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Preliminary association analysis of microsatellite polymorphism with body weight in Swiss albino mice population

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Abstract
Laboratory mice have been widely utilized in various biological researches worldwide due to their high degree of homology with humans. Inbred and outbred strains of mice are the two major classes of laboratory mice. Microsatellites remain highly informative and useful measures of genomic variation for linkage and association studies. The objective of this study was to investigate the association of microsatellites polymorphism with body weight in two different generations of Swiss albino mice population. Body weight (BW) was recorded individually for all the mice when mice from each generation reached at the age of around 2 and half months. The association with the body weights was evaluated by using ten microsatellites markers. All the microsatellite markers were found to be polymorphic. Effect of allelic variants of microsatellites (genotype) was determined on BW through PROC GLM module of SAS. The result reveals that two microsatellite loci (D9Mit27 and D10Mit180) showed a significant association with body weight of the mice. Although the present study should be considered preliminary, our results reveal for the first time that two microsatellite loci were associated with body weight traits in mice.

Keywords: Swiss albino mice, microsatellites, association, body weight

1. Introduction
Mice are common experimental animals in laboratory research of genetics, biology, psychology, medicine and other scientific fields primarily because they are mammals and also they share a high degree of homology with humans. Around 50-100 million vertebrates are used annually for research purposes but out of the total different species used, 53% is contributed by the mice globally. The reason behind wide use of mice are that, in particular, mice produce numerous offspring in a short period of time, are small and easily maintained, their genome has been sequenced and most importantly they are genetically similar to humans. Two major classes of laboratory mice include inbred and outbred strains. The first defines as a genetically homologous strain which the heterogeneity in the genome is <1% among the colony. This strain is produced by mating within a family over at least 20 generations. Due to this procedure, many genetic variants become fixed, and a specific and reliable genetic background is created in the colony. The use of such inbred mouse strains could decrease experimental variations and increase reproducibility and repeatability of *in vivo* tests.
Marker assisted selection (MAS), which combines traditional genetics and molecular biology has become a valuable tool in selecting animals for traits of interest *viz*. body size, fertility, tame behaviour and disease resistance. Microsatellites, or short tandem sequence repeats (STRs), are highly polymorphic repetitive DNA sequences 1–6 base pairs (bp) in length (Weitzmann *et al.*, 1998) [6] that are randomly distributed throughout eukaryotic genomes (Tautz and Renz, 1984) [5] and were discovered in the 1990s. During the 1990s and the first several years of this century, microsatellites were the workhorse genetic markers for hypothesis-independent studies in human genetics, and it is important to note that microsatellites remain highly informative and useful measures of genomic variation for linkage and association studies. Their continued advantage in complementing SNPs (single nucleotide polymorphisms) lies in their greater allelic diversity than biallelic SNPs. In fact, microsatellites have starred in association studies leading to widely replicated discoveries of type 2 diabetes (TCF7L2) and prostate cancer genes (the 8q21 region) (Gulcher, 2012) [2].
The information from the DNA testing combined with the observed performance records for

mice could improve the accuracy of selection and increase the possibility of identifying individuals carrying desirable traits at an earlier stage of breeding. The association of microsatellite marker genotype with the body weight of mice could be helpful for selection of mice for breeding in later generations. The present investigation was therefore undertaken to estimate the association of microsatellites with body weight in the Swiss albino mice population.

2. Materials and Method

2.1 Sampling and DNA isolation

Swiss albino mice maintained at Laboratory Animal Research (LAR) Section of Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar were used for the present study. Two generations of mice population were used for the study. F_0 stock was the foundation mice (outbred) population and F_1 inbred mice population were the first generation inbred mice, produced by full sib mating of the F_0 mice. Body weight (BW) was recorded individually for all the mice when mice from each generation reached at the age of around 2 and half months. Tissue collection was done by taking approval from Institute Animal Ethics Committee (IAEC) of ICAR-IVRI, Izatnagar. Tail tissues (1-1.5 cm in size) were randomly collected for DNA extraction; from 100 mice each of F_0 as well as F_1 inbred Swiss albino mice. Genomic DNA extraction was done from the tail tissue of mice using DNA Isolation kit (Qiagen DNeasy Blood & Tissue Kit) as per the manufacturer instructions. DNA concentration and purity (A260/A280 ratio) for each sample was assessed using a spectrophotometer and by 0.8% agarose gel electrophoresis containing 0.5 µg/mL ethidium bromide, and visualizing the samples under the UV light. The measured DNA samples were stored at –20°C until further analysis.

