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SINGLE NUCLEOTIDE SOMATIC VARIANTS OF *Tp53* GENE IN WIDESPREAD NEOPLASMS OF *CANIS FAMILIARIS*

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ABSTRACT

Sequence diversity of *Tp53* gene was analyzed in dog tumors to have an insight of accumulated somatic mutations in 18 samples belongs to 7 different types of cancers. Nine different polymorphic sites were observed in exon 3, 4 and 5, while 32 polymorphic sites are observed in intron 3, 4 and 9. Most of these alterations are heterozygous, locus c.76 in exon 3 is transversion with homozygous (G) variant in one of the mammary and CTVT samples, which is missense in nature and alter the leucine amino acid to valine. Similarly, c.356 locus in exon 5 is homozygous (C), that is also a transversion, which changed the lysine residue to threonine. Most of the observed cancer samples are heterozygous in intronic regions of this gene. Current study of sequence variations of cancerous somatic cells in the aforementioned gene confirm the involvement of this gene in cancer pathways and might serve as potential molecular diagnostic and prognostic marker for these cancer types in *Canis familiaris* and may be helpful for other animal species as well.

Keywords:*Tp53*variants,Dog tumor, *Canis familiaris* cancer.

INTRODUCTION

Tumor may arise due to chemical, radiation, genetic and genomic reasons. Here we explored somatic *Tp53* variations which might defect the whole genome and ultimately can cause cancer. Moreover, intermittent and random causes may also develop cancer. Total seven types of cancers were analyzed in this study including mammary tumors, which is one of the wide spread neoplasm of the aged female dogs (Withrow and Vail, 2007), Canine Transmissible Venereal Tumor (CTVT) or sticker's sarcoma which is sexually transmitting cancer of the genital organs in *Canis familiaris* (Eze *et al*., 2008; Ganguly *et al*., 2013; Vermooten, 1987), Perianal adenocarcinoma, which is tumor of hairless skin around anus (North and Banks, 2009), Fourth one is canine lymphoma, which involves the lymphocytes and lymphocytes storage organs (lymphoid tissues) which may include spleen, bone marrow and lymph nodes of head and neck region (Withrow and Vail, 2007), Oral squamous cell carcinomas are also being address and analyzed in this study, which are mostly malignant melanoma, squamous cell carcinoma or fibrosarcoma. Its other types may include osteosarcoma, chondrosarcoma, anaplastic sarcoma, hemangiosarcoma and mast cell sarcomas.

Somatic mutations in cancerous tissue can not pass through the offspring because they only accumulate in the neoplastic tissues, that's why these somatic mutations being considered potential markers for cancer diagnosis. Different molecular biomarkers are studied now a days, which have great implication in diagnosis and prognosis of different cancers. Among those *Tp53* gene is one of the highly significant and attention seeking biomarker in human and animal species (Paoloni and Khanna, 2007; Zoubeidi and Gleave, 2012), which is almost 60% mutated in all human cancers and have significant role in different neoplasm of *Canis familiaris* (Hollstein *et al*., 1991; Wakui *et al*., 2001). Function of this gene is to apply brakes to the cell cycle and halt the growth of the uncontrolled and spontaneously aberrant dividing cells. Wild-type Tp53 protein increases in response to DNA damage, resulting in the cell arrest at G1 interphase. It allows DNA repair before replication or apoptosis if the damage is irreparable. So, the pro-apoptotic function of this gene is fully established which are explored in this manuscript to have an idea of all accumulated mutations due to different seven types of cancers in this valued pet animal species.

MATERIALS AND METHODS

Sample Collection: Total 26 samples (cancerous = 21, normal = 5) and peripheral blood were collected from the Pet Center-University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan and few samples from other private pet clinics through the standard technique of sample collection. Out of total 21, eighteen samples were

successfully amplified and screened. Which includes 5 CTVT, 4 mammary carcinoma, 3 from each perianal adenocarcinoma and SCC, 2 from each lymphocytic lymphoma and granuloma, 1 from each melanoma and pelvic-warts. All samples were obtained through excisional biopsies resected tissues and immediately stored at -86°C (Table 1).

