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# Evaluation of Nonsynonymous Single Nucleotide Polymorphisms in the *DNMT1* Gene Associated With Gastric Cancer by an *In- Silico* Approach.

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**ABSTRACT**

The human *DNMT1* (NCBI GENE ID: 1786) gene encodes a DNA methyltransferase-1 which is also termed as *maintenance methyltranferase*. The *DNMT1* (DNA Methyl Tranferase-1) gene plays a substantial role in aberrant DNA methylation in carcinogenesis. Over-expression (Increased activity) and polymorphisms within the *DNMT1* gene is allied with a multiplied risk of gastric cancer. Most deleterious non synonymous single nucleotide polymorphisms in the coding region of the *DNMT1* gene were investigated using SNP databases, and detected nonsynonymous variants were analyzed *in silico* from the standpoint of relevant protein function and stability by PolyPhen-2, SIFT, PROVEAN and MUpro, I-Mutant2.0 tools respectively. A sum of 93 nonsynonymous SNPs comprising of 92 missense variations and one frameshift variations were found in the *DNMT1* gene. 10 of the 92 missense variants were predicted to be damaging or deleterious by three different software programs PolyPhen-2, SIFT, and PROVEAN, and 37 of them were predicted to be less stable using I-Mutant 2.0 and MUpro software. Total 5 variants namely c.4324C>T (p.Arg1442Trp), c.3913C>T (p.Arg1305Cys), c.3223C>T (p.Arg1075Cys), c.3097C>T (p.Arg1033Trp), c.1531T>C (p.Tyr511His) out of 92 missense variants were predicted to be both deleterious and reduced protein stability.Additionally, one frameshift variant (rs35600922) c.3475_3476insG (p.Asp1159Gly) was predicted to be both deleterious and less stable. Deleterious variants of *DNMT1* gene with reduced protein stability were suggested that a subset of nonsynonymous SNPs in the *DNMT1* gene might be associated with risk of cancer, including gastric cancer.

**Keywords:** PolyPhen-2, SIFT, PROVEAN, MUpro, I-Mutant2.0, CpG islands.

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## INTRODUCTION

The *DNMT1* gene (MIM#126375) is located on chromosome 19p13.2 with an aggregate size of 62 kb and is constituted of 40 exons. DNA (cytosine-5) methyltransferase1 has a part in the establishment and regulation of tissue specific patterns of methylated cytosine residues. *DNMT1* has been shown to possess a 10-40 fold preference for hemimethylated DNA (Pradhan *et al.*1999) and is that the most abundant methyltransferase in somatic cells. The *DNMT1* is the foremost enzyme responsible for maintenance of the DNA methylation pattern and often referred as *maintenance methyltransferase,* because it is supposed to be the primary enzyme responsible for copying methylation patterns after DNA replication [1]. *DNMT1* is situated at the replication fork and methylates newly biosynthesized DNA [2]. *DNMT1* localizes to replication foci throughout S-phase via numerous independent domains and interacts with the proliferating cell nuclear antigen (PCNA). Over expression of *DNMT1* has been identified in several human cancers including gastric cancer. Aberrant methylation patterns are allied with certain human tumors and developmental abnormalities. Aberrant methylation is associated with cancer as it alters the normal gene regulations and these alterations are of three types: hypermethylation, hypomethylation and loss of imprinting. This sort of aberrant methylation occurs mainly within the promoter CpG island regions which are unmethylated in the normal condition. DNA hypermethylation has been associated with transcriptional repression of tumour suppressive genes which results in tumour genesis [3]. Aberrant DNA methylation of CpG islands is a common epigenetic change found in gastric malignancy has been indicated to provoke alterations of DNA methylation in gastric mucosa [4].

Single nucleotide polymorphisms (SNPs) can result in small structural alterations in some important enzymes and thus changes in the susceptibility to cancer. As genomic variations among people, single nucleotide polymorphisms (SNPs) exist throughout the genome and can be divided into several groups. Among the different kinds of SNPs, a nonsynonymous SNP in the coding region of a gene is important because it alters the amino acid composition; consequently, such alterations can have an impact on protein structure, function, and subcellular localization. Although pinpointing the effects of the many nonsynonymous SNPs using biochemical analyses is challenging, computational analysis tools predicting their effect on protein activity and stability have been recently developed, such as Polymorphism phenotyping v2 (PolyPhen-2) [5], Sorting Intolerant From Tolerant (SIFT) [6], Protein Variation Effect Analyzer (PROVEAN) [7], I-Mutant 2.0 [8], and MUpro [9] software. Since the *DNMT1* plays an important role in DNA methylation patterns and genome maintenance, a dysfunctional ability of *DNMT1* as a result of nonsynonymous SNPs might be associated with susceptibility to diseases, including gastric cancer. Thus, in the current study, we searched for nonsynonymous SNPs in the *DNMT1* gene using genome databases and explored the effects of nonsynonymous SNPs on *DNMT1* protein function and stability using a computational approach.

