From phenotype to genotype: Major genes in chickens

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The relationships between phenotype and genotype were first described by Mendelian genetics in the case of genes with a major phenotypic effect. The remarkable improvement of biochemical and molecular tools has now made it possible to identify the molecular nature of major genes, and to understand better their mode of action. From a functional point of view, mutations of a given gene can be classified into three groups: loss of function, interference and dominant negative action and gain of function. From a structural point of view, a large diversity of molecular polymorphisms can be found, the consequences of which depend rather on their position than on their nature. Genetic abnormalities or mutations with obvious morphological effects provide the most didactic examples to understand the genotype-phenotype relationships. The example of sex-linked dwarfism in chicken is described in detail and the present state of knowledge on the molecular identification of major genes in the chicken is reviewed. Finally, a general discussion, based upon examples taken from human genetics and mouse genetics, draws lessons from the study of major genes in order to approach the study of complex phenotypes or genotype x environment interactions.

Key-words: chicken, mutation, DNA, phenotype, gene mapping

Introduction

A few major genes affecting a quantitative trait and many morphological variants have been described in the chicken at the phenotypic level, and several of them have been mapped in the so-called 'classical map' (Somes, 1984; Bitgood and Somes, 1993). Recently, a few of these mutations have been characterized precisely with the identification of a molecular defect in a specific gene. In this case, the link from the phenotype to the genotype has been established as well as the role of a single gene in a complex function. Unravelling the molecular nature of a phenotypic mutation opens a way towards the genetic dissection of the affected trait. In other words, the existence of a mutant with a major phenotypic effect indicates that a single gene plays a crucial role whatever the complexity of the function involved. A few basic definitions are first needed in order to describe the relationships between phenotype and genotype. Then, the example of sex-linked dwarfism in chicken will be described in details, current knowledge on the

molecular nature of other chicken major genes will be reviewed, and the final discussion will consider some examples of complex phenotypes, gene x gene interactions and gene x environment interactions.

Definitions

MENDELIAN TERMINOLOGY

These definitions were established to suggest a genetic determinism from the observation of a phenotype. They are useful to recall in the view of connecting them to current molecular knowledge of gene structure and expression. The first two statements corresponded to the simple situations of recessivity and dominance:

- Recessivity when two copies of a mutation are needed to produce a new phenotype;
- Dominance when a single copy of a mutation is enough to modify the phenotype.

Because reality was often not so simple, two other notions were introduced:

- Penetrance, which describes the frequency of the carriers of the mutation (one or two copies) showing the modified phenotype;
- Expressivity, which describes the variable degree of the phenotypic effect.

DNA POLYMORPHISMS

Following the discovery of DNA as the biochemical support for genes, the main characteristics of the structure of a eucaryotic gene were discovered. Considering the normal structure of a gene, three types of modifications can take place:

- Deletion can represent a loss of one to several thousand bases;
- Insertion can represent the addition of one to several thousand bases, which can be of various nature, for instance duplication of a pre-existing DNA sequence or insertion of a foreign sequence, such as a viral sequence;
- Substitution consists in the replacement of one base by a different one, with no change in total number of bases in the sequence.

Deletions or insertions may affect gene expression when they are located in non-coding regions, and will affect protein structure when they are located in coding regions. In the latter case, the protein may be simply incomplete. The gene product may be, however, completely modified in the case of a frame shift mutation, in this case the remaining coding sequence is not read properly, i.e. wrong ‘words’ are read and wrong amino acids are incorporated into the protein.

In the case of base substitutions, three subtypes can be identified:

- Non-sense mutation, where the replacement of one base by another creates a stop codon in place of a codon specifying an amino-acid;
- Mis-sense mutation, where the mutated codon specifies a different amino-acid;
- Splice mutation, where a splicing site is suppressed or created.

Depending on the position of the polymorphism within the gene, it may affect DNA transcription, RNA splicing, RNA translation, protein structure or quantity. Functional consequences at the phenotypic level may show various degrees.

FUNCTIONAL TERMINOLOGY

First of all, many DNA polymorphisms can just be neutral. In the case where mutations have functional consequences, three categories have been identified.

