CHARACTERISATION OF MONO AMINE OXIDASE A (MAOA) GENE AND TRANSCRIPT FROM ASIAN ELEPHANT *(Elephas maximus)*: A POSSIBLE CANDIDATE FOR RISK TAKING BEHAVIOUR

JISHNU M

(14-02MS-003)

Abstract of Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of

MASTER OF SCIENCE

(Wildlife Studies)

Faculty of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University

2016

CENTRE FOR WILDLIFE STUDIES

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

POOKODE, WAYANAD, KERALA, INDIA

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DECLARATION

I hereby declare that this dissertation titled **"Characterisation of Mono Amine Oxidase A (MAOA) Gene and Transcript from Asian Elephant *(Elephas maximus)*: A Possible Candidate for Risk Taking Behaviour"** is a bonafide record of research work done by me during the course of my Master's research program and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Pookode **JISHNU M.**

Date 14-02MS-003

Dr. Arun Zachariah

Assistant Professor
KVASU Centre for Wildlife Studies
College of Veterinary and Animal Sciences
Pookode, Wayanad, Kerala 673576

CERTIFICATE

Certified that this dissertation, titled **"Characterisation of Mono Amine Oxidase A (MAOA) Gene and transcript from Asian Elephant (*Elephas maximus)*: A Possible Candidate for Risk Taking Behaviour"** is a bonafide record of research work done independently by **Jishnu M. (14-02MS-003)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

Pookode — Dr. Arun Zachariah

Date : — (Guide)

CERTIFICATE

We, the undersigned members of the advisory committee of **Jishnu M. (14-02MS-003)**, a candidate for the degree of Master of Science in Wildlife Studies, agree that the dissertation titled, "**"Characterisation of Mono Amine Oxidase A (MAOA) Gene and transcript from Asian Elephant (*Elephas maximus)*: A Possible Candidate for Risk Taking Behaviour"**" may be submitted by **Jishnu M. (14-02MS-003)**, in partial fulfillment of the requirement for the degree.

Dr. Arun Zachariah

Assistant Professor
KVASU- Centre for Wildlife Studies
College of Veterinary and Animal Sciences
Pookode, Wayanad, Kerala 673576
(Guide)

Dr. Abdul Azeez C P
Assistant Professor
KVASU-Centre for Wildlife Studies
College of Veterinary and Animal Sciences
Pookode, Wayanad, Kerala 673576
(Member)

Dr. George Chandy
Officer in Charge
KVASU-Centre for Wildlife Studies
College of Veterinary and Animal Sciences
Pookod, Wayanad, Kerala 673576
(Course Director)

EXTERNAL EXAMINER

ACKNOWLEDGEMENTS

With profound gratitude and respect, I would like to depict my deep sense of obligation and heartfelt indebtedness to my major guide, ***Dr. Arun Zachariah,*** *Veterinary surgeon, Veterinary Poly Clinic, Sulthan Bathery, for his meticulous guidance, constant supervision, affectionate encouragement, personal interest, parental care and timely advise during the entire study period. I consider it my blessed privilege and matter of pride to work under the potential guidance of a versatile scientist.*

I am cordially indebted to ***Dr. Abdul Azeez,*** *Assistant Professor, Department of Veterinary Gynecology and Obstetrics, Pookode, Member of the advisory committee, for his never ending support, valuable corrections and friendly guidance given to me for the successful completion of this research work.*

I am extremely thankful to ***Dr. George Chandy,*** *Course Director, KVASU-Centre for Wildlife Studies, Pookode, for providing me persistent support during my entire course and research work.*

No word can pay my respect and deep sense of gratitude to ***Dr. B. Chandramohan,*** *Scientist, SciGenom Research Foundation, Adayar, Chennai, for his whole hearted support, extremely frank and any time ready to help nature, valuable suggestions, constructive review of my manuscript and constant encouragement.*

I am thankful to ***Sri. Dhaneshkumar,*** *DFO, Wayanad Wildlife Sanctuary and* ***Dr. V. I. Gigimon****, AFVO, Sulthan Bathery for providing me the freedom to work in Centre for Wildlife Research and Forensic Laboratory to complete my research work. I am very much thankful to* ***Mr. Krishnadas,*** *Range Forest Officer, Sulthan Bathery Range, Wayanad Wildlife Sanctuary for providing the accommodation facilities in department quarters during my research work.*

I gratefully acknowledge ***Dr. Prejit*** *and* ***Dr. Vinod,*** *Assistant Professor****,*** ***Dr. Pratheesh****, Senior Research Officer,* ***Dr. Mahesh*** *and* ***Dr. Nimisha,*** *MVsc. Students, Department of Veterinary Public Health, Pookode for providing laboratory facility and full freedom to work in the lab.*

I am indebted to ***Mr. Sam Santhosh,*** *CEO, SciGenom Labs Pvt. Ltd. for his whole hearted support given to me for the postgraduate research work.*

I am thankful to ***Mr. and Mrs. Jignasu Dolia,*** *for their precious advices, meticulous guidance and perennial support during the course work and for the completion of the research work.*

I am thankful to the ***Teaching and non teaching staffs,*** *Centre for Wildlife Studies, Pookode, for providing me the facilities for my research.*

Words posses no enough power to reflect my thankfulness to my batch mates ***Jayakrishnan, Sreejith, Sophia, Shilpa, Aswathy, Haritha*** *and* ***Angel*** *for their mental support whenever I was frustrated, their help during thesis writing, constant encouragement and for being with me for the last two years.*

I remember with gratitude to my ***Seniors and Juniors*** *for their help and valuable guidance whenever I was in doubt. Special thanks to* ***Vishnu O.*** *for his whole hearted and brotherly support, care and suggestions during this study.*

There are no words to express my gratitude to my ***Parents*** *and my beloved sister* ***Jiji (Unni)*** *for their immense support and prayer for making what I am. Their whole hearted affection, prayers, blessings, and financial support helped me to attain my goal.*

I thank the ever pervading essence of the Universe and the treasure house of all knowledge ***the Almighty****, who had kept me alive, blessings showered on me, flooded me with energy and hope and allowed me to complete this voyage.*

JISHNU M.

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1. INTRODUCTION

Elephants are considered to be a 'flagship' species in Asian countries, which are closely associated with the social and cultural aspects of people in Asian continent (Perera, 2005). They also play a key role in maintaining biodiversity of the ecosystems they inhabit. Hence, conservation of elephants will eventually ensure the conservation of other species that co-exist in the same habitat.

Globally, wild elephants inhabit 50 countries, in which 13 are in Asian and 37 are in African continent. The estimated population of Asian elephant lies between 35,000 and 52,000 individuals (Perera, 2005; Sukumar, 2006). India has the largest population, comprising 60 per cent of the global population. Poaching for ivory, human-elephant conflict, habitat fragmentation and habitat degradation are considered to be major factors in population decline of Asian Elephant (Santiapillai, 1997). Human-elephant conflict poses significant threat to elephants throughout the range countries. Crop raiding by elephants is observed to be a major issue among human wildlife conflict situations. Annually, elephants damage millions of dollars worth of crops and hundreds of people and elephants are killed as a result of this conflict (Sukumar, 2006).

All elephants are not crop raiders. In a herd only a few elephants have the tendency for crop raiding. However, there is a pattern observed in the conflicting animals. Most of the crop raiders are solitary males of sub adult and adult category and tend to do frequent crop raiding (Sukumar and Gadgil, 1988). The male elephants start to raid crop once they were detached from their families and a large proportion of elephants raid when they are approaching reproductive age (Chiyo and Cochrane, 2005). Crop raiding not only provides highly palatable and nutritious food than the wild forages and also provides rich source of food within shorter time and foraging distance (Sukumar, 1989; Chiyo and Cochrane, 2005).

The advantage of crop raiding is basically to develop the body mass and help in male to male competition for reproductive success. Any source of high nutrition will be used to build up the body size which may help the males to compete with other bulls for getting access to the oestrous females. The crop raiding bulls have longer period of musth when compared to the non-crop raiders. The males of polygynous species are having greater variance in the reproductive success than females. This selection pressure favours them to take the high risk-high gain strategy for enhancing reproductive success (Sukumar, 1991).

Crop raiding is a risk involved process and crop raiders are vulnerable to human interventions such as shooting, poisoning, electrocution and other malicious activities. In our country more than 200 elephants (crop raiders) have been killed in a span of five years from 2006-2011 (Baskaran *et al.,* 2011). The elephant which has the tendency to take risk may be rewarded with greater body size, extended period of musth and also reproductive success. The trend observed in conflicting elephants hypothesised that certain genetic group tends to have conflicting/crop raiding/ risk taking behaviour.

Risk taking behavior has a genetic background and may be inherited to the next generation. So far two genes (*MAOA* and *CDH13*) have been identified to have significant correlation with extreme violent or aggressive behaviour in humans and *Mono Amine Oxidase A* (*MAOA*) gene is extensively studied in mammals. The *MAOA* gene is located in the X-chromosome and comprises of 15 exons in humans and highly conserved in other mammals (Shih *et al.,* 1999). It encodes a mitochondrial catabolic enzyme which catalyzes the oxidative deamination of biogenic amines, making it a critical regulator of neurotransmitters like serotonin, norephinephrine and dopamine (Joshua and Meyer-Lindenberg, 2008). *MAOA* enzyme regulates neurotransmitter levels in the brain and balances the normal behaviour. Any alteration in *MAOA* gene may potentially alter its function (Scott *et al*., 2008). Elephants and humans have similar genetic architecture and social organization. Hence, it is assumed that *MAOA* gene may have a similar role in elephants also. Characterization of *MAOA* gene and its

transcript is a preliminary step to understand its involvement in violent and risk taking behaviour in elephants.

As per the available literature, no such studies have been reported in elephants. Hence, the present study has been conducted with the following objectives:

1. Identification and characterisation of *MAOA* gene in Asian Elephant (*Elephas maximus)*

2. Identification and characterisation of *MAOA* transcript in Asian Elephant (*Elephas maximus)*

3. To investigate the probable role of *MAOA* gene in Asian Elephant (*Elephas maximus)*

2. REVIEW OF LITERATURE

2.1. STATUS AND DISTRIBUTION OF ASIAN ELEPHANTS *(Elephas maximus)*

Perera (2005) stated that all around the world, 50 countries had wild elephants, which includes 13 Asian and 37 African countries. The population of wild Asian Elephants (*Elephas maximus*) was estimated to be 35000-50000. The author described the elephant as "Umbrella" and "Keystone species". The author also stated that the Asian Elephant categorized under 'Endangered' category in the Red List of the World Conservation Union (IUCN, 2008) and classified with the Convention for International Trade of Endangered Species (CITES) under Appendix I.

Sukumar (2006) reported that Asian Elephants inhabit in 13 countries with a discontinuous distribution in the Asian continent. The total wild population estimated to be between 38500-52000 individuals. The author also pointed that India held the highest population of wild Asian Elephants, which comprised 60 percentage of the total population. He also discussed the historic distribution of Asian Elephants from Mesopotamia to Southeast Asia and China and also reported that Asian Elephants had disappeared from 95 percent of their historic range.