Table 1. Samples collection procedure, mode of obtaining case-control tissues and representation of disease prevalence data in relation to their age, sex and breeds.

Sample ID	Breed	Tumor type/type of tissue	AgeYears	Sex	Tissue Collection Method
DP1	Non-descriptive	Canine Lymphoma	7	Male	Necropsy lymph node
DP3	English Springer	CTVT	6	Female	Chunk of tissues
DP5	German Shepherd	Mammary adenocarcinoma	8	Female	Surgical excisional
DP8	Labrador	Perianal adenocarcinoma	7	Female	Surgical excisional
DP9	Non-descriptive	Oral tumor/SCC	2	Male	Surgical excisional
DP10	Non-descriptive	CTVT	7	Female	Surgical excisional
DP11	German shepherd	Mammary adenocarcinoma	10	Female	Surgical excisional
DP12	German shepherd	Mammary adenocarcinoma	11	Female	Surgical excisional
DP14	Non-descriptive	Perianal adenocarcinoma	10.5	Male	Surgical excisional
DP15	German shepherd	Perianal adenocarcinoma	3.5	Female	Surgical excisional
DP17	Non-descriptive	Oral tumor/SCC	5	Male	Surgical excisional
DP18	Non-descriptive	Canine lymphoma	12	Male	Necropsy lymph node
DP19	Non-descriptive	Melanoma	10	Male	Chunk of tissues
DP20	Non-descriptive	Mammary adenocarcinoma	11	Female	Surgical excisional
DP21	Labrador	CTVT	2.5	Female	Chunk of tissues
DP22	Rottweiler	Granuloma	2	Female	Chunk of tissues
DP23	Non-descriptive	Granuloma	3	Male	Chunk of tissues
DP24	Sheep dog	CTVT	2.5	Male	Chunk of tissues
DP25	German shepherd	CTVT	2.5	Female	Chunk of tissues
DP26	German shepherd	Head &Neck SCC	2.5	Male	Surgical excisional
DP27	Labrador	Pelvic Warts	4 Month	Male	Chunk of tissues
Normal 1	Random bred	Lungs	5	Male	Necropsy samples
Normal 2	Random bred	Liver	6	Male	Necropsy samples
Normal 3	Random bred	Testes	8	Male	Necropsy samples
Normal 4	Random bred	Kidney	9	Male	Necropsy samples
Normal 5	Random bred	Endometrial	5	Male	Necropsy samples

Nucleic acid extraction: TaiGen genomic DNA tissue extraction kit (TaiGen Biotechnology Co., Ltd, Neihu, Taipe, Taiwan) was used to extract the DNA from all tissues according to manufacturer's guideline (Vogelstein, 1979). DNA concentrations and integrity were measured by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and gel electrophoresis methods respectively. High quality DNA of 50ng/uL concentration were used for downstream PCR amplifications.

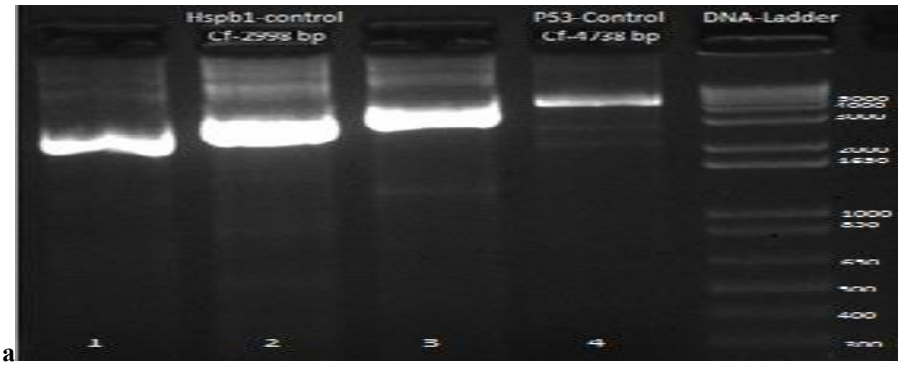
Primers: Long-range primer set was designed from the DNA sequence ID ENSCAFT00000026465 for *Tp53* gene taken from ensemble genome browser using primer3 and Net-Primer software (PREMIER Biosoft International, Palo Alto, CA) (Rozen and Skaletsky, 1999). This primer set amplified total 4738bp of *Tp53* gene. Then further internal primers were designed for sanger sequencing of the complete gene (Table 2). *Tp53* gene has 11 exons with a single splice variant of 2110 bp which encodes 283 amino acids.

