Identification of aneuploidy in dogs screened by a SNP microarray

Lisa G. Shaffer1,2, Bradley Hopp1, Marek Switonski3, Adam Zahand1, Blake C. Ballif1

Received: 29 May 2021 / Accepted: 14 July 2021 / Published online: 21 July 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
Microarray analysis is an efficient approach for screening and identifying cytogenetic imbalances in humans. SNP arrays, in particular, are a powerful way to identify copy-number gains and losses representing aneuploidy and aneusomy, but moreover, allow for the direct assessment of individual genotypes in known disease loci. Using these approaches, trisomies, monosomies, and mosaicism of whole chromosomes have been identified in human microarray studies. For canines, this approach is not widely used in clinical laboratory diagnostic practice. In our laboratory, we have implemented the use of a proprietary SNP array that represents approximately 650,000 loci across the domestic dog genome. During the validation of this microarray prior to clinical use, we identified three cases of aneuploidy after screening 2053 dogs of various breeds including monosomy X, trisomy X, and an apparent mosaic trisomy of canine chromosome 38 (CFA38). This study represents the first use of microarrays for copy-number evaluation to identify cytogenetic anomalies in canines. As microarray analysis becomes more routine in canine genetic testing, more cases of chromosome aneuploidy are likely to be uncovered.

Introduction
For nearly 2 decades, microarray analysis has been used to identify genomic gains and losses associated with chromosomal anomalies in humans, including autosomal and sex chromosome aneuploidies (Shaffer and Bejjani 2004; Ballif et al. 2006; Shaffer et al. 2012; Shaffer and Rosenfeld 2013; Gou et al. 2020). SNP array analysis, in particular, is a powerful way to directly assess individual genotypes in known disease loci while allowing for identification of copy-number gains and losses representing aneuploidy, aneusomy, and mosaicism (Conlin et al. 2010). Although now commonplace in human clinical genetic testing, microarrays have just recently been implemented for canine genetic testing for genotyping analysis of known variants (Donner et al. 2016, 2018, 2019), detection of cytogenetic anomalies in canine and feline cancers (Thomas et al. 2014, 2020), and more recently for the identification of sex chromosome aneuploidy in horses (Pirosanto et al. 2021).

Cytogenetic anomalies have been identified in many different mammalian species including cats (reviewed in Szczerbal and Switonski, 2020) and dogs (reviewed in Szczerbal and Switonski, 2021; Szczerbal et al. 2021). The dog karyotype consists of 38 acrocentric autosome pairs and the X and Y chromosomes (2n = 78). Karyotype and FISH analysis has shown sex chromosome anomalies including monosomy X, trisomy X, trisomy XXX, sex chromosomal mosaicism, or XX/XY chimeras in dogs primarily presenting with abnormal estrous cycles, infertility, sterility, or hypoplastic gonads (Szczerbal and Switonski 2021), although some dogs with mosaic karyotypes have shown normal estrus. Structural chromosome anomalies have been seen as well, mostly Robertsonian translocations that are apparently balanced. Although, some dogs did present with abnormal phenotypes (Szczerbal and Switonski 2021), it is unclear if these are associated with the cytogenetic anomalies identified. Even in the most experienced hands, the dog karyotype is difficult to reconstruct using chromosome banding techniques. Thus, the partner chromosomes involved in many of the Robertsonian translocations found have not been identified and reciprocal translocations are rarely reported due to the difficulties in karyotype analysis (Szczerbal and Switonski 2021).

Lisa G. Shaffer
Lshaffer@pawprintgenetics.com

1 Paw Print Genetics, Genetic Veterinary Sciences, Inc, 220 E Rowan, Suite 220, Spokane, WA 99207, USA
2 Center for Reproductive Biology, Washington State University, Pullman, WA, USA
3 Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Poznan, Poland
We report the first constitutional cytogenetic anomalies identified in dogs using a SNP array and the screening of more than 2000 dogs from various breeds.

**Methods**

We have developed a proprietary microarray representing genetic loci throughout the dog genome (Affymetrix, Santa Clara, CA, USA). The array design contains 1,402,677 SNP Probes representing about 475 known variants for diseases and traits in domestic dogs (6552 probe sets represented by 104,784 probes), as well as a backbone of about 642,580 markers, represented by 1,297,893 probes spaced approximately every 2–4 Kb across the genome. Each disease or trait locus is interrogated by 2–14 probe sets and each probe set is made up of 8–128 different probes, physically spaced throughout the array. Specifically, the X chromosome is represented by 12,815 probe sets consisting of 30,148 probes. The pseudoautosomal regions (PAR), shared by the X and Y chromosomes, are represented by 12,815 probe sets consisting of 30,148 probes.

