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Association of SNP markers in different candidate genes with growth performance of Landrace × Ghurrah crossbred pigs

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ABSTRACT

The objective of this study was to assess the polymorphic dispersion of SNP markers in candidate growth genes and their impact on growth performance of Landlly (Landrace × Ghurrah crossbred) pigs. PCR-RFLP procedure was utilized to differentiate the genotypes at marker loci. Most of the SNP loci [MC4R (AA, AG and GG); PGK2 (GG, GT and TT); CTSD (AA, AG and GG); CTSK (AA, AG and GG); SLC27A4 (AA, AG and GG); MYC (CC, CT and TT); and LEP (CC, CT and TT)] had 3 genotypes with varying frequency. Only 2 genotypes [AA (59%) and AG (41%)] were noticed for GHRL. IGF-1 and CTSZ SNPs were monomorphic for AA genotype. Allelic frequencies for these SNPs (MC4R, PGK2, CTSD, CTSK, CTSZ, SLC27A4, GHRL, MYC, IGF-1and LEP) were 0.51 and 0.49 (A/G), 0.66 and 0.34 (G/T), 0.55 and 0.45 (A/G), 0.48 and 0.52 (A/G), 1.00 (A), 0.69 and 0.31(A/G), 0.80 and 0.20 (A/G), 0.22 and 0.78 (C/T), 1.00 (A) and 0.47 and 0.53 (C/T), respectively. Impact of PGK2, CTSK, GHRL and LEP SNP was meaningful on the body weight at birth. MC4R had significant impact on body weight at 6 weeks. SLC27A4 and LEP SNP significantly affected body weight at 32 weeks. This investigation suggested MC4R, PGK2, CTSK, SLC27A4, GHRL and LEP SNPs as potential markers for improving growth performance in Landlly pigs.

Keywords: Candidate genes, Growth performance, Landlly crossbred pig, PCR-RFLP, SNP marker

Pigs are rapidly growing and prolific livestock species. Pork is a better source of biological protein and most widely consumed meat in the word (Wang *et al.* 2012). For a profitable pig industry, a good body growth is a significant attribute and body weight is a reliable measure for understanding the body growth rate. Growth traits are moderately heritable and can be enhanced by appropriate breeding strategies. Low growth performance is a major issue of swine industry. Knowledge of SNP markers of growth candidate genes has immense importance in swine breeding programs.

Melanocortin receptor 4 (MC4R) is a centrally expressed G-protein that mediates the effect of leptin (LEP) on food intake, energy homeostasis and body growth regulation in mammals (Muñoz *et al*. 2011). Phosphoglycerate kinase 2 (PGK2) plays a key role in central metabolic pathway of glycolysis and increases angiogenesis leading to building up of normal muscle mass formation (Jang *et al.* 2011, Wang *et al.* 2012). CTSD, CTSK and CTSZ SNP markers in cathepsin gene were linked with growth, fat deposition, and production characteristics in pig population (Fontanesi *et al.* 2010, Kim *et al*. 2015).

MYC (v-myelocytomatosis viral oncogene homologue) is a receptor encoding nuclear phosphoprotein gene which is involved in cell proliferation and cell growth (Oh *et al*. 2014). Insulin-like growth factor-1 (IGF-1) plays a prime role in regulating animal growth, development and metabolism. The porcine IGF-1 gene acts as a promising growth marker in pig breeds (Hao *et al*. 2011). Solute carrier family 27 member 4 (SLC27A4) is a kind of fatty acyl-CoA synthetase that generates very large fatty acid-CoA for lipid metabolic tracts, suggesting that it serves as a potential candidate gene for animal fat deposition traits (Xu *et al.* 2009). Ghrelin (GHRL) acts as appetite-stimulating hormone (Peino *et al*. 2000) that controls food intake and growth of tissue (Leite-Moreira *et al*. 2007), suggested as a promising marker for growth.

For the present investigation, SNP markers in candidate genes potentially relevant to porcine growth were taken for analysis in Landlly crossbred pigs.

