Title: Report on “Advanced Laboratory Skills in Field Biology” Course
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Abstract

The androgen receptor (AR) gene, plays a critical role in male phenotypes and behavioral patterns, particularly aggression. This study investigates the polymorphic variants of the AR gene in six participants, correlating genetic data with a chimpanzee personality test and a human personality test (TCI). The experiment also explores alcohol tolerance of the participants by genotyping the SNP types of ALDH2 gene and conducting a patch test. During this laboratory course, we collected buccal cell samples from 6 participants and genetically analyzed them using various methods including DNA extraction, PCR, Agarose gel electrophoresis, and sequencing. Additionally, we requested the participants to take multiple personality tests designed for human and non-human primates to evaluate the reliability of our genetic data. Our results indicate that the male participants, possessing one X chromosome, were homozygous in glutamine and glycine regions of the androgen receptor gene. Females on the other hand, with two X chromosomes, exhibited heterozygosity. Also, among the participants, shorter repeat numbers correlated with lower dominance and harm avoidance, while acquiring a higher neuroticism score in the Chinpanzee personality trait assessment. Alcohol tolerance, assessed by patch test and SNP detection of ALDH2 gene in buccal cell samples using PCR and agarose gel electrophoresis revealed that all participants, except one male individual, exhibited strong alcohol tolerance.

Introduction

The androgen receptor (AR) is a nuclear hormone receptor that is expressed in almost every tissue of the body [1], and has a significant function in the development of male phenotypes [2,3]. The gene encoding this receptor, which is also known to have an influence on behavioral patterns in terms of aggression [2], has eight exons in human [5] one of which contains microsatellite repeat regions that code for poly-glutamine and poly-glycine (hereafter referred to as poly-Q and poly-G respectively). These two regions are reported to be polymorphic in their length in both human and non-human primates [1]. Multiple studies have been done on the correlation between the polymorphism in poly-Q and poly-G sequence repeat number and personality traits including agression in human and other animal species; For instance, an study carried out by Konno and colleagues (2011) on Japanese Akita Inu dogs [6] suggests the existence of a link between the length of AR gene and aggression. According to the results of this study, male dogs with higher score in aggressiveness (based on a questionnaire taken by the dog's owners) had a shorter allele of poly-Q (CAG repeat region), which results in more activity of AR.

In this experiment, we investigated the AR gene polymorphism in six participants, ans compared the genetic data collected with the result of a personality test designed for chimpanzees [7,8], which is titled "Chimpanzee Personality Trait Assessment", to predict the participants' personality traits. We also asked them to take the TCI test [9] in order to analyze the reliability of our evaluations of personality their personality traits.

It has been reported that approximately 50% of East Asian people [10,11] experience severe reactions in case of alcohol consumption [12], due to a deficiency in their class 2 mitochondrial aldehyde dehydrogenase (ALDH2) activity. ALDH2 is an enzyme consisting of four sub-units, which is responsible for metabolyzing the acetaldehyde that is formed as a result of ethanol oxidation in the liver [10]. A single point mutation in exon 12 of ALDH2 coding region can lead to a dramatic reduction in its activity. As a result of this mutation, one code changes from G to A and consequently, glutamine substitutes lysine [13]. Deficiency in this enzyme not only causes unpleasant reactions to consuming alcoholic drinks, it also carries a significant risk of drinking-related cancers [11]. This defective variant could be genotyped by detecting the single nucleotide polymorphism (SNP) types in human genome [14]. During this laboratory course, and in addition to investigating the relationship between emotions and AR coding sequence variations, we also checked the alcohol tolerancy of the participants through a combination of genotyping the ALDH2 gene and performing patch test.

Methods

- **Androgen Receptor Genotyping**

Preparations Before and After the Experiment: Before and after each experimental session, the surface of the tables, tube stands, and other equipments were cleaned using first H2O and then ethanol.