## MATERIALS AND METHODS

### Collection of nonsynonymous SNPs

Data on nonsynonymous variations of the *DNMT1* gene were collected from the database of SNPs (dbSNP) located on the homepage of the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/SNP/) and from Ensembl genome browser (http:// http://www.ensembl.org/index.html). The reference Transcript ID and the reference Protein ID of *DNMT1* are NM_001379.2 and NP_001370.1, respectively.

### POLYPHEN-2 prediction

PolyPhen [10] is a computational tool for identification of potentially functional nsSNPs. Predictions are based on a combination of phylogenetic, structural, and sequence annotation information characterizing a substitution and its position in the protein. For a given amino acid variation, PolyPhen performs various steps: (a) extraction of sequence based features of the substitution site from the UniProt database, (b) calculating the profile scores for two amino acid variants, and (c) calculating the structural parameters and contacts of a substituted residue. PolyPhen-2 server discriminates nsSNPs into three main categories: scores were classified as “benign”, “possibly damaging” or “probably damaging” [12].

**SIFT prediction**

The Sorting Intolerant from Tolerant (SIFT) algorithm predicts the consequence of coding variants on protein function based on the degree of conservation of aminoacid residues in sequence alignments derived from closely related sequences [6]. It was first introduced in 2001, with a corresponding website that provides users with predictions on their variants. Since its release, SIFT has become one of the standard tools for characterizing missense variation. SIFT is based on the premise that protein evolution is correlated with protein function. Variants that occur at conserved alignment positions are expected to be tolerated less than those that occur at diverse positions. The algorithm uses a modified version of PSIBLAST [11] and Dirichlet mixture regularization [13] to construct a multiple sequence alignment of proteins that can be globally aligned to the query sequence and belong to the same clade. The underlying principle of this program is that it generates alignments with a large number of homologous sequences and assigns scores to each residue, ranging from zero to one. SIFT scores [12] categorised as potentially intolerant (0.051–0.10), intolerant (0.00–0.05), tolerant (0.201–1.00) or borderline (0.101–0.20). The higher the tolerance index of a particular amino acid substitution, the lesser is its likely impact.

**PROVEAN prediction**

PROVEAN (Protein Variation Effect Analyzer), which predicts the functional impact for all classes of protein sequence variations not only single amino acid substitutions but also insertions, deletions, and multiple substitutions on the alignment based score [7]. The score measures the change in sequence similarity of a query sequence to a protein sequence homolog between without and with an amino acid variation of the query sequence. If the PROVEAN score ≤-2.5, the protein variant is predicted to have a “deleterious” effect, while if the PROVEAN score is >- 2.5, the variant is predicted to have a “neutral” effect. Both types of software are available on the homepage of the J. Craig Venter Institute: the SIFT tool is at http://sift.jcvi.org, and the PROVEAN tool is at http://provean.jcvi.org.

**I-Mutant 2.0 prediction**

I-Mutant 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html) is a support vector machine-based tool for the prediction of protein stability changes upon nonsynonymous variations [8]. The tool evaluates the stability change upon nonsynonymous SNP starting from the protein structure or from the protein sequence. The DDG value (difference in free energy of mutation) is calculated from the unfolding Gibbs free energy value of the variant protein minus the unfolding Gibbs free energy value of the wild type (Kcal/mol), and scores <0 are predicted by the algorithm to indicate decreased stability, whereas scores >0 are considered to indicate increased stability.

**MUpro prediction**

MUpro (http://www.ics.uci.edu/~baldig/mutation.html) is also a support vector machine-based tool for the prediction of protein stability changes upon nonsynonymous SNPs [9]. The value of the energy change is predicted, and a confidence score between -1 and 1 for measuring the confidence of the prediction is calculated. A score <0 means the variant decreases the protein stability; conversely, a score >0 means the variant increases the protein stability.

## RESULTS

By examining SNPs in the *DNMT1* gene using the dbSNP, HGVD, ENSEMBL databases, a total of 93 nonsynonymous SNPs were found. These SNPs consisted of 92 missense variations, and one frameshift variation. To determine which missense variants are damaging or deleterious, PolyPhen-2, SIFT, and PROVEAN software were applied for the 92 missense variants of the *DNMT1* gene (Table 1). In the PolyPhen-2 analysis, 26 (28.2%) of the 92 variants were predicted to be probably damaging, and the others were predicted to be benign or possibly damaging. When the SIFT software was used, 21 variants (22.8%) were predicted to be damaging, and the others were predicted to be tolerated. In the PROVEAN analysis, 28 variants (30.4%) were predicted to be deleterious, but the others were neutral. Among the above 10 (10.8%) common *DNMT1* variants, namely, c.4324C>T (p.Arg1442Trp), c.3913C>T (p.Arg1305Cys), c.3519A>C (p.Gln1173His), c.3223C>T (p.Arg1075Cys), c.3097C>T (p.Arg1033Trp), c.3036C>G (p.Ile1012Met), c.2464G>A (p.Gly822Arg), c.1816G>T

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(p.Val606Phe), c.1532A>G (p.Tyr511Cys), c.1531T>C (p.Tyr511His), were found. Therefore, these variants are considered to be most likely damaging or deleterious.