Sukumar and Easa (2006) pointed out that Nagarhole, Bandipur, Wayanad, Muthumalai Protected Areas and Nilgiri North division had forest types of moist and dry deciduous and enclosed high elephant population and density. The South Indian ranges had the high elephant density in the country (6813 individuals in 11472 sq. km in area).

Oswin Perera (2009) observed that Project Elephant of India estimated 27669 – 27719 elephants in the wild and the country had 26 elephant reserves with a

total of 60000 sq. km area for the protection of elephants, their habitat and corridors.

Fernando and Pastorine (2011) observed that the average total population of Asian Elephants in range countries were 43445, in an area of 16413676 sq. km. The minimum population was 39463 and maximum was 47427. Authors mentioned that among all the range countries, India held the highest population of Asian Elephants. They also described various conservation threats, including habitat fragmentation and human-elephant conflict.

Baskaran *et al.* (2011) observed that the largest number of wild Asian Elephants inhabited in India and they had a significant role in Indian culture and mythology. The elephant distribution was confined to four regions, the foothills of Himalayas in the north, north eastern states, east central India and forested hilly tracts of western and eastern ghats. The elephant population in India was close to a large population decline because of habitat loss, mining, hydroelectric dams and human-elephant conflict.

Ramkumar *et al*. (2014) stated that Asian Elephants were categorized under 'Endangered' category of the World Conservation Union (IUCN) Red list and as a keystone species coming under Schedule I and Part I of the Indian Wildlife (Protection) Act 1972. The authors also noted human-elephant conflict as an important challenge for the conservation of Asian Elephants.

Baskaran and Sukumar (2015) reported that the Nilgiri Biosphere Reserve had the highest Asian Elephant density anywhere else in the Asian continent. The Biosphere Reserve encompasses a wide range of habitats as well as distinctive seasons, including dry season and wet season, making it an ideal habitat for these mega herbivores. The authors also discussed the threats to the survival of the species in the study area.

2.2. HUMAN-ELEPHANT CONFLICT

Sukumar (1990) observed crop raiding in Chamraj Nagar, Kollegal and Satyamangalam Forest Division and crop raiding was noticed during the natural movements of elephants. He also reported that some male elephants were habituated crop raiders. Some habituated crop raiders roamed near to the villages and raided crops regularly at night and returned to the forest in the morning. The crop damage occurred while elephants moved through agricultural lands in the dry season for water. The crop raiding was high in the dry season and also after the rain.

Hoare (1999) stated that the human activities which could cause the degradation of elephant habitat, also forced the wild elephants to come in direct contact with humans and their settlements causing human-elephant conflict.

Hoare (2000) described human-elephant conflict involve crop raiding, damage to wealth, problem for day to day activities of humans by elephants which could cause negative impact on both elephants and humans.

Bist (2002) pointed out that in every year about 300 human deaths, damage to 10000 – 15000 houses and destruction of 8 to10 hectars of crop were caused by elephants. About 200 elephant deaths were recorded due to poaching, poisoning, electrocution and train accidents in Assam.

Chiyo and Cochrane (2005) conducted a study at Kibale National Park, Uganda, suggested that males were the predominant raiders. An elephant start raiding crops at the age of 10 – 14 years and the major threat was at the age group of 20 – 24 years, at this age group elephants would be in post pubertic stage and approaching reproductive age.

Sukumar (2006) stated that human-elephant conflict had emerged as a serious conservation issue, which caused millions of dollars worth of crop damage and death of a hundred elephants annually. He also observed that cultivated crops

were more palatable and nutritious than wild forages. Some elephants continued to raid crops irrespective of the availability of natural forage. In the crop raiding incidents, sub adult and adult males contributed the majority.

Jayson and Cristopher (2008) conducted a study on Peppara Wildlife Sanctuary and observed that crop raiding by elephants depended on the type of crop cultivated. Whenever the palatable and nutritious crops like plantain, arecanut and coconut were planted, elephants attacked them. They observed that solitary males were frequent raiders and also the majority of the crop raids occurred in the night time. The conflict was high in summer due to lack of foliages and water in the forest. The conflict was high because of easy access to food and water from the cultivated lands. During the study time, four human deaths due to elephants were also recorded.

Baskaran *et al*. (2011) stated that the increase in human-elephant conflict needed proper attention for the conservation of the species. The authors also observed that from 1998 to 2001 there were 900 human deaths due to elephant attacks in the country, an average of about 250-300 people per year that had since increased to over 400 deaths in 2010. On the other side, 200 elephants were killed by poisoning and electrocution within a time span of 5 years from 2006-2011.

Webber *et al*. (2011) conducted a study in Cambodia and found that crop raiding was high in October–December because the male elephants would forage to attain high nutrition for entering into musth. The major reason in man-elephant conflict was the reduction in habitat due to human intervention.

Ekanayaka *et al*. (2011) conducted a study in Sri Lanka which revealed that banana, paddy and maize suffered major damage. Crop raiding was high because of the high nutrients and palatability of these crops than the wild food source. The peak crop raiding was found in rainy season. The cause of crop raiding was not depended on the decrease in fodder in the forest, rather on availability and choice

of cultivated crops. They also observed that males raid crops much more than herds.

Gubbi (2012) conducted a study on Nagarhole National Park which revealed that conflict was high in August – November, because of flowering of finger millets and ripening of maize and paddy which contained sodium and minerals. The peak was observed in post monsoon and not increasing in summer season.

Rohini *et al.* (2015) conducted a study on human-elephant conflict in the Moothedam Panchayath of Kerala. The study revealed that the majority of the crop raids were done by solitary animals. The authors also discussed the conflict incidents in the study area and conflict resolution methods which included multiple stakeholders from the area.

2.3. RISK TAKING BEHAVIOUR IN ASIAN ELEPHANTS

Kurt (1974) suggested that better nutrition and body condition enabled successful expression of musth in dominant bulls over non dominant bulls which provided increased access to oestrous females. Ultimately the better body size and expression of musth provided reproductive success for adult males.

Ralls (1977) reported that due to the high variance in the reproductive success, male elephants were prone to take high risk to attain body mass for their reproductive success. The high variability in the reproductive success of males in polygynous species like elephant were considered to be due to high competition for physical fitness to participate in reproduction

Clutton-Brock *et al*. (1982) studied Red Deer and stated that any increased nutrition other than natural forages obtained could be translated into a larger body size, which helped in the fighting abilities of adult bull during reproductive competition.

Sukumar and Gadgil (1988) stated that the crop raiding was considered to be sex biased. Crop raiding was undertaken more by males than females. The authors also hypothesized that high risk, high gain strategy in male elephants caused the animals to take a high risk to enter the cultivation land which provided highly nutritious food which would help in the future for younger ones and the inheritance of their genes to the next generation.

Poole (1989) said that the male elephants raiding on palatable, nutritious and digestible food had longer period of musth while other energy less males had shorter musth. Males with larger body size were preferred by females for mating. So the crop raiders tended to take much more risk than other males to build up their body to get access to females.

Sukumar (1990) discussed that the males of the polygamous species had to face a greater level of competition from other males as well as the dominant bulls. So the males needed to increase their body size to compete with other males. So, they adopted a risky strategy to raid the cultivated crops for better nutrition which helped them to succeed the selection process and also for reproductive success.

Chiyo *et al*. (2005) stated that male elephants started to raid crops once they detached from the families and a large number of males started crop raiding when they approached reproductive competition. They predicted that when the male elephants separated from the family, they would be in search of new feeding grounds and crops, even though it was risky.

Chiyo *et al.* (2011) suggested that the crop raiding was a risky foraging behaviour which could cause injuries or death. The authors reported that 10 percent of the crop raiders in the Amboselli elephant population was injured with spear. When foraging on crops, elephants were benefited with 38 percent of their daily intake in 10 percentage of foraging time when compared to foraging on wild plants. So, crop raiding helped to attain body weight and reproductive success.

The authors also found that crop raiding enabled elephants to grow larger in their body mass for their age than non crop raiders.

2.4. *MONO AMINE OXIDASE A (MAOA)* GENE

Archer (1991) stated that heritability of aggressiveness was related to gender. In humans, very clear cut distinctions between sexes made on the basis of crime revealed a correlation between testosterone levels and aggression.

Brunner *et al.* (1993) observed that a point mutation in the 8th exon in the coding gene of *MAOA* caused the insertion of a premature stop codon which resulted in non functioning of the gene. This point mutation caused increased aggressive and violent behaviour in the males of a Dutch family.

Kim *et al.* (1997) discussed that *MAOA* knockout mice were hyper aggressive and showed fear responses. In knockout mice serotonin (5HT), norephinephrine (NE) and dopamine (DA) levels in the brain increased which caused these abnormal behaviours.

Shih *et al*. (1999) pointed that *MAOA* gene was located on the X-chromosome and comprised of 15 exons. *MAOA* knock-out experiment in mice resulted in elevated brain levels of serotonin, norephinephrine, and dopamine and manifest aggressive behaviour similar to human males with a deletion of *MAOA*. The author stated that the *MAOA* sequence was highly conserved in humans and other mammals.

Nelson and Chiavegatto (2001) explained that the metabolic enzyme *MAOA* functioned to alter the neurotransmitter level and was responsible for aggressive behaviour. MAOA enzyme was said to be present in the catecholaminergic neurons of brain. *MAOA* knockout male mice showed aggression behaviour.

Geha *et al.* (2002) observed that monoamine oxidase was a flavo protein located at the outer membranes of mitochondria in neuronal, glial and other cells.

It could catalyze the oxidative deamination of monoamine neurotransmitters such as serotonin, norephinephrine and dopamine and appeared to play important roles in several psychiatric and neurological disorders.

Olivier (2004) pointed out that aggression behaviour was controlled by serotonin (5HT) system and 5HT receptors. Of the 14 5HT receptors the post synaptically located 5HT1B receptor had a high role in the regulation of aggressive behaviour.

Popova (2006) observed that the genes which produced enzymes for serotonin (5HT) metabolism like TPH2, MAOA, SERT and 2 types of serotonin receptors 5HT1A and 5HT1B were responsible for the genetic basis of aggressive behaviour. The decrease in the biosynthesis of serotonin (5HT) increased the male to male aggression in mice.

Scott *et al.* (2008) said that when *MAOA* gene was eliminated from mice, lack of function of the gene caused an increase in aggression and male to male conflict in mice. The authors also suggested that this elimination of *MAOA* gene also occurred spontaneously in humans.

Joshua and Meyer-Lindenberg (2008) discussed that *MAOA* gene encoded a mitochondrial catabolic enzyme which catalyzes the oxidative deamination of biogenic amines, making it a critical regulator of neurotransmitters like serotonin, norephinephrine and dopamine.

Beuno (2010) suggested that some genes and their variants had particular effects on aggressive behaviour and in human conflicts. The author also stated that aggressive and antisocial behaviour was the result of genetic as well as environmental factors.

