Identification of Aneuploidy in Dogs Screened by a SNP Microarray

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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 propriety 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 2,053 dogs of various breeds including monosomy X, trisomy X and an apparent, mosaic trisomy of canine chromosome 38 (CFA 38). 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 two 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 & 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; Donner et al. 2018; Donner et al. 2019), detection of cytogenetic anomalies in canine and feline cancers (Thomas et al. 2014; Thomas et al. 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 XXY, 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 translocation found have not been

identified and reciprocal translocations are rarely reported due to the difficulties in karyotype analysis (Szczerbal and Switonski, 2021).

We report the first constitutional cytogenetic anomalies identified in dogs using a SNP array and the screening of more than 2,000 dogs from various breeds.

Methods

We have developed a proprietary microarray representing for 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 (6,552 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 and the pseudoautosomal regions (PAR), shared by the X and Y chromosomes is represented by 1,765 probe sets with 3,187 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 KingFisher 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/ul was used according to Affymetrix Axiom® 2.0 Assay Manual. The samples were amplified using Target Prep Protocol QSCB1 (P/N 702990), 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 was 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, 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 2,053 samples analyzed, three were found to have apparent chromosome aneuploidies (Figure 1 and Figure 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 ounces at 1 week and 16 ounces 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 one 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 B allele frequencies (BAF) as either AAA, AAB, BBA or BBB.

Mosaic Trisomy 38 (78,XY/mos 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

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 2,053 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 2,000 dogs, the frequency is roughly 1 in 1,000, 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 24.1Mb 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 phenotype.

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 not be excluded in these pups. However, given the apparent mosaicism found in the sire, the possibility that this finding is due to a translocation is less likely. Cytogenetic analysis may have been informative in this case. However, karyotyping dog chromosomes have proven problematic (Szczerbal and Switonski, 2021) and only a buccal swab was available to us for molecular testing. In addition, testing more than one tissue type may have provided additional information about the extent of the mosaicism.

Given the inherent difficulties in karyotyping canine samples, microarray technologies have significant advantages to the identification of cytogenetic anomalies in dogs. In addition, PHA-stimulated peripheral blood cultures may produce a selection bias and some mosaicisms can be missed by chromosome analysis (Shaffer et al. 2006). Although we have implemented the use of a SNP array for genotyping known variants, cases can also be examined for copy number variations that may indicate segmental aneusomies and aneuploidy. The finding of three aneuploid cases in only 2,053 samples studied may indicate that aneuploidy is a relatively common occurrence in dogs that has previously only been recognized in the study of infertility. Continued screening of additional samples is likely to uncover other

cases. However, CFA38 is the smallest canine chromosome and the finding of trisomy in a mosaic state may indicate that autosomal trisomies are not well tolerated in dogs. On the contrary, autosomal aneuploidies may occur at similar rates as those observed in humans, but do not come to clinical attention due to fetal reabsorption or a neonatal death in which finding a cause is not pursued by the breeder. With the availability of microarrays in a few canine genetic testing laboratories, perhaps more cases of dogs with or without malformations will be investigated which may lead to an increased detection of cytogenetic anomalies including segmental aneusomies and whole chromosome aneuploidies.

Declarations

Funding

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

Conflicts of interest/Competing 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.

Availability of data and material

All relevant data generated in this study is included in this published article.

Code availability

Not applicable.

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.

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.

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Electronic

https://uswest.ensembl.org/Canis_lupus_familiaris/Location/Synteny?otherspecies=Homo_sapiens&r=38%3A1-1000

