ORIGINAL ARTICLE

Genetic background of lumbosacral transitional vertebrae in German shepherd dogs

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Objectives: **Estimation of genetic parameters of lumbosacral transitional vertebrae based on data derived from radiographic screening of 27,597 German shepherd dogs.**

Materials and Methods: **Results of radiographic screening for lumbosacral transitional vertebrae classified according to a published scheme were collected. Obtained data were used for estimating variance components in single and multiple trait linear animal models to obtain heritabilities and additive genetic correlations for different types of lumbosacral transitional vertebrae.**

Results: **Estimations indicated a moderate heritability of lumbosacral transitional vertebrae of $h^2 = 0.27$. Trait definitions reflecting the different types of lumbosacral transitional vertebrae revealed positive additive genetic correlations of $r_g > 0.5$ between those types usually categorised as pathologic.**

Clinical Significance: **Results of comprehensive genetic analyses enable the development of breeding measures against lumbosacral transitional vertebrae to reduce their prevalence and support management of potentially correlated diseases in German shepherd dogs.**

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INTRODUCTION

Lumbosacral transitional vertebrae (LTV) are congenitally malformed vertebrae located at the transition between the lumbar spine and the sacrum (Morgan 1968, Morgan 1999). Their morphology is highly variable and combines characteristics of both the lumbar and sacral segment of the spine (Morgan 1968, Larsen 1977, Winkler 1985, Damur-Djuric *et al.* 2006, Flückiger *et al.* 2009, Westworth & Sturges 2010). Previous studies suggested correlations with the development of clinical neurologic and orthopaedic diseases. The association of LTV with a higher incidence of Cauda equina syndrome (CES) has been reported by several authors (Morgan *et al.* 1993, Flückiger *et al.* 2006, Ledecký *et al.* 2007). Dogs with LTV were found to be eight times more susceptible for this neurologic condition and developed symptoms 1.6 years earlier compared to dogs without LTV (Flückiger *et al.* 2006). Moreover, LTV were surmised to cause or support the progression of canine hip dysplasia (CHD) and coxofemoral osteoarthrosis when they are asymmetric (Komsta *et al.* 2015, Flückiger *et al.* 2017). Although the role of LTV for CHD progression appears to be less evident compared with the close relationship of LTV with CES, both CES and CHD may supposedly be related to altered biomechanics of the lumbosacral junction.

A breed predisposition for LTV has been clearly documented for German shepherd dogs (GSDs) (Larsen 1977, Winkler 1985, Damur-Djuric *et al.* 2006, Fialová *et al.* 2014). Data regarding a possible sex predisposition were inconsistent and not statistically significant (Larsen 1977, Morgan 1999, Flückiger *et al.* 2006, Julier-Franz 2006). LTV are commonly detected as an incidental finding during radiographic examinations of the pelvis, spine and abdomen (Tellhelm & Brass 1994, Breit *et al.* 2003). Despite their clinical relevance, LTV are mostly still given minor attention. Due to the lack of consensus regarding their classification, data of different studies vary widely in terms of the prevalence of LTV. For GSDs, reported figures range between 4.3% and 40.0% depending on the scoring scheme (Larsen 1977, Lappalainen *et al.* 2012). Flückiger *et al.* (2009) proposed a straightforward radiographic classification scheme with four different types of LTV (0 to III) with the aim of obtaining valid and comparable data. Implementation of the scoring

scheme is based on radiographs taken for the official screening for CHD. The normal lumbosacral transition is referred to as LTV type 0. LTV type I presents a mild deviation from normal anatomy characterised by a separation of the spinous processes of the first sacral vertebra from the remainder of the sacrum. It is assumed, that dogs with this type of LTV are not or only slightly more prone to the development of aforementioned secondary diseases than dogs with normal lumbosacral transition. LTV types II and III show more obvious morphologic abnormalities, which increase the liability of affected dogs for developing correlated locomotor disabilities. LTV type II shows a symmetrical orientation of the transverse processes, whereas LTV type III has asymmetrical processes, which often facilitate its identification on a radiograph. Most frequently, transitional vertebrae show anatomic deviations at their transverse processes and vertebral arches (Morgan 1968). The described scheme, following Flückiger *et al.* (2009), is currently applied by the Swiss dysplasia commission and the Association for radiological diagnosis of genetically influenced skeletal diseases in small animals (GRSK) in Germany.