Tiihonen *et al.* (2014) conducted a study on the prisoners in Finland and commented that the majority of all violent crime was committed by a small group of antisocial recidivistic offenders. Their results showed that *monoamine oxidase*

A (*MAOA*) low-activity genotype (contributing to low dopamine turnover rate) as well as the *CDH13* gene (coding for neuronal membrane adhesion protein) were associated with extremely violent behaviour (at least 10 committed homicides, attempted homicides or battering) from the sample of two independent cohorts of Finnish prisoners. No substantial signal was observed for either *MAOA* or *CDH13* among non-violent offenders, indicating that these genes were specific for violent behaviour.

3. MATERIALS AND METHODS

3.1. COLLECTION AND PRESERVATION OF TISSUE SAMPLES

Tissues from brain, heart, lungs, kidney, liver and spleen were collected from dead Asian Elephant (n=4) during post-mortem investigation (Table 1). 100 mg of each tissue sample was sliced with a sterile surgical blade and immersed in 5 ml eppendorf® tube containing RNAlater® (Invitrogen, Bangalore) solution and absolute alcohol. The collected samples were initially stabilised at 10°C and stored at -80°C until nucleic acid isolation.

Table 1. Details of samples used in the present study

Sl. No.	Case No.	Age group	Cause of Death	Place
1	183	Adult	Poisoning	Rosemount, Kalikavu Range, Malappuram
2	185	Calf	Diseased	Muthanga, Wayanad
3	188	Adult	Gunshot	Kurichiyad, Wayanad
4	193	Adult	Electrocution	Neervaram, Wayanad

3.2. ISOLATION OF NUCLEIC ACID FROM TISSUE SAMPLES

3.2.1. Genomic DNA Isolation

Genomic DNA from absolute alcohol preserved elephant tissue sample was isolated using DNeasy Blood & Tissue Kit® (QIAGEN GmbH, Germany) according to the manufacturer's instructions. 25 mg of the tissue (up to 10 mg spleen) was finely chopped using sterile surgical blade and placed in a 1.5 ml eppendorf® tube. Buffer ATL 180 µl and 20 µl of proteinase K were added to it. The mixture was vortexed thoroughly and incubated at 56°C til the complete lysis of the tissue. The mixture was vortexed occasionally during incubation to disperse the tissue. Once the lysis was done, 200 µl buffer AL was added to the sample and mixed thoroughly by vortexing and proceeded to the next step.

Ethanol 200 µl (96–100%) was added and mixed thoroughly by vortexing. The mixture was pipetted into the DNeasy Mini spin column placed in a 2 ml collection tube. The set up was centrifuged at 6000 x g for 1 min and the flow-through and collection tube were discarded. The DNeasy Mini spin column was placed in a new 2 ml collection tube, 500 µl of buffer AW1 was added, and centrifuged at 6000 x g for 1 min. The flow-through and collection tube was discarded. Then the DNeasy Mini spin column was placed in a new 2 ml collection tube, 500 µl buffer AW2 was added and centrifuged at 20,000 x g for 3 min to dry the DNeasy membrane. The flow through and collection tube were discarded. The DNeasy Mini spin column was placed in a clean 1.5 ml or 2 ml eppendorf tube and 200 µl of buffer AE was added directly onto the DNeasy membrane. The setup was incubated at room temperature for 1 min, and then centrifuged at 6000 x g for 1 min to elute the genomic DNA. The elute containing genomic DNA was stored at -80^{o}C for further analysis.

3.2.2. Total RNA Isolation

Total RNA from RNAlater ® (Invitrogen, Bangalore) preserved elephant tissue sample was isolated using RNeasy Mini Kit® (QIAGEN GmbH, Germany) according to the manufacturer's instructions.

Thirty milligram of the tissue was disrupted and transferred into a sterile Eppendorf® tube. Buffer RLT 350 µl (1 ml Buffer RLT + 10 µl ßmercaptoethanol) was added to the lysate, vortexed and then centrifuged for 3 minutes at maximum speed. The supernatant was carefully removed by pipetting, and used in the next step. One volume of 70 percent ethanol was added to the lysate and mixed well by pipetting and preceded immediately to the next step. 700 µl of the sample was transferred to an RNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 x g for 15 seconds. The flow-through was discarded. 700 µl of Buffer RW1 was added to the RNeasy spin column and centrifuged for 15 seconds at 8000 x g. The flow-through was discarded. 500µl of Buffer RPE was added to the RNeasy spin column and centrifuged at 8000 x g

for 15 seconds. The flow-through was discarded. Again 500 µl of Buffer RPE was added to the RNeasy spin column and centrifuged at 8000 x g for 2 minutes. The RNeasy spin column was then placed in a new 1.5 ml collection tube to which 40 µl of RNasefree water was added and centrifuged at 8000 x g for 1 minute, to elute the RNA. The flow-through was saved and stored at -80°C for further analysis.

3.3. COMPLEMENTARY DNA (cDNA) SYNTHESIS

The first strand cDNA synthesis was done using High Capacity cDNA Reverse Transcription® kit (Applied Biosystem, USA). The master mix required for cDNA synthesis was prepared by mixing the ingredients given in Table 2 by pipetting and brief spin.

Table 2. Ingredients of Reverse Transcription Reaction Mix

Sl. No.	Master mix ingredients	Vol. required (µl)
1	10X RT Buffer	2.0
2	25 X dNTPs	0.8
3	OligodT Modified Primer	2.0
4	Multiscript Reverse Transcriptase	1.0
5	RNase inhibitor	1.0
6	Nuclease free water	3.2
	Total	**10.0**

The master mix was then aliquoted in required number of properly labelled 0.2 ml PCR tubes (10 µl each). 10 µl of total RNA was added and mixed well by gentle pipetting. The mixture was then incubated in BIORAD Thermal Cycler® (BIORAD, USA), with the cyclic condition given in Table 3.

Table 3. Thermal cyclic condition for cDNA synthesis

	Step 1	**Step 2**	**Step 3**	**Step 4**
Temperature (ºC)	25	37	85	4
Time	10 min	120 min	5min	Hold

3.4. PCR AMPLIFICATION OF *MONO AMINE OXIDASE A* (*MAOA*) FROM cDNA

Orthologous sequences of *MAOA* gene from mammals were retrieved from NCBI GenBank (http://www.ncbi.nlm.nih.gov/) and aligned with EMBL ClustalW (http://www.ebi.ac.uk//Tools/clustalw/) to identify conserved regions for the design of primers to amplify the coding region. The designed primers (Table 4) were synthesised in Sigma Aldrich, Bangalore. PCR amplification of complete coding sequence from cDNA was done with the primers MAOAF1/ MAOAR1 (Table 4) in 25µl of reaction mixture containing 10x PCR buffer, 0.2 mM dNTPs, 0.2 µm each primer, 1.25 unit *Taq* DNA polymerase and cDNA. The reaction was carried with the following cyclic condition: initial denaturation at 95^{o}C for 3 min followed by 35 cycles with denaturation at 95^{o}C for 45 sec, annealing temperature 62.5ºC for 40 sec, 72º C for 50 sec and final elongation at 72ºC for 7 min. The PCR products were electrophoresed on 1.5 percent agarose gel and viewed under UV transilluminator. The amplicons were amplified using the nested primers MAOAF2/MAOAR2 (Table 4) to discriminate the contaminants/false amplification.The positive amplicons were gel eluted, cloned in to TA vector and sequenced using Big Dye Termination method.

3.5. RAPID AMPLIFICATION OF cDNA ENDS (RACE) OF *MAOA* mRNA

The 3’ RACE was done using oligodT modified based cDNA synthesis method (Chandramohan *et al.*, 2013). The amplification was carried out with the

primers MAOAF2/NSTodt followed by the amplification with MAOAF1/ OdTmodi primers (Table 4). Next, 5'RACE was carried out according to the method reported in Dallmeier and Neyts (2013). The first strand cDNA was synthesised with gene specific 5' phosphorylated primer MAOAP, subsequent amplification was done with MAOAF/ MAOAR1 primers and semi nested amplification were performed by using MAOAF/MAOAR2 primer pair (Table 4). The amplification of *MAOA* cDNA ends was done in 25µl of reaction mixture containing 10x PCR buffer, 0.2 mM dNTPs, 0.2 µm each primer, 1.25 unit *Taq* DNA polymerase and cDNA. The reaction was carried with the following cyclic condition: initial denaturation at 98^{o}C for 2 min followed by 35 cycles with denaturation at 95^{o}C for 45 sec, annealing temperature 62.5ºC for 40 sec, 72º C for 50 sec and final elongation at 72ºC for 7 min. The PCR products were electrophoresed on 1.5 percent agarose gel and viewed under UV transilluminator. The positive amplicons were gel eluted, cloned in to TA vector and sequenced using Big Dye Termination method

3.6. SEQUENCE ANALYSIS

Nucleic acid protein data base searches were performed using blast at the NCBI server and visualized with Circoletto (Darzentas, 2010). The cDNA and DNA sequence data were analysed using DNASTAR 5.0 software (Dayhoff *et al.* 1978). The alignment of the amino acid sequence of *MAOA* was performed using bioinformatics software Clustal Omega.

3.7. WHOLE GENOME SEQUENCING OF ASIAN ELEPHANT USING NEXT GENERATION SEQUENCING

The genomic DNA with good quality and quantity was selected for Next Generation Sequencing (NGS). Initially the DNA was sheared and library was made with the adopters. Finally the library was sequenced in Illumina MiSeq.

The sample MAOAfastq files were initially quality checked using FastQC tool with default parameters. The raw read counts, base composition, base quality

distribution and GC contents were calculated and the reads with good quality (Phred score >= Q30) suggested by Illumina were considered for further downstream analysis. The good quality reads were taken for adapter removal and this was achieved using cutadapt, version 1.8. (-a) option was used to remove forward and reverse sequence adapters from both ends. The adapter removed reads were further taken for alignment. The adapter filtered reads were aligned against *MAOA* reference gene sequences. Alignment was done using Bowtie2 program with 1 mismatch. Further, alignment statistics was calculated using in-house PERL program. The aligned reads in SAM file format were converted into paired-end FASTQ files using Picard tools SamtoFastq option. Reads with less the 10 bp were ignored. The genome assembly was performed by using SPADES assembly tool using *MAOA* genes as reference. Manifest file was generated for the reference based assembly and assembly was performed with 31 k-mer size.

