

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,500

Open access books available

118,000

International authors and editors

130M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Cytogenomic Microarray Testing

Irene Plaza Pinto, Alex da Cruz, Emília Costa,
Samara Pereira, Lysa Minasi and Aparecido da Cruz

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.80514>

Abstract

Cytogenomic microarray testing allows the detection of submicroscopic genomic rearrangements, commonly denominated copy number variations (CNVs) that are implicated with many neurodevelopmental disorders, dysmorphic features, multiple congenital anomalies, hematological and solid tumors, and complex disorders and traits in both humans and animals. On the other hand, this approach is also widely used for the identification of structural variations that are applied as a biomarker in pharmacogenomics, agriculture, and animal selection and breeding. The chromosomal microarray analysis (CMA) has been applied for over a decade to screen for submicroscopic genomic gains and losses in DNA sample in both diagnostic and functional scenarios. Herein, we present an overview of the fundamental concepts of cytogenomics and its potential application in human genetic diagnosis, agrigenomics, mutagenesis, and pharmacogenomics.

Keywords: microarray analysis, SNP array, CNV, array CGH

1. Introduction

Since the use of high-resolution chromosome banding and molecular cytogenetic methodologies, several chromosomal aberrations have been identified. Despite genome-wide detection capacity of these technologies, the rearrangements still remain visibly undetectable, which can be explained by microscopic resolution limitations and the lack of knowledge regarding the regions under investigation.

In the 1990s, the array chromosome-based comparative genomic hybridization (array CGH) was established and began to be used for the detection of significant submicroscopic losses and gains with high sensitivity. It was initially applied to analyze copy number changes in

tumors, and it was able to detect changes as small as 50 kb in size, with a resolution up to 1000 times higher than the karyotype. Subsequently, the array CGH methodology was optimized and applied to detect unbalanced constitutional rearrangements [1–4]. Initially, bacterial artificial chromosomes (BACs) and fosmid clones were used in array CGH. However, it became clear that not only larger DNA insert constructed in BAC vector but also PCR products and oligonucleotide sequences were good targets for array CGH [5–7]. Microarray approaches offer a high resolution and relatively quick way for genome-wide analysis, increasing the potential possibilities of genomic scrutiny in clinical scenario as well as its potential application in many other distinct structural genome investigations. In addition, chromosomal microarray analysis (CMA) is useful to estimate the breakpoints of the DNA sequences that can reveal potential mechanisms and risk factors underlying the occurrence of chromosome rearrangement, especially in the case of recurrent rearrangements [8]. Nowadays, chromosomal microarray analysis (CMA) is used as a powerful tool to reveal copy number variants thought to play an important role in the pathogenesis of a variety of disorders or the development of complex traits. Thus, genomic variants can be used as a biological biomarker.

Herein, we present an overview of the fundamental concepts of cytogenomics and the potential application of this technology in human genetic diagnosis, agrigenomics, mutagenesis, and pharmacogenomics.

2. Fundamental concepts of cytogenomics: understanding the tool of arrays

Cytogenomic analysis comprises the use of microarray-based technologies for the investigation of specific loci and the entire genome [9]. It has been used for the detection of copy number variation (CNV), defined as genomic intervals that deviate from the normal diploid state that can vary in size ranging from a few base pairs to mega base pairs [10]. The microarray technologies are frequently nominated as chromosomal microarray analysis (CMA), known as comparative genomic hybridization (CGH) and SNP array (**Figure 1**). CMA could be used for a dual role in SNP (single nucleotide polymorphism) and CNV-based association studies and in humans for the evaluation of patients with various diseases and congenital malformations [11, 12].

Initially, the CMA was based on the same principles of chromosome-based comparative genomic hybridization. The CGH was developed in the early 1990s to screen for unbalanced rearrangements in whole genomes [3]. The CGH consists in the simultaneous hybridization of labeled test DNA and normal reference DNA onto normal metaphase chromosomes spread on glass slides. Metaphase CGH was widely used to identify chromosomal numerical alterations associated with solid tumors [13, 14]. Overall, the resolution of metaphase CGH is the same of G-banding cytogenetics, limited to 5–15 Mb. Moreover, experiments require cytogenetic expertise for the preparation of suitable metaphase chromosomes [3]. Due to its resolution limitations, the metaphase CGH became restricted to cancer research and did not demonstrate feasibility for analysis of genomic rearrangements in patients with developmental disorders. Subsequently, CGH was implemented as microarrays replacing the metaphases

CMA

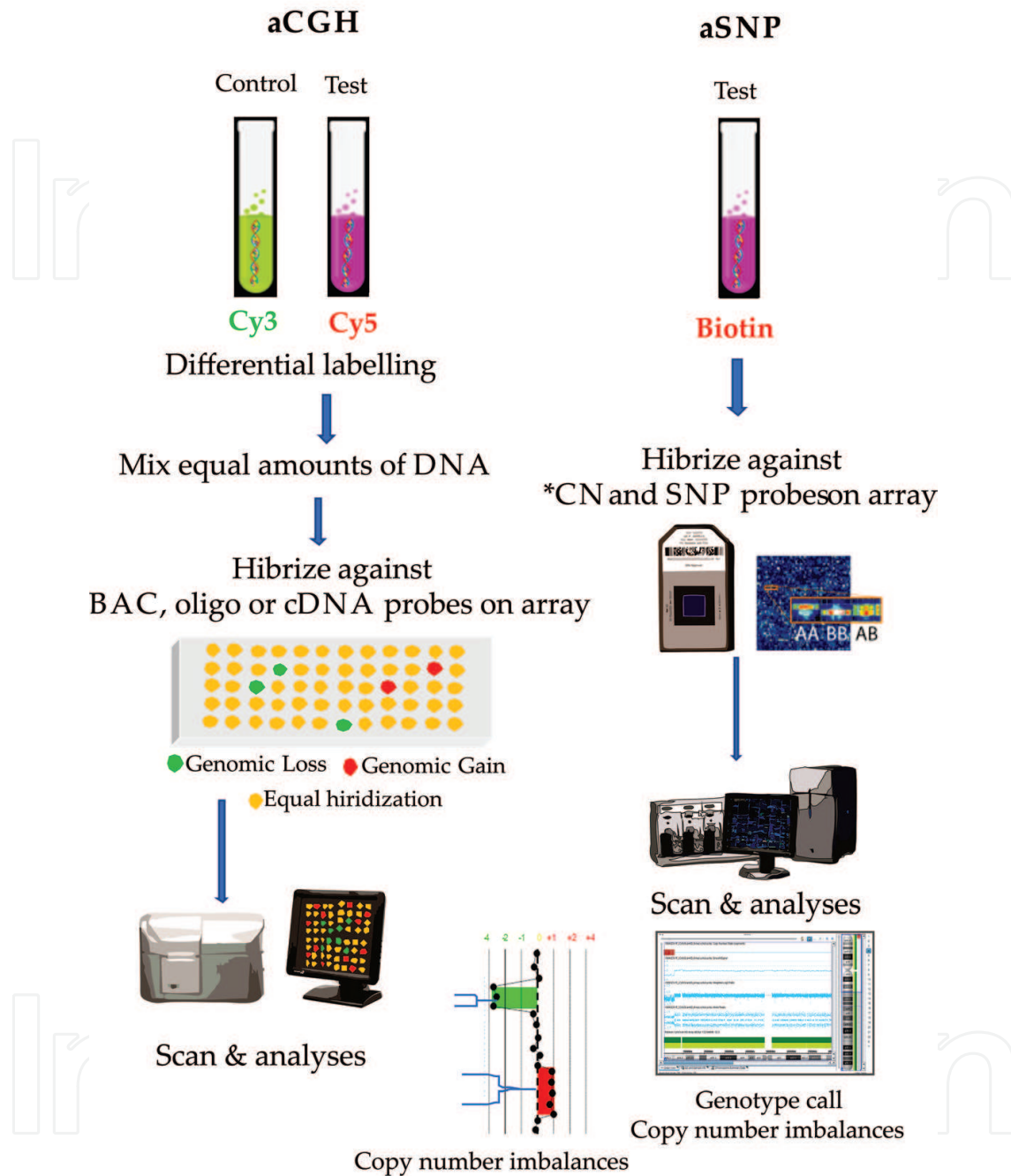


Figure 1. Fundamental concepts of chromosomal microarrays. Currently, the most widely used arrays are comparative genome hybridization arrays (aCGH) and single-nucleotide polymorphism arrays (aSNP). Both microarray-based technologies are able to detect genomic imbalances based on the spectral differences of the fluorescent dyes used to label test and reference DNA in aCGH and test DNA in aSNP. Sample analyses are possible with the aid of computational tools designed to call, view, summarize, and report chromosomal aberrations, including copy number gain or loss, across the genome. *CN: Copy Number.

