PCR-RFLP-Gene Study in Musculoskeletal Deformed Birds

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ABSTRACT

The present study was carried out in musculoskeletal deformed birds for screening the relation of their deformed growth with growth related candidate genes (TGF-b2, IGF-1, GHR, Myostatin, BMP-2) by PCR-RFLP. The overall incidence of musculoskeletal deformities in the hatch (nearly 1500 chicks) was 0.8 % associated with legs and wings (twisted leg, Perosis, reluctance to move and a stilted gait). The overall means of body weight at 3rd and 5th week of age were 402.19±51.62 and 854.39±96.07 gm, respectively. Candidate genes mediating growth promotion (TGF-ß2, IGF-1 and GHR) as well as gene exerting negative regulation on growth (myostatin) along with one gene (BMP-2) influencing bone formation were analysed in coloured broiler male line. At seventh weeks of age the hematobiochemicals (Ca, P, T3 and T4) did not show significant difference among genotypes of different candidate genes compared by t-test. There was no significant effect of genotypes of gene on the growth of deformed birds. These non-significant results of present study suggest that one should go for other restriction enzymes or some other genes may have role in the present musculoskeletal deformed birds.

Key words: TGF, BMP, GHR, IGF, PCR-RFLP, Poultry

The long term selection programmes in poultry have resulted into exhaustion of most of genetic variability of our high yielding stocks both in layer and broilers. Poultry geneticists are also facing additional challenges today because of negative correlation between production and fitness traits. Moreover, selection for faster and higher growth rates and muscling has also resulted into musculoskeletal deformities and meat quality defects like tibial dyschondroplasia, obesity, ascites (Dunnington and Siegel, 1996; Deeb and Lamont, 2002), pale soft & exudative (PSE) meat, focal myopathy, deep pectoral myopathy etc. The situation warrants for application of finer molecular tools for achieving desired gains in body weights vis a vis meat quality. With the advent of molecular techniques our knowledge of understanding the genetic architecture, gene function and gene interactions has increased tremendously. Many genes and gene products play key role in development and growth of birds. Muscle growth is a major determinant of performance in broiler chicken. Somatotropic axis involving GH-GHR-IGF genes has a major influence on growth and development in chicken. Myostatin gene a potent negative regulator of skeletal muscle growth was analysed in deformed birds.

The effect of various polymorphisms/ SNPs of these genes on array of economic trait recorded on deformed birds in a population of Coloured Synthetic Male Line (CSML) chicken for undertaking the molecular dissection of growth and related haematobiochemicals. The one of fine molecular technique like PCR-RFLP therefore, offer new way and means for augmentation of poultry production.

METHODOLOGY

The musculoskeletal defects like lameness, twisted legs, perosis, curled toe etc. were recorded based on visual observations in eleven CSML (coloured synthetic male line) broilers at 5 weeks. Body weights at 3rd and 5th week of age of birds were taken. Blood samples (approximately 200µl) were collected from individual birds through jugular vein to isolate the genomic DNA. The purity of DNA samples was checked spectrophotometrically. Candidate genes were amplified from the good quality DNA isolated from blood of

CSML birds as per the literature (Table 1). The amplified products were digested with the restriction enzymes having cutting sites within the region, as per the specification of manufacturers. Suitable software like Gene Tool/DNA Star/Web Cutter etc. was used to select the restriction endonucleases for PCR-RFLP analysis. The PCR products of different genes were digested with different restriction endonucleases (Table 2). Digested PCR products were run in 6% Acrylamide PAGE gel and silver stained to visualize the bands. Genotyping for different genes were done on the basis of band pattern observed on PAGE gel after silver staining.