Table 4. Primers used in the coding sequence amplification and RACE of *MAOA* gene

Sl.No.	Primer Name	Primer Sequence (5'-3')	Annealing Temperature(°C)
1.	MAOAF1	ATGTTCGAMGTAGTCGTGATAGGAG	66.0
2.	MAOAF2	CTCAGGATTRTCTGCTGCSAAACTC	65.4
3.	MAOAR1	GACCAGATCCRCCYACAAACTTCCG	68.8
4.	MAOAR2	CTTTGTCCAGCAGATTTTDTCGATG	68.3
5.	OdTmodi	GAGAGAGAGAGAGACAGAGAACTAGTCTCGAGTTTTTTTTTTTTTTTTT	74.9
6.	NSTodt	GAGAGAGAGAGAGACAGAGAACTAGTCTCGAG	68.0
7.	MAOAF	CACCCTGTTCTACTGTTACTTTC	58.4
8.	MAOAR1	GAAGATTCTGGTTTTGCAATTG	62.3
9.	MAOAR2	CTCAAACCGTCGTCTGTTAG	60.0
10.	MAOAP	p*CTTTCATTGTCATCTTGTCCCAC	

4. RESULTS

4.1. CHARACTERISATION OF THE *MAOA* FULL LENGTH TRANSCRIPT AND GENOMIC LOCUS

In total two transcripts were identified from total RNA purified from lungs of Asian elephant. The total length of identified transcript were 2083 (transcript - 1) (Figure 1) and 814 bp (transcript - 2) (Figure 2) and have a common 5'UTR of 212 bp . Transcript - 1 composed of 1596 bp open reading frame (ORF) and a 275 bp 3′UTR. The ORF has high similarity at the nucleotide level with horse (90%), cattle (88%), human (88%), orangutan (88%), gray wolf (87%), guinea pig (86%) and wild boar (85%) (Figure 3). It encodes a putative 531 amino acid (aa) protein, which is 91, 89, 88, 88, 88, 88, 87 and 87 percent identical to horse, grey wolf, cattle, orangutan, wild boar, mouse, rat and human MAOA respectively (Figure 4). The coded amino acid sequence has the characteristic signature for mammalian MAOA protein. On the contrary, transcript - 2 has a shorter ORF of 519 bp, and a 3′UTR of 83 bp when compared to the transcript-1and it encodes a putative 172 aa protein. The difference in encoded aa by transcript – 1 and 2 could be due to a large deletion in the ORF (517 -1080 bp) (Figure 1&2). The similarity search of nucleotide and protein sequences of transcript – 2 followed a similar trend to that of transcript -1.

```
ATGgcgagtcaggagaagagtcgggagaaggcgagtatctcgggccacatgttcgacgta
 M  A  S  Q  E  K  S  R  E  K  A  S  I  S  G  H  M  F  D  V
gtcgtgataggaggcggcatctcaggattgtctgctgccaaactcttggccgaacatgaa
 V  V  I  G  GG  I  S  G  L  S  A  A  K  L  L  A  E  H  E
gttaacgttttggtcttagaagcccgagatcgagttggagggagaacatatactgtgagg
 V  N  V  L  V  L  E  A  R  D  R  V  G  G  R  T  Y  T  V  R
aacgagcatgttaattacgtagatgttggcggagcatatgtgggaccaacccagaacagg
 N  E  H  V  N  Y  V  D  V  G  G  A  Y  V  G  P  T  Q  N  R
atcttacggctgtctaaggaactgggcttgaagacttacaaagtgaacgtcagtgaacgc
 I  L  R  L  S  K  E  L  G  L  K  T  Y  K  V  N  V  S  E  R
cttgttcaatatgtcaaggggaaaacttatccatttcggggtgcctttcctccagtgtgg
 L  V  Q  Y  V  K  G  K  T  Y  P  F  R  G  A  F  P  P  V  W
aacccgattgcatatctggattacaacaatctgtggcggacaatggataacatggggaag
 N  P  I  A  Y  L  D  Y  N  N  L  W  R  T  M  D  N  M  G  K
gagattccagctgatgcaccctgggatgccccacacgccgaaaagtgggacaagatgaca
 E  I  P  A  D  A  P  W  D  A  P  H  A  E  K  W  D  K  M  T
atgaaagatctcatcgataaaatctgctggacaaagacggctaggcagtttgcttatctc
 M  K  D  L  I  D  K  I  C  W  T  K  T  A  R  Q  F  A  Y  L
ttcgtgaacatcaacgtgacctccgagccccatgaagtctctgccttgtggttcttgtgg
 F  V  N  I  N  V  T  S  E  P  H  E  V  S  A  L  W  F  L  W
tacgtgaagcagtgcgggggcaccactcgaatcttctcgattaccaacgggggccaggaa
 Y  V  K  Q  C  G  G  T  T  R  I  F  S  I  T  N  G  G  Q  E
cggaagtttgtgggcggatctggtcaggtgagcgagcggataatggatcttcttggggaa
 R  K  F  V  G  G  S  G  Q  V  S  E  R  I  M  D  L  L  G  E
cgagtgaagctgaggtgccctgtcagctctattgaccagtcgggcgaaaatatcatcgta
```

```
 R  V  K  L  R  C  P  V  S  S  I  D  Q  S  G  E  N  I  I  V
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 E  T  L  N  H  E  V  Y  E  C  R  Y  V  I  S  A  I  P  PP
ttgacttctaagatccacttcagaccggaacttccatcagaaagaaaccaattaattcag
 L  T  S  K  I  H  F  R  P  E  L  P  S  E  R  N  Q  L  I  Q
cgtcttccgatggggtctatcattaagtgcatgatgtattacagggaagccttttggaag
 R  L  P  M  G  S  I  I  K  C  M  M  Y  Y  R  E  A  F  W  K
aagaaggattactgtggctgcatgatcattgaagatgaagaagccccaatatcaataacc
 K  K  D  Y  C  G  C  M  I  I  E  D  E  E  A  P  I  S  I  T
ctggatgacaccaaaccagatggatcgctgcctgccatcatgggctttatccttgcccga
 L  D  D  T  K  P  D  G  S  L  P  A  I  M  G  F  I  L  A  R
aaatctcatcgattcgctaaactccataaagaaataaggaaaaggaaaatctgtgagctc
 K  S  H  R  F  A  K  L  H  K  E  I  R  K  R  K  I  C  E  L
tatgccaaagtactgggatcccaagaagctttacaacccgtgcactatgaagagaagaac
 Y  A  K  V  L  G  S  Q  E  A  L  Q  P  V  H  Y  E  E  K  N
tggtgtgaggagcagtactctgggggctgctatacagcttacttccccctgggatcatg
 W  C  E  E  Q  Y  S  G  G  C  Y  T  A  Y  F  P  P  G  I  M
actcaatatggaagggtgattcgacaaccagtgggcaagatttactttgctggaactgag
 T  Q  Y  G  R  V  I  R  Q  P  V  G  K  I  Y  F  A  G  T  E
acagccacacagtggagcgggtacatggaaggagcagtcgaggccggggaaagggcagct
 T  A  T  Q  W  S  G  Y  M  E  G  A  V  E  A  G  E  R  A  A
agagagatcttgaatgctctggggaaggtagcaaagaaagacatctgggtcccagaaccg
 R  E  I  L  N  A  L  G  K  V  A  K  K  D  I  W  V  P  E  P
gaatcacaggatgtaccatctattgagatcacccactccttctgggaaaggaacctgcct
 E  S  Q  D  V  P  S  I  E  I  T  H  S  F  W  E  R  N  L  P
tcagtggcaggcctgctgaagatcattggcttttcaacatcaataactgccatgtgtgtt
 S  V  A  G  L  L  K  I  I  G  F  S  T  S  I  T  A  M  C  V
gtggtgtacaaatgcaaactgctgaaccgccccTGA
 V  V  Y  K  C  K  L  L  N  R  P  -
```

Figure 1. Nucleotide and deduced amino acid sequence of *MAOA* Transcript-1

```
ATGgcgagtcaggagaagagtcgggagaaggcgagtatctcgggccacatgttcgacgta
  M  A  S  Q  E  K  S  R  E  K  A  S  I  S  G  H  M  F  D  V
gtcgtgataggaggcggcatctcaggattgtctgctgccaaactcttggccgaacatgaa
  V  V  I  G  GG  I  S  G  L  S  A  A  K  L  L  A  E  H  E
gttaacgttttggtcttagaagcccgagatcgagttggagggagaacatatactgtgagg
  V  N  V  L  V  L  E  A  R  D  R  V  G  G  R  T  Y  T  V  R
aacgagcatgttaattacgtagatgttggcggagcatatgtgggaccaacccagaacagg
  N  E  H  V  N  Y  V  D  V  G  G  A  Y  V  G  P  T  Q  N  R
atcttacggctgtctaaggaactgggcttgaagacttacaaagtgaacgtcagtgaacgc
  I  L  R  L  S  K  E  L  G  L  K  T  Y  K  V  N  V  S  E  R
cttgttcaatatgtcaaggggaaaacttatccatttcggggtgcctttcctccagtgtgg
  L  V  Q  Y  V  K  G  K  T  Y  P  F  R  G  A  F  P  P  V  W
aacccgattgcatatctggattacaacaatctgtggcggacaatggataacatggggaag
  N  P  I  A  Y  L  D  Y  N  N  L  W  R  T  M  D  N  M  G  K
gagattccagctgatgcaccctgggatgccccacacgccgaaaagtgggacaagatgaca
  E  I  P  A  D  A  P  W  D  A  P  H  A  E  K  W  D  K  M  T
atgaaagatctcatcgataaaatctgctggacaaagTAG
M  K  D  L  I  D  K  I  C  W  T  K  -
```

Figure 2. Nucleotide and deduced amino acid sequence of *MAOA* Transcript-2

The gene structure of MAOA deduced by Next Generation Sequencing (NGS) platform displayed a 58366 bp of fragment after the alignment, which contains fifteen coding exons, in addition to fourteen intronic sequences. The genomic organization of the Asian