Sampling: To conduct this experiment, buccal cells of the participants was collected by rubbing a cotton bud on the buccal mucous membrane of the inside of the cheeks. To collect enough cells for this experiment, the cotton bud was rubbed 10 times on the inside of each cheek. Then, the cutton bud was washed in 1000µl saline (0.9% NaCl) inside a 1.5ml tube. In the next step, the solution containing buccal cells was centrifuged at 14000rpm for 1 minutes and 30 seconds, 800µl of supernatant was discarded, and 100-200µl of the remaining solution was used for DNA extraction. In total, 6 samples were prepared.

DNA Extraction: Students were divided into two groups of three, therefore, the measurements were prepared for three samples. 110µl saline and 22µl protease per sample were mixed. Then, 120µl of the solution containing cells was added to the mix and everything was vortexed together. Next, 200µl of Buffer AL was added and mixed by vortexing. The mix was then incubated at 56°C for 10min, vortexed, and spinned down. After adding 200µl of ethanol and mixing, 700µl of the solution was added to a spin column and centrifuged at 14000rpm for 1min. Then, the spin column was placed in a new collection tube, 500µl of Buffer AW1 was added and the whole mix was centrifuged at 8000rpm for 1min. The spin column was placed in a new collection tube, 500µl of Buffer AW2 was added and the whole mixture was centrifuged at 14000rpm for 4min. This longer period of centrifuging removes the remaining ethanol that was added previously, which could later inhibit the PCR process. After that, the spin column, now containing the DNA, was placed in a new 1.5ml tube, 50µl of H2O (previously incubated at 56°C) was added directly to the center of the column and the tube was incubated at 56°C for 5min. Next, the mixture was centrifuged at 8000rpm for 1min to separate the DNA from the filter inside the spin column, the column was discarded and the DNA was stored at 4°C.

Measuring DNA Concentration: To Nanodrop Spectrophotometer to measure the extracted DNA's concentration first, the device was calibrated by putting 1µl of either H2O or buffer on the device's sensor and running the analysis. Then, the surface was lightly wiped dry. Before puting the target DNA on the sensor for measurement, we tapped the tube to mix the DNA and then it was spinned down. Next, 1µl of DNA was placed on the nano drop sensor and the concentration was measured. After finishing measuring the DNA concentration of all 6 samples, the sensor was cleaned in the same way described previously.

PCR: To create the PCR mixture, the following materials were mixed in a 1.5ml tube under a clean bench, and 9µl of it was distributed into PCR tubes:

Table 1: PCR mixture measurements per sample.

Material	Measurement
2x GCI Buffer	5.0µl
H2O	1.8µl
sNTP	1.6µl
*Primer1 (20µl)	0.25µl
**Primer2 (20µl)	0.25µl
LA Taq	0.1µl
Template DNA	1.0µl
PCR mixture + Template DNA	10.0µl

***Primer1 sequence:** [ARhFF: 5'-TCCAGAATCTGTTCCAGAGCGTGC-3' (FAM labeled); ARhR: 5'-GCTGTGAGGGTTGCTGTTCCTCAT-3' ; Fragment size: around 280bp]; This primer is designed for glutamine (Q) repeat.

****Primer2 sequence:** [ARGFH: 5'-CAGTGCCGCTATGGGGACCTGGCGA-3' (HEX labeled); ARGR: 5'-GGACTGGGATAGGGCACTCTGCTCACC-3' ; Fragment size: around 320bp]; This primer is designed for glycine (G) repeat.

1µl of template DNA was later added to the tubes under a clean bench, and we ran the PCR reaction with the following condition: **PCR reaction (LA60C30): 95°C 2min (95°C 30s, 60/65°C 30s, 74°C 1min) x30/35, 74°C 10min, 10°C.**

Genotyping: Firstly, we diluted the PCR product by adding 100µl water and 1µl PCR product per well in a new 96 well plate, and then we spinned down the plate. In the next step, we made a mixture of Hi-Di Formamide and size standard (For 16 samples: 160µl Hi-Di Formamide and 1µl size standard). Hi-Di Formamide is a buffer that prevents H2O from evaporating when it is exposed to electricity during the sequencing process. Next, 10 µl of the mixture was added to a new 96 well plate, and 1µl of the mixture of water and PCR product that was prepared in the beginning was added to each well in the plate. The samples were heated 95°C for 5 min in order to denature the double-stranded amplicons and separate the strands, and then they were immediately placed on an ice box for 5 min. Before sunning the sequencer, we input samples' data to

the plate manager to associate them with the results of the sequencing, and next, we run the sequencer.

