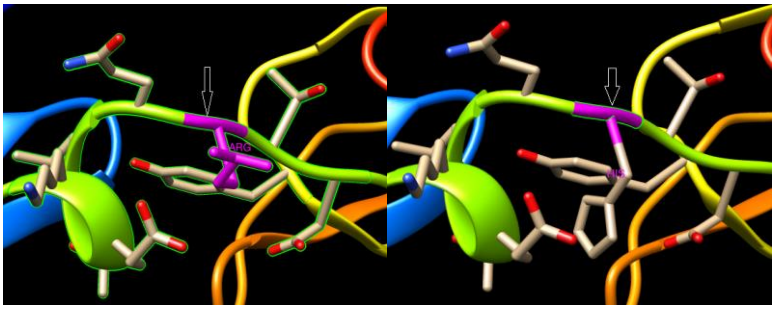
Where WT: Wild type amino acid, MT Mutant type amino acid, DDG: DG(New Protein)-DG(Wild Type) in Kcal/mol (DDG<0: Decrease stability, DDG>0: Increase stability), RI: Reliability index

## Modeling of amino acid substitution effects due to nsSNPs on protein structure

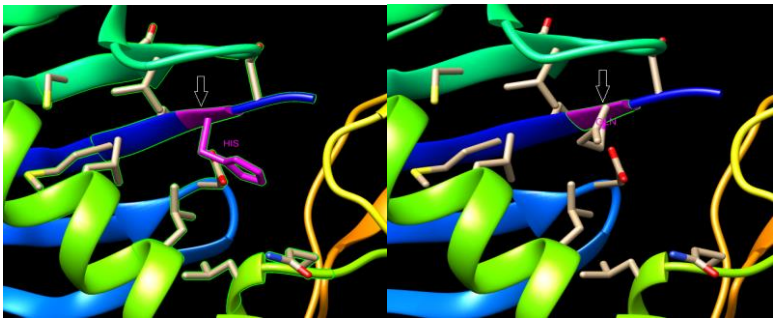
Protein sequences containing the nsSNPs were again submitted to CPH 3.2 server to get the protein 3D structure model, then Chimera program was to browse and respectively locate the 3D

structure of the each protein and to alter the native amino acid with a mutated one, the final outcome was a graphic model depicting the mutation.(**Fig. 2)**

