Preface

"Advances in Genetics Research" presents original research results on the leading edge of genetics discovery. Each article has been carefully selected in an attempt to present substantial research results across a broad spectrum. In this continuing series compilation, the authors present and discuss varied topical data such as late diagnosis in multiple X and Y chromosome disorders; the design and cloning strategies of recombinant allergens for diagnosis and specific immunotherapy; the advances in molecular cloning directing the production of plant proteins and their applications in pathogens control; the genetic basis of congenital heart disease;the large-scale evolution by generating new genes; characteristics of *MX* gene that shows resistance to vesicular stomatitis virus; the vertebrate fatty acid and retinoid binding protein and proteins; and the clonal evolution and cytogenetic changes in chronic myeloid leukemia.

Chapter 1 – *Background:* Sex chromosome aneuploidies (SCAs) are the most frequently occurring chromosomal abnormalities with an incidence of 1 in 400 births. Males with SCAs are known to have variability in their developmental profile. Sixty-four percent of males with 47,XXY are never diagnosed, 10% of these cases are diagnosed prenatally by amniocentesis, and 26% are diagnosed postnatally when they show developmental delay, behavioural problems, hypogonadism, gynecomastia, or infertility.

The aim of this paper is to evaluate how often developmental delay and behavioural problems can induce an early suspicion of this conditions.

Design and Methods: The sample was composed by 48 subjects (mean age=23,5 yrs, range:1-55) with karyotype 47,XXY (77%), 49,XXXXY (6,2%), 48,XXYY (6,2%), mosaicism 47,XXY/48,XXXY (2%), 47,XYY (4,1%), 48,XXXY (2%), 49,XXXYY (2%).

Primary caregiver completed a comprehensive questionnaire detailing birth, medical, developmental and psychological history (Tartaglia, 2008).

Results: Five subjects had a prenatal diagnosis (10,4%), 15 (31,2%) had a diagnosis before 10 yrs and 28 subjects (58,3%) had a late diagnosis (after 10 yrs). In the post-natal diagnosed group, patients were diagnosed for genital anomalies/dysmorphisms 32,5%; learning disabilities/attention disorders, behavioural disorders were diagnosed in 32,5%, hypogonadism/puberal delay 13,9 %, epilepsy 9,3%, recurrent infections 6,9%, infertility 2,3 %. *Conclusion:* Boys exhibiting developmental delay with learning and behavioural disorders should be considered for chromosomal analysis early in life. An early identification of the social and behavioural phenotypes in SCAs may enhance the clinical treatment, anticipatory guidance, and care throughout the lifespan.

Chapter 2 – Persons suffering from allergy (Type I hypersensitivity) produce immunoglobulin E against innocuous environmental antigens such as pollen, house dust, animal dander, food proteins. Diagnosis of allergy is based on the measurement of allergen – specific IgE antibodies and on provocation with allergens in skin prick test. Diagnostic reagents based on allergen extracts obtained from natural biological material often reveal unbalanced allergen content, a presence of nonallergenic components, and are difficult to standardize. Replacement of allergen extracts with a set of individual allergens in component-resolved diagnostics is regarded as a tool for patient selection for specific immunotherapy. The concept of using single recombinant allergens to determine the patient's sensitization profile was coined "component-resolved diagnosis" and is regarded as a precondition for patient-tailored immunotherapy, i.e., "component resolved immunotherapy".

To provide reliable, more specific reagents for allergy diagnosis and therapy recombinant DNA technology has been widely applied. The majority of recombinant allergens by far, have been produced in the prokaryotic expression system; however eukaryotic cells (yeast, plant, insect and mammalian cells) were also exploited. To avoid side effects in the course of immunotherapy various approaches in design of hypoallergenic molecules have been performed. This chapter will give an overview of the concepts and approaches in producing recombinant allergens for component resolved diagnosis and component resolved immunotherapy.

Chapter 3 – Plants are frequently exposed to adverse conditions that affects the crop production, mainly when are submitted to pathogens attack such as virus, bacterial or fungal. Like animals, plants have an innate immunity system composed by two defense layers at cellular and molecular level against pathogens attack. The first layer of the immune system is composed by extracellular surface receptors. These are transmembrane receptors or pattern recognition receptors (PRR`s) that recognize pathogen-associated molecular patterns (PAMPs). When the pathogens overcome the first layer, the second layer is activated, composed by genes that encode resistance proteins which recognize highly variable pathogen molecules called avirulence (Avr) effectors. Other molecules involved in defense against pathogen are vegetable proteins, such as lectins, peroxidases and Pathogenesis-Related proteins (PRs). All these biomolecules are of great biotechnological interest in plants breeding program. The molecular cloning allows us to study them deeply, by providing pure samples of genes that can be expressed later. It is possible due to rapid growth of bacterial, producing large amounts of identical DNA fragments which alone have no capacity to reproduce themselves. Once a gene has been cloned there is a lot of information that can be obtained about the structure and the expression of that gene. The popularization of cloned fragments has stimulated the improvement of methods for studying genes, with new approaches being used along the years. Recently, advances in molecular cloning allowed the study and expression of foreign genes and allowed the production of recombinant proteins in a large scale. This enabled the study of the biophysical and biochemical properties of proteins, as well as the elucidation of their roles in complex mechanisms, like in plant defense systems. Special types of cloning vectors and an expression system are needed for yielding recombinant proteins. The cloning vectors that provide a strong promoter and regulatory elements required for protein expression are called expression vectors. The expression system will determine which regulatory elements must be present in the expression vector. On the other hand, the choice of the appropriate system depends on several factors, among them yield, cost, and biochemical and functional properties of the protein to be expressed.