**Table 1: PolyPhen, SIFT and PROVEAN results for the 92 missense variants of *DNMT1* gene**

| Nucleotide | Position | dbSNP ID | Protein | POLYPHEN-2 prediction score | SIFT prediction score | PROVEAN prediction score |
|---|---|---|---|---|---|---|
| c.4894G>C | g.10133672 | rs147118268 | p.Asp1632His | (Probably Damaging) 0.995 | Deleterious (0.01) | Neutral (-1.178) |
| c.4884A>T | g.10133682 | rs370070174 | p.Glu1628Asp | (Benign) 0.003 | Tolerated (0.58) | Neutral (-0.783) |
| c.4876G>A | g.10133690 | rs201774098 | p.Glu1626Lys | (Benign) 0.030 | Tolerated (0.41) | Neutral (-0.204) |
| c.4444C>T | g.10137130 | rs147984942 | p.Arg1482Cys | (Benign) 0.011 | Tolerated (0.29) | Deleterious(-3.589) |
| c.4428T>G | g.10137146 | rs142647321 | p.His1476Gln | (Benign) 0.037 | Tolerated (0.07) | Deleterious(-2.707) |
| c.4324C>T | g.10137250 | rs139918621 | p.Arg1442Trp | (Probably Damaging) 0.999 | Deleterious (0.01) | Deleterious(-4.543) |
| c.4255G>A | g.10137870 | rs374047326 | p.Ala1419Thr | (Benign) 0.000 | Tolerated (0.13) | Neutral(0.592) |
| c.4221G>C | g.10137904 | rs367897930 | p.Glu1407Asp | (Benign) 0.073 | Tolerated (0.33) | Neutral(-1.465) |
| c.4193C>T | g.10137932 | rs375225009 | p.Ser1398Leu | Possibly Damaging (0.952) | Deleterious (0.03) | Deleterious(-3.822) |
| c.4108A>C | g.10138446 | rs201167482 | p.Ile1370Leu | (Benign) 0.001 | Tolerated (0.69) | Neutral(-0.633) |
| c.4061C>T | g.10138493 | rs141791913 | p.Ala1354Val | (Probably Damaging) 0.995 | Tolerated (0.13) | Deleterious(-2.753) |
| c.3913C>T | g.10139711 | rs200312526 | p.Arg1305Cys | (Probably Damaging) 1.000 | Deleterious(0.02) | Deleterious(-5.494) |
| c.3659C>T | g.10140193 | rs142648642 | p.Thr1220Ile | (Benign) 0.165 | Tolerated(0.19) | Deleterious(-2.926) |
| c.3656C>A | g.10140196 | rs201497993 | p.Thr1219Asn | (Benign) 0.030 | Tolerated(0.49) | Neutral(-1.206) |
| c.3519A>C | g.10140785 | rs151305495 | p.Gln1173His | (Probably Damaging) 1.000 | Deleterious(0.02) | Deleterious(-4.329) |
| c.3428C>T | g.10140876 | rs375976847 | p.Pro1143Leu | Possibly Damaging (0.826) | Tolerated(0.23) | Deleterious(-3.404) |
| c.3413C>G | g.10140891 | rs201308454 | p.Ser1138Cys | (Benign) 0.020 | Tolerated(0.06) | Neutral(-2.24) |
| c.3358C>T | g.10141141 | rs200950656 | p.Arg1120Cys | (Benign) 0.189 | Tolerated(0.12) | Deleterious(-2.64) |
| c.3353A>G | g.10141146 | rs150331990 | p.His1118Arg | (Benign) 0.340 | Tolerated(0.21) | Deleterious(-3.19) |
| c.3352C>T | g.10141147 | rs374676749 | p.His1118Tyr | (Benign) 0.069 | Tolerated(0.16) | Neutral(-2.363) |
| c.3293G>T | g.10142044 | rs377078524 | p.Arg1098Leu | Possibly Damaging (0.989) | Tolerated(0.24) | Deleterious(-6.008) |
| c.3278T>C | g.10142059 | rs370075258 | p.Met1093Thr | (Benign) 0.000 | Tolerated(0.48) | Neutral(0.121) |
| c.3223C>T | g.10142114 | rs140852137 | p.Arg1075Cys | (Probably Damaging) 0.999 | Deleterious(0.00) | Deleterious(-5.374) |
| c.3196G>A | g.10142141 | rs187394074 | p.Val1066Met | (Benign) 0.060 | Tolerated(0.10) | Neutral(-1.065) |
| c.3157G>A | g.10142180 | rs370786558 | p.Ala1053Thr | (Benign) 0.047 | Tolerated(0.18) | Neutral(-0.907) |
| c.3098G>A | g.10143784 | rs199827346 | p.Arg1033Gln | (Benign) 0.213 | Tolerated(0.42) | Neutral(-1.792) |
| c.3097C>T | g.10143785 | rs144533539 | p.Arg1033Trp | (Probably Damaging) 0.998 | Deleterious(0.00) | Deleterious(-5.318) |
| c.3077A>T | g.10143805 | rs373940840 | p.Asn1026Ile | Possibly Damaging (0.933) | Deleterious(0.02) | Neutral(-2.02) |
| c.3036C>G | g.10143846 | rs376854079 | p.Ile1012Met | (Probably Damaging) 1.000 | Deleterious(0.02) | Deleterious(-2.629) |
| c.2914G>A | g.10143968 | rs148038464 | p.Val972Met | (Benign) 0.268 | Tolerated(0.40) | Neutral(-1.084) |
| c.2728G>T | g.10146517 | rs150863675 | p.Val910Leu | (Benign) 0.000 | Tolerated(0.76) | Neutral(-0.698) |
| c.2693C>T | g.10148911 | rs201213597 | p.Thr898Ile | (Benign) 0.000 | Tolerated(0.19) | Neutral(-0.481) |
| c.2656G>C | g.10148948 | rs367681882 | p.Asp886His | (Benign) 0.268 | Tolerated(0.58) | Deleterious(- |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | 4.168) |
| c.2626G>A | g.10148978 | rs62621087 | p.Gly876Arg | (Probably Damaging) 1.000 | Tolerated(0.48) | Deleterious(-5.139) |