MATERIALS AND METHODS

Animals, sample collection and phenotypic information: Landlly crossbred (150; 75% Landrace and 25% Ghurrah) pigs, maintained at Swine Production Farm, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India; a unit of ICAR-All India Coordinated Research Project on Pigs were selected for this study. This center is situated at an altitude of 564 feet above the mean sea level, 28°N latitude and 79°E longitude. The temperature touches both the extremes (4-5°C in winter and 40-45°C in

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summer) and relative humidity ranges between 15 to 85%. Pigs were reared under uniform feeding and management conditions throughout the experimental period. Blood (5 ml) from each pig was collected from the anterior vena cava in a sterile 15 ml polypropylene centrifuge tube containing EDTA (0.5 ml/10 ml blood). Body weight of the piglet was recorded at birth, 2, 6 and 32 weeks of age.

DNA extraction: Phenol-chloroform extraction method (Sambrook and Russell 2001) was used to isolate genomic DNA from blood samples of all Landlly pigs. A spectrophotometer was used to evaluate the concentration and quality of DNA (A260/A280 ratio) for each sample. Until further study, DNA samples were preserved at -20°C.

PCR-RFLP analysis: Ten SNPs of porcine genes [MC4R (c.1426A>G), PGK2 (g.122T>G), CTSD (g.70G>A), CTSK (g.15G>A), CTSZ (g.37A>G), SLC27A4 (g.1777G>A), GHRL (93A>G), MYC (T906C), IGF-1(A440G) and LEP (T3469C)], showing association with growth in previous literature were taken in current study. Genotyping of SNPs was done by PCR-RFLP. Detailed information about SNPs along with primer sequence, annealing temperature, restriction enzymes and amplicon size of each primer is given in Table 1. Forward and reverse primers working solutions were made to achieve a final concentration (10 pmol) of each primer. Final reaction mix (25 µl) consisted of forward (1.0 µl) primer, reverse primer (1.0 µl), Dream Taq Green (2.5 µl), dNTPs (0.5 µl), DNA template (2 µl), Taq polymerase (0.2 µl) and nuclease-free water (17.8 µl). PCR was accomplished in a 25 µl reaction volume, which was kept constant for all reactions using thermo cycLEP (Bio-Rad, USA).

The optimization of appropriate annealing temperature in connection with each primer was decided by gradient PCR. The PCR conditions involved initial denaturation at 95°C (for 5 min), followed by 40 cycles with denaturation at 94°C (for 1 min), annealing at temperature between 52.5 and 62.0°C (for 45 sec) to specifically amplify a target region 1 and 2 and extension at 72°C (for 1 min) followed by a final extension at 72°C (for 5 min). The restriction enzyme (RE) digestion of PCR products were accomplished in 0.2 ml tube with a total reaction mixture of 23 µl by overnight incubation at a temperature, specified by enzyme manufacturer. The digested products were thereafter kept at -20°C till further study. On 3.5% agarose gel, the amplified and digested SNPs DNA fragments were segregated. The individual's genotype was assessed by evaluating their fragment size for each polymorphism.

Statistical analysis: Polymorphic information content (PIC), Hardy Weinberg equilibrium (HWE), heterozygosity and allelic diversity were estimated using proc allele module of SAS 9.3 software. The association analyses between SNPs and body weights were performed through PROC GLM module of SAS 9.3 using following model:

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

where y_{ij}, observation of body weight on j^{th} pig in i^{th} genotype; µ, overall mean; g_i, effect of i^{th} genotype; e_{ij}, random error ~NID (0, e^2).

Table 1. Allelic and genotypic frequency at different SNP sites in crossbred pigs

Gene (SNP)	Allele	Allelic frequency	Genotype	Genotypic frequency	PCR- RFLP pattern (bp)
MC4R (c.1426A>G)	A	0.51	AA	0.20	226
	G	0.49	AG	0.61	226, 156 and 70
			GG	0.19	156 and 70
PGK2 (g.122T>G)	G	0.66	GG	0.43	447 and 95
	T	0.34	GT	0.46	542, 447 and 95
			TT	0.11	542
CTSD (g.70G>A)	A	0.55	AA	0.19	117 and 67
	G	0.45	AG	0.72	184, 117 and 67
			GG	0.09	184
CTSK (g.15G>A)	A	0.48	AA	0.08	211 and 177
	G	0.52	AG	0.79	338, 211 and 177
			GG	0.13	338
CTSZ (g.37A>G)	A	1.00	AA	1.00	100
SLC27A4 (g.1777G>A)	A	0.69	AA	0.46	93 and 59
	G	0.31	AG	0.45	152, 93 and 59
			GG	0.09	152
GHRL (93A>G)	A	0.80	AA	0.59	343, 301 and 109
	G	0.20	AG	0.41	343, 301, 195, 148 and 109
MYC (T906C)	C	0.22	CC	0.13	209 and 123
	T	0.78	CT	0.18	332, 209 and 123
			TT	0.69	332
IGF-1 (A440G)	A	1.00	AA	1.00	459
LER (T3469C)	C	0.47	CC	0.39	397 and 89
	T	0.53	CT	0.15	486, 397 and 89
			TT	0.46	486