CGH by cloned DNA segments as genomic reference to be used as targets for the hybridization, improving the potential for the detection of small chromosomal imbalances and increasing its resolution in more than tenfold [14].

The pieces of information regard the DNA sequence of the human genome, which was obtained by the Human Genome Project, making it possible for the construction and characterization of DNA libraries that could be cloned using bacterial artificial chromosomes (BACs) and fosmids as vectors [15, 16]. The array CGH was introduced having known clinically relevant microdeletion genomic DNA segments in BAC clones [10]. However, the relatively large size of the initial clones (170 kb for BACs and 40 kb for fosmids) limited the resolution of the arrays which is dependent on the distances between probes, the length of the clones, and how both probes and clones are distributed across the genome [14, 17]. With the emerging of new protocols, different probes, including small insert clones (1.5–2.5 kb), cDNA clones (0.5–2 kb), PCR products (0.1–1.5 kb), and oligonucleotides (25–85 bp), have been used in the arrays. However, the oligonucleotides have been more appropriated targets for array CGH [5–7, 18]. The oligonucleotide array offers higher resolution and is better than BAC in measuring size of CNVs, increasing the detection of small CNVs. Most CGH arrays available are designed with oligonucleotides ranging from 50 to 70 base pairs (bp) on the probe [19].

According to the International System for Human Cytogenetic Nomenclature [20], in contrast to array CGH, in which DNAs from patient and control are labeled with different dyes and hybridized to a single slide for array CGH and the alterations in the ratio of the two fluorescent dyes indicate a different quantity of DNA in the test sample as compared with the control corresponding locations of CNVs, in the SNP arrays, only the patient's DNA is hybridized to the microarray and compared by computer analysis to a pool of genomic DNA from reference healthy individuals. Additionally, in SNP arrays, the size of the oligonucleotides is about 20 bp and was designed initially to detect genotypes for thousands to hundreds of thousands of SNPs across the entire genome with the focus on genome-wide association studies [19, 21]. SNP arrays can also enable the detection of CNVs, but opposed to array CGH, each probe is located at an SNP and can determine the genotype of the corresponding SNP, and the current SNP arrays with median inter-SNP distances of <0.7 kb ensure the high density of genome coverage [22, 23]. There are many commercial platforms for microarray analysis. Array CGH allows the detection of non-polymorphic region and has been manufactured by Agilent Technologies (Santa Clara, CA) and NimbleGen (Roche Nimble-Gen Inc., Madison, WI), and more recently, Agilent Technologies is offering array CGH with inclusion of SNP markers. The SNP arrays, manufactured by Illumina (San Diego, CA) and by Affymetrix (Santa Clara, CA), have markers for the detection of polymorphic and non-polymorphic regions.

Besides the detection of CNVs, SNP arrays have some advantages in relation to array CGH. The SNP markers can also detect long contiguous stretches of homozygosity (LCSH), low-level mosaic aneuploidies, and chimerism. The detection of LCSH could indicate uniparental isodisomies (UPD) and consanguinity. LCSH distributed in several regions of chromosomes is characterized by genetic identical by descent; on the other hand, when LCSH was identified in a single chromosome, this observation may indicate UPD [24]. The major disadvantage of SNP array and array CGH is the inability to detect balanced chromosome rearrangements because balanced rearrangements show no copy number alterations. Thus, array methodologies do not replace G-banding karyotype for the detection of balanced structural rearrangements. However, they detect abnormalities that are cytogenetically cryptic by G-banded

chromosome analysis. It is important to remember that CMA testing cannot detect balanced karyotypic rearrangements such as reciprocal translocations that could be clinically significant if they disrupt a critical gene. For clinical indications with increased risk for a balanced chromosome rearrangement, such as recurrent pregnancy loss, G-banding chromosome analysis should remain a primary diagnostic test [23]. Additionally, in agrigenomics, SNP arrays have facilitated marker-assisted selections, genome-wide association studies, quantitative trait loci analyses, parentage, and traceability, helping in genomic selection programs.

The primary focus for microarrays has been biomedical-related analyses. However, applications for array technology have broadened to include such fields as plant and animal genotyping and pharmacogenomics. The number of CNVs identified has increased as a function of the increased resolution used by the array technologies. The wide use of arrays has allowed their application in agrigenomics providing a powerful and flexible range of genotyping calls useful for genomic selection programs for plants and animals, helping researchers and breeders to develop healthier and more productive crops and livestock [25, 26]. Besides, array technologies can be used in pharmacogenomic research for the investigation of potential associations between genomic variation and individual drug response. Several SNP-based microarrays are intended to provide information about specific polymorphisms associated with variable drug responses within individuals in a population, which could increase treatment's overall efficacy and decrease the incidence of adverse events [27, 28]. Moreover, the extensively use of CMA has not only contributed to the identification of CNVs and SNP related to human variability but also contributed to the identification of rearrangements implicated in a variety of diseases such as lifestyle diseases, cancer, autoimmune diseases, and neurodevelopmental disorders, including intellectual disability, autism spectrum disorder, global developmental delay, and neuropsychiatric disorders such as schizophrenia, creating a new field of investigation which has transformed the clinical practice [16, 24, 29].

2.1. Application in the diagnosis of human diseases

Genomic gains and losses, defined as CNVs, often cause a wide variety of specific and complex phenotypes, resulting from alterations in the normal dosage of genes, which cause multiple malformation syndromes, neurodevelopmental disorders (NDD), multiple congenital anomalies (MCA), and dysmorphic features. Nowadays, the improved resolution of the microarray technologies has allowed the identification of cryptic chromosomal alterations, increasing the knowledge of the etiology of genomic disorders and offering potential advantages in the patient's follow-up and management [3, 30].

Since 2010, CMA is widely recognized and recommended as the first-tier cytogenomic diagnostic test for individuals with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASDs), or multiple congenital anomalies, increasing the diagnostic yield around 10–25% [16, 30–32]. DD and ID are defined as several significant delays in developmental areas, including cognitive, speech, social/personal, fine/gross motor, and daily activities. DD is described for children less than 5 years old, and ID is diagnosed at or after the age of 5 years old, with the intelligence quotient less than 70. ASD is a complex spectrum of neurodevelopmental disorders, including autism, Asperger syndrome, pervasive

developmental disorder, and childhood disintegrative disorder. MCAs are defined as multiple major structural malformations that cannot be explained by an underlying syndrome or sequence. These disorders might have a genetic etiology involving the gains and losses of CNVs and loss of heterozygosity (LOH), and the clinical consequences of these rearrangements are commonly associated with location, size, and the gene content (**Figures 2 and 3**) [32–34].