Prokaryotic and eukaryotic systems have been used successfully for protein expression. Prokaryotic expression systems are simpler, present high yields and are relatively low cost, but lack the posttranslational modifications present in eukaryotic systems. This technology has allowed advances in the control of important crops pests in the agriculture through the study of several proteins involved in plant defense systems.

Chapter 4 – Congenital heart disease (CHD) is the most common type of birth defect. Despite of the many advances in our understanding of cardiac development and many genes related to cardiac development identified, the fundamental etiology for the majority of cases of congenital heart disease remains unknown.

CHD is a multifactorial complex disease, with environmental and genetic factors playing important roles. A number of causative genes of selected congenital heart defects and genetic syndromes have been found. The molecular mechanisms of CHD may include mutations in components of the cardiac gene network, altered haemodynamics, regulatory pathway of cardiac genes, micro-RNA dysfunction, epigenetics, adult congenital heart diseases, and so on.

This review summarizes normal cardiac development, outlines the recent discoveries of the genetic causes of CHD, and provides possible strategies for exploring them. The molecular basis of CHD is an exciting and rapidly evolving field.

The continuing advances in the understanding of the molecular mechanisms of CHD will hopefully result in improved genetic counseling and care of affected individuals and their families.

Chapter 5 – In addition to Darwinian evolution selecting the advantageous mutants by the instantaneous judgment, the large-scale evolution by gene duplication is theoretically formulated on the basis of the concept of biological activity. This theory evaluates the fractions of satellite variants, which decline to the minor members in the population by having experienced gene duplication, and indicates the possibility that a new style organism arises from such satellite variants by generating new gene(s) from the counterpart of duplicated genes. This evolutionary route explains the punctuated mode of divergence of new and original styles of organisms. Moreover, the details of this large-scale evolution depend on the types of organisms which are different in their genome constitution and transmission. The monoploid organism such as prokaryote is suitable to generate one new gene step by step testing its biological function, but hardly generates many kinds of new genes simultaneously. The monoploid eukaryote, whose genome consists of the plural number of chromosomes, resolves this difficulty to produce a new style of organisms by receiving many kinds of new and large genes generated from different sources of gene duplication through the exchange of homologous chromosomes upon conjugation. The diploid eukaryote can also produce a new character expressed by multiple kinds of new genes accumulated through the successive hybridization of different variants, but its probability of accumulating such new genes is lower than in the monoploid eukaryote. Furthermore, the new style diploid organisms first appear as the heterozygotes concerning the new genes. If the new style diploid organisms have the higher increase rate, however, various variants carrying a partial set of new genes are also generated with the higher probability on the way to establish the new style organisms as homozygotes, and these variants hybridize with the other latent variants to yield various other new styles of diploid organisms. This tendency becomes outstanding especially when many kinds of new genes are required to express a character such as the advancement of the hierarchy of cell differentiation, and explicitly explains the explosive divergence of body

plans that has occasionally occurred in the diploid eukaryotes. These results also get an insight into the higher category of evolution that some of the monoploid eukaryotes have evolved to the diploid eukaryotes.

Chapter 6 – The antiviral *MX* gene is an interferon-induced GTPase, which is involved in inhibiting the multiplication of RNA viruses, such as vesicular stomatitis virus (VSV). Due to the severe threats of infectious spread to large numbers of livestock, animal breeding focused on innate immune-associated genes such as *MX* may play an important role in reducing the incidence of infection. In chicken, *Mx* gene was thought to lack antiviral activity. However, a specific single nucleotide polymorphism (SNP) in chicken *Mx* conferred antiviral activity against VSV and influenza virus. This SNP corresponded to amino acid position 631 (631 aa), and resulting in a serine to asparagine change at 631 aa. Therefore, this indicates that it is possible to breed infectious disease-resistant chickens based on the above-mentioned SNP, which could help to prevent the spread of infections. Furthermore, the SNP corresponding to 631 aa was shown to be involved in not only chicken *Mx* antiviral activity, but also in its intracellular localization. The SNP coincided with the cytoplasmic granular-like protein distribution, suggesting that the intracellular behavior of chicken Mx was likely responsible for its antiviral potential. The intracellular localization and protein behavior of human MxA and mouse MX1 have also indicated a role in viral inhibition. Some MX proteins show granular-like localization, similar to chicken Mx. Therefore, this pattern of chicken Mx localization may also contribute to the antiviral ability against VSV in other livestock animals from avians to mammals. This review focuses on the variety of *MX* genes among livestock animals and their function in resistance to infectious diseases, especially to VSV.