RESULTS AND DISCUSSION

PCR-RFLP pattern and frequency distribution: Landlly pigs (150) were genotyped by PCR-RFLP for 10 SNPs. Out of 10 SNPs, 8 were polymorphic {MC4R (c.1426A>G), PGK2 (g.122T>G), CTSD (g.70G>A), CTSK (g.15G>A), SLC27A4 (g.1777G>A), GHRL (93A>G), MYC (T906C) and LEP (T3469C)} and two were monomorphic {CTSZ (g.37A>G) and IGF-1(A440G)}. PCR-RFLP pattern along with allelic and genotypic frequencies at different SNP loci are shown in Table 1. PCR-RFLP pattern of observed genotypes at SNP loci was as per the expectation. The value of PIC, heterozygosity and allelic diversity showed that pig population under exploration was of intermediate diversity for these polymorphic SNP loci (Table 2). The χ^2 value for HWE further indicated that the population significantly deviated from HWE for these loci except PGK2 (g.122T>G) and SLC27A4 (g.1777G>A) SNP locus. PCR-RFLP profile of MC4R (c.1426A>G) in 3-3.5% agarose gel is given in Fig. 1.

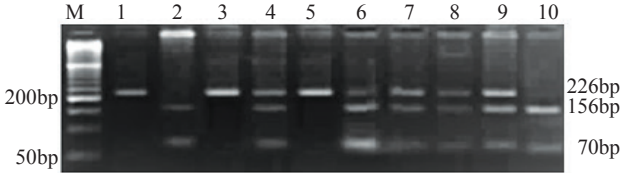

Fig. 1. PCR-RFLP profile of MC4R (c.1426A>G) SNP by using TaqI on 3-3.5% agarose gel. Lane M: 50 bp marker; Lanes 1, 3, 5: AG genotype; Lanes 4, 6-9: AG genotype; Lanes 2, 10: GG genotype.

MC4R (c.1426A>G) locus: Size of amplified PCR fragment (226 bp) was in agreement with Kim *et al.* (2015) and Saini *et al.* (2018). Frequency of homozygote AA (20%), heterozygote AG (61.33%) and homozygote GG (18.67%) was similar to that reported by Octura *et al.* (2014) and Kim *et al.* (2015). Wang *et al.* (2012) and Octura *et al.* (2014) observed 20 and 16.4% frequency of AA genotype in their study. However, Salajpal *et al.* (2009), reported lower frequency of AA (9.3%) genotype and higher frequency of GG (47.7%) genotype in offsprings of Swedish Landrace × Large White sows. The frequency of allele A (50.67%) and G (49.33%) at MC4R (C.1426A>G) locus in Landlly pigs was in agreement with findings of Dvořáková *et al.* (2011), Wang *et al.* (2012) and Kim *et al.* (2015). However, Octura *et al.* (2014) observed the frequency of G allele between 62.3 and 70% and frequency of A allele between 30 and 37.7% in Philippine native pigs.

PGK2 (g.122T>G) locus: Size of amplified PCR product was 542 bp, which corroborated with the findings of Jang *et al.* (2011) and Kim *et al.* (2015). Frequency of homozygote GG, TT and heterozygote GT (43.33, 10.67 and 46%) was different from that reported by Jang *et al.* (2011) and Kim *et al.* (2015) in Landrace and Duroc × Korean native pigs (GT genotype with 37.5 and 63% frequency and TT genotype with 62.5 and 31% frequency). Frequency of G allele (66.33%) was higher in this population while the frequency of T allele (33.67%) was lower as compared to the findings of Jang *et al.* (2011) and Kim *et al.* (2015). The variation in genotypic and allelic frequency might be due to difference in sample size and breed.