PCR protocol: Long-range polymerase chain reaction was conducted using Applied Biosystem thermo cycler with 94°C temperature for 2 minutes as initial denaturation, then 10 cycles of (94°C as cyclic denaturation for 10 sec, annealing at 61°C for 30 sec and extension at 68°C was adopted for 1 minute/kb). Then remaining 30 cycles were conducted at (94°C as cyclic denaturation for 10 sec, annealing at 59°C for 30 sec and extension at 68°C for 1 minute/kb with an increment of 20 sec per cycle), at the end, final extension was given at 72°C for 5 min and finally hold at 4°C. Optimized long-range PCR kit with dNTPs was used containing high-fidelity long range polymerase, 5 U/µL with final concentration of 1.8 U, PCR 10X enhancer A with final concentration of 1x and PCR additive dimethyl sulfoxide (DMSO) for the amplification of GC-rich region, 10X reaction buffer with final concentration of 1x was used to amplify the required gene of interest (Innis *et al*., 1990).

Gel electrophoresis and sequence analysis: PCR product was visualized through 1.5% agarose gel electrophoresis for 50 minutes (Figure 1), specific amplicon was obtained and cleaned with ExoSAP (ExoSAP-IT PCR Product Cleanup"Santa Clara, CA, USA) for downstream sequencing. Then sequencing was done with ABI BigDye terminator sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequence analysis was performed by "Sequencher software" ver. 5.2.3 to determine the sequence variants and its other statistical attributes (Gene Codes Corporation, Ann Arbor, MI, USA).

Table 2. Long-range and internal sequencing primer sequences of *Tp53* gene in *Canis familiaris.*

Primer Label	5' to 3' sequence	Total (bp)	Type /Purpose	Tm(°C)	GC%	Overall rating (%) (hairpin,self & cross diming)	Product size (bp)
P53-Cf-LRF	CCCTGGTATAATGTTGCTGGAAG	23	Long-range (Forward)	60.72	47.83	93	4738
P53-Cf-LRR	AACGTCACCTCCTTCCTCATCC	22	Long-range (Reverse)	61.89	54.55	88	
P53-Cf-E1F	GCTAACTGCTTGCCCTTACTTGTC	24	Exon1 (Forward)	61.93	50	100	286
P53-Cf-E1R	GTGACTCAGACAGGACTCATCTGG	24	Exon1 (Reverse)	60.18	54.17	86	
P53-Cf-1SF	CTGACCCTTGACTCTGGTCTCG	22	Internal (Forward)	61.12	59.09	83	
P53-Cf-2SF	GTTGAGCGTCTGCCTTTGGTT	21	Internal (Forward)	61.78	52.38	100	
P53-Cf-3SF	TGGTTTGGCATCTGAGGGGT	20	Internal (Forward)	62.3	55	100	
P53-Cf-4SF	TGTGGCTTCTCAATAGTCTGTAGGC	25	Internal (Forward)	62.1	48	100	
P53-Cf-E9F	GAGGAGGATGTGGGGCAGATAC	22	Internal (Forward)	62.23	59.09	100	
P53-Cf-E10R	GAGTAGAATGGAATCCTGTGGCTTT	25	Internal (Reverse)	62.38	44	100	
P53-Cf-6SF	AAAGCCACAGGATTCCATTCTACTC	25	Internal (Forward)	62.38	44	100	
P53-Cf-7SF	TTACTCCAGCCTTGGACCCTTC	22	Internal (Forward)	62.29	54.55	85	
P53-Cf-8SF	CCCTAAGACACTGAGTCATGAGCC	24	Internal (Forward)	62.02	54.17	82	