In a study with children with ID/DD, ASD, and/or MCAs from Hong Kong, the application of array CGH demonstrated a diagnostic yield of 11% for pathogenic and likely pathogenic CNVs [35]. Another study with a cohort of 339 patients with neurodevelopmental disorders and/or multiple congenital anomalies using the array CGH identified a detection rate of pathogenic CNVs of 20.6% [30]. Combining both array CGH and SNP array in a single platform, it is possible to make the most effective clinical diagnostic offering simultaneously

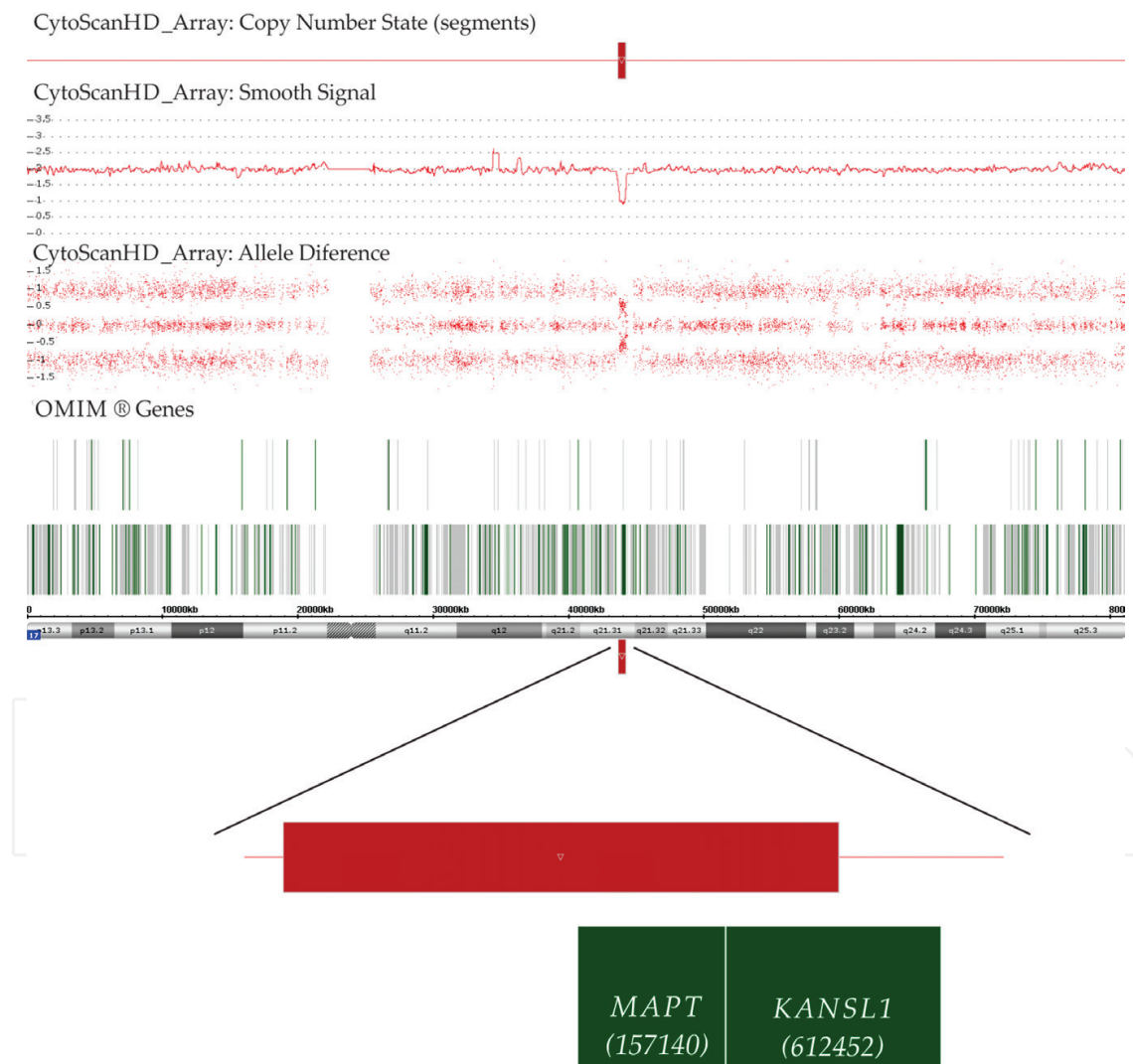


Figure 2. SNP array revealed a copy number loss of chromosome band 17q21.31 of approximately 0.56 Mb in size (arr[hg19] 17q21.31(43.703.801–44.212.416) × 1). This region involves 10 OMIM genes (*LOC644172*, *CRHR1*, *MGC57346*, *C17orf69*, *MAPT-AS1*, *SPPL2C*, *MAPT*, *MAPT-IT1*, *STH*, and *KANSL1*) related to Koolen-De Vries Syndrome (MIM610443). The red bar indicates the deleted region and the green bars indicated morbid genes.

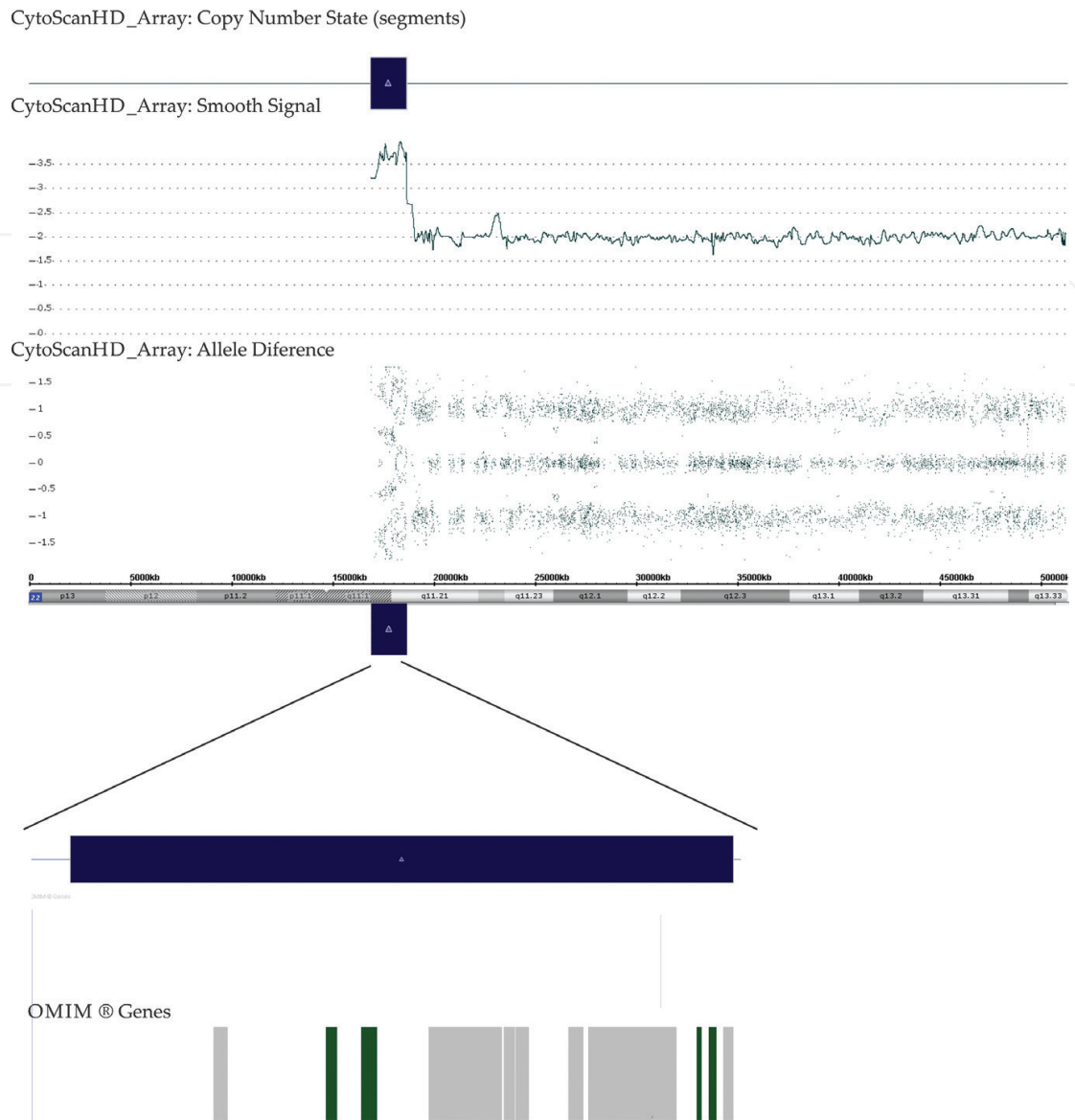


Figure 3. SNP array revealed a gain in copy number of chromosome band 22q11.1, spanning 1.75 Mb (arr[GRCh37] 22q11.1q11.21(16.888.899–18.644.773)×4). This gain as indicated in blue bars and comprise 12 OMIM genes (*XKR3*, *IL17RA*, *CECR1*, *CECR*), *SLC25A18*, *ATP6V1E1*, *BID*, *MICAL3*, *MIR648*, *PEX26*, *TUBA8*, and *USP18*), indicated with gray and green bars, related with Chromosome 22q11.2 Duplication Syndrome (OMIM608363).

identification of CNVs and LOH, as reported by different researches. A comparative study between high-resolution SNP arrays and CGH microarrays revealed that the use of SNP arrays increased the diagnostic yield in children with ID/MCA because these platforms permitted the identification of LOH, which can unravel recessive disorders [36]. Using the combining SNPs with customized exon-targeted oligonucleotide array in a cohort of 3240 patients, Wiszniewska et al. [24] provided a comprehensive approach for the identification of clinically relevant copy number neutral changes in addition to CNVs in a single assay. A study using CMA for 42 Korean patients with unexplained DD, ID, ASD, and MCA identified clinically relevant CNVs in 66.6% of patients [33]. Therefore, microarray-based

technologies have become a powerful tool for the identification of genomic rearrangements smaller than 5 Mb that are associated with neurodevelopmental disorders.