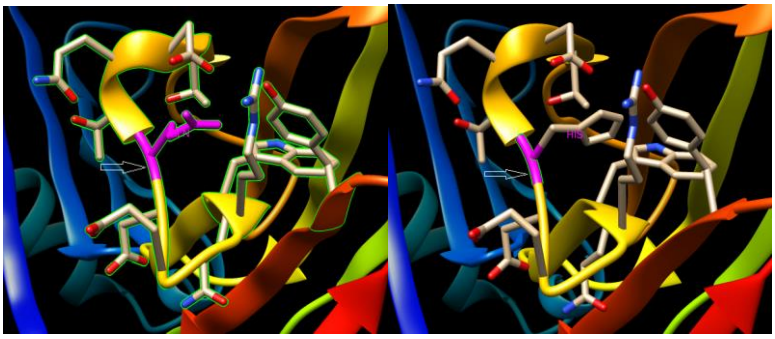
**Native residues** **New residues**



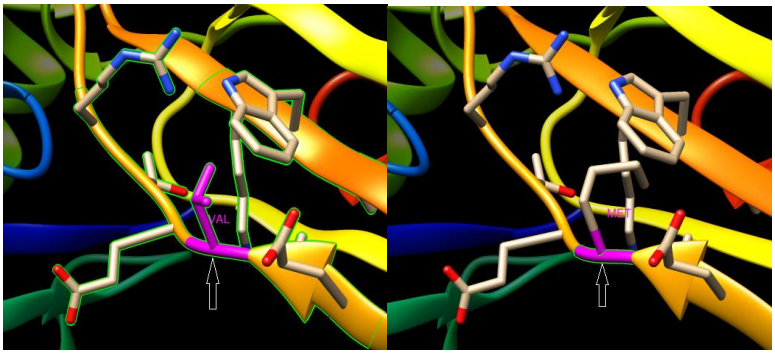

SNP ID: rs17185861, Protein position: 205 change from R to H



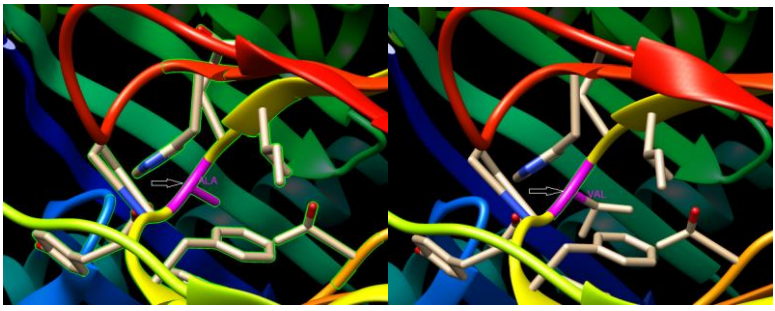

SNP ID: rs41541319, Protein position: 27 change from H to Q




SNP ID: rs138704952, Protein position: 248 change from Q to H




SNP ID: rs150028516, Protein position: 255 change from V to M




SNP ID: rs150135282 , Protein position: 235 change from A to V

**Fig 2. Shows position of native and new amino acid residues using Chimera programv1.8**

## Functional SNPs in 3 untranslated regions (UTR) predicted by PolymiRTS data base 3.0

SNPs within the boundaries 3UTR of *HLA-A* and *HLA-B* genes were submit it to PolymiRTS server, the result showed that among 18 SNPs in 3UTR region of *HLA-A* gene, nil functional SNP was predicted, but in other hand for *HLA-B* gene, through 35 SNPs, 18 functional SNPs predicted effected miRNA binding site, 13 allele disrupts a conserved of 42 miRNA site, and nine

derived allele creates a new site of 23 miRNA (ancestral allele with support >2). The results are listed in **Table 3.**

**Table (3): Prediction result of PolymiRTS data base**

| dbSNP ID | miR ID | Conservation | miRSite | Function Class |
|---|---|---|---|---|
| rs3177747 | hsa-miR-3202 | 5 | gagaaCCTTCCAg | D |
| | hsa-miR-4533 | 5 | gagaaCCTTCCAg | D |
| | hsa-miR-597-3p | 3 | GAGAACCttccag | D |
| | hsa-miR-148a-5p | 3 | gAGAACTTtccag | C |
| | hsa-miR-3978 | 5 | gagaaCTTTCCAg | C |
| | hsa-miR-875-3p | 4 | gagaacTTTCCAG | C |
| rs1058067 | hsa-miR-7110-3p | 3 | atttGAGAGAGca | D |
| | hsa-miR-130b-5p | 3 | atttGAAAGAGca | C |
| rs186928221 | hsa-miR-4424 | 8 | aaaTAACTCAatt | C |
| rs1057412 | hsa-miR-6083 | 3 | aATATAAAtttgt | D |
| rs1057151 | hsa-miR-5584-5p | 3 | TTCCCTActgaaa | D |
| | hsa-miR-6750-5p | 3 | TTCCCTActgaaa | D |
| | hsa-miR-6822-5p | 3 | TTCCCTActgaaa | D |
| rs114910620 | hsa-miR-6817-3p | 4 | CAGAGAGgtgggg | D |
| | hsa-miR-3191-5p | 4 | CAGAGAAgtgggg | C |
| | hsa-miR-4286 | 22 | cagagaAGTGGGG | C |
| rs361531 | hsa-miR-3191-5p | 4 | CCAGAGAggtggg | D |
| | hsa-miR-326 | 4 | CCAGAGAggtggg | D |
| | hsa-miR-330-5p | 4 | CCAGAGAggtggg | D |
| | hsa-miR-518c-5p | 4 | CCAGAGAggtggg | D |
| | hsa-miR-6764-3p | 4 | CCAGAGAggtggg | D |
| | hsa-miR-6817-3p | 4 | cCAGAGAGgtggg | D |
| | hsa-miR-6824-3p | 4 | CCAGAGAggtggg | D |
| | hsa-miR-2276-5p | 4 | cCAGAGGGgtggg | D |
| rs1056429 | hsa-miR-6768-5p | 5 | tgctgaCCTGTGT | D |
| rs112978603 | hsa-miR-1306-5p | 7 | tgtgaGGAGGTGg | D |
| | hsa-miR-5193 | 5 | tgtGAGGAGGtgg | D |
| | hsa-miR-1229-3p | 5 | tGTGAGAAggtgg | C |
| | hsa-miR-342-3p | 5 | TGTGAGAaggtgg | C |
| rs1054338 | hsa-miR-1182 | 3 | tcaAGACCCTctg | C |
| | hsa-miR-193a-5p | 3 | tcAAGACCCtctg | C |

miR ID : Link to miRBase, Conservation : Occurrence of the miRNA site in other vertebrate genomes in addition to the query genome, miRSite : Sequence context of the miRNA site. Bases complementary to the seed region are in capital letters and SNPs are highlighted in red, FuncClass : D: The derived allele disrupts a conserved miRNA site (ancestral allele with support > 2), C: The derived allele creates a new miRNA site.

## Conclusion

Our successful Insilco prediction of identification of several pathogenic SNPs in *HLA* Gene suggests the application of bioinformatics analysis tools as well as publicly available databases such as NCBI, dbSNP and HapMap for efficiently selecting a functional SNP for the conduct of genetic association studies. Finally we hope that our comprehensive investigation provide an empirical guideline for researchers to prioritize the known nsSNPs on the basis of molecular analysis. It is obvious from the results that the deployment of Insilco tools for application in biomedical research is highly effective and has a great impact on the ability to uncover the cause of genetic variation in different genetic disease. Important for future studies is to confirm this result in wet lab in order to obtain more information regarding possible novel SNPs involved in the renal transplantation rejection.

## Competing interests

The authors declare that they have no competing interests.

## Acknowledgements

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