Loss-of-function mutation. This type of mutation is characterized by a total absence of the gene product or the production of a totally inactive product. This type of mutation behaves generally in a recessive way, because the normal allele of a heterozygous carrier retains its function, and may even be transcribed at a higher level than in the normal homozygous. Thus, the heterozygous carrier will show an intermediate amount of the gene product, which can be sufficient to maintain the function. Variations may occur if the function is greatly impaired under a certain threshold level for the amount of the gene product. This latter phenomenon corresponds to a dosage effect (*Figure 1*). If the amount of the gene product in the heterozygous carrier is below the threshold, then the mutation will behave in a dominant way. This situation is also described as haploinsufficiency, where the presence of one normal allele is not sufficient to maintain the function. Because the normal activity of a gene can be disturbed in many different ways, one can expect an important molecular diversity at the origin of loss-of-function mutations.

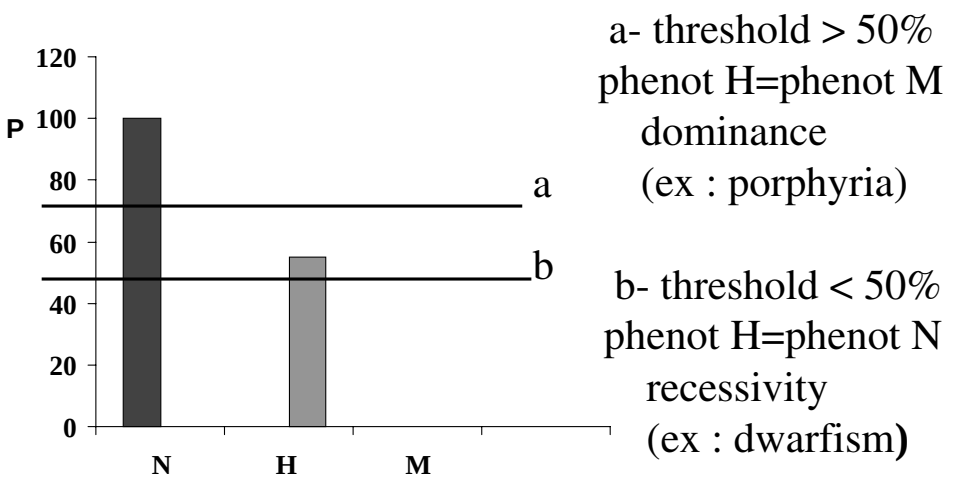

Figure 1 Schematic representation of the dosage effect for a loss-of-function mutation; P is the amount of gene product, a and b are threshold values, N represents the homozygous normal genotype, H the heterozygous genotype for the mutation, and M the homozygous mutant genotype.

Dominant negative mutation. This situation is observed when the gene product of the mutated allele is only partially active and may interfere with the normal gene product. This is particularly the case when the gene product acts as a cofactor or is involved in the formation of a dimer. The defect of one component in a dimer may be sufficient to impair the overall function because the abnormal allele 'disturbs' also the normal allele when both allelic products form a dimer. The mutation behaves in a dominant way because only one mutated allele is able to impair the gene function. This phenomenon is well illustrated in human genetics by mutations of the nuclear hormone receptors (Yen and Chin, 1994). The same applies when the dimer involves also the product of a different gene, then, the mutation of one gene has a negative epistatic effect on the function usually associated with the other gene.

Gain-of-function mutation. A new function can be obtained with the production of an aberrant protein or the expression of a normal protein in abnormal conditions, either at an unusual age or in an aberrant location. These mutations behave generally in a dominant way and may have severe phenotypic effects. For instance, the henny feathering mutation

in the chicken is due to an expression in the peripheral tissues of the aromatase, which converts testosterone into estrogen: although this is the normal function of this enzyme, it does not take place normally in the peripheral tissues. The enzyme activity in the skin is about half in the heterozygous as compared to the homozygous mutant, but this amount is enough to modify the phenotype, and induce the female plumage pattern in the male, thus the mutation is dominant (Somes *et al.*, 1984). Although a large molecular diversity may be expected for loss-of-function mutations, it may be speculated that only a specific abnormality of a gene can modify the function of the gene product in a specific way.