In addition to the usefulness of CMA to help increase the diagnostic rate for ID, DD, ASDs, and MCA, a variety of human conditions, such as epilepsy, schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder, have been reported in some individuals whose CNVs have an important causative role [23, 37–42]. Besides the roll of CNVs in the development of neurodevelopmental disorders, CNVs might also contribute to genetic variation in stature in a general population. This conclusion was driven from the observation that in children with short stature lower-frequency copy number variants play a role in the genetic basis of height [43]. Additionally, studies of CNVs in idiopathic short stature (ISS) individuals demonstrated that CMA is a promising approach for the identification of pathogenic CNVs in patients with ISS and could contribute for the recognition of candidate genes associated with growth pathways in humans [44, 45].

CMA testing, especially using SNP-based array, has improved the understanding of genetic causes of many types of human disease and added new pieces of information about gains and losses in the genome in a large variety of hematological malignancies and solid tumors. Additionally, loss of heterozygosity, which is frequently implicated in the tumorigenesis of a variety of cancers, could be detected using SNP array. So, CMA has played an important role to help in diagnosis, prognosis, risk stratification, and therapy for cancer patients [46, 47].

CMA has become a widespread strategy of genetic diagnosis in postnatal settings, especially evaluating children with neurodevelopmental disorders and multiple congenital malformations [48]. Moreover, the implementation of CMA in prenatal settings has helped physicians to identifying chromosomal abnormalities in fetuses harboring anatomical anomalies in the ultrasound, influencing on healthcare delivery in many countries [49]. CMA achieves nearly 100% accuracy rates when applied to identify common aneuploidies in prenatal specimens compared to G-banding karyotyping. Overall, studies showed that in pregnancies with fetal structural anomalies and karyotype with no numerical or structural alterations CMA increased the diagnostic yield around 7%. On the other hand, for all other indications, the increment in the diagnostic yield by CMA has remained around 2% [50, 51]. Taking into consideration the aforementioned information, in 2016 the American Congress of Obstetricians and Gynecologists (ACOG) recommended CMA as the first-tier test for the diagnostic evaluation of fetal structural anomalies. However, the challenge of CMA in prenatal settings is the adequate classification of CNVs as pathogenic and variants of unknown significance (VOUS) [50, 52]. To minimize the reporting of uncertain findings, the practice guideline from Canada issued by the Society of Obstetricians and Gynecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) recommended not to report VOUS smaller than 500 kb or VOUS smaller than 1 Mb for losses and gains, respectively [52].

For the diagnosis of human diseases, the microarray platforms should use probes derived from closely spaced genomic loci and have probes concentrated in clinically relevant genes and genomic loci, allowing the detection of smaller CNVs within disease-associated regions. The identification of pathogenic or likely pathogenic CNV by CMA offers benefits for the patient and family, bringing information about prognosis, allowing for appropriate genetic counseling, and adequate patient's management and follow-up for future disclosures [23].

2.2. Application in agrigenomics

2.2.1. Livestock microarray analysis

SNP-based genotyping technologies have become the first-tier methodology in programs of animal and plant breeding for genomic selection [53]. In this context, the use of SNP arrays in organisms of economic interest has facilitated the association between a given SNP with desired productive phenotypes, promoting a positive impact in the economy and contributing to consolidate the technology as a powerful tool to select animals and plants with higher genomic value [54].

The animal genomic is a reality in breeding programs, and the application's impact of these methodologies can be noticed in several areas of animal production [55]. The commercial use of genomic markers is driven based on the need to develop efficient selection and production systems. For instance, selection assisted by genomic markers has been applied to swine breeding aiming for little size and higher meat quality. On the other hand, in bovine, animal selection is used for meat tenderness and higher milk production. Still, for sheep, animal selection is used to increase reproductive efficiency and muscle deposition [56].

Although microarray analysis may reflect a promising future in the agricultural setting to economically produce labor and commodities, the efficiency of microarrays and other innovative methodologies applied to livestock production will always be affected by situations of difficult control, including measurements of traits, phenotypic variance, and low heritability [57]. Inadequate phenotyping could negatively affect, limit, and hinder the usefulness of genetic information in breeding strategies. Improvements and the use of SNP array, followed by cost reduction for genotyping and genome sequencing by Sanger or NGS, have created the possibility to use genomic information for the creation of livestock and supported the emergence of genomic selection programs [58].

The development and application of genomic selection in livestock breeding programs have benefited from the consolidated knowledge generated by classical breeding programs, in particular, information derived from mapping of the Quantitative Trait Loci (QTLs). QTL is characterized as a region of the genome responsible for the expression of a phenotypic trait, which has a continue distribution [25]. This observation is supported by the fact that the main phenotypic characteristics targeted by genomic and genetic breeding programs are polygenic and controlled by several loci, each one adding to the final effect observed in the phenotype. In the aforementioned scenario, genomic selection must be conceived as a process of making decisions regarding the selection of the best-fit animals based on their estimated genomic values. Genomic estimated breeding values (GEBVs) are most commonly obtained by Bayesian models, and it is nowadays considered to be an important step for the success of genomic selection [59]. In summary, GEBVs are the result of the presence of meaningful genetic markers, identified through a dense array of SNPs equally spaced throughout the whole genome, contained within all known QTLs from previously studied livestock [60].

There are different SNP arrays available for livestock genomic analysis. Schaefer et al. [61] designed two different genotyping platforms and demonstrated the application of customized SNP array for domestic horse. Júnior et al. [62] observed the importance of identification

of genomic region associated with puberty and early pregnancy to females of Zebu cattle. Gutierrez et al. [63] used a high-density ovine chip (700 K) to search signatures of selection related to dairy production in sheep and demonstrated the usefulness of the array in the identification of regions of economic interest in dairy sheep. The application of SNP array for genomic selection has been useful to determine genetic attributes and contributed for the genomic selection of traits of economic interest.

The SNP arrays available to estimate genomic values in farm animals can be divided into two categories, according to the time of their development and use. First, there were the SNP arrays from 2000 to 2012, characterized by the development of arrays based on the sequence of reference genomes of farm animals. Most of the arrays were developed by multinational companies, including genome Illumina, Affymetrix, ARK Genomics, and Applied Biotechnologies. Secondly, the arrays used from 2012 on are based on the genome of selected animals, including the possibility of customizing the array for a given property.

In general, the positive aspects for using commercially available SNP arrays are as follows: (a) genetic polymorphism can be estimated in different breeds, including synthetic cross breeds, and (b) the SNPP panels are able to discriminate genomic variability even in animals with close genetic makeup, especially considering the elevated level of inbreeding, for instance, in pigs and poultry lineages. With respect to the negative aspect of using SNP arrays, the following are noteworthy: (a) high cost per genotyped animal, (b) unavailability of personnel and laboratories qualified to generate and analyze genomic data, and (c) in the same array makers for different QTLs and different aptitudes reduction of the availability of genomic data for the trait of interest. Carroll and Charo [55] called the attention upon an array for bovine selection. The markers in that array were chosen to select meat and milk production. The total of informative SNPs was reduced based on the total number of SNPs in the array. Goddard [64] reported that perhaps the two most critical issues that hinder the usefulness of genomic selection in the agribusiness industry are the lack of qualified personnel and the cost per animal for their genotyping.