| c.2471C>T | g.10149568 | rs140993011 | p.Thr824Met | Possibly Damaging (0.973) | Tolerated(0.19) | Deleterious(-3.241) |
| c.2464G>A | g.10149575 | rs183555527 | p.Gly822Arg | Possibly Damaging (0.984) | Deleterious(0.00) | Deleterious(-7.127) |
| c.2443G>A | g.10149596 | rs373366822 | p.Ala815Thr | Possibly Damaging (0.987) | Tolerated(0.12) | Neutral(-0.601) |
| c.2387C>T | g.10149652 | rs148987580 | p.Thr796Met | Possibly Damaging (0.589) | Tolerated(0.12) | Neutral(-0.625) |
| c.2369T>C | g.10149865 | rs113497353 | p.Leu790Pro | Possibly Damaging (0.513) | Tolerated(0.30) | Deleterious(-4.051) |
| c.2315C>A | g.10149919 | rs142562681 | p.Thr772Asn | (Benign) 0.082 | Tolerated(0.48) | Neutral(-1.28) |
| c.2161G>A | g.10151502 | rs375620967 | p.Val721Ile | (Benign) 0.016 | Tolerated(0.20) | Neutral(-0.276) |
| c.2152G>A | g.10151511 | rs368319266 | p.Asp718Asn | Possibly Damaging (0.917) | Deleterious(0.05) | Neutral(-2.101) |
| c.1987G>A | g.10154325 | rs146467216 | p.Ala663Thr | (Benign) 0.005 | Tolerated(0.55) | Neutral(-1.004) |
| c.1816G>T | g.10154602 | rs397509391 | p.Val606Phe | (Probably Damaging) 1.000 | Deleterious(0.01) | Deleterious(-4.075) |
| c.1814G>C | g.10154604 | rs397509393 | p.Gly605Ala | (Probably Damaging) 1.000 | Tolerated(0.22) | Deleterious(-5.41) |
| c.1709C>T | g.10154709 | rs397509392 | p.Ala570Val | (Probably Damaging) 1.000 | Tolerated(0.07) | Deleterious(-3.606) |
| c.1678C>T | g.10154740 | rs375474222 | p.Arg560Cys | (Benign) 0.375 | Tolerated(0.08) | Deleterious(-3.506) |
| c.1648A>G | g.10154770 | rs368761660 | p.Thr550Ala | (Benign) 0.081 | Tolerated(0.62) | Neutral(-1.788) |
| c.1532A>G | g.10155017 | rs199473690 | p.Tyr511Cys | (Probably Damaging) 1.000 | Deleterious(0.00) | Deleterious(-8.114) |
| c.1531T>C | g.10155018 | rs199473692 | p.Tyr511His | (Probably Damaging) 1.000 | Deleterious(0.00) | Deleterious(-4.508) |
| c.1436A>C | g.10155909 | rs374027926 | p.Glu479Ala | (Benign) 0.208 | Tolerated(0.21) | Deleterious(-3.806) |
| c.1294C>T | g.10156496 | rs181300723 | :p.His432Tyr | (Benign) 0.001 | Tolerated(0.13) | Neutral(-2.117) |
| c.1223A>G | g.10159715 | rs367727676 | p.Asn408Ser | (Benign) 0.004 | Tolerated(0.22) | Neutral(-1.555) |
| c.1195A>G | g.10159743 | rs142673915 | p.Asn399Asp | (Benign) 0 | Tolerated(1.00) | Neutral(1.965) |
| c.1145A>T | g.10159867 | rs141562679 | p.Lys382Ile | (Benign) 0.243 | Tolerated(0.52) | Neutral(-1.708) |
| c.1064G>C | g.10160043 | rs201945078 | p.Arg355Pro | (Benign) 0.001 | Tolerated(0.30) | Neutral(-0.357) |
| c.1043C>T | g.10160384 | rs370592431 | p.Pro348Leu | (Benign) 0.011 | Tolerated(0.34) | Neutral(-0.805) |
| c.1003G>A | g.10162672 | rs200531080 | p.Glu335Lys | Possibly Damaging (0.493) | Tolerated(0.79) | Neutral(-1.295) |
| c.981T>G | g.10162694 | rs61758431 | p.Ile327Met | (Benign) 0.001 | Tolerated(0.11) | Neutral(-0.558) |
| c.979A>C | g.10162696 | rs2228612 | p.Ile327Leu | (Benign) 0.001 | Tolerated(0.30) | Neutral(-0.488) |
| c.977A>C | g.10162698 | rs143287044 | p.Gln326Pro | Possibly Damaging (0.467) | Tolerated(0.15) | Neutral(-0.583) |
| c.946G>A | g.10162729 | rs200601847 | p.Glu316Lys | (Benign) 0.008 | Tolerated(0.92) | Neutral(-0.108) |
| c.925C>G | g.10163327 | rs61758430 | p.Leu309Val | Possibly Damaging (0.933) | Tolerated(1.00) | Neutral(-0.083) |
| c.919A>G | g.10163333 | rs148831705 | p.Lys307Glu | (Benign) 0.144 | Tolerated(0.82) | Neutral(-0.015) |
| c.890A>G | g.10166599 | rs201025441 | p.Lys297Arg | (Benign) 0.001 | Tolerated(0.58) | Neutral(-0.448) |
| c.868G>A | g.10166621 | rs200024502 | p.Glu290Lys | (Benign) 0.165 | Tolerated(0.78) | Neutral(-0.257) |
| c.856G>A | g.10166633 | rs368960099 | p.Val286Met | Possibly Damaging (0.663) | Tolerated(0.06) | Neutral(-0.221) |
| c.827A>T | g.10166662 | rs372129479 | p.Glu276Val | Possibly Damaging (0.979) | Tolerated(0.26) | Neutral(-0.942) |
| c.740C>A | g.10173118 | rs370064676 | p.Thr247Asn | (Benign) 0 | Tolerated(0.33) | Neutral(-0.391) |
| c.736C>T | g.10173122 | rs201749864 | p.Arg246Cys | Possibly Damaging (0.618) | Deleterious(0.03) | Neutral(-0.054) |
| c.734C>T | g.10173124 | rs143598088 | p.Thr245Met | (Probably Damaging) 1.000 | Deleterious(0.00) | Neutral(-0.751) |
| c.731G>A | g.10173127 | rs150999369 | p.Gly244Glu | (Benign) 0.373 | Deleterious(0.04) | Neutral(0.728) |