To have an efficient data to further investigations, we filtered the data output by choosing specific peaks to be printed out. In other words, if there were two peaks next to each other, one shorter than the other, we removed the shorter one if it was as high as 50% of the other.

Repeat Number Calculation: Based on Table 2, the repeat numbers for each region were calculated. Firstly, the sequence lengths were rounded up/down (considering the fact that 3bp is required to create an amino acid code). Then, Since each amino acid code consists of 3bp, for every additional 3bp in the allele sequence, 1 number was added to the repeat number.

Table 2: The repeat number of ARG and ARQ based on their sequence length

Name of The Sequence	Sequence Length	Repeat No.
ARQ	273	23.00
ARG	314	17.00

ARQ: Poly-Glutamine; **ARG:** Poly-Glycine

Dominance, Neuroticism: To calculate these factors, we used the data reported by Weiss *et al*. (2009), which is provided in Figure 1, as a reference and first selected all the items related to each factor:

1. **Dominance:** Dominant, Persistent, Stable, Stingy/Greedy, Timid, Bullying, Aggressive, Manipulative, Inquisitive, Submissive, Dependent/ Follower, Decisive, Depressed, Defiant, Intelligent, Disorganized, Independent
2. **Neuroticism:** Fearful, Stable, Autistic, Excitable, Depressed, Clumsy, Erratic, Unemotional, Imitative)

Then, based on Figure 1, we changed the chimpanzees' and our scores of the test to positive and negative scores and summed the numbers of all the items in each category, divided them by the number of the items, and reported the final number in Tbale 6.

Figure 1: Structure of Chimpanzees in Japan Rotated to Structure from King and Figueredo (1997) [7]