Lately, the potential of array customization and the advancement of genome sequencing methodologies have boosted the applicability of genotyping farm animals and reduced the cost per animal. Moreover, NGS is a powerful tool to generate information on the whole genome of selected animals together with adequate animal phenotyping, which will contribute to adequately estimate true genomic breeding values for the livestock. Several authors have pointed out that the advancement of genome-wide association studies (GWAS) has providing excellent and efficient information to be used in livestock genomic selection programs (GSP) [54, 57, 65]. GWAS has allowed the identification of candidate genes potentially associated with phenotypes of economic interest (**Figure 4**). Thus, the piece of information generated for a given herd has become more powerful to predict its genomic merit and also to be used to assist adequate selection of the desired animal phenotype. Thereby, genomic breeding values have become even more useful and accurate, contributing to efficient decision-making by herd managers and producers.

Bosch et al. [67] have addressed the final cost for poultry genotyping considering two distinct arrays from the same company, namely, ChickenSNP50 and ChickenSNP600K, representing

both the early and the state-of-the-art arrays, respectively. The author reported that the genotyping challenge has remained the cost per animal, which has a negative impact on genomic selection. At first, genotyping as a whole has become cheaper. Nevertheless, the cost per animal has not reduced satisfactory. Manufactures claim that the new arrays will collect more significant SNPs to be used in breeding strategies (Figure 5), justifying the increase in the cost per animal. Similar arguments have been displayed by different authors [65, 67, 68] who also claim a steadfast increase in the use of high-density SNP arrays from both academic researchers and commercial facilities to assist with livestock breeding and genomic selection globally. Much debate around the SNP arrays customarily is used for bovine genotyping, especially for selection of meat and dairy animals. Specialists have claimed that low-density arrays has a reduced capacity to predict the phenotype in *Bos taurus indicus* mostly because the markers in the array are more representative of *Bos taurus Taurus*, affecting mainly the minor allele

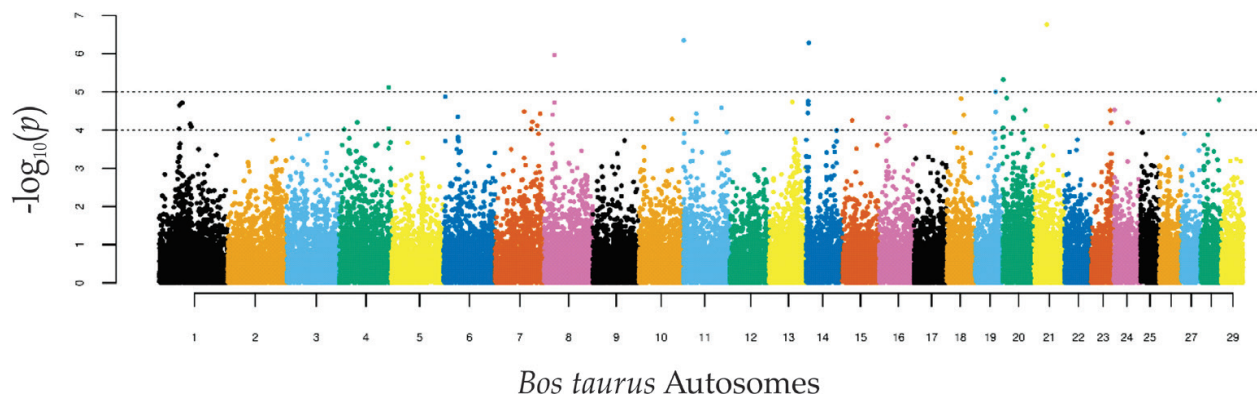


Figure 4. Manhattan plotting showing the distribution of the significant values of SNPs per bovine autosomal chromosomes with respect to 305-day milk yield in Girolando. The GWAS disclosed 7 SNPs associated with milk production trait in dairy cattle with a p value $<10^{-5}$ and a false discovery rate of 6.5% according to the study of milk production in Girolando [66].

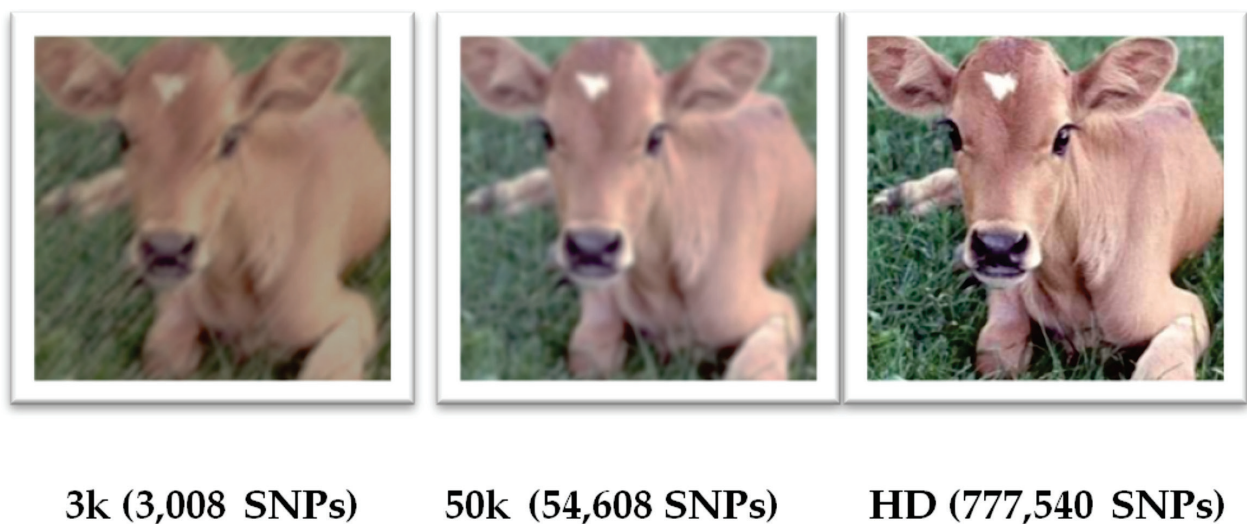


Figure 5. The resolution of the arrays. Illustration indicating the resolution of an array is based on the number of markers available to powerfully predict the breed genomic merit related to the desired economical trait.

frequency (MAF) of several SNPs for some breeds, rendering them non-useful for breeding selection [69]. Hickey [70], Auvray et al. [71], and Mrode et al. [68] have addressed the issue of development of future SNP arrays to be applied for bovine selection. They claim that new arrays must include a larger number of markers representative of distinct breeds and/or future arrays must be customized for a specific breed, having less markers, but all chosen based on their MAF. At last, it is also noteworthy to mention that array could be replaced by WGS, pending only on the cost per animal, a challenge that will be met by the manufacturers. Teng and Xiao [72], Bruford et al. [65], and Steyaert et al. [73] considered that as soon as WGS becomes economically competitive it will be accessible to promote a new revolution in the field of livestock genomic selection and breeding.

2.3. Application in research: from mutagenesis to pharmacogenomics

Microarray methodologies have been impacted in different fields of biological science, allowing the identification of genomic alterations applied in the area of mutagenesis, including toxicology, genetic toxicology, as well as pharmacogenomics [74, 75].

In the area of toxicology, gene expression profiles based on microarray analysis can help understand the multiple pathways and mechanisms on the action of toxic substances at the same time. Furthermore, microarray analyses are important to understand the effects of xenobiotics across the genome and the rapid identification of toxic risks of new drugs and chemicals. Thus, global analysis of gene expression has the importance of providing a more comprehensive view of toxicity than was previously possible, since toxicity usually involves changes not only in one or a few genes but is a cascade of gene interactions [74].

Understanding the function of genes is a major challenge in the post genomic era and in order to assign the role of genes in molecular networks, strategies such as proteomics, metabolomics, and transcriptomics have been implemented [76]. The gene expression profile of a cell determines its function, phenotype, and response to the environment. Thus, the analysis of gene expression becomes necessary for the in-depth study of biochemical pathways, regulatory mechanisms, and broader cellular function [77]. Some conventional analyses for the gene expression profile are optimized only for single-gene investigation. Microarrays have been developed as high-performance, efficient, and comprehensive tools for the simultaneous study of multiple genes [78]. Therefore, microarray methodologies are being used to study the transcriptional profile, leading to the research of new genes and molecular markers, having applicability in the field of pharmacogenomics for tracing changes in the expression of genes that are sensitive or resistant to a given drug; thus, it can be used to analyze differential profiles of gene expression that are induced or repressed by xenobiotics [79, 80].