Chapter 7 – Fatty acid binding proteins (FABP) and retinoid binding proteins (RBP) are members of a family of small, highly conserved cytoplasmic proteins that function in binding and facilitating the cellular uptake of fatty acids, retinoids and other hydrophobic compounds. Several human *FABP*-like genes are expressed in the body: *FABP1* (liver); *FABP2* (intestine); *FABP3* (heart and skeletal muscle); *FABP4* (adipocyte); *FABP5* (epidermis); *FABP6* (ileum); *FABP7* (brain); *FABP8* (nervous system); *FABP9* (testis); and *FABP12* (retina and testis). A related gene (*FABP10*) is expressed in lower vertebrate liver and other tissues. Four *RBP* genes are expressed in human tissues: *RBP1* (many tissues); *RBP2* (small intestine epithelium); *RBP5* (kidney and liver); and *RBP7* (kidney and heart). Comparative FABP and RBP amino acid sequences and structures and gene locations were examined using data from several vertebrate genome projects. Sequence alignments, key amino acid residues and conserved predicted secondary and tertiary structures were also studied, including lipid binding regions. Vertebrate *FABP*- and *RBP*-like genes usually contained 4 coding exons in conserved locations, supporting a common evolutionary origin for these genes. Phylogenetic analyses examined the relationships and evolutionary origins of these genes, suggesting division into three *FABP* gene classes: 1: *FABP1*, *FABP6* and *FABP10*; 2: *FABP2*; and 3, with 2 groups: 3A: *FABP4*, *FABP8*, *FABP9* and *FABP12*; and 3B: and *FABP3*, *FABP5* and *FABP7*. Several *FABP/RBP* gene evolutionary events are proposed: (1) ancient gene duplications within invertebrate genomes forming 3 major *FABP* (class 1, 2 and 3) genes and an *RBP* gene; (2) more recent gene duplications within ancestral vertebrate genomes forming multiple *FABP* class 1, *FABP* class 3 and *RBP* vertebrate genes; (3) duplications within the zebra fish (*Danio rerio*) genome forming multiple genes for *FABP1* and *FABP7* (by chromosomal duplication); and *FABP4* and *RBP1* (by tandem duplication); (4) loss of the lower vertebrate *FABP10* gene within the ancestral mammalian genome; and (5)

retrotransposon gene duplications for the mammalian *FABP5* genes, generating *FABP5* pseudogenes, particularly in primate genomes.

Chapter 8 – Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder. The disease has a progressive natural course from an indolent chronic phase (CP) followed by an accelerated phase (AP) which usually ends into a very aggressive blast crisis (BC). It accounts for approximately 5-20% of all cases of leukemia and is more common in elder people with a median age of 65 years at the time of diagnosis.

Approximately 85% of cases display a standard cytogenetically balanced translocation t(9;22)(q34;q11) known as Philadelphia chromosome (Ph). As a consequence of the reciprocal translocation, the *BCR-ABL1* chimeric gene is generated which is critical for the pathogenesis of CML. The remaining 15% of patients either harbor variant translocations, involving other chromosomes, or cryptic fusions seemingly a normal karyotype.

The t(9;22), the primary chromosomal abnormality, typically remains as the only abnormality along most of the CP. The progression of the disease is related with the clonal evolution and the acquisition of additional secondary cytogenetic abnormalities.

The most common secondary changes during the clonal evolution, in nearly 90% of cases, are: +8, +Ph, i(17q), and +19, which have been referred as the "major route". The "minor route" aberrations, includes other less frequent (<10%) observed changes: -Y, +21, -7, alterations of 3q, among others.

In the last fifteen years, the management of CML has been revolutionized by the development of *BCR-ABL* tyrosine kinase inhibitors (TKIs). The imatinib mesylate (IM) was introduced as first line therapy in 2000 for most newly diagnosed CML patients. Afterwards, second generation TKIs (dasatinib and nilotinib) were approved for the treatment of imatinib-intolerant or resistant patients, and they were proposed as a first line treatment option in 2012.

The great majority of patients receiving imatinib treatment remain in complete hematologic (98%) and cytogenetic response (87%) with a median follow-up time of >5 years. A small percentage (around 8%) develops additional cytogenetic changes in their Ph-positive cells during the course of treatment. These changes may be transient and disappear during the follow-up or when the treatment is modified including dose-increase or change to another TKIs, especially in those patients who initially achievied a complete cytogenetic response. The available data suggest that the cytogenetic evolution pattern in patients treated with TKI seems to follow the "major route" of evolution. In the authors' experience, 5% of cases in follow up treatment develop clonal evolution.

Due to the importance of cytogenetic studies to properly monitor CML patients, this chapter focuses on the cytogenetic profile of karyotypic evolution in the context of actual TKI treatment and its relationship with response, resistance and progression.