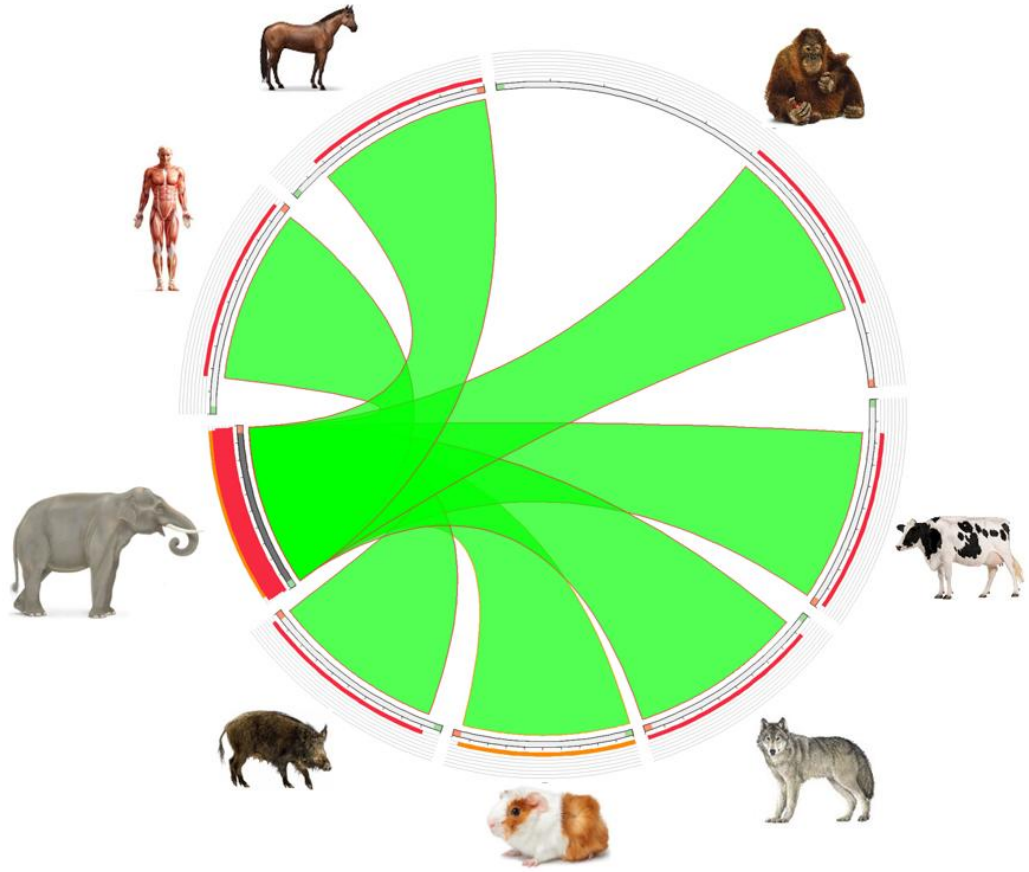


Figure 3. MAOA gene identities among the mammals are represented in Circoletto. The ribbons represent the local alignments produced by BLAST, the similarity correspond to the width, and the colors corresponds to alignment identity in four quartiles: blue for the first (i.e. worst) 25% of the maximum identity, green for the next 25%, orange for the third, and finally red for the top (i.e. best) identity between 75% and 100% of the maximum identity. High sequence similarity is observed with most of the organisms (mammals) at the nucleotide level.

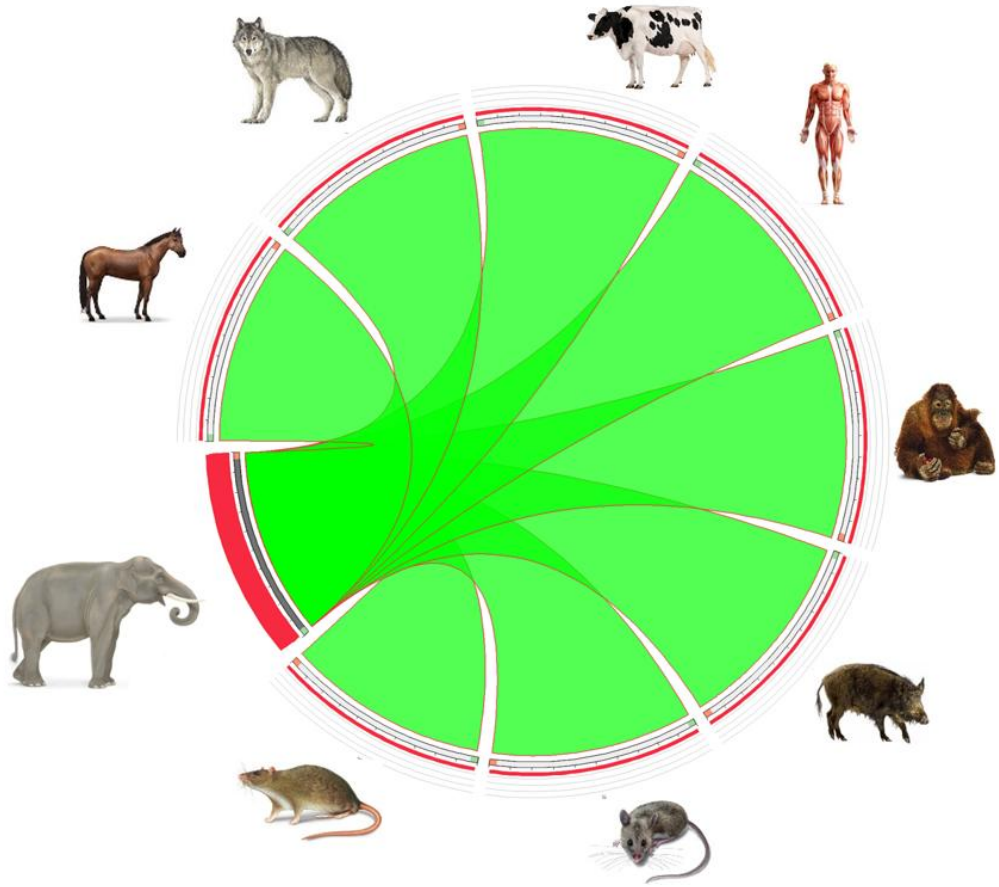


Figure 4. MAOA Amino Acid sequence similarities among the mammals are represented in Circoletto. The ribbons represent the local alignments produced by BLAST, the similarity correspond to the width, and the colors corresponds to alignment identity in four quartiles: blue for the first (i.e. worst) 25% of the maximum identity, green for the next 25%, orange for the third, and finally red for the top (i.e. best) identity between 75% and 100% of the maximum identity. High sequence similarity is observed with most of the organisms (mammals) at the protein level.

elephant MAOA gene is similar to that of African elephant and humans. The coding exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 are separated by 17895, 7420, 12006, 738, 1468, 2540, 286, 802, 6108, 4198, 1145, 1765, 202 and 185 bp intronic sequences, respectively (Figure 5). The MAOA gene structure is highly conserved in mammals.

4.2. MAOA GENE EXPRESSION IN DIFFERENT TYPES OF TISSUES

To analyse the wide spread expression of MAOA gene in different tissue of Asian elephant, total RNA from kidney, brain, heart, lungs, liver and spleen was used. Present analysis revealed, transcript -2 (truncated form) is expressed in all the tissues analyzed. On the contrary, none of the tissue showed amplicon for transcript – 1 (Figure 6).

4.3. MOLECULAR MODELLING OF MAOA

The homology modelling of MAOA protein deduced from the identified transcripts showed considerable alteration in the structure. The MAOA protein structure modeled from the aa sequence of transcript – 2 seems to be a truncated form. The model – template alignment of MAOA complete protein and truncated protein represented in the (Figure 7) and the 3D modelled structures of both the proteins are shown in the (Figure 8).

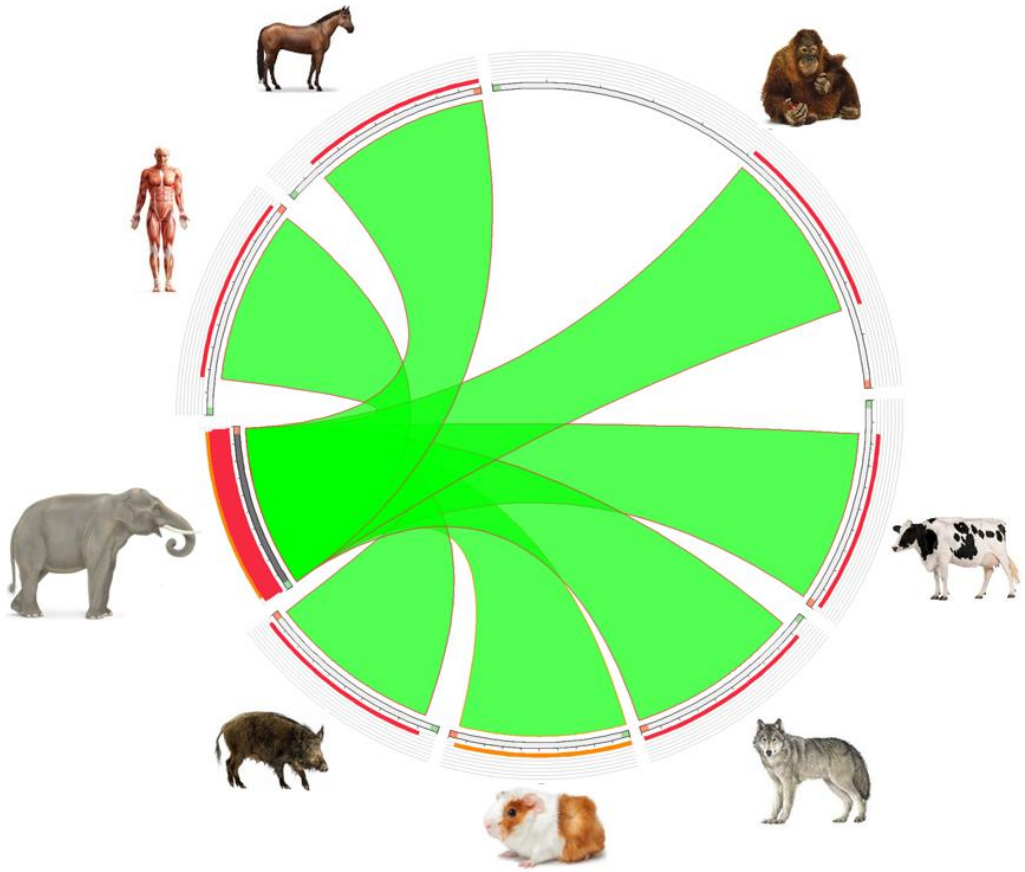


Figure 3. *MAOA* gene identities among the mammals are represented in Circoletto. The ribbons represent the local alignments produced by BLAST, the similarity correspond to the width, and the colors corresponds to alignment identity in four quartiles: blue for the first (i.e. worst) 25% of the maximum identity, green for the next 25%, orange for the third, and finally red for the top (i.e. best) identity between 75% and 100% of the maximum identity. High sequence similarity is observed with most of the organisms (mammals) at the nucleotide level.