Pharmacogenomic studies of genes and gene products (proteins) are essential for pharmacological or toxicological responses to pharmaceutical agents. In addition, it analyzes genetic determinants of enzymes, receptors, transporters, and targets that metabolize drugs and that influence drug efficacy, safety, and drug-related phenotypes [79]. A current focus of pharmacogenomic research explores the effect of interindividual genetic differences related to drug response by providing information that can be used to inform the appropriate selection of individual drugs or dosing regimens [27, 79, 81]. Pharmacogenomic research involves scanning the entire genome to find single nucleotide polymorphisms (SNPs) that may be

associated with drug responses [79]. Genetic polymorphism studies are performed to classify individuals according to their drug metabolism or disease response capabilities [81].

In a pilot study, Liljedahl et al. [82] developed a microarray genotyping system for multiplex analysis of a panel of SNPs in genes encoding proteins involved in the regulation of blood pressure, demonstrating their viability in response to hypertensive drugs. Therefore, microarray-based tests have provided a useful tool for simultaneous measurement of relative levels of expression of a large number of clinically relevant genes in the context of disease or drug responses [83]. Moreover, the application of the technique in the field of pharmacogenomics characterizes and validates new therapeutic targets, their mechanism of action, metabolic pathways, undesirable side effects, sensitivity, and toxicity to certain drugs [84].

To date, there is a scarcity of studies on the induction of germ line mutations in humans. However, SNP-based arrays can also be applied to monitor individuals exposed to ionizing radiation, and it has been proven to be a useful strategy to evaluate potential health risks related to environmental mutagens. Costa et al. [85] presented results of the analysis of the effect of accidental exposure to low doses of ionizing radiation on the formation of de novo, nonrecurrent CNVs in the progeny of a human population accidentally exposed to cesium-137 during the radiological accident in Goiânia, Brazil. The high-density SNP array used in that study allowed the observation of de novo mutations induced in the germ line of parents exposed to very low doses of ionizing radiation. Although the study of Costa et al. [85] is the pioneer in the field and requires validation, it shed light on the potential of SNP arrays to unravel CVS to be used as useful germ line biomarkers to characterize the exposure of biological systems to mutagenic agents. Thus, a new era of possibilities of using CMA to resolve a variety of biological questions is upon us and once again the future keep on looking promising.

Conflict of interest

The authors declare no conflict of interest.

Author details

Irene Plaza Pinto^{1,2}, Alex da Cruz¹, Emília Costa¹, Samara Pereira¹, Lysa Minasi¹ and Aparecido da Cruz^{1,2,3*}

*Address all correspondence to: acruz@pucgoias.edu.br

1 Pontifical Catholic University of Goiás, School of Agricultural and Biological Sciences, Genetics Master Program, Replicon Research Group, Goiânia, Goiás, Brazil

2 Federal University of Goiás, PhD Program in Biotechnology and Biodiversity, Goiânia, Goiás, Brazil

3 Human Cytogenetics and Molecular Genetics Laboratory, State Laboratory of Public Health Dr. Giovanni Cysneiros, Secretary of Goiás State for Public Health, Goiânia, Goiás, Brazil

References

- [1] Albertson DG, Pinkel D. Genomic microarrays in human genetic disease and cancer. *Human Molecular Genetics*. 2003;**12**:R145-R152. DOI: 10.1093/hmg/ddg261
- [2] Cai WW, Mao JH, Chow CW, Damani S, Balmain A, Bradley A. Genome-wide detection of chromosomal imbalances in tumors using BAC microarrays. *Nature Biotechnology*. 2002;**20**:393-396. DOI: 10.1038/nbt0402-393
- [3] Shaffer LG, Bejjani BA. A cytogeneticist's perspective on genomic microarrays. *Human Reproduction Update*. 2004;**10**:221-226. DOI: 10.1093/humupd/dmh022
- [4] Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, et al. Microarray based comparative genomic hybridization (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *The Journal of Medical Genetics*. 2004;**41**:241-248. PMCID: PMC1735726
- [5] Brennan C, Zhang Y, Leo C, Feng B, Cauwels C, Aguirre AJ, Kim M, Protopopov A, Chin L. High resolution global profiling of genomic alterations with long oligonucleotide microarray. *Cancer Research*. 2004;**64**:4744-4748. DOI: 10.1158/0008-5472.CAN-04-1241
- [6] Dharni P, Coffey AJ, Abbs S, Vermeesch JR, Dumanski JP, Woodward KJ, Andrews RM, Langford C, Vetrie D. Exon array CGH: Detection of copy-number changes at the resolution of individual exons in the human genome. *American Journal of Human Genetics*. 2005;**76**:750-762. DOI: 10.1086/429588
- [7] Wolf M, Mousset S, Hautaniemi S, Karhu R, Huusko P, Allinen M, et al. High-resolution analysis of gene copy number alterations in human prostate cancer using CGH on cDNA microarrays: Impact of copy number on gene expression. *Neoplasia*. 2004;**6**:240-247. DOI: 10.1593/neo.3439
- [8] Wechselblatt B, Rudd MK. Human structural variation: Mechanisms of chromosome rearrangements. *Trends in Genetics*. 2015;**31**:587-599. DOI: 10.1016/j.tig.2015.05.010
- [9] Liehr T, editor. *Fluorescence In Situ Hybridization (FISH) - Application Guide*. 2nd ed. Jena: Springer; 2017. 588 p
- [10] Harel T, Lupski JR. Genomic disorders 20 years on-mechanisms for clinical manifestations. *Clinical Genetics*. 2018;**93**:439-449. DOI: 10.1111/cge.13146
- [11] Haeri M, Gelowani V, Beaudet AL. Chromosomal microarray analysis, or comparative genomic hybridization: A high throughput approach. *MethodsX*. 2015;**3**:8-18. DOI: 10.1016/j.mex.2015.11.005
- [12] Wang K, Bucan M. Copy Number Variation Detection via High-Density SNP Genotyping. *CSH Protoc*. 2008; pdb.top46. DOI: 10.1101/pdb.top46

- [13] Kallioniemi A, Kallioniemi OP, Sudar DA, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*. 1992;**258**:818-821. DOI: 10.1126/science.1359641
- [14] Redon R, Carter NP. Comparative genomic hybridization: Microarray design and data interpretation. *Methods in Molecular Biology*. 2009;**529**:37-49. DOI: 10.1007/978-1-59745-538-1_3
- [15] Morozova O, Marra MA. From cytogenetics to next-generation sequencing technologies: Advances in the detection of genome rearrangements in tumors. *Biochemistry and Cell Biology*. 2008;**86**:81-91. DOI: 10.1139/O08-003
- [16] Nowakowska B. Clinical interpretation of copy number variants in the human genome. *Journal of Applied Genetics*. 2017;**58**:449-457. DOI: 10.1007/s13353-017-0407-4
- [17] Manning M, Hudgins L, Professional Practice and Guidelines Committee. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genetics in Medicine*. 2010;**12**:742-745. DOI: 10.1097/GIM.0b013e3181f8baad
- [18] Carter NP. Methods and strategies for analyzing copy number variation using DNA microarrays. *Nature Genetics*. 2007;**39**:S16-S21. DOI: 10.1038/ng2028
- [19] Beaudet AL. The utility of chromosomal microarray analysis in developmental and behavioral pediatrics. *Child Development*. 2013;**84**:121-132. DOI: 10.1111/cdev.12050
- [20] McGowan-Jordan J, Simons A, Schmid M, editors. *An International System for Human Cytogenomic Nomenclature (ISCN)*. 1st ed. Switzerland: Karger; 2016. 139 p
- [21] Ko JM. Chromosomal microarray: Application for congenital heart diseases. *Korean Circulation Journal*. 2018;**48**:233-235. DOI: 10.4070/kcj.2018.0032
- [22] Keren B. The advantages of SNP arrays over CGH arrays. *Molecular Cytogenetics*. 2014;**7**(Suppl1):I31. DOI: 10.1186/1755-8166-7-S1-I31
- [23] Rosenfeld JA, Patel A. Chromosomal microarrays: Understanding genetics of neurodevelopmental disorders and congenital anomalies. *Journal of Pediatric Genetics*. 2017;**6**:42-50. DOI: 10.1055/s-0036-1584306
- [24] Wiszniewska J, Bi W, Shaw C, Stankiewicz P, Kang SH, Pursley AN, et al. Combined array CGH plus SNP genome analyses in a single assay for optimized clinical testing. *The European Journal of Human Genetics*. 2014;**22**:79-87. DOI: 10.1038/ejhg.2013.77
- [25] Boichard D, Chung H, Dassonneville R, David X, Eggen A, Fritz S, et al. Design of a bovine low-density SNP array optimized for imputation. *PLoS One*. 2012;**7**:e34130. DOI: 10.1371/journal.pone.0034130
- [26] Bovine HapMap Consortium, Gibbs RA, Taylor JF, Van Tassell CP, Barendse W, Eversole KA, et al. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. *Science*. 2009;**324**:528-532. DOI: 10.1126/science.1167936