	Factor						
Item	D_{CH}	E_{CH}	C_{CH}[a]	A_{CH}	N_{CH}[a]	O_{CH}	Item Cong.
Dominant	**0.82**	0.04	−0.30	0.02	0.18	−0.15	0.94
Submissive	**−0.78**	−0.13	−0.01	0.23	−0.16	0.28	0.89
Dependent	**−0.68**	0.20	−0.15	0.06	−0.22	−0.01	0.95
Independent	**0.43**	−0.29	−0.21	−0.10	−0.31	0.17	0.80
Fearful	−0.39	−0.04	0.05	−0.03	**0.74**	0.19	0.72
Decisive	**0.55**	−0.08	−0.01	0.36	−0.20	**0.45**	0.78
Timid	**−0.59**	**−0.41**	**−0.40**	0.04	−0.03	0.07	0.66
Cautious	−0.25	−0.30	0.29	0.22	−0.04	**0.51**	0.72
Intelligent	**0.47**	0.14	−0.01	**0.54**	−0.07	**0.46**	0.93
Persistent	**0.56**	0.29	−0.28	0.15	−0.03	0.35	0.98
Bullying	**0.57**	−0.12	**−0.59**	−0.19	0.03	−0.05	0.96
Stingy	**0.52**	−0.14	**−0.46**	−0.19	0.10	0.07	0.87
Solitary	−0.26	**−0.77**	−0.08	−0.05	−0.14	0.23	0.94
Lazy	−0.15	**−0.71**	−0.05	0.22	−0.34	−0.28	0.91
Active	−0.01	**0.71**	**−0.43**	−0.07	−0.04	0.26	0.97
Playful	−0.07	**0.71**	−0.19	0.04	0.03	**0.50**	0.88
Sociable	−0.04	**0.63**	0.08	**0.58**	0.02	−0.06	0.94
Depressed	**−0.42**	**−0.44**	−0.03	−0.13	**0.53**	0.18	0.70
Friendly	−0.42	0.22	0.32	**0.56**	−0.25	−0.06	0.77
Affectionate	−0.04	0.38	0.05	**0.72**	0.16	−0.08	0.86
Imitative	−0.36	**0.44**	−0.26	0.17	**−0.40**	0.28	0.88
Impulsive	−0.08	−0.25	**−0.72**	−0.02	0.27	0.12	0.85
Defiant	**0.55**	−0.01	**−0.69**	−0.14	−0.11	0.00	0.96
Reckless	−0.12	0.08	**−0.76**	−0.17	−0.17	−0.15	0.86
Erratic	−0.32	−0.18	−0.28	−0.06	**0.66**	0.10	0.65
Irritable	0.34	−0.24	**−0.68**	−0.12	0.25	0.05	0.95
Predictable	0.19	**−0.44**	0.23	0.31	−0.27	−0.07	0.77
Aggressive	**0.59**	0.01	**−0.68**	−0.13	0.04	−0.03	0.96
Jealous	0.33	0.06	**−0.68**	−0.04	0.07	0.11	0.88
Disorganized	**−0.40**	−0.14	**−0.71**	0.10	−0.19	−0.13	0.80
Sympathetic	0.09	0.30	0.19	**0.81**	0.12	−0.20	0.86
Helpful	0.29	0.28	0.02	**0.74**	0.11	−0.18	0.77
Sensitive	0.28	0.00	0.04	**0.63**	−0.10	**0.41**	0.80
Protective	−0.03	0.12	−0.02	**0.69**	−0.21	−0.04	0.84
Gentle	−0.23	0.13	0.37	**0.74**	−0.14	−0.10	0.91
Stable	0.28	0.05	**0.40**	0.28	**−0.44**	0.09	0.92
Excitable	0.00	−0.32	**−0.63**	0.11	**0.41**	0.00	0.81
Unemotional	−0.12	−0.33	−0.04	**0.41**	**−0.62**	−0.22	0.74
Inventive	−0.06	**0.48**	−0.13	0.14	−0.18	**0.69**	0.92
Inquisitive	0.03	**0.48**	−0.11	0.11	−0.03	**0.72**	0.97
Manipulative[b]	**0.66**	0.04	−0.38	0.25	0.02	0.10	0.87
Clumsy[b]	−0.23	−0.40	**−0.52**	0.10	**−0.50**	−0.17	0.76
Autistic[b]	−0.28	−0.18	−0.20	0.10	**0.57**	0.05	0.66
Factor Cong.	0.89	0.91	0.89	0.89	0.69	0.71	0.85

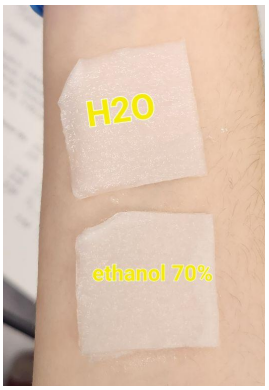
[a]Loadings have been reflected.
[b]Items not included in the factor analysis presented in King and Figueredo (1997). D_{CH} = Dominance; E_{CH} = Extraversion; C_{CH} = Conscientiousness; A_{CH} = Agreeableness; N_{CH} = Neuroticism; O_{CH} = Openness; Item Cong. = Item Congruence Coefficient; Factor Cong. = Factor Congruence Coefficient. Absolute loadings ≥ 0.40 are indicated in boldface.

- **ALDH2 Genotyping**

Sampling: DNA amplicons of the PCR reaction for the previous experiment was used in ALDH2 genotyping.

Patch Test: Two pieces of 1.5cm square cut paper towels were soaked in water and 70% ethanol separately. The patches were next placed on the inside of participants' arms (Figure 2) for 10min, and 10min after their removal, the skin was observed for redness in the point of touch with the alcohol patch. A red skin would indicate lower alcohol tolerancy. The H2O patches were used as control.

Figure 2: H2O and ethanol patches on the inside surface of arms.



PCR: PCR: To create the PCR mixture, the following materials were mixed in a 1.5ml tube outside of a clean bench, and 8µl of it was distributed into PCR tubes:

Table 1: PCR mixture measurements per sample.