a

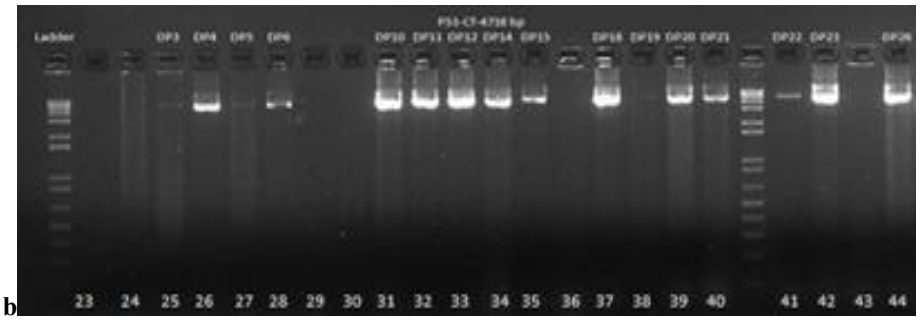


Figure 1. Long-range PCR amplified product of *Tp53* gene in control samples (a) and in tumor samples with 1Kb GeneRuler DNA ladder (b).

RESULTS AND DISCUSSION

Sequence diversity analysis of *Tp53* gene: *Tp53* is one of the most renowned tumor suppressor genes, which protect the whole genome but if this gene is transformed due to any reason, whole genome may be at risk and prone to develop malignancies. So, the whole gene was characterized including exonic and interonic regions for complete analysis of coding, non-coding and splicing regions.

Exon 3 mutational landscape: Exon 3 of *Tp53* found altered almost in all cancer cases analyzed in this. Coding position c.76 was found homozygous (G) in one CTVT sample (DP10) and heterozygous (C/G) in sample (DP3) and (C/T) heterozygous in another CTVT sample (DP6), which appeared as non synonymous mutation and alter the leucine residue to valine in one case and phenylalanine in other case. Similarly, at coding position c.79 and c.80 in CTVT sample (DP3) is observed heterozygous (C/T), which change the proline amino acid into serine residue. Two mammary tumor samples (DP12, DP20) found homozygous (G), which cause the change of leucine residue to valine, while in other two cases ID (DP4, DP5) were found heterozygous (C/T) on the same position.

Two mammary tumor samples (DP12, DP20), two CTVT samples (DP6, DP10) and one perianal adenocarcinoma cases (DP15) are heterozygous (C/A) at position c.87, which appeared as missense mutation and change the asparagine amino acid to lysine. Most of the mammary tumors among all studied cancer types are widely mutated at different loci, e.g. c.90 position is found heterozygous (T/G) in one mammary lacerationin sample (DP20) and two CTVT samples (DP3, DP6), which are also non-synonymous mutation and changed the asparagine to lysine residue.

CDS position c.92 is found (T/G) heterozygous in two of mammary adenocarcinoma samples (DP12, DP20) and one CTVT sample (DP10), which is also appeared as a non-synonymous mutation and change the valine to glycine. Another locus c.93 in exon 3 of *Tp53* gene in CTVT case of dog (DP3) revealed heterozygous (T/G), which is synonymous mutation.

Mutational landscape of Exon 4: As far as exon 4 of *Tp53* is concerned, CDS position c.103 is (G/A) heterozygous in one of the lymphoma sample (DP18) and one perianal adenocarcinoma sample (DP15), which is appeared as non-synonymous point mutation and changed the glutamic acid residue to lysine.