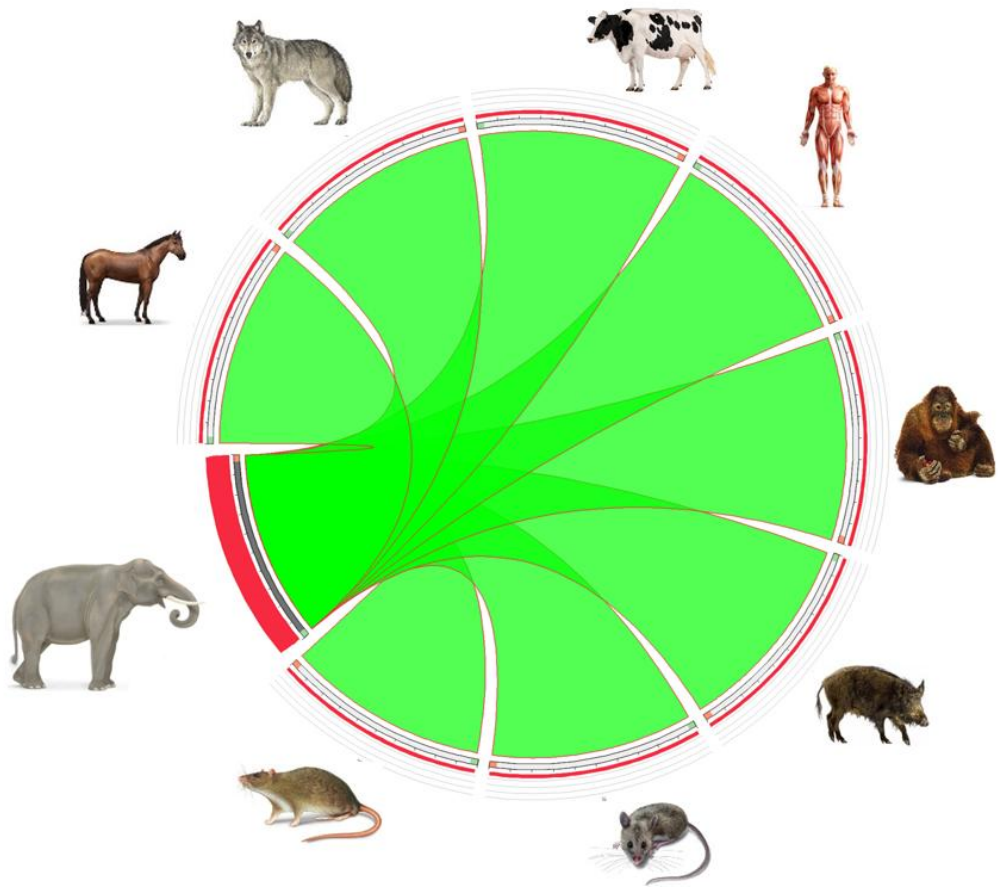


Figure 4. MAOA Amino Acid sequence similarities among the mammals are represented in Circoletto. The ribbons represent the local alignments produced by BLAST, the similarity correspond to the width, and the colors corresponds to alignment identity in four quartiles: blue for the first (i.e. worst) 25% of the maximum identity, green for the next 25%, orange for the third, and finally red for the top (i.e. best) identity between 75% and 100% of the maximum identity. High sequence similarity is observed with most of the organisms (mammals) at the protein level.

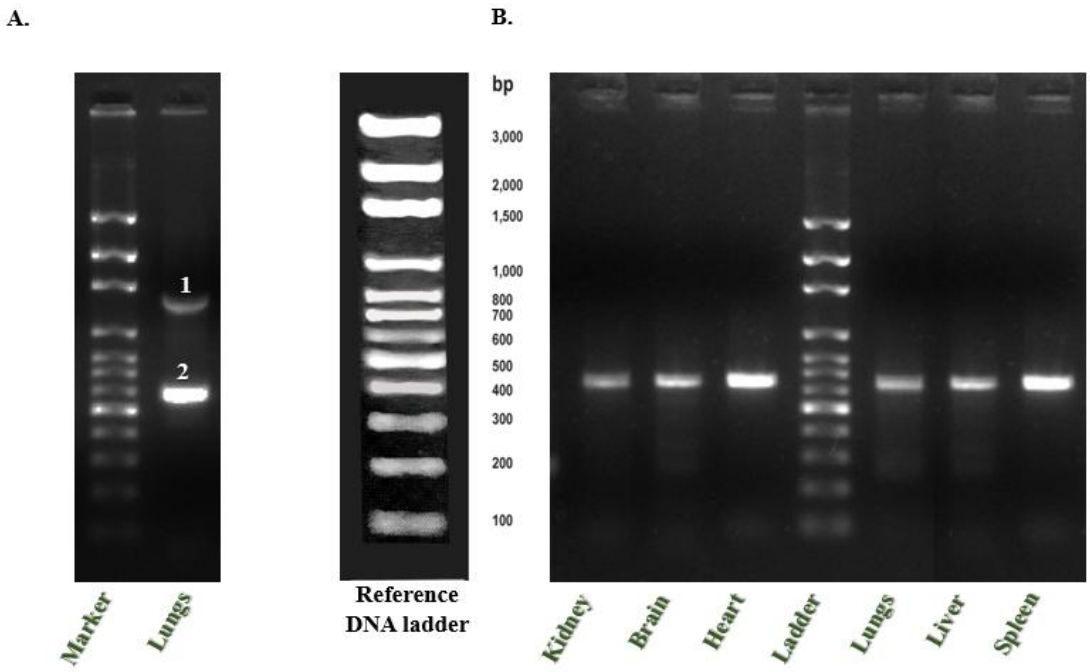
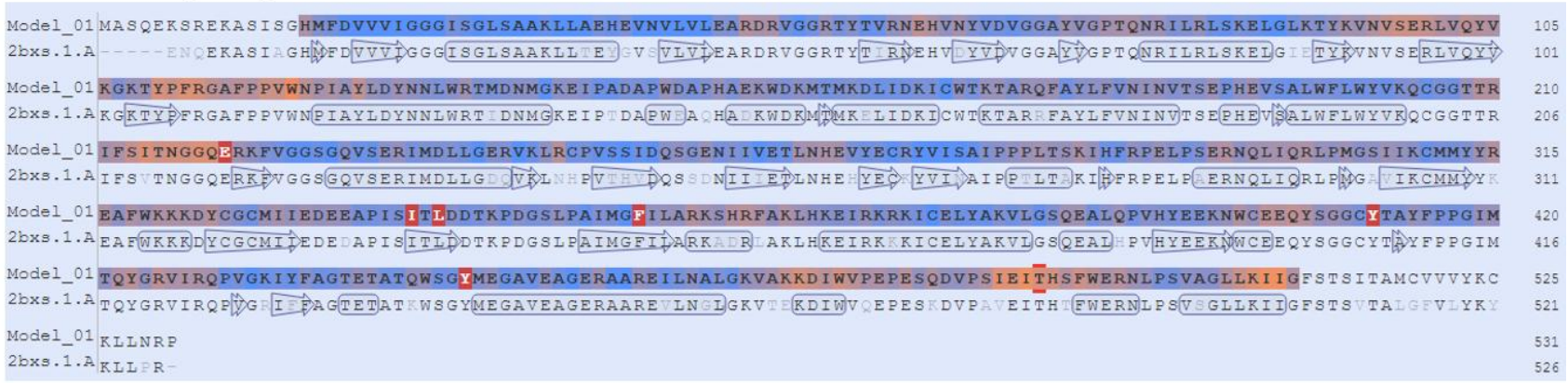


Figure 6. Representative gel image of *MAOA* transcript amplification. **A.** Represents full length transcript amplification (excluding 5'UTR) of *MAOA*. (1) Transcript – 1 and (2) transcript 2 amplified from lungs. **B.** Represents the wide spread expression of transcript – 2 in Kidney, Brain, Heart, Lungs, Liver and Spleen of Asian Elephant.

A. MAOA Model – Template Alignment



B. MAOA (Truncated) Model – Template Alignment

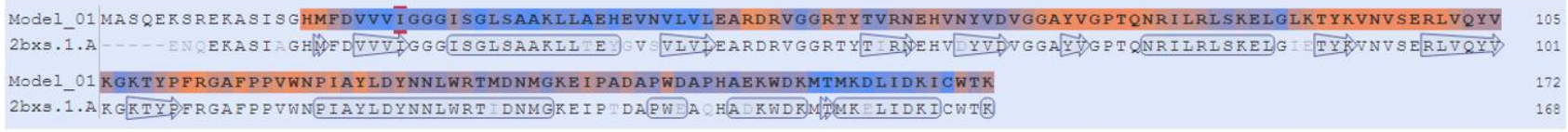


Figure 7. MAOA protein model – template alignment. **A.** Represents model template alignment between the protein sequence deduced from transcript -1 and the available characterized MAOA protein. **B.** Represents model template alignment between the protein sequence deduced from transcript -2 and the available characterized MAOA protein.The symbol ⇨ indicates beta strand and ▭ indicates alpha helix.

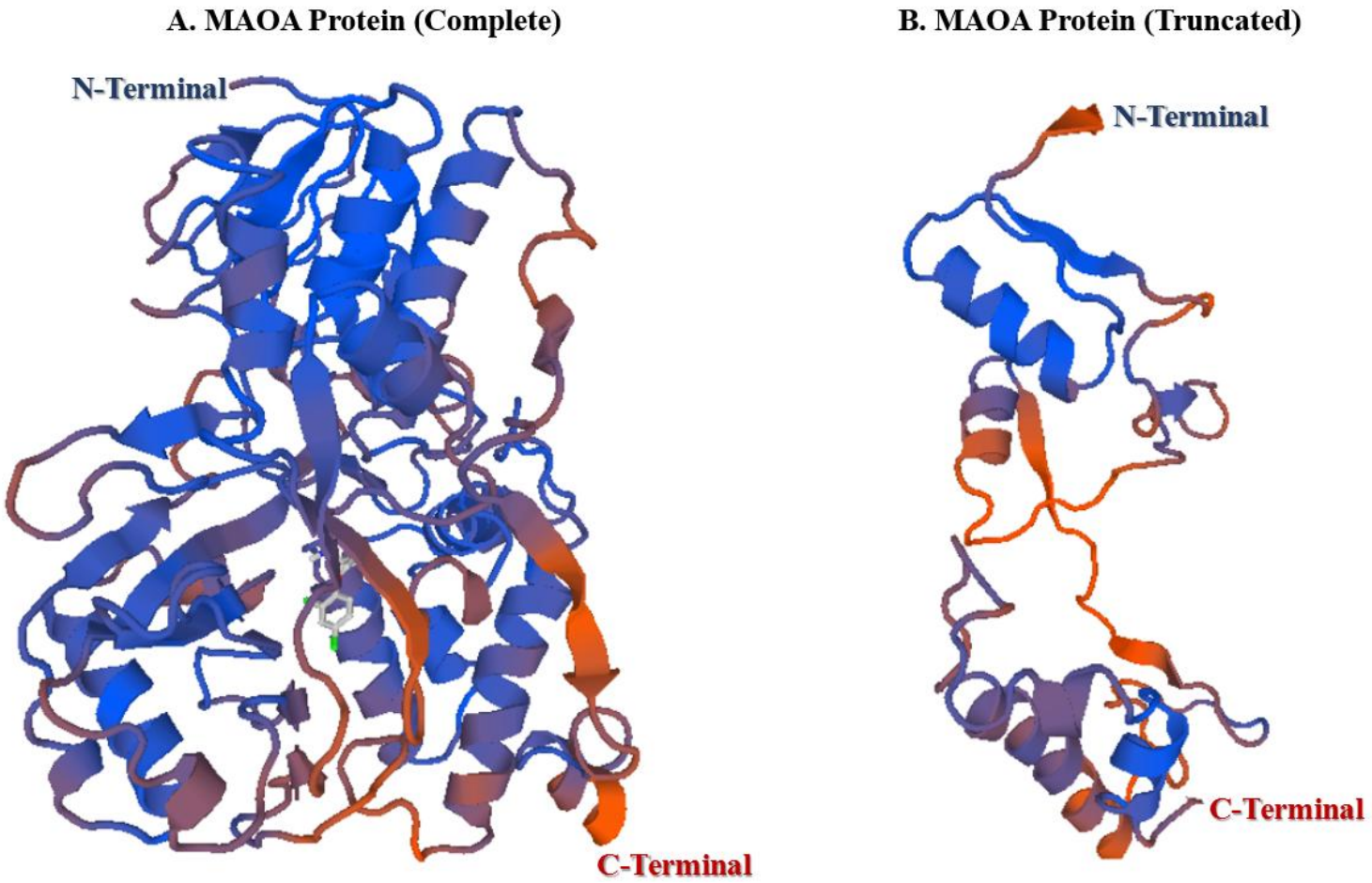


Figure 8. MAOA protein (predicted) 3D structure. **A.** MAOA protein modeled from the deduced amino acid of transcript – 1 (complete transcript). **B.** MAOA protein modeled from the deduced amino acid of transcript – 2 (truncated transcript).