Mutations of GH receptor gene in sex-linked dwarf chickens

Molecular defects. The recessive sex-linked dwarf gene (*DW*) provides a well-documented example that will be presented in detail. The *DW* gene has been known for many years (Hutt, 1959) and described in a variety of chicken lines. Extensive studies on performance, physiology and endocrinology of young and adult dwarf chickens were cumulated over many years, and led to the suggestion that GH receptor was the best candidate for the primary defect in dwarfs (Tixier-Boichard *et al.*, 1989; Decuypere *et al.*, 1991). The proof came when a molecular tool became available, i.e. when the gene for chicken GH receptor (*cGHR*) was cloned (Burnside *et al.*, 1991). Three different mutations of the *cGHR* gene have now been fully characterized in dwarf chickens (*Table 1*).

Several lessons can be drawn from the data shown in *Table 1*:

- different abnormalities of the *cGHR* gene have been found in independent populations where the *DW* gene resulted from a different mutational event, thus sex-linked dwarfism shows molecular heterogeneity and more alleles could be present in other dwarf populations;
- Because the finding of a modified gene structure is not a proof that the causal mutation for *DW* was identified, gene expression and protein function were investigated in order to establish the causal relationship between the molecular defect and the phenotype.
- The only common point between the three different mutations of *cGHR* is the lack of a significant GH binding activity on liver membranes; the associated decrease in body weight appeared to vary with the strain, either because of a different mutation in the *cGHR* or because of a different background for other genes controlling growth.
- Sex-linked dwarfism appears to be a loss-of-function mutation, with a very limited dosage effect, as shown by the slight decrease in body weight for heterozygous carriers; it must be noticed that the decrease in GH binding activity was much more pronounced than the decrease in body weight for the heterozygous carriers.
- The study of gene expression may not be sufficient to identify a causal mutation within a gene, the mutant of the WL strain had a normal expression pattern, only complete sequencing of the cDNA of *cGHR* made possible to detect the base substitution which dramatically impairs the protein function.
- Sequencing the cDNA may not be sufficient either, as shown by the GA mutation located at the junction between an intron and an exon, a genomic clone including this junction had to be sequenced, because the mutation was not found in the cDNA.
- Although the protein structure is abnormal in the CT/OB mutation, it does not correspond to a gain of function.

Search and proof for a causal mutation. When looking for the gene responsible for a major phenotypic effect, physiological studies of the function involved can propose candidate genes. In the case of the *DW* mutation, the investigations on growth regulation

Table 1 Molecular defects of the growth hormone receptor gene and body weight reduction in sex-linked dwarf chickens.

Origin of the chicken line		GA (USA) 'meat type '		CT (USA) 'meat type'	OB (France) 'egg type brown egg'	WL (France) 'egg type white egg'	
References		Huang et al. (1993)		Agarwal et al. (1994)		Duriez et al (1993)	
Molecula defect		substitutionr (TÆC)		1773 pb deletion		substitution (GÆT)	
Position of the mutation		Splicing site:2 bases after position 352 of the coding sequence 352+2(TÆC)		end of exon 10 and 3' unstranslated region, position 1744 to 3516 nt1744(del 1773)		position 679 of the coding sequence, 679(GÆT)	
Expected effect		abnormal splicing		frameshift mutation		missense mutation Ser199ÆIle	
Molecular diagnostic. test		sequencing		RFLP or PCR test		sequencing, PCR-ASO test	
Genotype under study	normal	hetero zygous	homo-zygous	Hetero-zygous	homozygous	Hetero-zygous	homo-zygous
Major RNA transcripts (kbp)	4.3	4.3 / 0.8	0.8	expected 4.3 / 2.5	2.5	4.3	4.3
Protein structure, and localisation	592 aa cytokine receptor family		not detectable		abnormal: loss of 27 aa (hydrophilic) and gain of 53 aa (hydrophobic)		normal but not expressed at the cell surface
GH binding activity	100	56	ε (detection limit)	not done	ε (detection limit)	not done	0
Phenotype: body weight	100	91	57	93-98	70	92	61

