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Progress and promises of candidate gene association studies for improvement of fish complex traits

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Abstract
In fisheries, optimization of the desirable traits such as resistance to diseases, meat quality and body growth is very essential. These traits have a high influence on the profitability of fish farmers. Several generations of selection are needed to improve in these traits through traditional phenotype-based selection method and complex qualitative trait like meat quality is tough to measure for proper selection. In candidate gene approach, some pre-specified genes responsible for the trait of interest are selected. Then genetic variations within these genes are used as a molecular marker to study their association with the desirable phenotypes. It is different from genome-wide association studies (GWAS) where the entire genome is scanned for common genetic variation, but here it only focused on the target genes. As the genetic variation directly impacts the function of the gene in question so the biological function of the genes and its impact on the trait should be known to be selected. Here genetic variation in the candidate gene of target individuals is compared with the control individuals to study the variation responsible for the target phenotype. The allele frequency of a candidate gene is calculated in case and control to check the frequency level in target individuals compared to control. Improvement of performance traits through candidate gene approach is fast and more accurate and allows us to understand the genetic mechanism affecting performance traits in fishes.

Keywords: Candidate gene association selection, selective breeding, genetic improvement, single nucleotide polymorphism, next generation sequencing

Introduction
The correlation between genetic variations in the target gene with performance traits of fishes is used in a candidate gene association study to increase the aquaculture yield, improve incomes of farmers and enhances food security. Many selective breeding programs in fish were carried out, but it takes much time to optimize the target traits. Neira (2010) [1] reported the numbers of selective breeding program in commercially important aquaculture species for genetic improvement in developing countries, Table 1 summarized their report with species name, number of program and number of selected traits. It shows that the present number of family-based selection programs in aquaculture now exceeds 100. They reported that the highest number of breeding programs is reported for tilapia (27), followed by rainbow trout (13) and Atlantic salmon (13), while among the important most reared group of species (cyprinids) only common carp (8) and rohu carp. However, phenotype-based selection needed considerable time to optimize the traits, so researcher are now moving from phenotype-based selection to genotype-based selection. The lacking of a molecular marker is the main limiting factor for the realization of genotype-based selection potentials in fishes. However, with the advent of DNA-based genetic markers in the late 1970s and now the ease of the marker discovery through the next generation sequencing allowed the researchers to identify large numbers of markers spreads throughout the genome of any species of interest. The dramatic development of molecular genetics laid the groundwork for genomics that has introduced a new generation of molecular markers for use in the genetic improvement of farm animals. These markers provide more accurate genetic information and a better understanding of the animal genetic resources (Samarai and Kazaz, 2015) [2]. Molecular markers have been used in many different aspects of genetic management in aquaculture. Type and characteristics of the marker, ease and expense of application, abundance in the genome and polymorphic information content (PIC) are some of the major properties that make one molecular marker more useful than others under different situations.

Nowadays the SNP became very popular in the fishery to develop the trait associated marker that can be used for the selection of better performing individuals for the selective breeding programme. In case of fish, the candidate gene association study was reported by Jung *et al*. (2014) [3] in *M. rosenbergii* by Sanger sequencing. Thanh *et al.* (2010) [4] studied on SNPs in actin and CHH genes of *M. rosenbergii* in Vietnam and found one SNP located in the third intron of the CHH gene had a positive correlation with growth.

SNP as a marker in candidate gene association study

A new molecular marker technology named single nucleotide polymorphism (SNP) was proposed by Lander (1996) [5]. Liu and Cordes (2004) [6] described SNPs as those caused by point mutations giving rise to different alleles containing alternative bases at a given nucleotide position within a locus. This sort of polymorphism includes single base transitions, transversions, insertions and deletions. To be considered an SNP the least frequent allele should have a frequency of 1% or greater (Lander, 1996) [5]. Theoretically, one SNP within a locus can produce as many as two alleles, each containing one of two possible base pairs at the SNP site. Therefore, SNPs have been regarded as bi-allelic. SNP markers are inherited as co-dominant markers. SNPs are mostly used as a molecular marker for measuring genetic variance in case and control group in candidate gene association study.