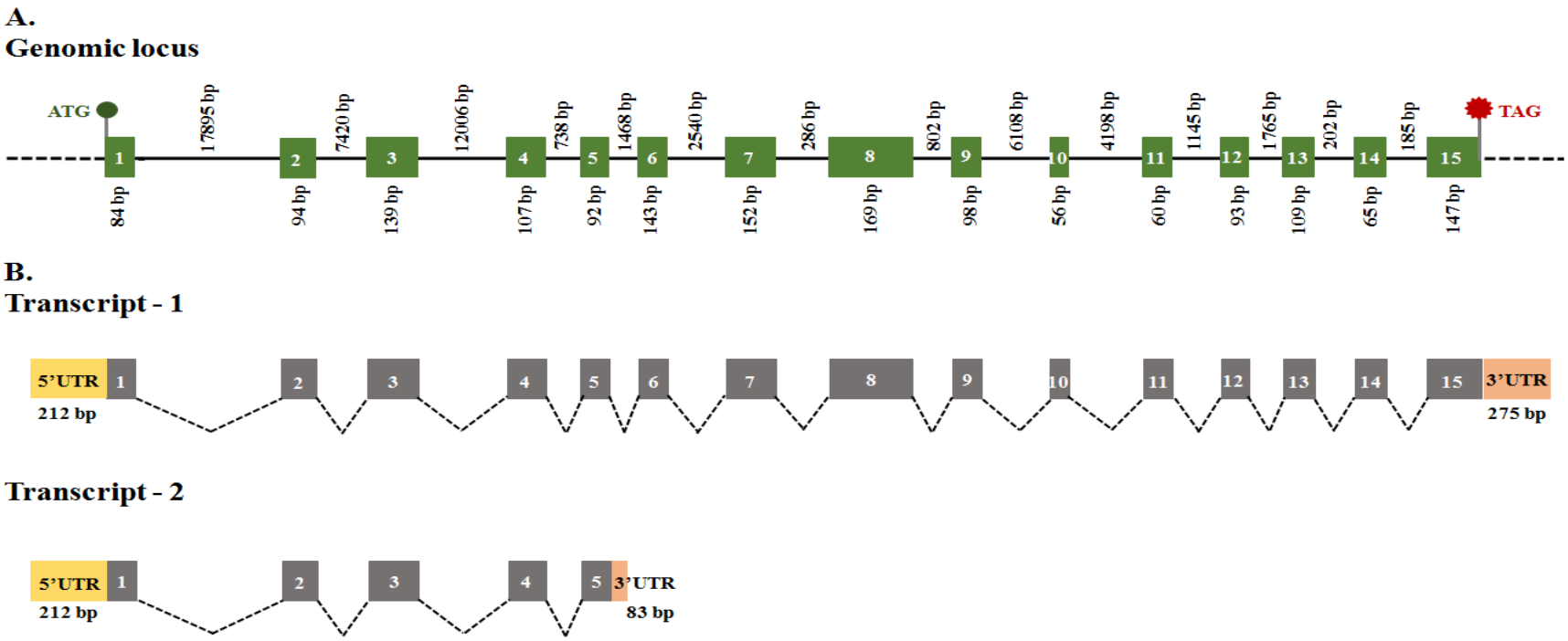


Figure 5. The structure of Asian Elephant *MAOA* genomic locus and its transcripts. **A.** The structure of Asian Elephant genomic locus and polymorphisms. Green boxes indicate protein coding sequences (exons 1 to 15). The *MAOA* main start codon (ATG) and the stop codon (TGA) are indicated by the green circle ( ) and red sparkle ( ) symbols, respectively. Numbers above and below the gene structure indicate the length of exons and introns in bp. The symbol ( ) indicate unknown sequence. **B.** Structure of *MAOA* transcripts. Transcript 1 and 2 are represented under the corresponding genomic locus. The position of coding exons (1 to 15), introns (2 to 14), 5' and 3′UTR were deduced from this study.

5. DISCUSSION

Asian elephants and humans observed to have a similar social organization, and also have comparable physiology and genetic architecture. Risk taking (crop raiding) behavior in elephants and humans are comparable. Like in humans, only a few elephants tend to have crop raiding/conflicting behavior. In Asian Elephants a trend has been observed in conflicting animals, only a few have the tendency to take risk and the trait could likely be inherited. In human risk taking behavior has a genetic background (Brunner *et al*. 1993; Tiihonen *et al*. 2014; Joshua & Meyer-Lindenberg, 2008). Plenty of genes have been studied to get correlation with risk taking behavior, among those a gene called *Mono Amine Oxidase A* (*MAOA*) has a significant correlation with the behavior (Beuno, 2010; Tiihonen *et al*. 2014). *MAOA* manifest abnormal aggressiveness (Brunner *et al*. 1993) by catalyzing the oxidative deamination of a number of biogenic amines in the brain and peripheral tissues by the production of hydrogen peroxide (H_2O_2) (Shih 1991, Thorpe *et al*. 1987). It is speculated that the *MAOA* may have a similar effect in Asian elephant.

5.1. CHARACTERISATION OF THE *MAOA* FULL LENGTH TRANSCRIPT AND GENOMIC LOCUS

In this thesis, the genomic structure of *MAOA* gene locus, full length transcripts and its probable role in risk taking behavior in Asian Elephants were reported. The results from the present research revealed that the *MAOA* gene organization is similar to human *MAOA* gene. The comparative analysis of human and elephant genomes shows that the elephant *MAOA* gene is embedded in a conserved syntenic block. The RT-PCR and RACE experiments presented here clearly show that in the Asian elephant, the *MAOA* gene produces two (1 and 2) different sized transcripts in lungs that differ in the ORF and 3'UTR. The sequence comparison of *MAOA* gene and transcripts revealed that the transcript -1 contains

typical characteristics of *MAOA* undergone with a normal splicing mechanism which is evident by the presence of all 15 exons in the transcript. Moreover, the translation of ORF sequence into amino acid sequence resulted in complete MAOA protein which is highly conserved among mammals. The strong conservation of amino acid sequence of MAOA among mammalian species may reflect evolutionary pressure to maintain the specific physiological function.

Surprisingly transcript – 2 is shorter than transcript -1, a large portion of ORF and 3'UTR sequence have been deleted in this transcript. Further more detailed analysis unveiled that during the splicing mechanism skipping of 10 exons and inclusion of a portion of intron 5 sequence as 3'UTR which is very clear from the characteristics of the transcript. The amino acid sequence deduced from the ORF showed truncated form of MAOA protein.

In our study, two transcripts have been identified, transcript -1 has a complete and transcript – 2 has a truncated mRNA structure. Moreover, the deduced amino acid from the coding sequence of transcript -1 and transcript – 2 showed a complete and truncated protein respectively. Surprisingly, the transcript – 1 (complete) was detected only in one animal (young calf) that too in lungs tissue. On the contrary, transcript – 2 (truncated) was detected in all the tissues irrespective of animals sample studied. Because of the truncated transcript animals would have produced truncated MAOA enzyme which may be nonfunctional. Any alteration in *MAOA* gene results in the expression of functional/non functional protein, which may significantly, affects the resulting phenotype. Abrogate *MAOA* expression are associated with violent, criminal, impulsive behavior in humans (Brunner *et al*. 1993; Chen *et al*. 2004). Functional MAOA isoenzyme metabolise biogenic amines level in the brain, and the same may increase in the absence of nonfunctional *MAOA* (Kim *et al*. 1997; Shih *et al*. *1999*). As a result varying biogenic amine levels result in both a unique biochemical and behavioral phenotype (Chen *et al*. 2004). Here increased level of

biogenic amines might have resulted in conflicting/crop raiding behavior in Asian Elephants. Furthermore, the history of death of elephants revealed an interesting fact that all the animals under study died (gun shot, poisoning and electrocution) due to conflict issues.

In order to clarify much about the fact, more samples have to be included especially adult males and animals not involved in conflict. Moreover, screening of *MAOA* gene for polymorphism, copy number variation and gene duplication would help in unveiling the situation better.

5.2. *MAOA* GENE EXPRESSION IN DIFFERENT TYPES OF TISSUES

MAOA predominately expressed in human brain, placenta and liver (Jahng *et al*. 1997, Masine-Repiso *et al*. 1986; Shih *et al*. 1999). In contrast, *MAOA* transcript – 2 is expressed in all elephant tissues analysed and non-showed expression for transcript -1 except for elephant calf's lungs. Even though the transcript is truncated, the observed wide spread expression seems transcript - 2 may have specific role in regulating physiology in elephants. Above all, the role of transcript – 1 and the reason for its absence in tissue is unknown.

5.3. MOLECULAR MODELLING OF MAOA PROTEIN

Homology modeling of a protein would help in studying/comparing 3D structure of two variants. The modeling of two variants obtained in the present investigation showed considerable variation in its structure. The protein sequences deduced from the transcript-1 showed complete structure in comparison with the human MAOA PDB structure. But the protein sequence translated from transcript -2 showed a considerable amino acid sequence deletion which may affects its normal physiological function. The truncated protein predicted from transcript–1 impaired

function. Further in-vitro functional validation is needed for the two *MAOA* variant to know its exact function in Asian elephant.

6. SUMMARY

The present study was conducted to elucidate the genetic basis for the risk taking behaviour (crop raiding) in Asian Elephants (*Elephas maximus*). Candidate gene approach has been used to address the issue by considering *MAOA* as probable gene. *MAOA* genomic locus and transcripts sequences were deduced.

In total two transcripts were identified from total RNA purified from lungs of the Asian elephant. The total length of identified transcript were 2083 (transcript - 1) and 814 bp (transcript - 2) and have a common 5'UTR of 212 bp. Transcript - 1 composed of 1596 bp open reading frame (ORF) and a 275 bp 3′UTR. On the contrary, transcript - 2 has a shorter ORF of 519 bp, and a 3′UTR of 83 bp when compared to the transcript-1and it encodes a putative 172 aa protein. The difference in encoded aa by transcript – 1 and 2 could be due to a large deletion in the ORF (517 -1080 bp).The similarity search of nucleotide and protein sequences of transcript – 2 followed a similar trend to that of transcript -1.