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|---|---|---|---|---|---|---|
| c.694C>A | g.10173164 | rs374856119 | p.Pro232Thr | (Benign) 0.066 | Tolerated(0.55) | Neutral(0.148) |
| c.575C>T | g.10175613 | rs62621089 | p.Ala192Val | (Benign) 0.328 | Tolerated(0.13) | Neutral(-0.525) |
| c.527A>G | g.10177334 | rs374440818 | p.Lys176Arg | (Benign) 0 | Tolerated(0.73) | Neutral(-0.088) |
| c.520A>C | g.10177341 | rs201319352 | p.Thr174Pro | Possibly Damaging (0.955) | Tolerated(0.10) | Neutral(-1.156) |
| c.440C>G | g.10180355 | rs1140470 | p.Ala147Gly | (Benign) 0.066 | Deleterious(0.02) | Neutral(-0.32) |
| c.410C>T | g.10180385 | rs377146699 | p.Thr137Met | Possibly Damaging (0.981) | Tolerated(0.10) | Neutral(-0.701) |
| c.406C>T | g.10180389 | rs138841970 | p.Arg136Cys | (Benign) 0.003 | Tolerated(0.07) | Neutral(-1.053) |
| c.386C>G | g.10180409 | rs370207020 | p.Pro129Arg | Possibly Damaging (0.836) | Tolerated(0.16) | Neutral(-0.894) |
| c.382C>A | g.10180413 | rs146601335 | p.Pro128Thr | (Benign) 0.29 | Tolerated(0.80) | Neutral(-0.312) |
| c.358G>C | g.10180437 | rs75616428 | p.Val120Leu | (Benign) 0.264 | Tolerated(0.26) | Neutral(-0.363) |
| c.357A>C | g.10180438 | rs373923585 | p.Arg119Ser | (Benign) 0.023 | Tolerated(0.70) | Neutral(-0.166) |
| c.355A>G | g.10180440 | rs146516082 | p.Arg119Gly | Possibly Damaging (0.165) | Tolerated(0.67) | Neutral(-0.743) |
| c.353G>A | g.10180442 | rs149362098 | p.Arg118His | Possibly Damaging (0.981) | Tolerated(0.19) | Neutral(-0.064) |
| c.328G>A | g.10180467 | rs376894659 | p.Gly110Arg | (Probably Damaging) 1.000 | Deleterious(0.01) | Neutral(-1.484) |
| c.301C>T | g.10180494 | rs369196079 | p.Arg101Trp | (Probably Damaging) 0.997 | Deleterious(0.02) | Neutral(-0.655) |
| c.290A>G | g.10180505 | rs16999593 | p.His97Arg | (Benign) 0.437 | Tolerated(0.82) | Neutral(-1.088) |
| c.206G>A | g.10180797 | rs61750053 | p.Arg69His | (Benign) 0 | Tolerated(0.38) | Neutral(0.044) |
| c.163A>G | g.10180840 | rs375585911 | p.Thr55Ala | (Probably Damaging) 0.996 | Tolerated(0.82) | Neutral(-0.431) |
| c.97A>G | g.10182061 | rs374817622 | p.Arg33Gly | (Probably Damaging) 0.996 | Deleterious(0.02) | Neutral(-0.365) |