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Tables

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

Figures

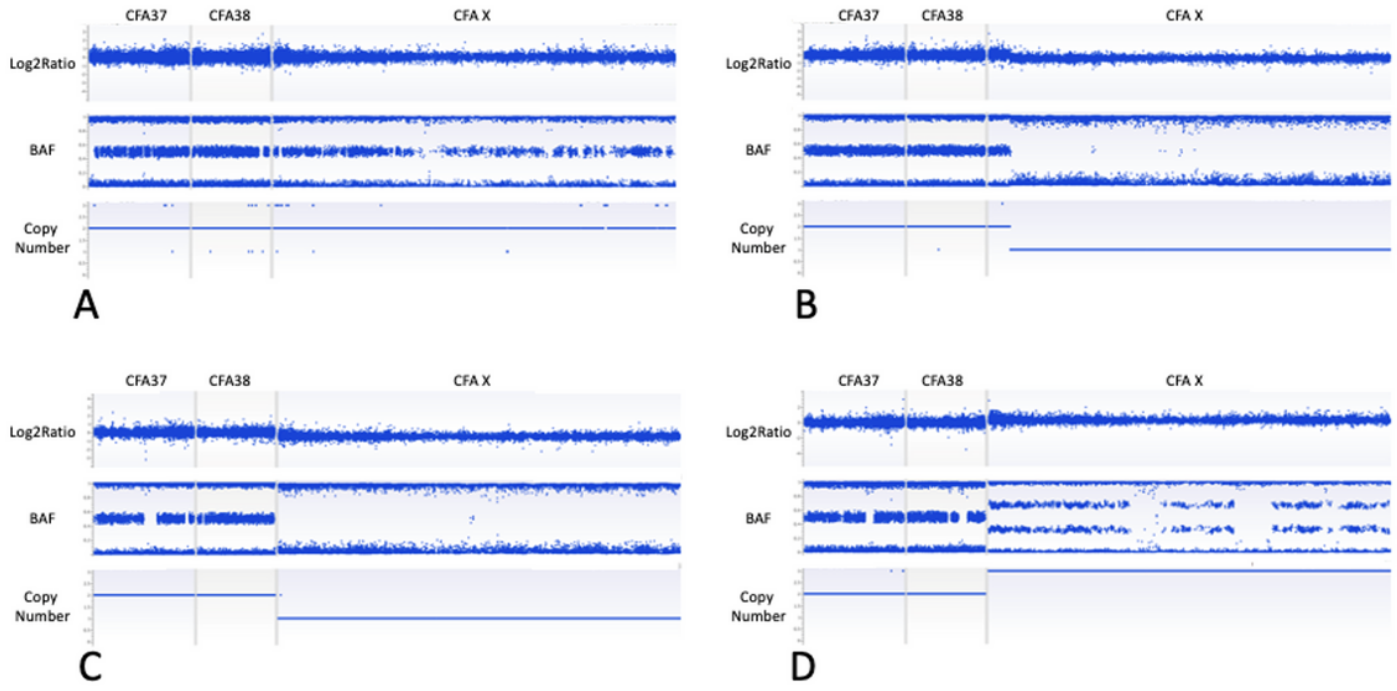

Figure 1

Microarray Results for Monosomy X and Trisomy X. Shown in each panel, from top to bottom, is the Log2Ratio, B Allele Frequency (BAF), and copy number for chromosomes 37, 38 and X. Panel A shows a normal 78,XX female. Note the AA, AB and BB genotype calls in the BAF line and the copy number of two. Panel B shows a normal 78,XY male. Note the AA, AB and BB genotype calls for the pseudoautosomal region (PAR) and the A and B only calls for the remainder of the X chromosome and a copy number of one. Panel C shows the monosomy X dog, showing A or B calls for the entire X chromosome including the

PAR. Panel D shows the trisomy X dog, showing AAA, AAB, ABB and BBB calls for the entire X chromosome and a copy number of three.