The gene structure of *MAOA* deduced by Next Generation Sequencing (NGS) platform displayed a 58366 bp of fragment after the alignment, which contains fifteen coding exons, in addition to fourteen intronic sequences. The genomic organization of the Asian elephant *MAOA* gene is similar to that of African elephant and humans. The coding exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 are separated by 17895, 7420, 12006, 738, 1468, 2540, 286, 802, 6108, 4198, 1145, 1765, 202 and 185 bp intronic sequences, respectively. The *MAOA* gene structure is highly conserved in mammals.

To analyse the wide spread expression of *MAOA* gene in different tissue of Asian Elephant, total RNA from kidney, brain, heart, lungs, liver and spleen was used. Present analysis revealed, transcript -2 (truncated form) is expressed in all the tissues analyzed. On the contrary, none of the tissue showed amplicon for transcript – 1. The homology modelling of MAOA protein deduced from the

identified transcripts showed considerable alteration in the structure. The MAOA protein structure modelled from the aa sequence of transcript – 2 seems to be a truncated form.

In our study, two transcripts have been identified, transcript -1 has a complete and transcript – 2 has a truncated mRNA structure. Moreover, the deduced amino acid from the coding sequence of transcript -1 and transcript – 2 showed a complete and truncated protein respectively. Surprisingly, the transcript – 1 (complete) was detected only in one animal (young calf) that too in lungs tissue. On the contrary, transcript – 2 (truncated) was detected in all the tissues irrespective of animals sample studied. Because of the truncated transcript animals would have produced truncated MAOA enzyme which may be non-functional. Any alteration in *MAOA* gene results in the expression of functional/non functional protein, which may significantly, affects the resulting phenotype.

In conclusion, the transcripts identified in the present study is highly correlated with the elephant's conflicting behaviour, hence *MAOA* gene may have a role in it. The study also speculates that there could be involvement of environmental factor in conflicting behaviour. Moreover, gene analysis for polymorphism, duplication and copy number has not been done due its size (58366bp) and time. The analysis on genomic locus may discriminate the cause for conflicting behaviour in elephants (regulated by genes or epigenetic factors or environmental factors). Above all, analysis of *MAOA* gene and its transcript may provide base line information on gene and transcripts structural organisation in Asian Elephants.

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8. ABSTRACT

Various studies in humans and other mammals showed the role of *Mono Amine Oxidase A (MAOA)* gene in violent and aggressive behaviour. The aggression and violent behaviour in case of humans is also a kind of risk taking. Hence we hypothesise that *MAOA* gene may have a role in the risk taking (crop raiding) behaviour of Asian Elephants. From the four autopsy samples of Asian Elephants, *MAOA* genomic locus and its transcripts were identified and characterised. The total length of identified transcript were 2083 (transcript - 1) and 814 bp (transcript - 2) and have a common 5'UTR of 212 bp. Transcript - 1 composed of 1596 bp open reading frame (ORF) and a 275 bp 3′UTR. On the contrary, transcript - 2 has a shorter ORF of 519 bp, and a 3′UTR of 83 bp when compared to the transcript-1and it encodes a putative 172 aa protein. The similarity search of nucleotide and protein sequences of transcript – 2 followed a similar trend to that of transcript -1. The gene structure of *MAOA* deduced by Next Generation Sequencing (NGS) platform displayed a 58366 bp of fragment after the alignment, which contains fifteen coding exons and fourteen introns. The genomic organization of the Asian Elephant *MAOA* gene is similar to that of African elephant and humans. In the wide spread expression analysis of *MAOA* gene in different tissues, transcript -2 (truncated form) was expressed in kidney, brain, heart, lungs, liver and spleen. On contrary, none of the tissue showed amplicon for transcript – 1 except the lung sample of a young male calf. The homology modelling of MAOA protein deduced from the identified transcripts showed considerable alteration in the structure. The MAOA protein structure modelled from the amino acid sequence of transcript – 2 seems to be a truncated form. The transcripts identified in the present study is highly correlated with the elephant's conflicting behaviour, hence *MAOA* gene may have a role in it. Above all, analysis of *MAOA* gene and its transcript may provide base line information on gene and transcripts structural organisation in Asian Elephants.

Academic Form NO: 19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Faculty of College of Veterinary and Animal Sciences

PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

(Vide Rule 25(b) of Post Graduate Regulations 1988)

Title of Thesis: Characterisation of Mono Amine Oxidase A (MAOA) gene and transcript from Asian elephant *(Elephas maximus)*: A possible candidate for risk taking behaviour.

1. **2a. Title of departmental/KVASU research project of which this forms a part**

   Centre for Wildlife Studies

   2b. Code No. if any, and order by which the department/KVASU research project is approved

   Nil

   3a. Name of the student

   Jishnu M

   3b. Admission No.

   14-02MS-003

   4a. Name of Major Advisor

   Dr. Arun Zachariah

   4b. Designation

   Assistant Professor

   KVASU-Centre for Wildlife studies

   College of Veterinary and Animal Sciences, Pookode , Wayanad.

5. Objectives of the study

1. Identification and characterisation of *MAOA* gene in Asian Elephant (*Elephus maximus*).
2. Identification and characterisation of *MAOA* transcript in Asian Elephant (*Elephus maximus*).
3. to investigate the probable role of *MAOA* gene in Asian Elephant (*Elephus maximus*).

6. Practical/Scientific utility

Human – elephant conflict is of greater importance in forest adjoining areas of India and

elephant inhabiting countries throughout the world. Crop raiding by elephants is observed to be a major issue among the human wildlife conflict. It was observed that all elephants are not raiding crops; instead some elephants are tending to be habituated with crop raiding. However there is a pattern observed in the conflicting elephants. Crop raiding is observed to be sex biased in which Males tend to do crop raiding than females. Within the male elephants sub adult and adult males are the major crop raiders. Solitary animals are the frequent crop raiders as well.

The advantage of crop raiding is basically to develop the body mass and help the male to male competition for its reproductive success. Better palatability and enhanced nutrition from the cultivated crops can provide high levels of nutrition than the natural forages. This enables them to develop the body mass. It is observed that the crop raiding elephants having longer period of musth than other non crop raiders. The males with large body size and longer period of musth are preferred by females. Ultimately the crop raiding provides access to oestrous females and reproductive success. However certain risk involve in the crop raiding. They are vulnerable to human interventions to safe guard their crop. It includes shooting, poisoning, electrocution and application of other malicious activities.

It was observed that some elephants are tending to take the risk and they were rewarded with reproductive success.

In this project we are focussing on the risk taking behaviour in elephants by elucidating genetic background for the same. While looking in to risk taking behaviour in mammals particularly humans, Finland researchers have identified few genes (*MAOA* and CDH13) associated with extreme violent and risk taking behaviour in humans. Moreover, the *MAOA* gene knock out experiments in mice (as spontaneously occurs in some humans), causing lack of function of the corresponding gene, showed the contribution of some of its alleles to impulsive

aggressiveness. We also hypothesized that there could be a genetic basis behind the risk taking behaviour in elephants.

Important publications on which the study is based

Patrick and Erica (2005) conducted a study on elephants of Kibale National Park; Uganda suggested that male elephants are the predominant crop raiders.

Poole (1989) said that male elephants raiding on palatable, nutritious, digestible food would have longer period of musth while other energy less males will have shorter musth. Males with larger body size are preferred by females for mating.

Sukumar and Gadgil (1988) hypothesised that high risk high gain strategy in male elephants to take a high risk to enter cultivation land which provide highly nutritious food which will help in the future for more young ones and the inheritance of their genes to next generation.

Chiyo P.I. *et al.* (2011) suggested that the crop raiding is a risk taking which can cause up to death but it will help to attain body weight. Crop raiding enables elephants to grow large for their age.

Sukumar (1990) pointed that the males of a polygynous species will take risk for high gain which lead them to satisfy the selection and also for reproductive success.

David Beuno (2010) suggested that some genes and its variants have particular effects on aggressive behaviour.

Nina.K.Popva (2006) observed that the genes which produce enzymes for serotonin (5HT) metabolism like TPH2, MAOA, SERT and 2 types of serotonin receptors 5HT1A and 5HT1B are responsible for the genetic basis of aggressive behaviour. The decrease in the bio synthesis of serotonin (5HT) increases the male to male aggression in mice.

The elimination of *MAOA* gene in mice resulted in an increase aggression and this event also occurs spontaneously in humans (Scott *et al*. 2008).

Joshua.W.B. and Andreas M.L. (2008) observed that *MAOA* susceptible alleles alter the structure and function of brain which in turn induces anti social or aggressive behaviour when it combines with other factors.

Brunner H.G et al. (1993) observed that a point mutation in the coding gene of *MAOA* caused increased aggressive and violent behaviour in the males of Dutch family.

1. Outline of the technical programme

The identification and characterisation of *MAOA* gene will be deduced from the DNA isolated from the absolute alcohol preserved elephant tissue samples by Next Generation Sequencing technique.

Initial characterisation of MAO A gene transcript will be done using total RNA isolated (Qiagen, RNAeasy mini kit, Germany) from RNA Later (Invitrogen, Bangalore) preserved elephant tissue. Intron-exon boundaries of MAOA gene transcript will be deduced using ensemble *Loxodonta africana* genome data base.

The isolated transcripts will be correlated with the individual profile of the dead elephant. Finally the sequence variation if any will be correlated with the behaviour of the elephants from which the samples has been collected.

2. Main items of observation to be made:

i. DNA isolation.
ii. RNA isolation.
iii. MAO A gene transcript.
iv. MAO A intron – exon boundaries.

10. Facilities

Existing: All facilities available in KVASU Centre for Wildlife Studies and the facilities in Centre for Wildlife Research and Forensics, Department of Forest and wildlife, Wayanad Widlife sanctuary and SciGenom Research foundation, Kakkanad, Kochi.

Additional facilities required: Nil

11. Duration of study: One Semester

12. Financial estimate:

Head	Jan-Jun	Total
Travel (hiring of vehicle for forest field surveys and bus charges)	4000	4000
Chemicals and consumables	39000	39000
RNA & DNA Isolation Kits		30000
Sequencing charge	40000	40000
Total		113000

Total- Rs. 1,13000

13. Signature of student

14. Signature of Major Guide

Place:

Date:

15. Name, designation and signature of members of the Advisory Committee Members

1. Dr. Arun Zachariah

(Major Guide)

Assistant Professor

KVASU-Centre for Wildlife studies

College of Veterinary and Animal Sciences, Pookode.

2. Dr. Abdul Azeez (Member)

Assistant Professor

KVASU-Centre for Wildlife studies

College of Veterinary and Animal Sciences, Pookode

Appendix I

References

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Appendix II

Time frame of Work

Semester I

1. Collection of literature

Semester II

1. Collection of literature

Semester III

1. Collection of literature
2. Review of literature
3. Planning of programme of research work
4. Preparation of synopsis

Semester IV

1. Analysis of results
2. Interpretation of results
3. Preparation of thesis and submission

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animals Act (Amendment, 1998)

Place

Date

Dr. Arun Zachariah

Guide