Mutational landscape of exon 5: Exon 5 coding position c.356 is mutated with homozygous (C) in one of the mammary tumor sample (DP11) instead of homozygous (A) in control and reference sequence (Figure 2a). This point mutation changed the lysine residue to threonine in its polypeptide chain of amino acid (Table 3).

Intronic mutational landscape: *Tp53* intronic mutations are also detected in different canine tumors under study as compared to normal tissues and reference sequence. Intron 2, 3, 9 and 10 are found altered at different seven loci. Intron 2 showed deletion of (A) in all screened cases, including normal control, but reference control from database has (A) at 9114 position. Intron 3 showed heterozygosity on its three mutated loci. Altered gene position 9246 in exon 3 found heterozygous (T/G) in all screened samples, including normal instead of homozygous (T) in reference sample. The locus 9248 is highly polymorphic as it is homozygous (G) in one of the CTVT (DP6) and two mammary tumor sample (DP12, DP20), while heterozygous (T/G) in eight cases, three of them are mammary, two were perianal adenocarcinoma, one from each CTVT, lymphoma and head & neck tumor. Another locus 9263 was found heterozygous (C/G) in all screened cases. As far as intron 9 is concerned, insertion of (T) and (C) were observed in all cases and control instead of reference sequence of two positions 11696, 11698, while one sample (DP26) was heterozygous (G/C) at position 12399 in intron 10 instead of homozygous (G) in all other cases including normal and reference controls (Table 4, Figure 2b

Table 3. Signature exonic mutations of *Tp53* gene in different tumor cases in *Canis familiaris.*

Animal ID	Phenotype/ Tissue type	Exon 3							Exon 4	Exon 5
		C.76 C>G>T	C.79 C>T	C.80 C>T	C.87 C>A	C.90 T>G	C.92 T>G	C.93 T>G	C.103 G>A	C.356 A>C
Reference	Normal	C	C	C	C	T	T	T	G	A
Control	Normal/Blood	C	C	C	C	T	T	T	G	A
DP1	Case/Lymphoma	C	C	C	C	T	T	T	G	A
DP2	Case/MT	C	C	C	C	T	T	T	G	A
DP3	Case/CTVT	C>G	C>T	C>T	C	T>G	T	T>G	G	A
DP4	Case/MT	C>T	C	C	C	T	T	T	G	A
DP5	Case/MT	C>T	C	C	C	T	T	T	G	A
DP6	Case/CTVT	C>T	C	C	C>A	T>G	T	T	G	A
DP10	Case/CTVT	G	C	C	C>A	T	T>G	T	G	A
DP11	Case/MT	C	C	C	**C**	T	T	T	G	C
DP12	Case/MT	G	C	C	C>A	T	T>G	T	G	A
DP14	Case/Perianal Adenocarcinoma	C	C	C	C	T	T	T	G	A
DP15	Case/Perianal Adenocarcinoma	C	C	C	C>A	T	T	T	G>A	A
DP17	Case/Oral (SCC)	C	C	C	C	T	T	T	G	A
DP18	Case/Lymphoma	C	C	C	C	T	T	T	G>A	A
DP20	Case/MT	G	C	C	C>A	T>G	T>G	T	G	A
DP21	Case/CTVT	C	C	C	C	T	T	T	G	A
DP22	Case/Granuloma	C	C	C	C	T	T	T	G	A
DP23	Case/Granuloma	C>T	C	C	C	T	T	T	G	A
DP26	Case/Head & Neck T.	C	C	C	C	T	T	T	G	A
a.a change		Leucine to Valine / phenylalanine	Proline to serine	Proline to Leucine	Asparagine to Lysine	Asparagine to Lysine	Valine to Glycine	Synonymous	Glutamic acid to lysine	Lysine to threonine