2.2 Polymerase chain reaction amplification and microsatellite genotyping

Ten microsatellites loci (D1Mit15, D2Mit51, D2Mit61, D3Mit55, D5Mit18, D7Mit323, D8Mit14, D9Mit27, D10Mit180 and D11Mit167) were employed based on good polymorphisms having been reported. Primer sequences of the microsatellite loci were used according to the Mouse Locus List (http:// www.informatics.jax.org). Selective amplification of different microsatellite markers was kept constant for all reactions and was performed using the thermocycler (Bio-Rad, USA). The PCR mixture contained 50 ng genomic DNA, 10 ng of each primer, 0.2 µM dNTPs mixture, 1X of 10X Taq buffer with KCl, 1.5 mM Magnesium chloride, 1.0 unit of Taq DNA polymerase (1U/µl) and nuclease free water to make the final reaction volume of 25 µl. The optimization of appropriate annealing temperature with respect to each primer was determined by gradient PCR. PCRs were performed in a programmable thermocycler with the following protocol: 95°C for 5 minutes; followed by 40 cycles of 95°C for 1 min, desired primer annealing temperatures for 45 seconds and 72°C for 1 minute; with a final extension step of 72°C for 5 minutes. Amplicons of the different microsatellite markers was confirmed by running the PCR products on ultra-high-resolution agarose (4%; at 70-80 V; for 3-5 h) to differentiate alleles as per their length (in bp) and visualizing under UV(ultra violet) rays using gel documentation system (Genesnap, Syngene) system.

2.3 Statistical analysis

The size of allele of microsatellite markers was determined by using the software Gel Analyzer (2010). The program POPGENE (Version 1.31) was used to determine the polymorphism information content (PIC), number of alleles at each locus, allelic frequency, effective number of alleles reflecting the interrelationship among different alleles, and observed heterozygosity (H_0).

The association between various allelic variants of microsatellites with body weight of mice from two generation (F_0 and F_1 inbred) was analyzed using different procedures of SAS 9.3.Effect of allelic variants of microsatellites (genotype) was determined on BW through PROC GLM module of SAS 9.3 using following model:

$$y_{ij} = \mu + g_i + e_{ij}$$

Where, y_{ij} = observation of BW on j^{th} mouse in i^{th} genotype; μ = overall mean; g_i = effect of i^{th} genotype; e_{ij} = random error ~ NID (0, e^2).

3. Results and Discussion

In total, 34 alleles were observed for the 10 microsatellite loci analysed in both generations. Complete lists of the allelic frequencies for each microsatellites marker are given in Table 1. A fairly typical amount of polymorphisms for the mice populations was discernible from the allele frequency data. The total number of alleles per locus ranged from 3 (D2Mit61, D3Mit55, D8Mit14, D9Mit27, D10Mit180, D11Mit167) to 4 (D1Mit15, D2Mit51, D5Mit18, D7Mit323) in both F_0 and F_1 inbred population, with a mean value of 3.4 indicating polymorphism in all 10 loci in both the generation mice. The representative image of gel electrophoresis of a microsatellite (D2Mit61) locus in 4% agarose gel is shown in Fig 1.

The PIC average value was 58.9% for F_0 and 56.0% for F_1 inbred mice. The PIC was originally introduced by Botstein *et al.* (1980) [1] and it refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency and has been proved to be a general measure of how informative a marker is. Higher is the PIC value the more informative a marker is. In the present study, D2Mit51 (68.2%) and D1Mit15 (67.4%) in F_0 mice population and D1Mit15 (66.48%) in F_1 inbred, appeared as the most informative microsatellite markers, whereas D3Mit55 locus was the less informative locus with the PIC value of 47.7% and 44.7%, respectively in F_0 and F_1 inbred mice population. While in a study conducted by Purohit *et al.* (2015) [3] the maximum PIC value was 0.4486 obtained in D1Mit16 and, minimum value of 0.2923 obtained in D1Mit356, with a mean PIC of 0.179 for 14 microsatellite loci in different strains of mice.

