Molecular characterization of CMO

A canine model of the Caffey syndrome, a human rare bone disease

(Report summarised by Dr P. Bamas)

Abstract

Dog CMO disease (Cranio Mandibular Osteopathy) is a clinical equivalent to an infantile cortical hyperostosis (Caffey Disease).

The study shows various experiments investigating the clinic-pathological features and genetic causes of CMO, and leading to identification of a pathogenic variant in the canine SLC37A2 gene, a gene coding for a protein transporting glucose-6-phosphate.
This gene appears as a new candidate gene for the Caffey disease, which is not characterized yet.

Introduction

This study addressed the clinical and genetic background of CMO in West Highland White Terriers (WHWT), Cairn Terriers and Scottish Terriers.

CMO is a self-limiting proliferative bone disease seen in young dogs [9].

It manifests between 4 to 8 months of age with typical signs including swelling of the jaw, periodical fever, lack of appetite, pain, difficulty opening the mouth and dysphagia. The excessive proliferation causes bony lesions primarily on the skull bones, especially on the mandible and tympanic bulla, but occasionally also on the metaphyses of long bones. Signs of the disease usually resolve with time, when the growth period is finished.

CMO exists in several breeds with the highest frequency in WHWT and has been previously suggested to be an autosomal recessive trait [10–12].

Results

The researchers performed a genome-wide association study (GWAS) to map the CMO locus in a cohort of 51 WHWTs, including 10 affected cases (diagnosed by radiography; Fig 1A) and 41 healthy controls.

Genetic analyses in canine CMO identify a variant in exon 15 of the *SLC37A2* gene (a gene of the chromosome 5), resulting in a truncated protein.