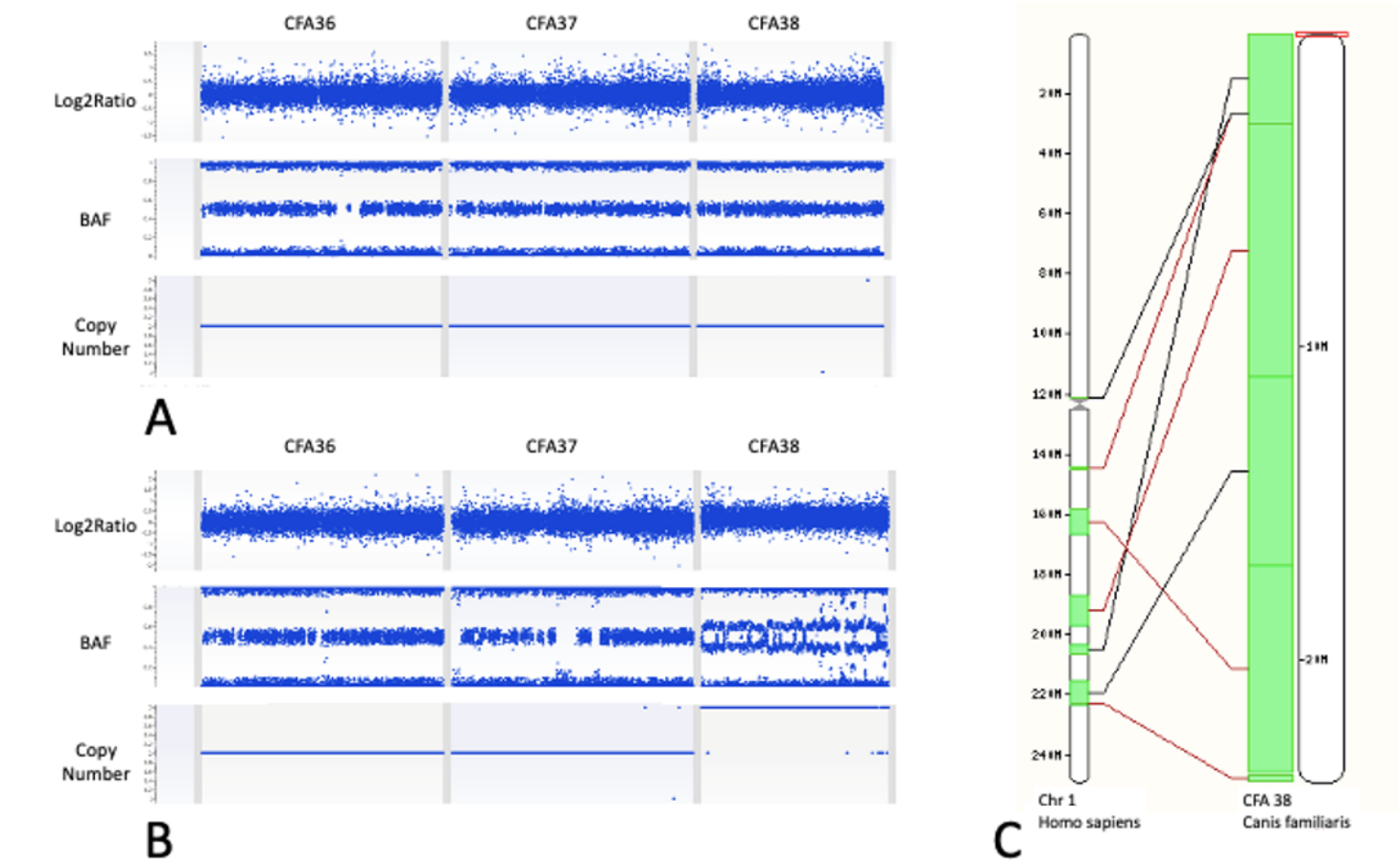

Figure 2

Microarray Results for Mosaic Trisomy 38. Shown in A and B, from top to bottom, is the Log2Ratio, B Allele Frequency (BAF), and copy number for chromosomes 36, 37 and 38. Panel A shows normal plots for chromosomes 36, 37 and 38. Panel B shows the plots for the mosaic CFA38 dog. Chromosomes 36 and 37 show typical BAF of AA, AB and BB and a copy number of two, whereas for CFA38, the BAF shows AAA, AAB, ABB and BBB calls and a copy number of three. However, the AAB and ABB calls are intermediate to what would be expected for a full (nonmosaic) trisomy (refer to Fig. 1D of the trisomy X case for comparison). C. Schematic showing the regions of synteny between human chromosome 1 and CFA38.