CTSD (g.70G>A) locus: The amplified PCR product was 184 bp in length, which was similar to the findings of Russo *et al*. (2008) and Kim *et al*. (2015). Homozygote AA, GG and heterozygote AG were observed with frequency of 18.67, 9.33 and 72%, respectively in present population. Kim *et al*. (2015) observed AA, GG and AG genotypes with 64, 2 and 34% frequency in Landrace pigs. Frequency of A and G allele was 54.67 and 45.33%, respectively. However, Russo *et al.* (2008) and Kim *et al.* (2015), reported higher A allele frequencies (93.5 and 80.8) and lower G allele frequencies (6.5 and 19.2%).

CTSK (g.15G>A) locus: The length of amplified PCR fragment was 388 bp, which was in agreement with the findings of Fontanesi *et al.* (2010) and Kim *et al.* (2015). Frequency of homozygote AA, GG and heterozygote AG was 8, 12.7 and 79.33% in present population. Kim *et al.* (2015), however, observed higher frequency of GG (75.5%) and lower frequency of AG (24.5%) genotype in Landrace pigs. Frequency of allele A (47.67%) and G (52.33%) at CTSK (g.15G>A) locus in present investigation did not show uniformity with the findings of previous studies. Kim *et al.* (2015) observed lower frequency of allele A (12.3%) and higher frequency of allele G (87.7%) in their study.

SLC27A4 (g.1777G>A) locus: The amplified PCR product (152 bp) was identical to the findings of Xu *et al.* (2009). This locus showed 3 genotypes; homozygote AA (46%), heterozygote AG (45.33%) and homozygote GG (8.67%). Xu *et al.* (2009), however, observed only 2 genotypes viz. homozygote GG (55%) and heterozygote AG (45%) in Landrace pigs. Frequency of allele A (68.67%) and G (31.33%) at SLC27A4 (g.1777G>A) SNP in Landlly pigs was dissimilar to that reported by Xu *et al.* (2009). They reported higher frequency of G (75%) allele in Landrace pigs.

GHRL (93A>G) locus: Length of amplified PCR fragment (778 bp) and frequency of homozygote AA (59.33%), heterozygote AG (40.67%), allele A (79.67%) and allele G (20.33%) were similar to the findings of Ropka-Molik *et al*. (2011). They observed frequency of AA and AG genotype as 56 and 44% in Polish Large Whites.

MYC (T906C) locus: Amplified PCR fragment (332 bp) was in accordance to the findings of Oh *et al.* (2014). However, at this locus, the observed frequency of homozygote CC (13.33%), TT (68.67%) and heterozygote CT (18%) differed from the previous studies. Oh *et al.* (2014) observed lower frequency of homozygote CC (7.1%) and higher frequency of heterozygote CT (38.1%) in Duroc, Landrace and Yorkshire pig breeds. They obtained only 1 genotype (TT) in Landrace pigs. At this locus, frequency of allele C (22.33 %) and T (77.67 %) was almost similar to that reported by Oh *et al.* (2014) in Duroc, Landrace and Yorkshire pig breeds (26 and 74% for C/T).

LEP (T3469C) locus: The length of amplified PCR

Table 2. Least squares means of growth traits across the genotypes at different SNP sites