Paw Print Genetics has performed genetic analysis on more than 200,000 dogs since 2013. DNA was selected from 2,053 samples representing more than 95 different purebred and mixed breed dogs. All dogs in the study were privately owned and samples were collected by the owners. Most samples were collected using the PERFORMAgene PG-100 (DNAGenotek, Ottawa, Ontario, Canada) buccal swabs to collect cheek cells. DNA was extracted using the King-Fisher Flex Purification System (ThermoFisher Scientific, Waltham, MA, USA). DNA was extracted from a few samples from tissues sent in by the dog owner (blood, dewclaws, umbilical cords, docked tails, or semen samples) using previously described methods (Shaffer et al. 2015; 2016; 2017). DNA concentration was quantified with a Varioskan LUX Multimode Microplate Reader (ThermoFisher Scientific) using the Quant-iT dsDNA HS and BR Assay Kits (ThermoFisher Scientific). For microarray analysis, 20 μl of genomic DNA with concentration of 10 ng/μl was used according to Affymetrix Axiom® 2.0 Assay Manual. The samples were amplified using Target Prep Protocol QSCB1 (P/N 702,990), fragmented, hybridized on the chip followed by single-base extension through DNA ligation and signal amplification. The Affymetrix GeneTitan® Multi-Channel Instrument was used for staining, washing, and scanning of the chip signals as per the manufacturer’s protocol (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017740_Axiom_96F_NIMBUS_UG.pdf). The CEL files from the Affymetrix GeneTitan® Multi-Channel Instrument were imported into Axiom Analysis Suite and CNV Discovery workflow was performed for the custom array according to the Axiom Analysis Suite v5.1 User Guide https://downloads.thermofisher.com/Axiom_Analysis/Axiom_Analysis_Suite_v5.1_User_Guide.pdf. Data were exported in IGV format with separate files created for Log2Ratio, BAF, and Copy Number. IGV formatted files were imported into Golden Helix SVS software for visualization. Log2Ratio, B Allele Frequency (BAF), and Copy Number information was plotted in SVS using the GenomeBrowse built in feature https://doc.goldenhelix.com/SVS/latest/svs_index.html.

**Results**

From the 2053 samples analyzed, three were found to have apparent chromosome aneuploidies (Figs. 1, 2). Specifically, one dog was found with monosomy X (Fig. 1C), one dog with trisomy X (Fig. 1D), and one dog with probable mosaic trisomy CFA38 (Canis Familiaris chromosome 38) (Fig. 2B). Four puppies from the mosaic trisomy 38 dog were tested on the SNP array and all had normal diploid results for CFA38 (data not shown).

**Case reports**

**Monosomy X (77,X)**

The puppy was one of five pups born to a miniature poodle mother and a goldendoodle (golden retriever poodle mix) father. The pup was visibly smaller than littermates, weighing 9.5 oz at 1 week and 16 oz at 2 weeks of age (Table 1). The puppy was able to nurse, but required supplemental bottle or tube feeding for the first week of life. None of the other pups in the litter required supplemental feeding. Physical exam at 2 months, 23 days showed persistent, bilateral, pupillary membranes, but otherwise healthy. The puppy was spayed at this time prior to the first estrous. Breeder reported that the pup, now 1 year of age at the time of this report, has caught up with her siblings for both height and weight. Examination of photographs provided by the breeder, in comparison to siblings, showed no obvious dysmorphic features (data not shown). Figure 1C shows the plots for the SNP array data. As in human microarray data, monosomy X shows complete homozygosity for the A or B allele, including probes in the PAR.

**Trisomy X (79,XXX)**

The puppy is a Papillon born in a litter of three pups. She is currently an intact female, 18 months old, and has not entered into a first estrous, which normally occurs around 9–11 months of age. As compared to her brother and sister, she is of appropriate size and has no known health problems. The breeder reports that the pup is nondysmorphic...
and meets the breed standard for conformation with the exception of a low-set tail, which is considered a fault in the breed. Developmentally, the pup seems lagging as compared to her sibs and other Papillons in the household, according to the breeder who is also a human developmental specialist. Behaviorally, the pup is sweet, docile, and not as quick to learn or train in the breeder’s experience. Figure 1D shows the plot for the trisomy X dog. As with human microarray data, the entire X chromosome, including the PAR, shows the BAF as either AAA, AAB, ABB, or BBB.