**Table 2: I-Mutant 2.0 (DDG) and MUpro prediction results for the 92 missense variants of *DNMT1* gene**

| Protein | I-MUTANT 2.0 prediction DDG | MUpro prediction score |
|---|---|---|
| | | |
| | | |
| p.Asp1632His | Increase (0.22) | Decrease(-0.10903844) |
| | | |
| p.Glu1628Asp | Decrease (-0.59) | Decrease(-0.52108761) |
| | | |
| p.Glu1626Lys | Decrease (-1.1) | Increase ( 0.42257611) |
| | | |
| p.Arg1482Cys | Decrease (-1.41) | Decrease(-0.65875544) |
| | | |
| p.His1476Gln | Decrease (-1.07) | Increase (0.15517414) |
| | | |
| p.Arg1442Trp | Decrease (-1.04) | Decrease( -0.65662057) |
| | | |
| p.Ala1419Thr | Decrease (-0.73) | Decrease( -0.3106366) |
| | | |
| p.Glu1407Asp | Increase (0.71) | Decrease(-0.93424268) |
| | | |
| p.Ser1398Leu | Increase (0.07) | Increase (1.00) |
| | | |
| p.Ile1370Leu | Decrease (-0.71) | Decrease(-0.20012688) |
| | | |

| | | |
|---|---|---|
| p.Ala1354Val | Increase (0.37) | Increase(0.4574522) |
| | | |
| p.Arg1305Cys | Decrease (-1.19) | Decrease (-1) |
| | | |
| p.Thr1220Ile | Decrease (-0.42) | Decrease(-0.32585604) |
| | | |
| p.Thr1219Asn | Decrease (-0.44) | Increase (0.21924097) |
| | | |
| p.Gln1173His | Increase (0.05) | Decrease ( -0.02099037) |
| | | |
| p.Pro1143Leu | Increase (0.28) | Increase (0.49908364) |
| | | |
| p.Ser1138Cys | Decrease (-1.07) | Decrease(-0.12061732) |
| | | |
| p.Arg1120Cys | Decrease (-0.75) | Decrease(-0.55790231) |
| | | |
| p.His1118Arg | Decrease (-0.2) | Increase ( 0.56244423) |
| | | |
| p.His1118Tyr | Increase (0.51) | Increase (0.51835535) |
| | | |
| p.Arg1098Leu | Decrease (-0.32) | Increase (0.57209445) |
| | | |
| p.Met1093Thr | Decrease (-2.1) | Decrease( -0.51850139) |
| | | |
| p.Arg1075Cys | Decrease (-1.19) | Decrease(-0.10784229) |
| | | |
| p.Val1066Met | Decrease (-1.81) | Decrease(-0.27486652) |
| | | |
| p.Ala1053Thr | Decrease (-1.01) | Decrease( -0.32267644) |
| | | |
| p.Arg1033Gln | Decrease (-2) | Decrease(-1) |
| | | |
| p.Arg1033Trp | Decrease (-1.34) | Decrease(-0.91990014) |
| | | |
| p.Asn1026Ile | Increase (1.11) | Increase (0.079030455) |
| | | |
| p.Ile1012Met | Decrease(-0.53) | Increase (0.081750989) |
| | | |
| p.Val972Met | Decrease(-2.33) | Decrease(-0.21734536) |
| | | |
| p.Val910Leu | Decrease(-0.39) | Decrease(-0.3568134) |
| | | |
| p.Thr898Ile | Decrease(-0.92) | Decrease( -0.7727123) |
| | | |