The effective number of alleles has an average value of 2.935 and 2.733, respectively in F_0 and F_1 inbred mice population. An average observed heterozygosity per locus was estimated at 0.460 and 0.390 respectively for F_0 and F_1 inbred mice population. Such findings were comparable with Yu and Peng (2002) [7] who observed that effective number of alleles and expected heterozygosity ranged from 3.17 to 8.50 and from 0.35 to 0.83, respectively for six microsatellite loci polymorphism study in house mice in Taiwan. Shang *et al.* (2009) [4] reported that the mean number of alleles and mean of expected heterozygosity for each microsatellite locus were 5.933 and 0.572, respectively in two Chinese Kunming mice population.

The association between different allelic variants of microsatellites and BW is shown in Table 2. However, out of the ten microsatellite loci studied only two loci (D9Mit27 and D10Mit180) showed a significant association with body weight of the mice. The D9Mit27 locus showed a statistically significant association ($P< 0.05$) with BW in both the generation of mice population studied. The highest BW (33.00 ± 1.52) was observed in F_0 populations for genotype 190/178 and in F_1 inbred populations the highest BW (34.44 ± 1.65) was observed for the genotype190/190. The lowest BW was observed for the genotype 158/158 in both the mice population studied.

The D10Mit180 locus showed a statistically significant association between genotypes and BW only in the F_0 population. In the F_0 population, the highest BW (34.66 ± 1.01) was observed for genotype 224/158, which was significantly higher as compared to their contemporary genotypes. The lowest BW was observed for the genotype 158/138 in the F_0 mice population studied. Though non-significant, in F_1 inbred populations D10Mit180 locus showed the highest BW (33.35 ± 1.53) for the genotype 224/158, similar to that of the F_0 population. For other genotype, no significant differences in BW were observed (Table 2).

To our knowledge association between the polymorphism of microsatellites and body weight traits in mice has not been studied yet. Scanty resources were available for this study in literatures. Although a comparison with other species can be biased due to the different marker sets used by different authors, it may be noted that in a study conducted by Zatoń-Dobrowolska *et al.* (2014) [8] Polymorphism of 30 canine-derived microsatellites was studied in a group of 200 red foxes kept on 2 Polish farms. 22 out of 30 microsatellites were selected to study association between marker genotypes and body weight, two microsatellite loci FH2613 and ZUBECA6 had been found to be significantly associated with the body weight.

Table 1: Allelic frequency distribution at 10 microsatellite loci in F_0 and F_1inbred population of Swiss albino mice

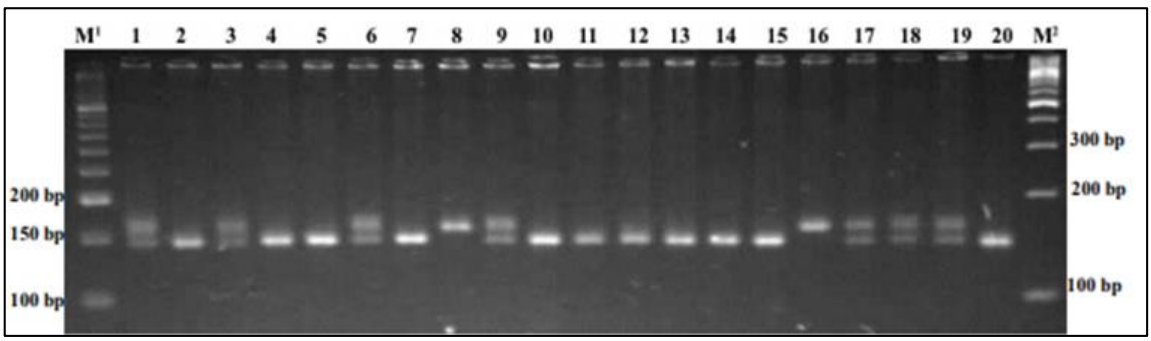
Microsatellite Locus	Allele	F_0			F_1		
		N	Frequency	SE	N	Frequency	SE
D1Mit15	205	51	0.255	0.030	51	0.255	0.034
	190	76	0.380	0.047	79	0.395	0.043
	175	40	0.200	0.035	34	0.170	0.027
	160	33	0.165	0.026	36	0.180	0.030
D2Mit51	156	36	0.180	0.027	13	0.065	0.022
	144	37	0.185	0.030	77	0.385	0.043
	136	66	0.330	0.039	26	0.130	0.034
	128	61	0.305	0.036	84	0.420	0.045
D2Mit61	168	47	0.235	0.031	41	0.205	0.033
	156	50	0.250	0.040	31	0.155	0.034
	146	103	0.515	0.043	128	0.640	0.039
D3Mit55	162	41	0.205	0.027	23	0.115	0.021
	148	33	0.165	0.034	46	0.230	0.031
	142	126	0.630	0.039	131	0.655	0.037
D5Mit18	246	40	0.200	0.028	14	0.070	0.017
	238	29	0.145	0.028	53	0.265	0.033
	232	27	0.135	0.031	46	0.230	0.037
	220	104	0.520	0.038	87	0.435	0.039
D7Mit323	128	64	0.320	0.033	45	0.225	0.032
	112	26	0.130	0.026	17	0.085	0.022
	106	21	0.105	0.027	35	0.175	0.033
	100	89	0.445	0.040	103	0.515	0.038
D8Mit14	160	38	0.190	0.028	50	0.250	0.035
	150	92	0.460	0.048	94	0.470	0.047
	144	70	0.350	0.042	56	0.280	0.038
D9Mit27	190	47	0.235	0.030	38	0.190	0.032
	178	64	0.320	0.036	81	0.405	0.042
	158	89	0.445	0.036	81	0.405	0.042
D10Mit180	224	50	0.250	0.032	63	0.315	0.039
	158	64	0.320	0.036	76	0.380	0.040
	138	86	0.430	0.037	61	0.305	0.037
D11Mit167	144	60	0.300	0.036	54	0.270	0.035
	128	67	0.335	0.043	116	0.580	0.044
	122	73	0.365	0.042	30	0.150	0.030