Mosaic Trisomy 38 (78,XY/79,XY,+38)

The intact, adult male dog is of mixed breed origin. The dog has proven fertility in that he has sired two litters. He is reportedly in good health. Examination of photographs did not show any obvious dysmorphic features (data not shown). Figure 2B shows the plots for chromosomes 36, 37, and 38. The BAF for CFA38 are intermediate to those expected for a full (nonmosaic) trisomy (refer to Fig. 1D for comparison with the trisomy X case), indicating likely mosaicism. Cytogenetic or FISH analysis was not possible, so the mosaicism could not be confirmed and an unbalanced Robertsonian translocation or isochromosome could not be excluded.

Discussion

Chromosome abnormalities have been recognized in many mammalian species. Among domesticated animals, sex chromosome aneuploidies are the most frequently identified. Overall, monosomy X appears to be relatively rare, with the exception of mares (Bugno-Poniewierska and Raudsepp 2021). A relatively high incidence of monosomy X in mares and a low incidence in cattle, pigs, dogs, and cats may be explained by length differences of the PAR, as suggested by Raudsepp and colleagues (2012). In horses and humans, the PAR is relatively short as compared to a longer length in cats and dogs and a much longer length in cattle and pigs, allowing for better pairing, recombination, and proper segregation during meiosis. Trisomy X and autosomal aneuploidies have been reported rarely in domestic mammals with the vast majority of cases found in cattle and horses (Bugno-Poniewierska and Raudsepp 2021; Iannuzzi et al. 2021).

We recently acquired a GeneTitan (Affymetrix, CA) microarray system and have developed a proprietary microarray that will be used for genotyping known variants contributing to diseases and traits in dog, and for copy-number assessment. During the validation of this array, three cases of aneuploidy were identified out of 2053 samples screened. While monosomy X and trisomy X have been reported many times before in dogs (Szczerbal and...
Switonski 2021), this report is the first time that microarray analysis has been used in dogs to uncover aneuploidy and the first report of trisomy 38. Figure 1 shows the results of the monosomy and trisomy X dogs as compared to a normal female and a normal male result. Based on our findings, sex chromosome aneuploidies may be quite common in dogs. In our unbiased screening of more than 2000 dogs, the frequency is roughly 1 in 1000, which is similar to that reported for monosomy and trisomy X in humans.

The finding of mosaic trisomy 38 (CFA38) was surprising in an apparently normal, fertile male dog. CFA38 has synteny with distal human chromosome 1 bands 1q23.2, 1q23.3, 1q32.1, and 1q41 (Fig. 2C). CFA38 is estimated to be about 24.1 Mb in size and contains 502 genes and 47 pseudogenes. Trisomy or duplication of distal 1q in humans is associated with dysmorphic features, proportionate short stature, and intellectual disability (reviewed in: Morris et al. 2015). To our knowledge, the dog identified with mosaic trisomy 38 did not have any obvious phenotypic abnormalities. Because this is the first dog to be identified with trisomy 38 mosaicism, we do not know the long-term, clinical implications for this dog.

Four puppies from this dog’s most recently sired litter were examined by microarray analysis and all had normal results (data not shown). Because microarray analysis cannot reveal balanced translocations, a Robertsonian translocation, found previously in many mammals including dogs, could

Table 1 Comparison of weights for the first 14 days of life for the litter including the monosomy X puppy

| Puppy          | Sex | Week 1 | Week 2 |
|----------------|-----|--------|--------|
| Blue collar (Monosomy X) | Female | 9.5 oz | 16 oz  |
| Pink collar    | Female | 20 oz  | 30 oz  |
| Purple collar  | Female | 17 oz  | 26 oz  |
| Yellow collar  | Male  | 16 oz  | 26 oz  |
| Green collar   | Male  | 16 oz  | 28 oz  |
Acknowledgements The authors express their appreciation to all of the dogs and their owners who participated in this study. Special thanks to Corinne Seganos, Paw Print Genetics, Genetics Veterinary Sciences, Inc., for obtaining the samples from the mosaic trisomy 38 dog and his puppies.

Authors' contributions LGS, BH, AZ, and BCB contributed to the study conceptualization and design. Any additional sample recruitment was arranged by LGS. Microarray design was performed by AZ and reviewed by LGS and BCB. Microarray testing and results analysis was performed by BH. BH and LGS produced the figures. LGS wrote the first draft of the manuscript. MS provided critical review of the manuscript and additional literature review. All authors commented on and approved the final manuscript.