RESULTS AND DISCUSSION

The musculoskeletal defects like lameness, twisted legs, perosis, curled toe etc. were recorded based on visual observations in eleven CSML (coloured synthetic male line) broiler at 5 weeks (Fig.6). Somatotropic axis involving GH-GHR-IGF genes has a major influence growth and development in chicken. Hinf I-PCR-RFLP of IGF-I gene (813 bp) exhibited two genotypes AA and AB (Fig.1). PCR-RFLP of GHR (544 bp) region with Alu I yielded two genotypes AA and AB (Fig.7). The AluI PCR-RFLP of exon 1 region of TGF-ß2 (316) produced single genotype AA as a monomorphic pattern (Fig.5). Myostatin gene a potent negative regulator of skeletal muscle growth was analysed in deformed birds. PCR-RFLP analysis of mysotatin gene (2517 bp) with Taq I enzyme revealed two alleles A and B and three genotypes AA, AB and BB (Fig.2).

PCR-RFLP of BMP-2 gene exon region (824 bp) with BcnI produced single genotype AA and only one allele A in all the birds (Fig.3). Bme1390I PCR-RFLP also produced only one AA genotype with only one allele A in all the birds screened (Fig.4). The experimental deformed birds therefore, showed monomorphic pattern with both the enzymes.

The IGF-AA genotype (456.67±32.69 gm) had approximately 60 gm higher body weight as compared to IGF-AB genotype (360±155.56 gm) whereas GHR-AA genotype (450±34.21 gm) had 80 gm higher body weight as compared to GHR-AB genotype (330 gm). The MYO-AB genotype (335±61.95 gm) had over than 100 gm body weight as compared to MYO-AA (335±61.95 gm) and MYO-BB (400±98.99 gm) genotype deformed birds (Table 23).

In the present investigation, overall means for body weights at 3 and 5 weeks were higher than reported by Pramod (2005) in coloured broilers and by Nischal (2001) in frizzle broiler line. At 7th wk of age the hematobiochemicals were studied in deformed birds (Picture 1). The mean±SE (Table 3) for effect of genotype of different genes (IGF-1, GHR and Myostatin) on Triiodothyronine (T3), Thyroxine (T4) serum calcium (Ca) and phosphorus (P) activity were studied. The means of different hematobiochemicals, as compared by t-test, did not show significant difference among genotypes of different candidate genes. The results obtained in the present investigation were in normal range (Raghuramulu et al., 1983). There was no significant effect of genes on the deformity in CSML birds.

CONCLUSION

Candidate genes mediating growth promotion (TGF-ß2, IGF-1 and GHR) as well as gene exerting negative regulation on growth (myostatin) along with

Table 1 Gene and Primer data

Gene	Primer sequence	Length of primer (bp)	Anneal-ing Temp (°C)	PCR product length (bp) (Accession Number)
GHR	5' GCA ACA TCA GAA TCG CTT TT 3'	20	58.0	544bp (AJ506750)
	5' TCC CAT CGT ACT TGA ATA TCC 3'	21		
IGF-1	5' CAT TGC GCA GGC TCT ATC TG 3'	20	58.0	813bp (M74176)
	5' TCA AGA GAA GCC CTT CAA GC 3'	20		
TGF-b2	5' TGC ACT GCT ATC TCC TGA G 3'	19	60.0	316bp (X58071)
	5' ATT TTG TAA ACT TCT TTG GCG 3'	21		
BMP-2	5' ACA TGT TGG ACC TCT ATC GCC 3'	21	58.0	824bp (AY237249)
	5' TCA GCG GCA CCC GCA GCC CTC 3'	21		
Myostatin	5' AGT AGC GAT GGC TCT TTG GA 3'	20	60.0	2517bp ((DNA)
	5' CTG GGA ATG TGA CAG CAA GA 3'	20		

ble 2 Digestion condition for analysis of different gene using different REs

Candidate gene	Restriction Enzyme	Enzyme specificity (5'---3')	Enzyme in units	Incubation	
				Temp.(^{0}C)	Time
GHR	*Eco*721	CAC↓ GTG	0.5	37	Overnight
	Alu I	AG↓ CT	0.1	37	Overnight
IGF-1	*Hinf I*	G↓ ANTC	0.5	37	Overnight
TGF-b2	*Alu I*	AG↓ CT	0.1	37	Overnight
BMP-2	*Bcn I*	CC↓ SGG	0.2	37	Overnight
	*Bme*13901	CC↓ NG	0.2	37	Overnight
Myostatin	*Taq I*	T↓ CGA	0.3	65	Overnight

N = Any nucleotide; S = C or G.