Methods of SNP genotyping

Several approaches have been used for SNP discovery including SSCP analysis, hetero-duplex analysis, real time PCR, microarray and DNA sequencing. DNA sequencing has been the most accurate and most used approach for SNP discovery and has also become cost effective with the advent of new generation sequencing (NGS) techniques (Vignal *et al*., 2002) [7]. For most accurate detection of SNPs, Sanger sequencing method is the gold-standard sequencing technology, making it ideal for confirmation of novel variants. The high throughput Sanger DNA sequencing service gives a high quality resolution of polymorphisms, insertions and deletions in exonic and promoter regions and is useful in resequencing the gene of interest. Resequencing of coding sequences of genes in large populations has previously been shown to be useful for identifying multiple rare variants affecting quantitative traits. RNA-Seq is a recently developed approach to transcriptome profiling that uses deep-sequencing technologies.

Body growth association study using SNPs:

There are few trait association studies in fish have been conducted, table 2 shows some of the studies and it is clearly detectable that most of studies have been conducted for improvement of growth and disease resistance. Jung *et al*. (2014) [3] characterized 47 candidate loci in 4 cultured and 8 wild samples of *M. rosenbergii* by Sanger sequencing and detected 342 putative SNPs. Among these, 28 SNPs were selected from 23 growth-related candidate genes and genotyped in 200 animals selected for improved growth performance in an experimental GFP culture line in Vietnam. The associations between SNP markers and individual growth performances were then examined. Statistical analysis revealed that two exonic SNPs in glycogen phosphorylase and peroxidasin had additive effect, while one exonic SNP in HSP90 and one intronic SNP in ankyrin repeats-like protein had additive and dominant effect on growth. Five intronic SNPs in rolling pebbles, transforming growth factor-β induced precursor and UTP-glucose-1-phosphate uridylyl transferase 2 genes had dominant effect on growth. A study on SNPs in actin and CHH genes of *M. rosenbergii* was carried out in Vietnam by Thanh *et al*. (2010) [4]. SNPs identified in actin gene amplified from three different stocks obtained from Dong Nai, Mekong and Hawaii, were 7, 8 & 4, respectively. SNPs identified in the CHH gene were found to be present in intronic, exonic and 5' untranslated regions. Twenty two SNPs (including 15 in introns, 4 in exons and 3 in 5' UTR) were identified in Dong Nai stock, while the Mekong and Hawaii stocks had 16 and 9 SNPs, respectively. Among the SNPs identified in both the genes, the SNPs of actin gene are found to be unrelated to growth, but the SNP located in the third intron of the CHH gene had a positive correlation with growth. A genome wide association experiment was carried out by Tsai *et al*. (2015) [8] to predict association of the putative SNPs with growth traits in Atlantic salmon. A total of 111,908 SNPs was genotyped in 622 fish (from 61 full sibling families) and reported no SNP showed genome wide significant level association with growth. Only 1 SNP mapping to chromosome 17 surpassed the chromosome wide significance level for length and explained ~7% of the variation. The authors concluded that the study did not reveal any major QTL. Tao *et al*. (2003) [9] tested SNPs in five candidate genes for study their associations with the growth hormone axis and the age-specific growth rate of Arctic charr (*Salvelinus salpinus L*. : Salmonidae). They reported that SNPs were identified in 10 proposed candidate genes known to be linked to the growth hormone axis. All the individuals in the two backcross families were genotyped for these SNP markers using PCR-RFLP or bidirectional amplification of specific alleles (Bi-PASA). A significant association between a particular SNP allele and early growth was found for the locus containing the growth hormone-releasing hormone and pituitary adenylatecyclase-activating polypeptide genes (GHRH/PACAP2, P=0.00001).