| | | |
|---|---|---|
| p.Asp886His | Decrease(-0.2) | Decrease(-0.63334686) |
| | | |
| p.Gly876Arg | Decrease(-1.07) | Decrease(-0.5232877) |
| | | |
| p.Thr824Met | Increase (0.14) | Increase (0.60580589) |
| | | |
| p.Gly822Arg | Decrease(-2.31) | Increase(0.43076103) |
| | | |
| p.Ala815Thr | Decrease(-1.15) | Increase (-0.39906648 |
| | | |
| p.Thr796Met | Decrease(-0.58) | Increase (0.65700508(I) |
| | | |
| p.Leu790Pro | Decrease(-1.07) | Decrease(-1) |
| | | |
| p.Thr772Asn | Decrease(-0.56) | Decrease(0.12964373) |
| | | |
| p.Val721Ile | Decrease(-0.51) | Decrease(-1) |
| | | |
| p.Asp718Asn | Decrease(-0.81) | Decrease( -0.074145978) |
| | | |
| p.Ala663Thr | Decrease(-1.03) | Decrease(-0.84092604) |
| | | |
| p.Val606Phe | Decrease(-1.95) | Increase (0.1003691) |
| | | |
| p.Gly605Ala | Decrease(-0.65) | Decrease( -0.35296908) |
| | | |
| p.Ala570Val | Increase (0.34) | Increase (1.00) |
| | | |
| p.Arg560Cys | Decrease(-1.35) | Decrease(-0.88868736) |
| | | |
| p.Thr550Ala | Decrease(-1.53) | Decrease(-1) |
| | | |
| p.Tyr511Cys | Increase (1.01) | Decrease(-1) |
| | | |
| p.Tyr511His | Decrease(-1.21) | Decrease(-1) |
| | | |
| p.Glu479Ala | Decrease(-1.22) | Decrease( -0.66101422) |
| | | |
| p.His432Tyr | Increase (0.53) | Increase (0.38227554) |
| | | |
| p.Asn408Ser | Decrease(-0.37) | Decrease(-0.89456556) |
| | | |
| p.Asn399Asp | Decrease(-1.55) | Decrease(-0.44215936 |
| | | |

| | | |
|---|---|---|
| p.Lys382Ile | Decrease(-0.54) | Decrease(-1) |
| | | |
| p.Arg355Pro | Decrease(-1.68) | Decrease(-1) |
| | | |
| p.Pro348Leu | Increase (0.56) | Increase (0.69297658) |
| | | |
| p.Glu335Lys | Decrease(-1.46) | Decrease(-0.84073241) |
| | | |
| p.Ile327Met | Decrease(-1.17) | Decrease(-0.48730767) |
| | | |
| p.Ile327Leu | Decrease(-1.07) | Decrease( -0.57145761) |
| | | |
| p.Gln326Pro | Decrease(-2.28) | Increase ( 0.061387435) |
| | | |
| p.Glu316Lys | Decrease(-1.35) | Increase ( 0.061387435) |
| | | |
| p.Leu309Val | Decrease(-1.07) | Increase ( 0.061387435) |
| | | |
| p.Lys307Glu | Decrease(-0.06) | Increase ( 0.061387435) |
| | | |
| p.Lys297Arg | Decrease(-0.71) | Increase ( 0.061387435) |
| | | |
| p.Glu290Lys | Decrease(-0.98) | Increase ( 0.061387435) |
| | | |
| p.Val286Met | Decrease(-0.46) | Increase ( 0.061387435) |
| | | |
| p.Glu276Val | Decrease(-0.15) | Increase ( 0.061387435) |
| | | |
| p.Thr247Asn | Decrease(-0.39) | Increase ( 0.061387435) |
| | | |
| p.Arg246Cys | Decrease(-0.03) | Increase ( 0.061387435) |
| | | |
| p.Thr245Met | Increase (0.29) | Increase ( 0.061387435) |
| | | |
| p.Gly244Glu | Decrease(-0.59) | Increase ( 0.061387435) |
| | | |
| p.Pro232Thr | Decrease(-1.55) | Increase ( 0.061387435) |
| | | |
| p.Ala192Val | Decrease(-0.5) | Increase ( 0.061387435) |
| | | |
| p.Lys176Arg | Increase (0.14) | Increase ( 0.061387435) |
| | | |
| p.Thr174Pro | Decrease(-0.75) | Increase ( 0.061387435) |
| | | |

| | | |
|---|---|---|
| p.Ala147Gly | Decrease(-0.95) | Increase ( 0.061387435) |
| | | |
| p.Thr137Met | Decrease(-0.95) | Increase ( 0.061387435) |
| | | |
| p.Arg136Cys | Decrease(-0.5) | Increase ( 0.061387435) |
| | | |
| p.Pro129Arg | Decrease(-0.3) | Increase ( 0.061387435) |
| | | |
| p.Pro128Thr | Decrease(-1.27) | Increase ( 0.061387435) |
| | | |
| p.Val120Leu | Increase (0.63) | Increase ( 0.061387435) |
| | | |
| p.Arg119Ser | Decrease (-2.25) | Increase ( 0.061387435) |
| | | |
| p.Arg119Gly | Decrease (-1.58) | Increase ( 0.061387435) |
| | | |
| p.Arg118His | Decrease (-0.33) | Increase ( 0.061387435) |
| | | |
| p.Gly110Arg | Decrease (-0.12) | Increase ( 0.061387435) |
| | | |
| p.Arg101Trp | Decrease (-0.62) | Increase ( 0.061387435) |
| | | |
| p.His97Arg | Decrease (-0.11) | Increase ( 0.061387435) |
| | | |
| p.Arg69His | Decrease (-0.89) | Increase ( 0.061387435) |
| | | |
| p.Thr55Ala | Decrease (-1.25) | Increase ( 0.061387435) |
| | | |
| p.Arg33Gly | Decrease (-1.03) | Increase ( 0.061387435) |