- [27] Berm EJ, Loeff MD, Wilffert B, Boersma C, Annemans L, Vegter S, Boven JF, Postma MJ. Economic evaluations of pharmacogenetic and pharmacogenomic screening tests: A systematic review. Second update of the literature. *PLoS One*. 2016;**11**:e0146262. DOI: 10.1371/journal.pone.0146262
- [28] Matson RS. Applying genomic and proteomic microarray technology in drug discovery. 1st ed. Boca Raton: CRC Press: Taylor & Francis e-Library; 2005. 72 p
- [29] Takumi T, Tamada K. CNV biology in neurodevelopmental disorders. *Current Opinion in Neurobiology*. 2018;**48**:183-192. DOI: 10.1016/j.conb.2017.12.004
- [30] Maini I, Ivanovski I, Djuric O, Caraffi SG, Errichiello E, Marinelli M, et al. Prematurity, ventricular septal defect and dysmorphisms are independent predictors of pathogenic copy number variants: A retrospective study on array-CGH results and phenotypical features of 293 children with neurodevelopmental disorders and/or multiple congenital anomalies. *Italian Journal of Pediatrics*. 2018;**44**:34. DOI: 10.1186/s13052-018-0467-z
- [31] Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, et al. Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *The American Journal of Human Genetics*. 2010;**86**:749-764. DOI: 10.1016/j.ajhg.2010.04.006
- [32] Pratte-Santos R, Ribeiro KH, Santos TA, Cintra TS. Analysis of chromosomal abnormalities by CGH-array in patients with dysmorphic and intellectual disability with normal karyotype. *Einstein (Sao Paulo)*. 2016;**14**:30-34. DOI: 10.1590/S1679-45082016AO3592
- [33] Lee SH, Song WJ. Chromosomal microarray testing in 42 Korean patients with unexplained developmental delay, intellectual disability, autism spectrum disorders, and multiple congenital anomalies. *Genomics and Informatics*. 2017;**15**:82-86. DOI: 10.5808/GI.2017.15.3.82
- [34] Ooki S. Multiple congenital anomalies after assisted reproductive Technology in Japan (between 2004 and 2009). *ISRN Epidemiology*. 2013:1-9. DOI: 10.5402/2013/452085
- [35] Tao VQ, Chan KY, Chu YW, Mok GT, Tan TY, Yang W, et al. The clinical impact of chromosomal microarray on paediatric care in Hong Kong. *PLoS One*. 2014;**9**:e109629. DOI: 10.1371/journal.pone.0109629
- [36] D'Amours G, Langlois M, Mathonnet G, Fetni R, Nizard S, Srouf M, et al. SNP arrays: Comparing diagnostic yields for four platforms in children with developmental delay. *BMC Medical Genomics*. 2014;**7**:70. DOI: 10.1186/s12920-014-0070-0
- [37] Chen J, Calhoun VD, Perrone-Bizzozero NI, Pearlson GD, Sui J, Du Y, Liu J. A pilot study on commonality and specificity of copy number variants in schizophrenia and bipolar disorder. *Translational Psychiatry*. 2016;**6**:e824. DOI: 10.1038/tp.2016.96
- [38] Chen YH, Lu RB, Hung H, Kuo PH. Identifying potential regions of copy number variation for bipolar disorder. *Microarrays (Basel)*. 2014;**3**:52-71. DOI: 10.3390/microarrays3010052
- [39] Lowther C, Merico D, Costain G, Waserman J, Boyd K, Noor A, et al. Impact of IQ on the diagnostic yield of chromosomal microarray in a community sample of adults with schizophrenia. *Genome Medicine*. 2017;**9**:105. DOI: 10.1186/s13073-017-0488-z

- [40] Mefford HC. CNVs in epilepsy. *Current Genetic Medicine Reports*. 2014;**2**:162-167. DOI: 10.1007/s40142-014-0046-6
- [41] Thygesen JH, Wolfe K, McQuillin A, Viñas-Jornet M, Baena N, Brison N, et al. Neurodevelopmental risk copy number variants in adults with intellectual disabilities and comorbid psychiatric disorders. *The British Journal of Psychiatry*. 2018;**212**:287-294. DOI: 10.1192/bjp.2017.65
- [42] Vlaskamp DRM, Callenbach PMC, Rump P, Giannini LAA, Dijkhuizen T, Brouwer OF, van Ravenswaaij-Arts CMA. Copy number variation in a hospital-based cohort of children with epilepsy. *Epilepsia Open*. 2017;**2**:244-254. DOI: 10.1002/epi4.12057
- [43] Dauber A, Yu Y, Turchin MC, Chiang CW, Meng YA, Demerath EW, et al. Genome-wide association of copy-number variation reveals an association between short stature and the presence of low-frequency genomic deletions. *American Journal of Human Genetics*. 2011;**89**:751-759. DOI: 10.1016/j.ajhg.2011.10.014
- [44] Hu G, Fan Y, Wang L, Yao RE, Huang X, Shen Y, Yu Y, Gu X. Copy number variations in 119 Chinese children with idiopathic short stature identified by the custom genome-wide microarray. *Molecular Cytogenetics*. 2016;**9**:16. DOI: 10.1186/s13039-016-0225-0
- [45] Singh H, Tiwari P, Bhavi V, Chaudhary PS, Suravajhala P, Mohan MK, Mathur SK. Application of chromosomal microarray for evaluation of idiopathic short stature in Asian Indian children: A pilot study. *Indian Journal of Endocrinology and Metabolism*. 2018;**22**:100-106. DOI: 10.4103/ijem.IJEM_202_17
- [46] Mukherjee S, Sathanoori M, Ma Z, Andreatta M, Lennon PA, Wheeler SR, et al. Addition of chromosomal microarray and next generation sequencing to FISH and classical cytogenetics enhances genomic profiling of myeloid malignancies. *Cancer Genetics*. 2017;**216-217**:128-141. DOI: 10.1016/j.cancergen.2017.07.010
- [47] Nowak D, Hofmann WK, Koeffler HP. Genome-wide mapping of copy number variations using SNP arrays. *Transfusion Medicine and Hemotherapy*. 2009;**36**:246-251. DOI: 10.1159/000225372
- [48] de Jong A, Dondorp WJ, Macville MV, de Die-Smulders CE, van Lith JM, de Wert GM. Microarrays as a diagnostic tool in prenatal screening strategies: Ethical reflection. *Human Genetics*. 2014;**133**:163-172. DOI: 10.1007/s00439-013-1365-5
- [49] Callaway JL, Shaffer LG, Chitty LS, Rosenfeld JA, Crolla JA. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: A review of the literature. *Prenatal Diagnosis*. 2013;**33**:1119-1123. DOI: 10.1002/pd.4209
- [50] Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. *Fertility and Sterility*. 2018;**109**:201-212. DOI: 10.1016/j.fertnstert.2018.01.005
- [51] Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *The New England Journal of Medicine*. 2012;**367**:2175-2184. DOI: 10.1056/NEJMoa1203382
- [52] Armour CM, Dougan SD, Brock JA, Chari R, Chodirker BN, DeBie I, et al. Practice guideline: Joint CCMG-SOGC recommendations for the use of chromosomal microarray