Immune genes association study using SNPs

Mohindra *et al*. (2016) [10] conducted a study to identify the gene responsive to hypoxic stress in *Clarias magur*. A total of 12 subtracted cDNA libraries (six each forward and reverse) were constructed from different tissues like liver, muscle, brain, heart, head kidney, and spleen and a total of 2020 clones were sequenced for screening and a total of 1805 high quality expressed sequence tags (ESTs) was obtained from this experiment. They identified genes that are involved in vast majority of pathways/processes affecting metabolism, cellular processes, signal transduction and/or immune functions through the annotation of these differentially expressed ESTs. Additionally, 18 potential novel genes expressed in hypoxia stress exposed fish were also identified. Singh *et al*. (2012) [11] also generated 1937 high quality EST from 2045 single-pass sequenced clones in *Clarias magur*. Based on sequence similarities, 65 ESTs were found to be associated with immune functions. Six EST-SSRs and three SNPs were found associated with eight immune-relevant genes. These markers associated with important immune genes would be useful for the identification of trait associated alleles for marker-assisted selection. A total of 48 SNPs were discovered by Zou *et al*. (2015) [12] in the partial sequence of MmSAA (Serum amyloid A) gene from the clam *Meretrix meretrix* and examined their association with Vibrio-resistance and growth traits. The single SNP association analysis indicated that 5 SNPs were significantly associated with Vibrio-resistance (P<0.05).

Use of molecular markers

Molecular markers have been widely used for identification of strains and species, detection of inter and intra-specific hybridization, parentage and kinship analysis, assessment of parental contribution in mass spawning, estimation of effective population size and level of inbreeding, preventing inbreeding, mapping of quantitative trait loci (QTLs) and selective breeding. With regard to rehabilitation aquaculture, genetic markers have found a role in comparison of farmed strains and wild populations, choice of donor population, detection of genetic changes in hatchery-reared fishes over generations and monitoring the impact of reared animals after release to the wild (Cross, 2000) [13]. Last three decades have seen major advances in development of genetic markers. There is now an entire range of markers (low, moderate or highly polymorphic) to choose from depending on the evolutionary range of species being studied. These include isozymes, mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites, expressed sequence tags (ESTs) and single nucleotide polymorphism (SNP). The markers have been classified into two categories: type I are markers associated with genes of known function, while type II markers are associated with anonymous genomic segments. Now a day, the molecular markers showing higher polymorphism like SNPs or microsatellites, are highly used in candidate gene association approach to link the genetic variation (SNPs) with the target traits in economically significant aquaculture species.

Advantages and Limitation of Trait association study in fish

For an aquaculture scientist the trait association study is more easy way to develop an improve fish strain of target trait than the conventional selection methods especially for the traits having low heritability. In present time, the improvement of performance traits through traditional selection integrated with molecular tools. This method is fast and more accurate and allows us to understand the genetic mechanism affecting performance traits. But the main drawback of traditional breeding program is that it needs several progenies to optimize traits and in some case the progeny testing scheme requires a long generation interval. Also through selective breeding, it is difficult to optimize the traits which are difficult or expensive to measure or the traits expressed lately in individuals. Here the trait association study is very useful tool to reduce generation interval through early selection, even before maturity of the fish and to select those traits which are restricted to only one sex. But the main restriction in trait association study is the lack of complete genotype information of the fishes and high sample size is needed for SNPs detection. A limited number of trait association studies conducted in fishes. There are a few number of genes fully sequenced and studied for a performance traits in fish and also lack of confidence of users to associate this gene with the molecular marker leads to lower the accuracy of linkage between the trait with a molecular marker and thus it affect the potentials of the candidate gene association in fishes. So more studies need to conduct in fish for candidate gene association study so that number of improved performed fish species can be developed in less time.

Table 1: Status of selective breeding program in commercially important fishes

Species	No. of programs	No. of families Per program	Average no. of traits selected
Tilapia	27	495	11
Atlantic salmon	13	280	5
Rainbow trout	13	206	5
Common carp	8	76	2
Coho salmon	4	133	3
Rohu carp	1	65	2

(Modified from Neira, 2010)