Material	Measurement
AmpliTaq Gold 360 Master Mix	5.0µl
H2O	2.5µl
*F Primer	0.25µl
R/*R2 Primer	0.25µl
Template DNA	2.0µl
PCR mixture + Template DNA	10.0µl

***F Primer sequence:** (ALDH F): CAAATTACAGGGTCAACTGCT; This primer is designed for both Glu and Lys.
****R Primer sequence:** (ALDH R): CCACACTCACAGTTTTCACTTC; This primer is designed for Glu.
****R Primer sequence:** (ALDH R): CCACACTCACAGTTTTCACTTC; This primer is designed for Lys.

2µl of template DNA was later added to the tubes under a clean bench, mixed with vortex, and spinned down before running the PCR reaction with the following condition: **PCR reaction (GT65C35): 95°C, 9:00 (94°C, 0:30; 65°C, 1:00; 72°C, 1:00) 35 cycles 72°C, 5:00.** In this thermal cycle, in addition to the 6 samples previously collected, 2 control DNA templates were also amplified.

Agarose Gel Electrophoresis: We first prepared a 1.5% agarose gel by measuring 60ml of 1xTBE, and adding 0.9g of agarose powder to it until the mixture weighed 60g. Then, we dissolved the powder in 1xTBE by heating it in the microwave for 1 to 2min. While heating, some of the mixture evaporates, therefore, we added H2O to both restore the original volum and cool down the liquid to 60°C. We then placed the comb in the gel tray and poured the mixtures in it to harden for 30min. Before removing the comb and running the PCR amplicons in the gel, we added a smal amount of 1xTBE to avoid damages to the wells and gel. In the next step, we filled the electrophoresis tank with 400ml of 1xTBE, and put the gel in it. To load the samples in the gel, we put 1µl of dye to a piece of parafilm stuck on the table, added 6µl of PCR products to it and pipeted them together before loading everything to the wells. We also loaded a mixture of 1µl dye with 3µl size marker in the middle well. We ran the electrophoresis at 100V for 20min until the dye moves to the middle of the gel. Later, to preserve the data, we took a photo of the gel on the UV Transilluminator (Figure 2).

Results

DNA Concentration: Nanodrop Spectrophotometer device was used to check whether each sample's DNA concentration was desirable. The DNA concentration for all of the samples is shown in Table 3. For all of the samples, an increase was observed between 250nm and 280nm. Moreover, the 260/280 factor for sample number 3 was 1.91.

Table 3: DNA concentration measured by nanodrop spectrophotometer

Sample No.	DNA Concentration
1	38.1
2	17.1
3	9.1
4	13.7
5	19.0
6	21.7

Genotyping: The androgen receptor gene was sequenced in the regions encoding glutamine and glycine amino acids to check their hetrezygocity/homozygocity. Since this gene is placed on the X chromosom, it only had one allele for each of the amino

acids in the male participants. In other words, all of the male samples were homozygote for both glutamine and glycine. On the other hand, three out of four female samples were heterozygote in glutamine region, while one of them was homozygote. The results of the female samples for the glycine region were the opposite, meaning that only one female was heterozygote and the four other female individuals were homozygote (Table 4). Based on Table 2, repeat numbers of each sample in the Glutamine and Glycine alleles were calculated, and the results are shown in Table 5.

Table 4: Genotyping results for glutamine and glycine region in androgen receptor gene

Sample No.	Sex	ARQ1	ARQ2	Heterozygote /Homozygote	ARG1	ARG2	Heterozygote/ Homozygote
1	F	279.11	284.86	Hetro	323.20	331.11	Hetro
2	M	276.42		Homo	331.20		Homo
3	F	276.71	276.71	Homo	331.27	331.27	Homo
4	F	279.40	282.20	Hetero	331.16	331.16	Homo
5	F	276.61	288.00	Hetro	331.26	331.26	Homo
6	M	276.29		Homo	331.33		Homo