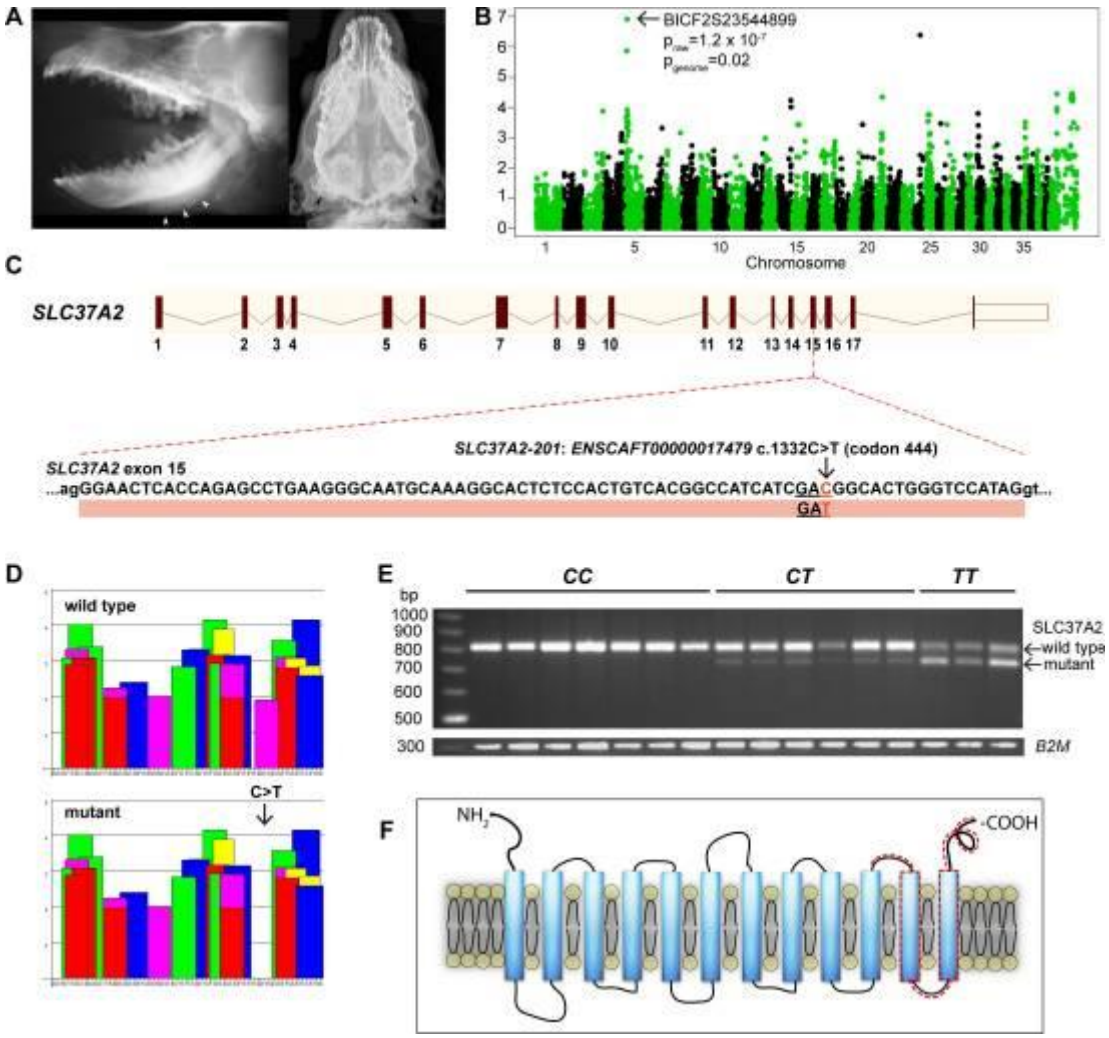

Fig 1

As an additional independent verification to avoid potential targeted capture biases, they performed whole genome sequencing in one CMO-affected WHWT and 188 control dogs from other breeds to compare the variants in the associating region.

Deeper investigations indicated that both wild-type and mutant transcripts were expressed in even the CMO affected dogs, although the expression of the wild-type transcript was significantly reduced in the affected homozygous dogs (CMO-2)(Fig 1E).

The reduction of the wild type transcript level was more moderate in healthy heterozygous carrier dogs (CMO-1) than in affected dogs.

None of the examined wild-type dogs (CMO-0) expressed the mutant transcript.

To investigate the segregation and frequency of the variant across the CMO affected breeds, researchers performed a large variant screening by genotyping the c.1332C>T variant altogether in 1052 dogs, including 695 WHWT, 249 Scottish Terriers and 108 Cairn Terriers (S3 Table).

They found 123 homozygous dogs (CMO-2) in the WHWT breed, of which 48% had been reported with CMO.

About 40% of WHWT (275 dogs) carried the pathogenic variant, of which 10 dogs (3,6%) were reported with CMO.

In Scottish Terriers, 10 dogs (4%) were homozygous (CMO-2)

In Cairn Terriers, 9 dogs (8%) were homozygous and reported with CMO, and 15 dogs (14%) carried the pathogenic variant, from which 3 dogs (20%) were reported with CMO.

The researchers found one wild-type dog (CMO-0) in both Scottish and Cairn Terriers with CMO, and screened the coding regions of the entire *SLC37A2* gene in these two dogs for possible other pathogenic variants, but did not find any. This suggests phenocopies, misdiagnoses or genetic heterogeneity.

The analysis of the pathogenic variant in the three main breeds with 96 cases resulted in a highly significant association with CMO.

In addition to the above three breeds, the c.1332C>T variant was also screened in 458 dogs in 124 breeds, but only a single heterozygous carrier dog in Jack Russell Terrier breed was found (S3 Table). The phenotype information for this dog was not available.

The variant was also screened in the known CMO cases from seven breeds (two Bull Terriers, one Curly Coated Retriever, two Border Collies, one Australian Terrier, one Basset, one German Wirehaired Pointer, one Old English Sheepdog), but they did not have it.

S3 Table. Summary of validation data for the *SLC37A2* mutation (c.1332C>T).

Dog breed		Total	Wild Type C/C	Heterozygous C/T	Homozygous T/T
WHWT	CMO affected	69		10	59
	Population control*	626	297	265	64
	Total	695	297	275	123
Scottish terrier	CMO affected	14	1	3	10
	Population control*	235	195	40	
	Total	249	196	43	10
	CMO affected	13	1	3	9

Cairn terrier	Population control*	95	83	12	
	Total	108	84	15	9
124 other breeds *		458	457	1**	
Total (all breeds)		1385	909	334	142

*Clinically confirmed phenotype information in regard to CMO was not available for population controls and other breeds.

**Jack Russell Terrier

Collectively, results suggest that CMO is inherited as dominant disease with incomplete penetrance.

Identification of the canine CMO variant implicates a sugar-phosphate transporter in hyperostosis.

This study unraveled a physiological function of SLC37A2 and provided new insights into infantile swelling diseases, which may be related to disturbances in the intracellular glucose homeostasis during bone development.

SLC37A2 represents a new functional candidate gene. It belongs to the SLC37 family of four ER-associated glucose-phosphate transporters [18]. SLC37A2 is ubiquitously expressed, but transcript and protein levels are particularly high in bone-related tissues such as bone marrow and hematopoietic cell linages such as osteoclasts and macrophages [19, 20]. Murine *Slc37a2* was shown to be one of the genes strongly involved in the osteoclast differentiation, suggesting that it plays a role in osteoclast function and differentiation [20]. Therefore, SLC37A2 may play a central role in glucose homeostasis in the key cell types that participate in osteogenesis. For example, an impaired function of might disturb proper glucose supply in the osteoclasts, decreasing their overall activity, which in turn would result in an imbalance between osteoblastic and osteoclastic functions in the developing bones eventually leading to hyperostosis.