- analysis for prenatal diagnosis and assessment of fetal loss in Canada. *Journal of Medical Genetics*. 2018;**55**:215-221. DOI: 10.1136/jmedgenet-2017-105013
- [53] Bhat SA, Malik AA, Ahmad SM, Shah RA, Ganai NA, Shafi SS, Shabir N. Advances in genome editing for improved animal breeding: A review. *Veterinary World*. 2017;**10**: 1361-1366. DOI: 10.14202/vetworld.2017.1361-1366
- [54] Gonen S, Jenko J, Gorjanc G, Mileham AJ, Whitelaw CB, Hickey JM. Potential of gene drives with genome editing to increase genetic gain in livestock breeding programs. *Genetics, Selection, Evolution*. 2017;**49**:3. DOI: 10.1186/s12711-016-0280-3
- [55] Carroll D, Charo RA. The societal opportunities and challenges of genome editing. *Genome Biology*. 2015;**16**:242. DOI: 10.1186/s13059-015-0812-0
- [56] Blendon S, Gorski M, Benson J. The public and the gene-editing revolution. *The New England Journal of Medicine*. 2016;**374**:1406-1411. DOI: 10.1056/NEJMp1602010
- [57] Murray JD, Maga EA. Genetically engineered livestock for agriculture: A generation after the first transgenic animal research conference. *Transgenic Research*. 2016;**25**: 321-327. DOI: 10.1007/s11248-016-9927-7
- [58] Fleming A, Emhimad AA, Christian M, Christine FB. Invited review: Reproductive and genomic technologies to optimize breeding strategies for genetic progress in dairy cattle. *Archives Animal Breeding*. 2018;**61**:43-57. DOI: 10.5194/aab-61-43-2018
- [59] Davoudi P, Abdollahi-Arpanahi R, Nejati-Javaremi A. The impact of QTL allele frequency distribution on the accuracy of genomic prediction. *Archives Animal Breeding*. 2018;**61**:207-213. DOI: DOI.org/10.5194/aab-61-207-2018
- [60] de Los Campos G, Vazquez AI, Fernando R, Klimentidis YC, Sorensen D. Prediction of complex human traits using the genomic best linear unbiased predictor. *PLoS Genetics*. 2013;**9**:e1003608. DOI: 10.1371/journal.pgen.1003608
- [61] Schaefer RJ, Schubert M, Bailey E, Bannasch DL, Barrey E, Bar-Gal GK, et al. Developing a 670k genotyping array to tag ~2M SNPs across 24 horse breeds. *BMC Genomics*. 2017;**18**:565. DOI: 10.1186/s12864-017-3943-8
- [62] Júnior GAO, Perez BC, Ferraz JBS. Genomics applied to puberty in beef cattle (*Bos Indicus*). *Revista Brasileira de Reprodução Animal*. 2017;**41**:264-269
- [63] Gutiérrez-Gil B, Arranz JJ, Pong-Wong R, García-Gámez E, Kijas J, Wiener P. Application of selection mapping to identify genomic regions associated with dairy production in sheep. *PLoS One*. 2014;**9**:e94623. DOI: 10.1371/journal.pone.0094623
- [64] Kemper KE, Goddard ME. Understanding and predicting complex traits: knowledge from cattle. *Human Molecular Genetics*. 2012;**21**(R1):R45-51. DOI: 10.1093/hmg/dds332
- [65] Bruford MW, Ginja C, Hoffmann I, Joost S, Orozco-terWengel P, Alberto FJ, et al. X. Prospects and challenges for the conservation of farm animal genomic resources, 2015-2025. *Frontiers in Genetics*. 2015;**21**:314. DOI: 10.3389/fgene.2015.00314

- [66] da Cruz, Alex Silva. Estudo de associação ampla do genoma bovino para lactação ajustada em 305 dias em Girolando [thesis]. Goiânia: Universidade Federal de Goiás; 2015. p. 106
- [67] Bosch P, Forcato DO, Alustiza FE, Alessio AP, Fili AE, Olmos Nicotra MF, Liaudat AC, Rodríguez N, Talluri TR, Kues WA. Exogenous enzymes upgrade transgenesis and genetic engineering of farm animals. *Cellular and Molecular Life Sciences*. 2015;**72**: 1907-1929. DOI: 10.1007/s00018-015-1842-1
- [68] Mrode R, Tarekegn GM, Mwacharo JM, Djikeng A. Invited review: Genomic selection for small ruminants in developed countries: How applicable for the rest of the world? *Animal*. 2018;**12**:1333-1340. DOI: 10.1017/S1751731117003688
- [69] Uemoto Y, Sasaki S, Kojima T, Sugimoto Y, Watanabe T. Impact of QTL minor allele frequency on genomic evaluation using real genotype data and simulated phenotypes in Japanese black cattle. *BMC Genetics*. 2015;**16**:134. DOI: 10.1186/s12863-015-0287-8
- [70] Hickey JM. Sequencing millions of animals for genomic selection 2.0. *Journal of Animal Breeding and Genetics*. 2013;**130**:331-332. DOI: 10.1111/jbg.12054
- [71] Auvray B, McEwan J, Newman SA, Lee M, Dodds K. Genomic prediction of breeding values in the New Zealand sheep industry using a 50K SNP chip. *Journal of Animal Science*. 2014;**92**:4375-4389. DOI: 10.2527/jas.2014-7801
- [72] Teng X, Xiao H. Perspectives of DNA microarray and next-generation DNA sequencing technologies. *Science in China. Series C, Life Sciences*. 2009;**52**:7-16. DOI: 10.1007/s11427-009-0012-9
- [73] Steyaert W, Callens S, Coucke P, Dermaut B, Hemelsoet D, Terryn W, Poppe B. Future perspectives of genome-scale sequencing. *Acta Clinica Belgica*. 2018;**73**:7-10. DOI: 10.1080/17843286.2017.1413809
- [74] Aardema MJ, MacGregor JT. Toxicology and genetic toxicology in the new era of “toxicogenomics”: Impact of “-omics” technologies. *Mutation Research*. 2002;**499**:13-25. DOI: 10.1016/S0027-5107(01)00292-5
- [75] Guindalini C, Tufik S. Use of microarrays in the search of gene expression patterns - application to the study of complex phenotypes. *Revista Brasileira de Psiquiatria*. 2007;**29**:370-374. DOI: 10.1590/S1516-44462007000400014
- [76] Saei AA, Omid Y. A glance at DNA microarray technology and applications. *BioImpacts: BI*. 2011;**1**:75-86. DOI: 10.5681/bi.2011.011
- [77] Chavan P, Joshi K, Patwardhan B. DNA microarrays in herbal drug research. *Evidence-based Complementary and Alternative Medicine*. 2006;**3**:447-457. DOI: 10.1093/ecam/nel075
- [78] Bumgarner R. DNA microarrays: Types, applications and their future. *Current Protocols in Molecular Biology*. 2013;**22**:1-17. DOI: 10.1002/0471142727.mb2201s101

- [79] Ventola CL. Role of Pharmacogenomic biomarkers in predicting and improving drug response part 1: The clinical significance of pharmacogenetic variants. *P&T*. 2013;**38**: 545-551. PMID: 24273401
- [80] Wang Z, Du Q, Wang F, Liu Z, Li B, Wang A, Wang Y. Microarray analysis of gene expression on herbal glycoside recipes improving deficient ability of spatial learning memory in ischemic mice. *Journal of Neurochemistry*. 2004;**88**:1406-1415. DOI: 10.1046/j.1471-4159.2003.02258.x
- [81] Shahandeh A, Johnstone DM, Atkins JR, Sontag JM, Heidari M, Daneshi N, Freeman-Acquah E, Milward EA. Advantages of array-based technologies for pre-emptive pharmacogenomics testing. *Microarrays*. 2016;**5**:1-11. DOI: 10.3390
- [82] Liljedahl U, Karlsson J, Melhus H, Kurland L, Lindersson M, Kahan T, Nyström F, Lind L, Syvänen AC. A microarray minisequencing system for pharmacogenetic profiling of antihypertensive drug response. *Pharmacogenetics*. 2003;**3**:7-17. PMID: 12544508
- [83] Anderson DC, Kodukula K. Biomarkers in pharmacology and drug discovery. *Biochemical Pharmacology*. 2014;**87**:172-188. DOI: 10.1016/j.bcp.2013.08.026
- [84] Meloni R, Khalfallah O, Biguet NF. DNA microarrays and pharmacogenomics. *Pharmacological Research*. 2004;**49**:303-308. DOI: 10.1016/j.phrs.2003.06.001
- [85] Costa EOA, Pinto IP, Gonçalves MW, da Silva JF, Oliveira LG, da Cruz AS, et al. Small de novo CNVs as biomarkers of parental exposure to low doses of ionizing radiation of caesium-137. *Scientific Reports*. 2018;**8**:1-13. DOI: 10.1038/s41598-018-23813-5