Table 2: Candidate gene association studies in fish

Species	Target trait	Author/Year
Macrobrachium rosenbergii	Body growth	Jung *et al.* (2014) [3]
Macrobrachium rosenbergii	Body growth	Thanh *et al.* (2010) [4]
Atlantic Salmon *(Salmo salar)*	Body growth	Tsai *et al.* (2015) [8]
Arctic charr (*Salvelinu salpinus*)	Body growth	Tao *et al.* (2003) [9]
Clarias magur	Disease resistance	Mohindra *et al.* (2016) [10]
Clarias magur	Disease resistance	Singh *et al.* (2012) [11]
Meretrix meretrix	Disease resistance	Zou *et al.* (2015) [12]
Clarias batrachus	Disease resistance	Singh *et al.* (2013) [14]
Atlantic Salmon *(Salmo salar)*	Growth rate and age at sexual maturation	Gutierrez *et al.* (2015) [15]
Sole *(Solea solea*).	Growth and maturation	Diopere *et al.* (2013) [16]

Conflict of interest statement

Author declares that there is no conflict of interest in the manuscript.

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References

1. Neira R. Breeding in aquaculture species: genetic improvement programs in developing countries. In Proceedings of the 9th World Congress on Genetics Applied to Livestock Production. 2010; 8.
2. Samarai FR, Al-Kazaz AA. Applications of Molecular Markers in Animal Breeding: A. American Journal of Applied Scientific Research. 2015; 1:1-5.
3. Jung H, Lyons RE, Li Y, Thanh NM, Dinh H, Hurwood DA, *et al.* A candidate gene association study for growth performance in an improved giant freshwater prawn (*Macrobrachium rosenbergii*) culture line. Marine Biotechnology. 2014; 16:161-80.
4. Thanh NM, Barnes AC, Mather PB, Li Y, Lyons RE. Single nucleotide polymorphisms in the actin and crustacean hyperglycemic hormone genes and their correlation with individual growth performance in giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 2010; 301:7-15.
5. Lander ES. The new genomics: global views of biology. Science. 1996; 274:536.
6. Liu ZJ, Cordes JF. DNA marker technologies and their applications in Aquaculture Genetics. Aquaculture. 2004; 238:1-37.
7. Vignal A, Milan D, Cristobal M, Eggen A. A review on

SNP and other molecular markers and their use in animal genetics. Genetics Selection Evolution. 2002; 34:275-305.
8. Tsai HY, Hamilton A, Tinch AE, Guy DR, Gharbi K, Stear MJ, *et al*. Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. BMC genomics. 2015; 16:969.
9. Tao WJ, Boulding EG. Associations between single nucleotide polymorphisms in candidate genes and growth rate in Arctic charr (*Salvelinus alpinus* L.). Heredity. 2003; 91:60-69.
10. Mohindra V, Tripathi RK, Singh A, Patangia R, Singh RK, Lal KK, *et al.* Hypoxic stress-responsive genes in air breathing catfish, *Clarias magur* (Hamilton 1822) and their possible physiological adaptive function. Fish & Shellfish Immunology. 2016; 59:46-56.
11. Singh A, Sood N, Chauhan UK, Mohindra V. EST-based identification of immune-relevant genes from spleen of Indian catfish, *Clarias batrachus* (Linnaeus, 1758). Gene. 2012; 502:3-59.
12. Zou L, Liu B. Identification of a Serum amyloid A gene and the association of SNPs with Vibrio-resistance and growth traits in the clam *Meretrix meretrix*. Fish & shellfish immunology. 2015; 43:301-309.
13. Cross TF. Genetic implications of transaction and stocking of fish species with particular reference to Western Australia. Aquaculture. 2000; 31: 83-94.
14. Singh A, Barman AS, Sood N, Mohindra V. Transcriptome generation and analysis from spleen of Indian catfish, *Clarias magur* (Linnaeus, 1758) through normalized cDNA library. Molecular biology reports. 2013; 40:6965-6975
15. Gutierrez AP, Yáñez JM, Fukui S, Swift B and Davidson WS. Genome-wide association study (GWAS) for growth rate and age at sexual maturation in Atlantic salmon (*Salmo salar*). PLoS One. 2015; 10:e0119730.
16. Diopere E, Hellemans B, Volckaert FA and Maes GE. Identification and validation of single nucleotide polymorphisms in growth-and maturation-related candidate genes in sole (*Solea solea* L.). Marine genomics. 2013; 9:33-38.