F: Female; **M:** Male; **ARQ:** Glutamine; **ARG:** Glycine

Table 5: Repeat numbers of ARQ and ARG for 6 samples

Sample No.	ARQ1	ARQ2	ARG1	ARG2	ARQ1 Repeat No.	ARQ2 Repeat No.	Average Repeat No. for ARQ	ARG1 Repeat No.	ARG2 Repeat No.	Average Repeat No. for ARG
1	279.00	285.00	323.00	332.00	25.00	27.00	26.00	20.00	23.00	21.50
2	276.00	—	332.00	—	24.00	—	24.00	23.00	—	23.00
3	276.00	276.00	332.00	332.00	24.00	24.00	24.00	23.00	23.00	23.00
4	279.00	282.00	332.00	332.00	25.00	26.00	25.50	23.00	23.00	23.00
5	276.00	288.00	332.00	332.00	24.00	28.00	26.00	23.00	23.00	23.00
6	276.00	—	332.00	—	24.00	-	24.00	23.00	—	23.00

Additionally, the average number between ARG 1 and 2, and ARQ 1 and 2 was calculated (Table 5). This number was later used to put the samples into two groups of short and long sequence length. Based on this result, samples 2, 3, and 6 were reported short, whereas samples 1, 4, and 5 were reported to be in the long group (Table 7). Moreover, the dominance, neuroticism, and harm avoidance indices were calculated for the 6 participants of this experiment, using the Chimpanzee Personality Assesment (Table 6). Then, the average number of these indices in each group were calculated and their relevance to the sequence length was investigated (Table 6). Based on the results, individuals with a shorter sequence length are less dominant and avoidant of harmful situation, and have a higher neuroticism index compared to the individuals with a higher sequence length.

Table 6: Dominance, Neuroticism, and Harm avoidance index of human samples based on the Chimpanzee Personality Assessment and TCI test.

Sample No.	Dominance	Neuroticism	Harm Avoidance
1	2.00	-0.33	80.00
2	2.18	-0.33	Not reported
3	1.35	0.44	49.00
4	2.53	-0.11	40.00
5	2.12	-0.22	40.00
6	2.00	-0.44	68.60

Table 7: The average number of Dominance, Neuroticism, and Harm avoidance and their relevance to the sequence length.

Sequence length	Sample No.	Dominance	Neuroticism	Harm avoidance
Short	2,3,6	1.84	-0.11	39.20
Long	1,4,5	2.22	-0.22	60.00

To investigate the alcohol tolerance of the participants, two seperate experiments were done and their results were aligned to determine whether the participant have low or high alcohol tolerancy. Firstly, we surveied the participants' alcohol tolerance using patch test; 2 male and 3 females. For this stage, we only tested 5 individuals since one of the participants was absent for this laboratory session. Among the five individuals, only one male participant developed redness in part of the skin in contact with alcohol patch (Table 8). The rest of the participants showed no change in their skin color after being exposed to 70% ethanol. Among the people with negative results in the patch test, 25% were male (one individual) and 75% were female (three individuals).

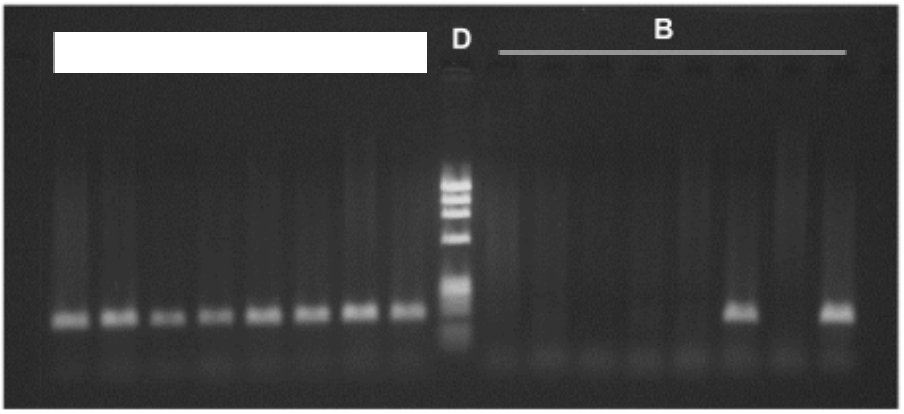
Table 8: Patch test results and the electrophoresis results of the presence/absence of Glu and Lys coding sequences for investigating alcohol tolerance.