focused on abnormalities of the thyroid and somatotroph axis. Because the mutant phenotype was known to be caused by a single gene, a single factor was to be found in order to explain both abnormalities. The GH receptor appeared to be the first candidate to meet this condition. Thus, the candidate gene strategy was successful in this case. In other instances, candidates can be suggested from the knowledge of a homologous mutation with a similar phenotypic effect in another species where molecular tools are more advanced. Most often, there may be either too many candidates or not a single one, and the search for the mutation will start with mapping the gene with anonymous markers. Then the genetic map will be used to suggest candidate genes based upon their chromosomal position and their function. If the marker appears to be very close to the mutation, molecular techniques can be used in order to identify an expressed gene in the closely linked region. This strategy called positional cloning is very heavy; it involves a wide range of molecular tools as well as extensive sequencing and cannot be presented here. It has been used successfully to identify some human genetic disorders.

In the case of *DW* gene, mapping information for the *cGHR* gene was obtained independently by FISH mapping (Suzuki *et al.*, 1999a) and linkage with anonymous markers (Levin *et al.*, 1993), which showed a position at 52 cM on the short arm of Z chromosome. Furthermore, complete linkage between the RFLP and the dwarf mutation of the OB line, as well as between the PCR-ASO test and the dwarf mutation of the WL line, were observed in segregating families produced in our experimental facilities.

In the mouse, the definitive proof for a causal mutation comes when animals made transgenic for the modified gene exhibit the mutated phenotype. This tool has not been used in the chicken but gene transfer can be realized in cell culture, and the protein function investigated at the cellular level, provided that this function can be observed in the particular cell type used for gene transfer. In the case of the WL dwarf mutant, the functional importance of the base substitution was confirmed by reproducing the same defect in the human gene for GHR (*hGHR*): the mutated *hGHR* gene was transiently expressed in cell culture and showed a defective cell surface expression responsible for a markedly reduced binding activity of human GH (Duriez *et al.*, 1993).

Molecular identification of other chicken major genes

Candidate genes for other mutations of the chicken have been already identified or suggested by physiological or genetic studies (*Table 2*). Feather colour genes provide an example where homology between species may be a useful strategy. In the case of the *C* locus, both physiological data (Oetting *et al.*, 1985) and homology with albino gene in mammals led to the same candidate gene, the tyrosinase, which has been localised on chromosome 1 (Suzuki *et al.*, 1999b). The *Extension* locus which affects coat colour has been shown to code for the receptor of the alpha-melanocyte-stimulating-hormone in the mouse (Robbins *et al.*, 1993), in cattle (Joerg *et al.*, 1996), pig (Kijas *et al.*, 1998) and probably also chicken (Takeuchi *et al.*, 1996). In this case, a similar phenotype would be regulated by the same gene across species.

Reciprocally a mutation of a given gene may not always have the same phenotypic consequences: for instance a point mutation in the ryanodine receptor gene *RYR1* is responsible for the malignant hyperthermia syndrome in pig (Fujii *et al.*, 1991), whereas the lack of expression of the alpha isoform of the ryanodine receptor, *RYR1*, causes severe skeletal muscle dysgenesis and prevents normal embryonic development in the chicken, as found in the crooked neck dwarf mutant (Airey *et al.*, 1993).

Finally, when a mutation has been mapped in chicken but its mode of action remains unknown, comparative mapping appears the most powerful strategy to propose candidate genes that are located in the homologous chromosomal region in a reference species, most often the mouse, and may have phenotypic effects similar to those observed for the chicken mutation. This was illustrated for the autosomal dwarf mutation (Ruyter-Spira *et al.*, 1998).

Although the number of chicken mutations showing linkage with a molecular marker is still rather limited, it may increase in the future because linkage analysis can be done at reduced costs in a family of reasonable size (about 75 half-sibs) by pooling DNA samples and using bulked segregant analysis (Ruyter-Spira *et al.*, 1998; Pitel *et al.*, 2000). An alternative way, currently preferred to map human rare genetic disorders, is the identical-by-descent strategy, where a few affected individuals are sampled in families, which share a common ancestor carrier of the genetic defect. This approach might be applied to chicken pedigrees, particularly in closed experimental lines or fancy breeds, which derive usually from a limited number of founder animals.