Locus	Genotype	W0	W2	W6	W32
MC4R	AA	1.15±0.08 (30)	3.82±0.32 (30)	11.06^{a}±0.70 (30)	105.31±2.08 (30)
	AG	1.11±0.04 (92)	3.64±0.19 (92)	9.66^{b}±0.41 (92)	105.90±1.26 (63)
	GG	1.11±0.07 (28)	3.43±0.28 (28)	8.85^{c}±0.62 (28)	104.81±2.22 (12)
PGK2	GG	1.02^{b}±0.06 (65)	3.46±0.25 (65)	9.48±0.56 (65)	102.10±1.84 (40)
	TG	0.99^{c}±0.06 (69)	3.54±0.27 (69)	10.38±0.60 (69)	105.04±1.96 (49)
	TT	1.36^{a}±0.10 (16)	3.88±0.42 (16)	9.72±0.91 (16)	108.88±2.78 (16)
CTSD	AA	1.02±0.10 (28)	3.23±0.44 (28)	8.73±0.96 (28)	104.74±2.82 (28)
	AG	1.07±0.12 (108)	3.06±0.50 (108)	8.44±1.09 (108)	104.12±3.20 (63)
	GG	1.28±0.17 (14)	4.54±0.70 (14)	12.40±1.53 (14)	107.16±4.47 (14)
CTSK	AA	1.18^{b}±0.18 (12)	3.24±0.73 (12)	8.60±1.59 (12)	105.82±4.65 (12)
	GA	0.91^{c}±0.09 (119)	3.50±0.39 (119)	9.69±0.85 (119)	107.83±2.50 (74)
	GG	1.28^{a} ±0.11 (19)	4.15±0.45 (19)	11.28±0.99 (19)	102.36±2.90 (69)
SLC27A4	AA	1.33±0.16 (69)	4.11±0.68 (69)	10.72±1.47 (69)	119.30^{a}±4.93 (24)
	GA	0.97±0.12 (68)	3.09±0.53 (68)	8.78±1.14 (68)	100.66^{b}±3.58 (68)
	GG	1.07±0.22 (13)	3.69±0.90 (13)	10.08±1.96 (13)	96.02^{c}±5.84 (13)
GHRL	AA	1.00^{b}±0.07 (89)	3.44±0.30 (89)	9.87±0.67 (89)	105.75±2.03 (44)
	AG	1.25^{a}±0.05 (61)	3.82±0.22 (61)	9.85±0.48 (61)	104.93±1.50 (61)
MYC	CC	1.13±0.09 (20)	3.52±0.39 (20)	9.67±0.85 (20)	106.42±2.50 (20)
	TC	0.95±0.08 (27)	3.06±0.35 (27)	10.09±0.77 (27)	107.54±2.32 (27)
	TT	1.29±0.11 (103)	4.31±0.48 (103)	9.82±1.03 (103)	102.06±3.09 (58)
LER	CC	1.38^{a}±0.10 (59)	4.32±0.41 (59)	11.09±0.88 (59)	111.86^{a} ±2.71(59)
	TC	0.95^{c}±0.09 (22)	3.52±0.38 (22)	9.62±0.83 (22)	110.54^{ab}±3.30 (21)
	TT	1.03^{b}±0.11 (69)	3.06±0.48 (69)	8.86±1.05 (69)	93.61^{b}±4.40 (25)

Superscripts with different symbol indicates the significant difference of mean. Figures in parentheses indicate number of observations.

fragment was 486 bp, which corroborated with the findings of Deoliveira- Peixoto *et al.* (2006). Frequency of homozygote CC, TT and heterozygote CT in this population was 39.33, 46 and 14.67%, respectively. Deoliveira-Peixoto *et al.* (2006), however, reported only 2 genotypes viz. TT (86.36%) and CT (13.64%). Frequency of allele C and T was 46.67 and 53.33% in this study. However, Deoliveira *et al.* (2006), reported higher frequency of allele T (86.36%).

Association between SNP genotypes and body weights: Least squares analysis of variance for body weights with SNP effect in model and least squares means of body weights across the genotypes at different SNP sites is given in Table 2. The least squares analysis revealed significant association of MC4R (C.1426A>G) with body weight at 6th week, SLC27A4 (g.1777G>A) with weight at 32 weeks, GHRL (93A>G), PGK2 (g.122T>G) and CTSK (g.15G>A) with birth weight and LEP (T3469C) with weight at birth and 32 week of age. These results are in agreement with the findings of Xu *et al*. (2009), Jang *et al*. (2011) and Kim *et al*. (2015). Xu *et al*. (2009) noticed marked association of SLC27A4 (g.1777G>A) SNP with body weight at birth and weaning in Landrace pig population. Jang *et al.* (2011) observed striking effect of PGK2 (g.122T>G) on birth weight and weight at 3 weeks in Korean native and Landrace pigs.