N indicates sample size

Table 2: Association of microsatellite loci with BW for F_0 and F_1 inbred mice population

Microsatellite locus	Least square means of body weight (grams)		Micosatellite locus	Least square means of body weight (grams)	
	F_0	F_1 Inbred		F_0	F_1 Inbred
D1Mit15			D7Mit323		
205/205	32.66^{b}±3.25(5)	31.57^{a} ±1.50(11)	128/128	31.76^{a}±1.51(10)	31.63^{a}±1.36(8)
205/190	31.80^{b}± 1.45(3)	-	128/112	29.37^{a} ±1.24(9)	34.53^{a}±6.08(2)
205/175	32.60^{b}±1.65(12)	34.01^{a} ±1.18(19)	128/106	33.20^{a}±1.87(9)	33.12^{a}±3.05(5)
205/160	32.34^{b}±0.81(26)	29.69^{a} ±1.47(10)	128/100	32.19^{a}±0.80(26)	33.37^{a}±0.87(22)
190/190	31.91^{b}±0.75(35	31.11^{a} ±0.86(28)	112/112	32.78^{a}±3.42(4)	32.78^{a}±6.29(3)
190/175	33.10^{a}±0. 00(1)	31.81^{a} ±1.33(9)	112/100	33.45^{a}±1.06(9)	31.01^{a}±1.65(9)
190/160	29.53^{b}±0.98(2)	33.16^{a} ±1.43(14)	106/106	32.33^{a}±1.63(6)	32.23^{a}±1.05(10)
175/175	29.56^{b}±1.02(13)	30.20^{a} ±2.26(3)	106/100	-	33.26^{a}±1.84(10)
175/160	27.35^{b}±0.00(1)	-	100/100	31.00^{a}±0.96(27)	30.39^{a}±0.79(31)
160/160	31.78^{b}±0.79(2)	32.27^{a} ±1.95(6)	D8Mit14		
D2Mit51			160/160	32.54^{a}±2.81(4)	30.92^{a}±1.36(12)
156/156	30.08^{ab}±2.50(3)	33.01^{a}±1.97(4)	160/150	32.93^{a}±1.83(8)	33.84^{a}±2.11(7)
156/144	34.10^{a}±0.00(1)	32.88^{a}±2.19(5)	160/144	31.15^{a}±0.97(22)	31.41^{a}±1.17(19)
156/136	31.20^{ab}±1.09(19)	-	150/150	32.31^{a}±0.74(42)	32.01^{a}±0.73(41)
156/128	31.0^{ab}±1.12(10)	-	150/144	-	32.64^{a}±2.45(5)
144/144	29.12^{b}±1.95(6)	30.80^{a}±0.95(28)	144/144	31.04^{a}±0.79(24)	32.25^{a}±1.23(16)
144/136	33.32^{ab}±0.55(5)	-	D9Mit27	*	*
144/128	30.81^{ab}±0.89(19)	32.76^{a}±1.07(16)	190/190	32.04^{ab} ±1.22(6)	34.44^{a}±1.65(9)
136/136	32.95^{ab}±1.18(19)	32.14^{a}±1.37(13)	190/178	33.00^{a}±1.52(11)	33.35^{ab}±1.53(10)
136/128	29.38^{b}±1.00(4)	-	190/158	31.9^{ab}±0.83(24)	31.78^{ab}±1.79(10)
128/128	34.36^{ab}±1.42(14)	32.21^{a}±0.87(34)	178/178	31.40^{ab}±1.06(15)	32.20^{ab}±0.97(28)
D2Mit61			178/158	32.09^{ab}±0.86(23)	33.02^{ab}±1.22(15)
168/168	32.46^{a}±0.99(7)	31.24^{a}±1.74(9)	158/158	28.37^{b}±1.79(21)	29.93^{b}±0.70(28)
168/156	32.97^{a}±1.63(10)	-	D10Mit180	*	