Table 3 Genotypewise mean±SE of body weights and haematobiochemicals in deformed birds

Gene	Genotypes	3rd Wk BW(gm)	5th Wk BW(gm)	T3 (ng/dl)	T4 (ng/dl)	Ca (mg/100ml)	P (mg/dl)
IGF-I	A A	456.67	951.11	189.48	385.43	12.38	6.71
	(9)	±32.69	±40.64	±37.73	±40.18	±0.64	±0.44
	AB	360.00	890.00	166.97	623.82	11.54	7.78
	(2)	±155.56	±56.57	±22.78	±218.27	±0.83	±0.28
GHR	A A	450.00	947.00	179.12	440.96	12.34	7.14
	(10)	±34.21	±36.79	±32.99	±53.01	±0.57	±0.32
	AB (1)	330.00	870.00	248.09	306.88	11.13	4.55
MYO	A A	335.00	883.33	212.22	387.73	12.55	±7.11
	(3)	±61.95	±51.15	±96.98	±53.17	±1.98	±1.08
	AB	504.17	995.00	161.91	411.37	12.26	7.09
	(6)	±29.51	±50.50	±42.63	±59.61	±0.65	±0.37
	BB	400.00	860.00	215.58	542.52	11.63	6.07
	(2)	±98.99	±14.14	±45.97	±333.25	±0.71	±2.14
Overall mean ± SE		402.19 ±51.62	854.39 ±96.07	171.87 ±25.36	421.47 ±62.57	10.96 ±1.21	6.29 ±0.74

one gene (BMP-2) influencing bone formation were analysed in muskulo-skeltal deformed coloured broiler male line birds. In the present study, we have not found any significant effect of genotypes of these genes on the growth of deformed birds. These non-significant results of present study suggest that one should go for other restriction enzymes or some other genes may have role in the present musculoskeletal deformed birds.

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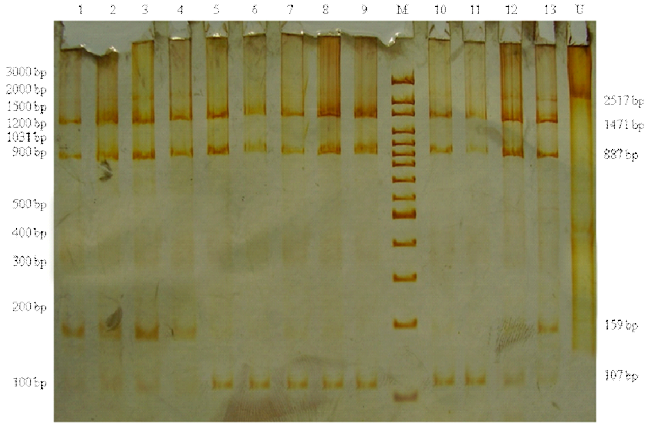
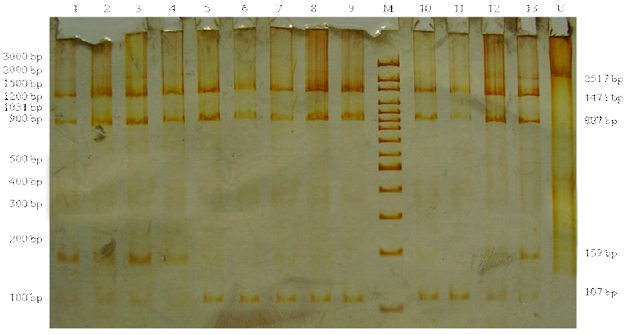
Fig 2: *Taq*I-PCR RFLP of Myostatin gene in muskulo skeletal deformed CSML birds.