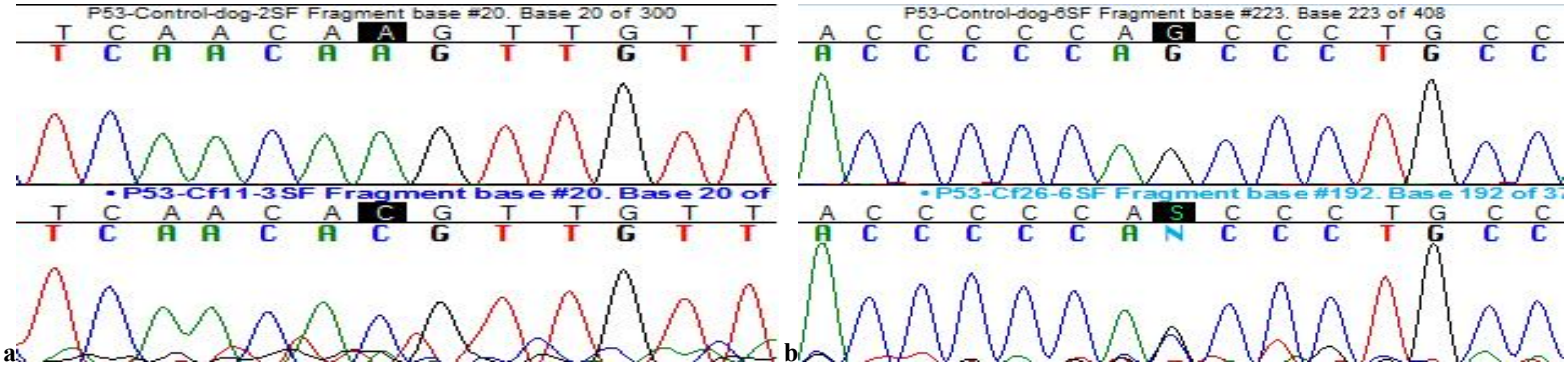


Figure 2. (a) Chromatogram of position c.356 in exon 5 of *Tp53* gene in *Canis familiaris* showing (C) substitution in mammary tumor sample DP11 instead of (A) in control mentioned in (Table 1).(b) Chromatogram of position 12399 in intron 10 of *Tp53* gene in *Canis familiaris* showing (G/C) heterozygosity in H&N SCC sample (DP26) instead of (G) in control mentioned in (Table 2).

Table 4. Signature intronic mutations of *Tp53* in different tumors in *Canis familiaris.*

Sample ID	Phenotype / Tissue Type	Intron 1	Intron3			Intron 9		Intron 10
		9114 Del (A)	9246 T>G	9248 T>G	9263 C>G	11696 Ins (T)	11698 Ins (C)	12399 G>C
Reference	Normal	A	T	T	C	-	-	G
Control	Normal/Blood	-	T/G	T/G	C/G	T	C	G
DP2	Case/MT	-	T/G	T	C/G	T	C	G
DP3	Case/CTVT	-	T/G	T	C/G	T	C	-
DP4	Case/MT	-	T/G	T/G	C/G	T	C	G
DP5	Case/MT	-	T/G	T/G	C/G	T	C	G
	Case/Blood	-	-	-	-	T	C	-
DP6	Case/CTVT	-	T/G	G	C/G	T	C	G
DP10	Case/CTVT	-	T/G	T	--	T	C	G
DP11	Case/MT	-	T/G	T/G	C/G	T	C	G
DP12	Case/MT	-	T/G	G	C/G	T	C	G
DP14	Case/Perianal Adenocarcinoma	-	T/G	T/G	C/G	T	C	G
DP15	Case/Perianal Adenocarcinoma	-	T/G	T/G	C/G	T	C	G
DP18	Case/Lymphoma	-	T/G	T/G	C/G	T	C	G
DP20	Case/MT	-	T/G	G	C/G	T	C	G
DP21	Case/CTVT	-	T/G	T/G	C/G	T	C	G
DP23	Case/Granuloma	-	T/G	T/G	C/G	T	C	G
DP26	Case/Head & Neck T.	-	T/G	T	C/G	T	C	G/C

A study by Robinson found that cancer development is micro-evolutionary process, in which merely a single point mutation is not responsible for cancer outbreak, but single mutation can start the process of chaos in the genome. The tumor suppressor *Tp53* gene was selected here, which has pro-apoptotic activities. Normally, mutations damage the proto-oncogene and trigger them to become oncogene, these mutations might keep the cell cycle acceleration on, which should remain stop all the time in a fully functional proto-oncogenes. On the other hand, mutations alter the tumor suppressing function of these genes and lose of function may accure due to cell growth brakes, which usually tells the cell to stop growing as their life span has ended (Souza *et al*., 2012).