Kim *et al.* (2015) observed significant link of MC4R (C.1426A>G) locus with birth weight in crossbreds of Korean native pig × Duroc pigs. Muñoz *et al.* (2011) and Ropka-Molik *et al.* (2011), however, did not notice marked effect of MC4R (C.1426A>G) and GHRL (93A>G) on growth in pigs. Kim *et al.* (2015) also did not observe significant association of CTSK (g.15G>A) SNP with body weight at birth, weaning and market age.

AA genotype was heavier (11.06±0.70 kg) than AG (9.66±0.41 kg) and GG (8.85±0.62 kg) genotype at MC4R (C.1426A>G) for body weight at 6th week. AA genotype of SLC27A4 (g.1777G>A) SNP was heavier (119.30±4.93 kg) than GA (100.66±3.58 kg) and GG (96.02± 5.84 kg) genotype at 32 weeks. TT genotype of PGK2 (g.122T>G) was heavier (1.36±0.10 kg) than TG (0.99±0.06 kg) and GG (1.02±0.06 kg) genotype at birth. GG genotype was heavier (1.28±0.11 kg) than GA (0.91±0.09 kg) and AA (1.18±0.18 kg) genotype at CTSK (g.15G>A) locus for birth weight. AG genotype of GHRL (93A>G) locus was heavier (1.25±0.05 kg) than AA genotype (1.00±0.07 kg) at birth. CC genotype of LEP (T3469C) SNP was heavier (1.38±0.10 and 111.86±2.71 kg) than TC (0.95±0.09 and 110.54±3.30 kg) and TT (1.03±0.11 and 93.61±4.40 kg) genotype at birth and 32 weeks.

Heavier weight of AA genotype for MC4R (C.1426A>G) at 6 weeks, AA genotype for SLC27A4 (g.1777G>A) at 32 weeks, AG genotype for GHRL (93A>G) at birth, TT genotype for PGK2 (g.122T>G) at birth, GG genotype for CTSK (g.15G>A) at birth and CC genotype for LEP (T3469C) at birth and 32 weeks in this investigation revealed that these genotypes may be applied in genetic selection program as compared to their contemporaries for improving growth performance in Landlly crossbred pigs.

Deoliveira *et al.* (2006), however, reported that pigs with TT genotype at LEP (T3469C) were heavier than other genotypes. Xu *et al.* (2009) noticed that pigs with AG genotype of SLC27A4 (g.1777G>A) SNP had significantly higher body weight at birth as compared to GG genotype in Landrace pigs. Kim *et al.* (2015) observed superiority of GG genotype of PGK2 (g.122T>G) SNP as compared to TG and GG genotype for body weight at birth. Sampling error, genotype-environmental interaction, linkage, and genetic background could be the cause of observed inconsistency.

CTSD (g.70G>A) and MYC (T906C) SNP had non-significant association with body weight at all the ages. Results are contrary to the findings of Russo *et al*. (2008), Oh *et al*. (2014) and Kim *et al.* (2015). Kim *et al.* (2015) observed significant association of CTSD (g.70G>A) with birth weight in crossbred pigs (Korean native pig × Duroc). Russo *et al.* (2011) also observed marked association of CTSD (g.70G>A) SNP with growth traits in Italian Large White pigs. Oh *et al.* (2014) reported significant association of MYC (T906C) SNP with body weights in Duroc, Landrace and Yorkshire pigs.

Landlly crossbred pig population under present study was polymorphic for 8 SNP loci [MC4R (c.1426A>G), PGK2 (g.122T>G), CTSD (g.70G>A), CTSK (g.15G>A), SLC27A4 (g.1777G>A), GHRL (93A>G), MYC (T906C), LEP (T3469C)] and was monomorphic for 2 SNP loci [CTSZ (g.37A>G) and IGF-1(A440G)]. Significant association of MC4R (c.1426A>G) locus with weight at 6 weeks; SLC27A4 (g.1777G>A) with weight at 32 weeks; LEP (T3469C) with weight at birth and 32 weeks; and PGK2 (g.122T>G), CTSK (g.15G>A) and GHRL (93A>G) with weight at 6 weeks, revealed that MC4R, PGK2, CTSK, SLC27A4, GHRL and LEP SNPs may act as potential candidate markers for improving growth performance of Landlly crossbred pigs.

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