Sample No.	patch test	Glu	Lys	Alcohol tolerance
1	−	+	−	Storong
2	−	+	−	Storong
3	−	+	−	Storong
4		+	−	Storong
5	−	+	−	Storong
6	+	+	+	Medium

In the next step, DNA samples extracted from participants' buccal cells were genotyped in the ALDH2 gene, looking for SNP in the Glu and Lys coding regions. The result of running the DNA samples on Agarose Gel Electrophoresis (Figure 3) shows that all of the samples contained the Glu coding sequence, while only sample No. 6 had the coding sequence of Lys. Based on this result, All of the participants had strong alcohol tolerance, except for sample 6 (Male participant) who had a medium alcohol tolerance (Table 8).

TCI Test: Since the TCI (Temperament and Character Inventory) test was available in Japanese at the time of experiment, I took the IPIP equivalent to TCI test [15]. The test results are shown on Table 9.

Figure 3: The result of gel electrophoresis detecting the presence of Glu and Lys.



A: Amplicons of Glu (From left to right: samples 1, 2, 3, 4, 5, 6, *Control 1, **Control 2); **B:** Amplicons of Lys (From left to right: samples 1, 2, 3, 4, 5, 6, Control 1, Control 2); **C:** Size marker.
*The combined results of control 1 indicates a person with strong alcohol tolerance (Glu: +/ Lys: –).
**The combined results of control 2 indicates a person with medium alcohol tolerance (Glu: +/ Lys: +).

Table 9: IPIP equivalent to TCI test results for participant No.3.

Personality Domain	Score
Idealistic	32
Novelty seeking	63
Harm avoidance	49
Reward dependence	47
Persistence	56
Self-directedness	70
Cooperativeness	61

Discussion

In this experiment, all of the DNA samples extracted from male participant were homozygote in both glutamine and glycine regions. This happens as a result of the androgen receptor gene being located on the X chromosome [4], and since male individuals are XY, they only possess one copy of this gene. Therefore, they can only be homozygote for our target regions on the androgen receptor gene. On the other hand,

samples collected from female individuals could be either homozygote or heterozygote in the glutamine and glycine regions, because they are XX and have two copies of the androgen receptor gene. In our study, 75% of the female samples were heterozygote in glutamine, while only 25% of them were heterozygoete in glycine region. Moreover, individuals with shorter repeat numbers in the glutamine and glycine regions, had lower dominance factor and harm avoidance index, with a lower neuroticism. On the other hand, individuals with a longer repeat number in the target regions were more dominant and were expected to avoid harm more than the other group, while being more neurotic (based on the Chimpanzee Personality Assessment). In previous studies[8], it is mentioned that the shorter sequence length of Poly-Q is associated with more aggression in male dogs, and they also reported no evidence of a correlation between AR sequence length and aggression in female dogs. However, in our experiment, the group with short AR coding sequence consisted of both male and female individuals, and according to their average dominance factor, they are less aggressive compared to the group with a longer sequence length(Table 7). For future experiments, I suggest the use of a personality assessment test designed for humans. Since the personality test we used in this study was originally designed for chimpanzees, it could decrease the accuracy of the experiment when comparing the test results with genotyping data. For instance, while taking the test myself, I had difficulty interpreting some of the questions from how a chimpanzee would behave to their human counterpart.

In the second part of our experiment, we investigated the alcohol tolerance of the participants by combining the results of two methods; Firstly, we conducted the patch test to check the participants' alcohol tolerance through observing their skins' reaction to being exposed to alcohol. The result of this test aligned perfectly with what was determined through gel electrophoresis, showing that 17% of the participants (1 male) had medium tolerancy, while 83% of them (4 female, 1 male) were strongly tolerant to alcohol.

References

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