It is also observed in the current results that *Tp53* tumor suppressor gene is highly mutant especially in intronic regions. A similar study was conducted successfully, which depicts that exon 4 and 8 in *Tp53* gene have different mutations in dog mammary tumors cases, in which CDS Positions 147, 248, 266 and 287 proved missense and changes the valine residue to serine, arginine to proline, glycine to proline and glutamic acid to valine respectively. It is also demonstrated that codon 176TGC>TTC, 236TAC>AAC and 245GGC>GCC found mutated in dog mammary tumor cell lines which is also in conformity to human codons(Van Leeuwen *et al*., 1996).Another study was conducted on sixty three benign and malignant mammary carcinoma on dog's *Tp53* gene, in which four missense mutations were observed in 38 benign cases, five missense mutation in 25 mammary carcinomas and one nonsense mutation was found in 25 mammary carcinomas(Muto *et al*., 2000). According to another Mexican study in which TVT cases in dogs were screened with *Tp53* gene and four new polymorphism were found(Sánchez-Servín *et al*., 2009).In another study which demonstrated that TVT is one of the oldest and most mutated transmissible cancer genome in animals which has almost 10,000 non-synonymous mutation among its 1.9 million alteration in total, while 646 genes were lost in its genome(Murchison *et al*., 2014).found that CTVT samples included in this study showed (G) change in two samples (DP3, DP10) while another sample (DP6) was found changed with (T) at c.76 position which was (C) in control and reference sample. Similarly, in another study of *Canis familiaris* mammary carcinoma, in which exon 4 and 8 of *Tp53* gene found hotspot regions(Souza *et al*., 2012). (Wakui *et al*., 2001) showed that prognostic and diagnostic implications of *Tp53* gene was also addressed, in which 17% canine mammary adenocarcinoma cases were found to be altered in 5-8 exons. Similarly, (Setoguchi *et al*., 2001; Veldhoen *et al*., 1998) demonstrated that canine lymphoma samples were sequenced directly for studying the *Tp53* somatic variants. In one of the similar type of study, seven lymphoma cases were found aberrant on one or both alleles. In the current studied samples, exon 4 and 5 at CDS position 103, 356 were found altered, which fall into 34 and 118 number codon respectively. These are non-synonymous alterations and change the glutamic acid amino acid to lysine and lysine to threonine respectively (Table 5).

As for as the human cancer in the context of comparative genomics are concerned, (Murakami *et al*., 1996) highlighted few of human lungs cancer cases, (Heide *et al*., 1997) found colorectal cancer, (Ooyama *et al*., 2006) demonstrated the breast cancer and (Nakamori *et al*., 1995) revealed 1bp deletion in pancreatic adenocarcinoma.at the same CDS 76 position in 26 codon of exon 3. The same position was also found altered (C>G/T) in our eight samples (DP3, DP4, DP5, DP6, DP10, DP12, DP20, DP23) of mammary, CTVT and granuloma tumors cases (Table 5). This position is at the end of exon 3 and may involve splicing site. In above mentioned human studies, this position appeared non-synonymous similar to our samples, but in human studies it appeared as frame shift point mutation, while in our dogs' samples, it changed the valine residue to phenylalanine amino acid.