Table 2 Mutations with major phenotypic effects in the chicken: candidate genes and/or linkage with molecular markers.

'Classical mutant'	Chromoso-mal position	Candidate gene and/or physiological effect	Molecular defect	References
Auto5somal albinism	?	tyrosinase		Oetting *et al.* (1985)
	TYR gene maps on 1		6 base deletion	Tobita-Teramoto *et al.* (2000) Suzuki *et al.*, (1999b)
Autosomal dwarfism	1p	*HMGI-C* (homology with mouse pygmy mutation)		Ruyter-Spira *et al.* (1998)
Blue egg-shell	1p	biliverdin pigment in shell	?	Bitgood *et al.* (1980) Bartlett *et al.* (1996)
Crooked neck dwarf	E25C31 linkage group	lack of expression of alpha-ryanodine receptor	?	Airey *et al.* (1993) Groenen *et al.* (2000)
Dermal melanin inhibitor	Z position 214	?	?	Levin *et al.* (1993)
Dominant white	E22 Linkage group	defect in eumelanin synthesis and death of pigment cell		Ruyter-Spira *et al.* (1997)
Extension of eumelanin	1 ?			Carefoot (1990)
	MC1R maps on a micro-chromosome	alpha-MSH receptor *(MC1R)*	point mutation	Takeuchi *et al.* (1996) Sazanov *et al.* (1998)
Henny feathering	E29 linkage group	aromatase		Somes *et al.* (1984) Dunn *et al.* (1999)
Late-feathering *K* and *ALVE21*	Zp	?	duplication and retroviral insertion	Bacon *et al.* (1988) Levin and Smith (1990) Iraqi and Smith (1995)
Naked neck	3qter	?	?	Pitel *et al.* (2000)
Nanomelia	E29 linkage group	aggrecan	point mutation	Li *et al.* (1993) Primorac *et al.* (1994)
Pea-Comb	1p	?	?	Zartmann (1973) Bitgood (1985) Bartlett *et al.* (1996)
Polydactyly	2p	comparative mapping: *Hx* mouse polydactyly mutation	?	Pitel *et al.* (2000)
Restricted ovulator	Z	oocyte vitellogenesis receptor *(OVR)*	point mutation	Bujo *et al.* (1995)
Riboflavinuria	?	riboflavin-binding protein	point	Maclachlan *et al.* (1993)
			mutation	Ramanathan *et al.* (1980)
Sex-linked white skin	Z	HDL deficiency	?	Poernama *et al.* (1990)
Sex-linked dwarfism	Zp position 52	GH receptor	See Table 1	see Table 1 and text

General discussion

The finding of genetic factors underlying known phenotypes brings a lot of new information on gene expression and on relationship between genotype and phenotype.

ALLELIC DIVERSITY

This is the most general conclusion that can be drawn from current data on the molecular characterization of mutations with major phenotypic effects. Different abnormalities of a given gene may not always lead to the same phenotype. Variable expressivity of a mutation may in fact be due to different molecular defects of the same gene that can only be distinguished by appropriate molecular tools. In some cases, various abnormalities may be combined within a single allele and give rise to a new phenotype. Composite heterozygous carriers are individuals carrying two different mutated alleles, which may lead to a 'new' phenotype, different from either homozygous mutants. For instance, the CFTR gene responsible for mucoviscidosis can show up to 400 different molecular defects, some being associated with different phenotypic effects. The location of a molecular defect in regulatory regions may also change the expression pattern of a gene in a way that is very difficult to understand at the phenotypic level. In the case of the agouti mutant of the mouse the production of yellow hairs can be normal, limited to the ventral region or totally absent (recessive black phenotype), depending on insertions of variable length located upstream of the gene coding region (Siracusa, 1994).