**Table 3: Frameshift variation of *DNMT1* gene**

| Nucleotide | Position | dbSNP ID | Protein | SIFT | PROVEAN | POLYPHEN | I-MUTANT | MUpro |
|---|---|---|---|---|---|---|---|---|
| c.3475_3476insG | g.10140828 | rs35600922 | p.Asp1159Gly | Damaging (0) | Deleterious | Probably | Decrease | Decrease |
| | | | | | (-6.614) | Damaging (1.000) | (-1.29) | (-1) |

Next, the changes in the protein stability of the missense variants were examined using I-Mutant 2.0 and MUpro software (Table 2). In the I-Mutant 2.0 (DDG) prediction, 77 (71.61%) of the 92 variants were predicted to be decreasing the protein stability and the others were predicted to be increasing the protein stability, in case of MUpro prediction, 42 (39.06%) of the 92missense variants were predicted to be reducing the protein stability and others were predicted to be increasing the stability of protein. A total of 37 variants (40.2%) out of the 92 missense variants were common. Regarding the one frameshift variation in the *DNMT1* gene, the variant c.3475_3476insG (p.Asp1159Gly) was also predicted to be both deleterious or damaging and less stable by using Polyphen-2, SIFT, PROVEAN, I-MUTANT and MUpro software (Table.3). Out of 93 *DNMT1* variants a total of 6 variants namely c.4324C>T (p.Arg1442Trp), c.3913C>T (p.Arg1305Cys), c.3475_3476insG (p.Asp1159Gly), c.3223C>T (p.Arg1075Cys), c.3097C>T (p.Arg1033Trp), c.1531T>C (p.Tyr511His) were predicted to be damaging or deleterious by using Polyphen-2, SIFT, PROVEAN software and predicted to be

less stable using both I-Mutant and the MUpro software.

## DISCUSSION

Our analysis revealed 93 non synonymous variants out of which 92 missense and one frame shift variants. 5 variants namely c.4324C>T (p.Arg1442Trp), c.3913C>T (p.Arg1305Cys), c.3223C>T (p.Arg1075Cys), c.3097C>T (p.Arg1033Trp), c.1531T>C (p.Tyr511His) out of 92 missense variants showed a high deleterious scores by SIFT, PROVEAN, PolyPhen and decreasing the protein stability upon their aminoacid changes by I Mutant 2.0 and MUpro. Additionally one framehift variant c.3475_3476insG (p.Asp1159Gly) was also predicted to be both deleterious or damaging and less stable by using Polyphen-2, SIFT, PROVEAN, I-MUTANT and MUpro software. These variants are considered to be most likely damaging or deleterious and less stable. DNA methylation has turn into the subject of intense investigation in cancer cells. It has been reported that the incidence and degree of DNA hyper methylation was exaggerated in other groups of cancers such as gastric carcinoma, lung cancer, hepato cellular carcinoma, hepatitis and liver cirrhosis compared with the normal mucosa [14-16]. It was reported that DNMT1 mRNA over expression correlates significantly with CpG island methylator phenotype in gastric and colorectal cancers [17]. As compared with normal cells, the malignant cells show major disruptions in their DNA methylation patterns. Aberrant methylation patterns may also ultimately affect gene activity with the disruption of the transcription– translation process by escalating the possibility for a mutational event to take place and reducing overall chromosomal stability, leading to the manufacture of a dysfunctional protein product. To those conducting large-scale population based epidemiologic studies; the idea of prioritizing nsSNPs in the investigation of association of SNPs with disease risk is of great interest. The use of these servers to select potentially polymorphic nsSNPs for epidemiology studies can be an efficient way to investigate the role of genetic variation in disease risk and to curtail cost. Furthermore, predicted impact of these nsSNPs can be tested with the use of animal models or cell lines to determine if functionality of the protein has indeed been altered.

## CONCLUSION

A total of 93 nonsynonymous SNPs consisting of 92 missense variations, and 1 frameshift variations were found in the *DNMT1* gene by searching dbSNP and ENSEMBL databases in this study. Eleven of the 93 missense variants were predicted to be damaging or deleterious by the PolyPhen-2, SIFT, and PROVEAN software programs, and 38 of the variants were predicted to be less stable by both the I-Mutant 2.0 and MUpro software programs. Out of 93 *DNMT1* variants a total of 6 variants that were both deleterious and decreased protein stability will be studied further based on Insilico and population studies. These results suggested that alleles that encode functionally reduced or less stable *DNMT1* proteins may exist in humans. These *DNMT1* alleles might be associated with an increased risk of diseases, including gastric cancer. We therefore concluded this nsSNP as the potential functional polymorphic.

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