Results link SLC37A2 to bone physiology and disease, and propose *SLC37A2* as an excellent candidate for genetic screening in Caffey patients. Meanwhile, the affected dogs provide unique resources for future experiments to address SLC37A2-related mechanisms in osteogenesis biology.

A recent study in hematopoietic cells identified *SLC37A2* as a primary vitamin D target with a conserved vitamin D receptor-binding site [24]. This may open investigations to study the opportunity to use vitamin D as a therapeutic booster to regulate diminished expression of wild type expression of *SLC37A2* in the affected dogs to alleviate clinical signs.

Caffey disease is an autosomal dominant disease with incomplete penetrance, although rare cases of recessively inherited Caffey disease have also been reported [25].

The determination of the exact mode of inheritance in dogs is not straightforward due to the nature of the variant and mild self-limiting phenotype that may remain unobserved and prevent retrospective diagnosis. They found some dogs that were homozygous for the variant (CMO-2) but had no reported clinical signs. However, we observed a considerable level of the wild-type *SLC37A2* transcript in homozygous dogs (CMO-2) in the peripheral blood due to the splicing leakage, suggesting that the leaky expression is sufficient to avoid a clinical phenotype in some cases.

They also found several heterozygous dogs (CMO-1) that had developed CMO. They found that heterozygous dogs (CMO-1) had lower levels of wild-type *SLC37A2* transcript compared to the unaffected dogs with individual variation of expression between dogs.

This result suggested a dominant disease with incomplete penetrance that could help to explain the reported differences in the severity and duration of CMO among the affected dogs, although alternative models of inheritance cannot be completely ruled out yet.

The dominant phenotype could be due to a dominant-negative effect, but this hypothesis requires further experimental validation to better understand the details of the gene, its regulation and protein function, including potential pairing with other proteins as described for SLC37A4/G6Pase complexes [18]. The *in vivo* function of SLC37A4 has been

shown to depend upon its ability to couple functionally with either G6Pase-a or G6Pase-b [18, 26].

Comments about the scientific publication on CMO: (Dr P Bamas)

The publication is bringing to us important information about the CMO mutated gene:

1-About the mechanisms the mutated gene is acting to produce the disease.

This part is important as it can lead to new tracks for future treatments. Vitamin D is evocated in the publication as a drug to test as a therapeutic booster to help controlling the disease.

2-About the statistics of the mutated gene and the disease in our breed population.

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Same statistics with a different view/presentation:

		WHWT		SCOTTISH T		CAIRN T		Total of the 3 breeds	
Total		**695**	%	**249**	%	**108**	%	**1052**	%
CMO 0	**total**	**297**	**42,7%**	**196**	**78,7%**	**84**	**77,8%**	**577**	55 %
	affected	0	0	1	0.5%	1	1,2%	2	

CMO 1	**total**	**275**	39,6%	**43**	17,3%	**15**	13,9%	**333**	32 %
	affected	10	**3.6% of CMO-1**	3	**20% of CMO-1**	3	**20% of CMO-1**	16	
CMO 2	**total**	**123**	17,7%	**10**	4%	**9**	8,3%	**142**	13 %
	affected	59	8,5% **48% of CMO-2**	10	**100% of CMO-2**	9	**100% of CMO-2**	78	
CMO 1 +CMO 2	**total**	**398**	**57,3%**	**53**	**21,3%**	**24**	**22,2%**	**475**	45 %
	affected	69	9,9% 17% of CMO1+2	13	5,2% 24,5% of CMO1+2	12	11% 50% of CMO1+2	94	19,8%
Total affected dogs		69	**9,9%**	14	**5,6%**	13	**12%**	96	9,1 %

Synonymous denomination for dogs and genotypes regarding to CMO status

CMO-0	Normal	Wild type=wt	Homozygous=hom wild	C/C	N-N
CMO-1	Carrier .		Heterozygous=het	C/T	N-CMO
CMO-2	Mutated	Homozygous mutated	Homozygous	T/T	CMO-CMO

1-The mechanisms

The gene involved is coding for a protein transporter for glucose-6-phosphate in some cells, like osteoclasts (bone cells). The CMO variant for this gene is a mutation of the nucleic base C (Cytosine) which is replaced by the T (Thymine) in the DNA sequence. This DNA sequence is normally responsible for the building of the transporter protein, and the DNA mutation leads to the building of a truncated protein which doesn't make her transporter job properly. For this reason, Osteoclasts are lacking Glucose6phosphate, so they cannot fill their function which is to remove the excess of bone production.