GENE X GENE INTERACTIONS

One particular case is observed when a major molecular defect involves two neighbour genes, for instance a deletion may impair two genes, and the resulting phenotype will involve two physiological pathways and may be quite impossible to interpret as a single gene defect. One such example has been described in the mouse, at the pink-eyed locus, where one mutation was associated with reduced pigmentation, as expected, but also with cleft palate and neurological disorders. This complex phenotype was better understood when this allele was shown to consist in a large deletion affecting also a cluster of 3 neighbor genes (Nakatsu *et al.*, 1993). Because these genes were coding for subunits of the receptor to a neurotransmitter (gamma-amino-butyric acid), their dysfunction was causing the neurological disorders.

Interactions will take place when different gene products participate in the same function, either because of the formation of a multimer or because of complementary actions at different levels. One interesting case that has not yet been characterized at the molecular level is the hyper-pigmented silky fowl. This phenotype is determined by the combination of a sex-linked recessive mutation at the *ID* locus and an autosomal dominant mutation at the fibromelanosis (*FM)* locus. Thus, the penetrance of the fibromelanotic mutation is null in individuals carrying the wild type dominant allele at the *ID* locus, and complete in individuals homozygous for the recessive mutant allele at the *ID* locus. Several alleles have been described, however, for *ID* and the penetrance of the fibromelanotic mutation is not well known for composite heterozygous carriers of mutant alleles at the *ID* locus.

Finally, interactions may take place between one gene and the background genome. Although the molecular basis for these interactions is not well understood, they have been demonstrated experimentally in transgenic mice, where phenotype associated with the same transgene could vary depending on the recipient genome (Doetschmann, 1999; Muller, 1999).

GENE X ENVIRONMENT INTERACTIONS

A given mutation may be necessary but not sufficient to determine a phenotypic change. An obvious example is provided by phenylketonuria, a genetic disease due to a loss-of-function mutation of an enzyme, which leads to an accumulation of phenylalanin with neurotoxic effects. Phenylalanin is found in animal proteins but not in plant proteins, consequently, individuals who do not eat animal proteins will not show the abnormal

phenotype, the penetrance of the mutation will be null in this case. Besides this situation, where an environmental factor interacts directly with the gene product, interactions may be often explained by pleiotropic effects of a mutation. In this case, the primary metabolic or phenotypic effect of the mutation has indirect consequences on the physiological balance of the individual, whatever the molecular basis of the mutation.

A particular case of gene x environment interaction is found when gene expression depends on the parental origin of the inherited allele, either paternal or maternal. The phenomenon, known as imprinting in mammals, has not been reported yet in birds. Imprinting is based upon parentally controlled gene methylation; the phenomenon would involve similar mechanisms as random X inactivation. In bird, random Z inactivation is not observed in the homogametic sex, which could suggest that imprinting might also not take place in birds. This should be confirmed, however, by experimental data. Another situation where parental origin seems to affect phenotypic expression of a mutation, is illustrated in human genetics by dominant mutations due to trinucleotidic expansion, such as Huntington's disease, which appears later in life when transmitted by the mother.

Conclusions

There is no general rule that can help to predict the phenotypic consequence of a mutation. Generally, the gain of a new function alters the phenotype in a more severe way than the loss of a function. Several observations are worthwhile remembering:

- At a given locus, a dominant or a recessive form of the same defect may be found,
- Several types of information need to be accumulated in order to identify a causal mutation: genetic linkage and comparative mapping, gene homology, gene expression, gene sequence, protein function; the single finding of either a normal gene expression or a normal coding sequence can not exclude totally the gene from being responsible of a mutant phenotype,
- Phenotypic consequences of a given molecular polymorphism improve our understanding of gene function and of the role of one gene in a complex function, there must be a regular exchange of information between physiological studies, either *in vivo* or *in vitro*, and molecular genetics,
- When several genes interact in a complex function, the consequences of variability at one locus are more difficult to predict, that is why the candidate gene approach can be very tedious, unless a key factor can be identified in the metabolic pathway.

In most situations where the relationship between a molecular abnormality and a major gene has been clearly established, the modification of the phenotype was very obvious, either on a qualitative scale or because of a large quantitative effect. Molecular diversity that can be revealed in these extreme situations may also contribute to phenotypic variability of quantitative traits, however these traits are probably more subject to epistatic interactions, gene redundancy and gene x environment interactions, so that the phenotype-genotype relationship may be more difficult to establish.

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