168/146	32.11^{a}±0.96(23)	31.78^{a}±0.85(23)	224/224	32.88^{ab}±1.53(8)	31.76^{a}±1.25(18)
156/156	32.89^{a}±1.17(19)	34.17^{a}±1.76(12)	224/158	34.66^{a}±1.01(12)	33.15^{a}±1.03(13)
156/146	31.03^{a}±1.48(2)	32.58^{a}±2.32(7)	224/138	31.53^{ab}±0.98(22)	31.30^{a}±1.25(14)
146/146	30.73^{a}±0.71(39)	31.55^{a}±0.65(49)	158/158	30.76^{b}±1.19(15)	32.79^{a}±1.57(23)
D3Mit55			158/138	29.86^{b}±0.76(22)	31.10^{a}±0.85(17)
162/162	29.18^{a}±0.83(2)	-	138/138	32.85^{ab}±1.14(21)	31.25^{a}±1.30(15)
162/148	31.12^{a}±1.70(7)	33.70^{a}±1.70(10)	D11Mit167		
162/142	32.63^{a}±0.94(30)	33.07^{a}±1.59(13)	144/144	31.93^{a}±1.66(14)	30.55^{a}±0.88(12)
148/148	33.48^{a}±1.30(12)	31.36^{a}±2.47(7)	144/128	32.3^{a}±1.08(14)	33.37^{a}±0.91(17)
148/142	31.55^{a}±5.95(2)	31.89^{a}±1.03(22)	144/122	30.75^{a}±0.93(18)	30.54^{a}±1.76(13)
142/142	31.00^{a}±0.60(47)	31.42^{a}±0.61(48)	128/128	31.13^{a}±0.84(25)	32.25^{a}±0.75(48)
D5Mit18			128/122	33.26^{a}±2.85(3)	30.55^{a}±1.71(3)
246/246	31.44^{a}±2.69(4)	-	122/122	32.68^{a}±0.991(26)	32.24^{a}±1.57(7)
246/238	-	31.36^{a}±2.38(4)			
246/232	30.39^{a}±1.75(7)	31.36^{a}±2.23(10)			
246/220	31.73^{a}±0.95(25)	-			
238/238	33.36^{a}±1.38(5)	28.41^{a}±0.97(9)			
238/232	34.22^{a}±0.92(2)	-			
238/220	32.50^{a}±1.24(17)	33.28^{a}±0.73(31)			
232/232	31.76^{a}±1.45(9)	32.08^{a}±1.31(15)			
232/220	-	31.43^{a}±2.09(6)			
220/220	31.47^{a}±0.83(31)	32.00^{a}±0.97(31)			

Means with different superscript differs significantly ($P \leq 0.05$); Number in parenthesis indicates sample size; *Significant at $P \leq 0.05$.



Lane M1: 50 bp marker
Lane M2: 100 bp marker
Lanes 1-20: resolved PCR products (Animal no. 1 to 20)

Fig 1: D2Mit61 microsatellite allele profiling using ultra resolution agarose in Swiss albino mice (F_1 inbred)

4. Conclusions

Although the present study should be considered preliminary since the analyses were performed on a limited number of animals and few number of microsatellite markers, our results reveal for the first time that two microsatellite loci (D9Mit27 and D10Mit180) showed a significant association with body weight traits of the mice.

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