Funding Funding for this study was provided by Genetic Veterinary Sciences, Inc.

Data availability All relevant data generated in this study are included in this published article.

Code availability Not applicable.

Declarations

Conflicts of interest LGS is the owner of Genetic Veterinary Sciences, Inc., DBA Paw Print Genetics which provides genetic testing on a fee-for-service basis. The remaining authors have no conflicts of interest to declare.

Ethics approval Not applicable.

Consent to participate All canine samples included in this study were obtained through consent of the individual owners or were obtained from otherwise discarded DNA samples after clinical testing at Paw Print Genetics.

Consent for publication Not applicable.

References

Ballif BC, Kashord CK, Saleki R, Sundin K, Beijani A et al (2006) Detecting sex chromosome anomalies and common triploidies in products of conception by array-based comparative genomic hybridization. Prenat Diagn 26:333–339. https://doi.org/10.1002/pd.1411

Bugno-Poniewierska M, Raussepp T (2021) Horse clinical cytogenetics: recurrent themes and novel findings. Animals 11:831–857. https://doi.org/10.3390/ani11030831

Conlin LK, Thi B, Bonnemann CG, Medne L, Ernst LM, Zackai EH et al (2010) Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. Hum Mol Genet 19:1263–1275. https://doi.org/10.1093/hmg/ddq003

Donner J, Kaukonen M, Anderson H, Müller F, Kyöstilä K, Sankari S et al (2016) Genetic panel screening of nearly 100 mutations reveals new insights into the breed distribution of risk variants for canine hereditary disorders. PLoS ONE 11:e0161005. https://doi.org/10.1371/journal.pone.0161005

Donner J, Anderson H, Davison S, Hughes AM, Bouirmeane J, Lindqvist J, Lytle KM, Ganesan B, Ottka C, Ruotanen P, Kaukonen M, Forman OP, Fretwell N, Cole CA, Lohi H (2018) Frequency and distribution of 152 genetic disease variants in over 100,000 mixed breed and purebred dogs. PLoS Genet 14:e1007361. https://doi.org/10.1037/journal.pgen.1007361

Donner J, Anderson H, Davison S, Hughes AM, Bouirmeane J, Lindqvist J et al (2019) Correction: frequency and distribution of 152 genetic disease variants in over 100,000 mixed breed and purebred dogs. PLoS Genet 15:e1007938. https://doi.org/10.1037/journal.pgen.1007938

Gou L, Liu T, Wang Yi WuQ, Hu S, Dong B et al (2020) Clinical utilization of chromosomal microarray analysis for the genetic
analysis in subgroups of pregnancy loss. J Matern Fetal Neonatal Med 23:1–8. https://doi.org/10.1080/14767058.2020.1849126

Iannuzzi A, Parma P, Iannuzzi L (2021) Chromosome abnormalities and fertility in domestic bovids: a review. Animals 11:802–829. https://doi.org/10.3390/ani11030802

Morris ML, Baroneza JE, Teixeira P, Medina CT, Cordoba MS, Versiani BR et al (2015) Partial 1q duplications and associated phenotype. Mol Syndromol 6:297–303. https://doi.org/10.1159/000443599

Pirosanto Y, Laseca N, Valera M, Molina A, Moreno-Millán M, Bugno-Poniewierska M et al (2021) Screening and detection of chromosomal copy number alterations in the domestic horse using SNP-array genotyping data. Anim Genet. https://doi.org/10.1111/age.13077

Raudsepp T, Das PJ, Avila F, Chowdhary BP (2012) The pseudoautosomal region and sex chromosome aneuploidies in domestic species. Sex Dev 6:72–83. https://doi.org/10.1159/000330627

Shaffer LG, Beijani BA (2004) A cytogeneticist’s perspective on genomic microarrays. Hum Reprod Update 10:221–226. https://doi.org/10.1093/humupd/dmh022

Shaffer LG, Rosenfeld JA (2013) Microarray-based prenatal diagnosis for the identification of fetal chromosome abnormalities. Expert Rev Mol Diagn 13:601–611. https://doi.org/10.1586/14737159.2013.811912

Shaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC et al (2006) Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. J Peds 149:98–102. https://doi.org/10.1016/j.peds.2006.02.006

Shaffer LG, Dabell MP, Fisher AJ, Coppring J, Bandholz AM, Ellison JW et al (2012) Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies. Prenat Diagn 32:976–985. https://doi.org/10.1002/pd.3945

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.