Another human study where (Nakamori *et al*., 1995) reportedc.79 position in the same exon 3 at 27th codon was found altered. (Fransén *et al*., 2004), (Jones *et al*., 2008) and (Lee *et al*., 2010) revealed the following four studies in human pancreatic cancer, colorectal adenocarcinoma, pancreatic tumor and in non-small cell lungs cancer. In the earlier three studies, this codon showed non-synonymous frame shift mutations, while in the fourth one this c.79 locus in codon 27 changed from CCT to ACT codon, which replaced the proline amino acid to threonine. In our studied tumor cases of dog *Tp53*, this c.79 locus in 27th codon changed from (C>T) in one of the CTVT sample ID (DP3) and this non-synonymous mutation replaced the proline to serine amino acid (Table 3).

According to (Curtis *et al*., 2012) exon 3 c.80 locus in human breast tumor showed a change of CCT to CTT, which replaced the proline amino acid into leucine, the same c.80 locus in one of our studied CTVT sample ID (DP3) revealed the same change of (C>T) as observed in the human and replaced the proline amino acid to leucine residue (Table 3).

(Grenier, 2002) revealed that CDS position 87 was also found altered in another human breast cancer study, in which AAC29AAG codon change was observed, which replaced the asparagine amino acid to lysine, exactly the same position was noticed in our two mammary tumors (DP12, Dp20), two CTVT samples (DP6, DP10) and one perianal adenocarcinoma sample (DP15) in dog, in which c.80 locus was found altered (C>A) which changed the asparagine to lysine, same as in the human cases (Table 3).

c.103 locus in exon 4 is also found altered in three human studies, two studies were conducted by (Mitsudomi *et al*., 1992)on NSCLC, in which insertion were observed and appeared as frame shift mutations, another study by (Lee *et al*., 2010) showed the same results, while one study conducted by (Temam *et al*., 2003) on human head and neck SCC which showed deletion at the same locus. Our study on these veterinary species also found this locus as a hotspot and observed (G>A) change at this locus, which changed the glutamic acid amino acid to lysine (Table 3).

(Bäckvall *et al*., 2004) demonstrated that according to Codon 119 of *Tp53* gene at c.356 position in our dog mammary tumor (DP11) was found altered in accordance to two of the human skin basal cell carcinoma in which GCC>GAC transversion mutation was observed(Lind *et al*., 2007), which changed the alanine amino acid into asparagine (p.A119D) and other one was human NSCLC, which changed c.356C>G, this mutation transformed the polypeptide chain from alanine to glycine (p.A119G).

Current study can be extended by gene expression profiling in this tumor suppressor gene which can be coupled with sequence diversity data for the purpose of establishing fidelity in the field of diagnostic, two of similar studies were conducted on *Tp53* markers in CTVT cases, which augment the diagnostic confidence on the basis of these polymorphism and expression based techniques(Chu *et al*., 2001; Stockmann *et al*., 2011). Cancer treatment by inhibiting *Hspb27*gene product is also getting acceptance in the scientific community to combat this cancer type in different species(Gleave *et al*., 2009).

Cross-tissue comparison: As a subsidiary finding, comparison of neoplastic tissues and blood of the same diseased animal were compared, in which *Tp53* gene was amplified in one of the mammary tumor sample (DP5) to ascertain the comparison of mutational variations in blood and tumorous tissues of the animal to have an idea of germ line and somatic transmitted mutations. As somatic cell mutations in neoplastic tissues are those which are due to cancer outbreak, while the germ line mutations have familial history and transfer generations to generations. It has been observed that, *Tp53* c.76 position in dog mammary tumor (DP5) tissues has somatic mutation of (T), while same position has a germ line mutation (C) at the same locus (Table 3). Similarly, *Tp53* intron 3 positions 9246, 9248 and 9263found polymorphic/heterozygous (T/G), (T/G) and (C/G) in cancerous tissues and found homozygous (T), (T) and (C) in germ line tissues in sampleID (DP5) (Table 4).

Conclusion: In nutshell, seven different loci in intron 2, 3, 9 and 10 were found altered. It is also predominantly deduced that most of heterozygous variants are observed in intronic regions rather than exonic part of the gene.

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