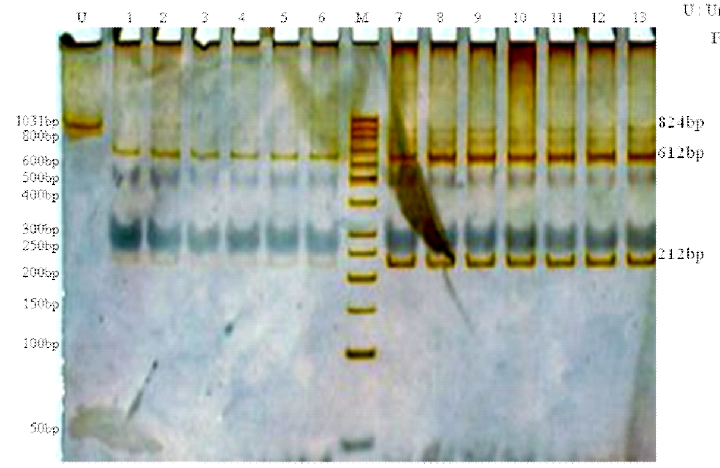
U : Uncut-PCR product ofMyostatin gene (2517bp), Lane 1, 2, 3, 4, 13 : AB genotype, Lane 5, 6, 7, 8, 9, 10, 11 : AA genotype, M : Marker DNA lader (100 bp).

Fig 2: *Taq*I-PCR RFLP of Myostatin gene in muskulo skeletal deformed CSML birds.



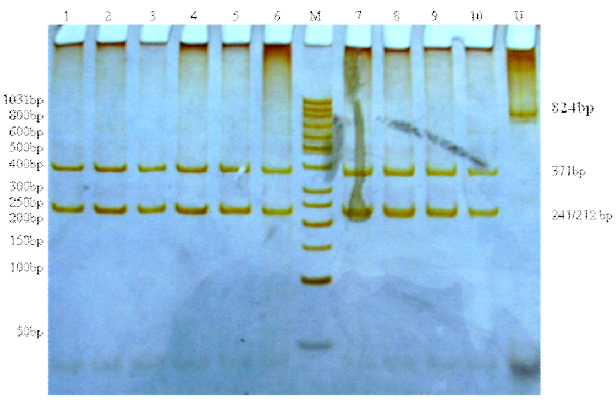
U : Uncut-PCR product ofMyostatin gene (2517bp), Lane 1, 2, 3, 4, 13 : AB genotype, Lane 5, 6, 7, 8, 9, 10, 11 : AA genotype, M : Marker DNA lader (100 bp).

Fig 3: *Bcu*I-PCR RFLP of BMP2 gene in muskulo skeletal deformed CSML birds.



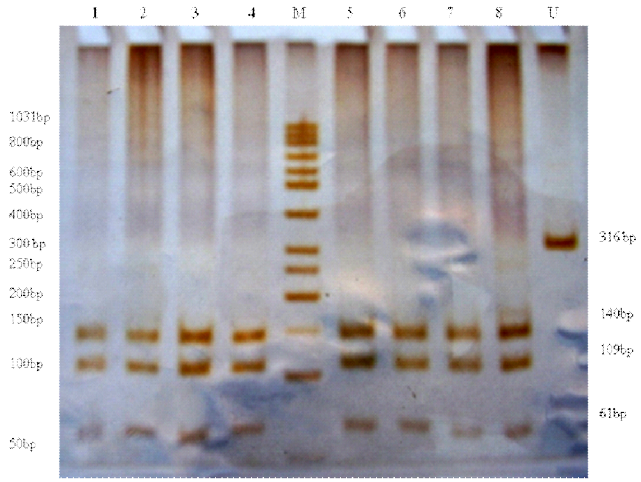
1 to 13 : AA genotype, M : Marker O'gene ruler (50 bp), U : Uncut-PCR product of BMP2 gene(824bp)

Fig 4: *Bme*1390I-PCR RFLP of BMP2 gene in muskulo-skeltal deformed CSML birds.



1 to 10 : AA genotype, M : Marker O'gene ruler (50 bp), U Uncut-PCR product of BMP2 gene(824bp),

Fig 5: *Alu*I-PCR RFLP of TGF-β2 gene in muskulo-skeletal deformed CSML birds.



U : Uncut-PCR product of TGF-β2 gene (316bp), 1 to 8 : AA genotype M : Marker O'gene ruler (50 bp)

Fig 6: Bone deformity in both leg (Twisted leg) of coloured broiler.