2-The statistics

We can try to get an analysis of the numbers. We can see that the situation seems to be very different between the three breeds, and especially between WHWT on one side and Cairn and Scotties on the other side.

Even if we don't know the way "proven affected" dogs and "supposed non affected" dogs (control dogs) were segregated, nor in what country they were coming from, we can suppose that this could have had an impact on some statistics, and specially on the total affected dogs number comparatively to the total population number.
For this reason, it is much more interesting to analyze the differences between the three breeds because it should remains significant.

a-the mutated gene is much more frequent in WHWT population than in Scotties and Cairn, but there is less affected Westies (9,9% of the

population of the study) than Cairns (12% of the population of the study).

b-the frequency of affected dogs among the CMO-1 and CMO-2 populations is much smaller in WHWT than in Scotties and Cairn (CMO-2= 48%, 100%, 100%, and CMO-1= 3.6%, 20%, 20%)

Conclusion:

This state of fact leads to the conclusion that the management of the mutated variant in the WHWT population must be done with care.

A dominant variant with incomplete penetrance is always a challenge for a breed, but especially if the penetrance is low and the frequency of the mutated variant is high, which seems the case in our breed.

Although the goal of this challenge remains to eradicate the CMO gene from the breed gene pool,

Its high frequency (57.3% of the westies are either CMO-1 or 2) indicates that it would be a nonsense to do it quickly because it would lead to a real genetic bottleneck to remove all these westies from the population. The decrease of genetic diversity that would occur is not desirable in terrier breeds and in our breed particularly.

This high frequency of the mutated gene is certainly a bad news for the breed, **but the good news is that affected dogs are low among CMO-1 and CMO-2 populations**, with **only 3.6% and 48% affected among them,** which is less than what the few available statistics until now were showing.

What does it mean?

It means that the goal of breeding is still to produce:

-no CMO-2 puppies

-the least CMO-1 puppies as possible

Only 3.6% of the CMO-1 puppies will fall ill, so the risk doesn't justify losing the precious affected lines gene pool:
That's what breeders would call throwing the baby out with the bathwater,

and I think that the statistics are giving reason to experienced westie breeders that have the instinctive desire to still work with the mutated gene for a while.

Virtually:

By talking with some breeders, the first question I heard was: " what to do with CMO-2 dogs and bitches?"

These CMO-2 are probably the result of bad luck, or the result of lines that are heavy carriers of the CMO mutation.

If the girl or the boy is healthy (of course no skin problem, no cryptorchidism, no Legg Perthes, no uterus problems, no CMO disease, no liver disease) and if he/she is a good exemplary of the breed, the breeder will want to save his good genes, and would be right to do so.
If it's a bitch, the breeder will have to find a CMO-0 stud to mate her, and keep a bitch from her. This bitch will be CMO-1 and will be a part of the future breeding program only if she is not affected by CMO.

If it's a male, the breeder will have to find a CMO-0 bitch to mate him. I don't think that other breeders will agree to use this dog at stud, and I think it is not desirable for the breed!
So if the breeder does not own a CMO-0 bitch that could be mated to this stud, he will have to acquire one. Then he will have to select a CMO-1 girl in the litter for his future breeding program, etc…

The goal is not to have lots of puppies from the CMO-2 stud, the breeder just need to select one (or a few) daughters for the future.

The second question is what to do with CMO-1 girls and dogs?

As for the CMO-2, they have to be mated only with CMO-0.

If a breeder has a CMO-1 bitch, he will find a CMO-0 dog to mate her.

Then he will select a puppy in the litter, giving the priority to CMO-0 puppies.

If a breeder has a CMO-1 male, he will have to mate her with a CMO-0 bitch.

If he doesn't own a CMO-0 bitch that can be mated by his CMO-1 dog, he will have to acquire one or to find an agreement with a breeder that have a CMO-0 bitch.

Cooperation between breeders is always a key to the future of the breed.

In the same way, the breeder will select a puppy, giving the preference to CMO-0 pups.

How long could it last to remove the CMO gene from the breed?

Given the small size of litters in our breed, given the fact that CMO-2 could be used, it could take several years to reach that goal and probably a decade.

There are three ways for breeders to work:
-testing the breeding stock before mating, which is the most preferable.

-testing the puppies before selecting, which is preferable than after.

-testing the puppies after selecting, which will lead to some time lost to approach the goal.

The breeder that is testing the breed stock before breeding and the puppies before selection will have to give some information to the future buyers, especially for the CMO-1 buyers.

Concerning the breeders who choose not to test before breeding, I hope that prospective buyers will find information about the CMO gene from breed clubs.

Dr P Bamas.