In terms of genetics, previous analyses, based on data from a radiographic screening of 4,386 dogs, have indicated a moderate heritability of $h^2 = 0.2–0.3$ of LTV in the population of the German breeding association for GSDs [Verein für Deutsche Schäferhunde (SV) e.V.] (Wigger *et al.* 2009). Ondreka *et al.* (2013) obtained even higher estimates of heritability ($h^2 > 0.6$), based on data from the radiologic examination of the lumbosacral junction of 572 GSDs. Although LTV can be of great welfare concern for affected individuals (Summers *et al.* 2010), breeding measures have not yet been established to reduce their frequency. Present recommendations rely upon regulations confined to the severely altered LTV types II and III. The genetic basis of LTV type I and its relation to other LTV types are uncertain. Although this type is supposed not to have direct clinical relevance, genetic similarities (shared genetic background) to LTV types II and III would imply reasonable importance for disease management by breeding. LTV type I is by far the most common anomalous form of LTV (Fialová *et al.* 2014). In case of a common genetic basis between LTV types, mating of LTV type I affected dogs would have an inherent risk to increase the number of progeny prone to locomotor disabilities (precipitated by LTV types II and III). Consequently, a genetic correlation between the clinically insignificant LTV type I and LTV types II and III would have major implications on the practical implementation of breeding programmes. To develop effective and sustainable breeding measures against LTV, it is crucial to perform refined genetic analyses on the different types of LTV. Improved understanding of the genetics of LTV and their characteristics may allow implementing successful breeding strategies to reduce their prevalence and possibly the frequency of related secondary diseases. Therefore, the objective of this study was to estimate genetic parameters of the different LTV types (0 to III) using single and multiple trait linear animal models based on data of the radiographic screening of 27,579 GSDs (born between 2006 and 2015) to be able to give specific recommendations for the development of breeding measures.

MATERIALS AND METHODS

Data acquisition

The online database vetsXL.com (Veterinärmedizinisches Dienstleistungszentrum (VetZ) GmbH, Isernhagen, Germany) was searched for radiographic records of GSDs of the kennel club SV radiographed between the years 2007 and 2016. The database is used by the GRSK and SV for the collection and processing of data originating from official radiographic screening procedures. Inclusion criteria were complete information on basic data of the dog (date of birth, kennel name, sex, age at the time of radiologic examination, transponder-ID) and the hip joint status as regulated by the Fédération Cynologique Internationale (FCI). Records regarding the presence of LTV according to the proposed scheme by Flückiger *et al.* (2009) complemented the list of inclusion criteria. The underlying radiographic screening for both, CHD and LTV, was performed by the designated scrutineer for GSDs of the SV (one author). Additionally, complete pedigree data were provided for each dog by the kennel club.

Statistical analysis

Descriptive analysis was performed using the procedures FREQ and MEANS of the software package Statistical Analysis System (SAS), version 9.2 (SAS Institute Inc.). Simple and multiple analyses of variance were performed in general linear models using the SAS procedure GLM, which allowed model development for the subsequent genetic analyses.

Genetic analyses

For the genetic analyses, all LTV types were considered and dogs being affected by a particular type were opposed to all other LTV types. Trait definitions are illustrated in Table 1. The final model was the same for all LTV traits analysed and included the following fixed effects: sex (male, female), season of birth (November–January, February–April, May–July, August–October), year of radiologic examination (until 2011, single years between 2012 and 2016) and age at time of radiologic examination (up to 12 months, 13 to 14 months, 15 to 18 months, >18 months). The random additive genetic effect of the animal and the random residual completed the linear animal models for univariate and multivariate variance component estimation:

$$y_{ijklmn} = \mu + Sex_i + Season_j + Year_k + Age_l + a_m + e_{ijklmn}$$

with y_{ijklmn}=radiographic finding,
μ=model constant,

Table 1. Definitions of traits considered within the genetic analyses

Trait	Trait values	Definition of trait
nLTV	0-3	LTV-classification (all types)
LTV0	0-1	0=LTV types I-III, 1=LTV type 0
LTV1	0-1	0=not LTV type I, 1=LTV type I
LTV2	0-1	0=not LTV type II, 1=LTV type II
LTV3	0-1	0=not LTV type III, 1=LTV type III
LTV23	0-1	0=not LTV type II/III, 1=LTV type II or III

Sex_i=fixed effect of i-th sex (i = 1–2),
$Season_j$=fixed effect of j-th season of birth (j = 1–4),
$Year_k$ = fixed effect of k-th year of radiologic examination (k = 1–6),
Age_l=fixed effect of l-th age group at time of radiologic examination (l = 1–4),
a_m=random additive genetic effect of m-th dog and
e_{ijklmn}=random residual.

For the estimation of genetic parameters pedigree information on up to four generations was considered, resulting in a relationship matrix of up to 61,978 dogs. Genetic analyses were performed using the software packages PEST (Groeneveld *et al.* 1990) and VCE6 (Variance Component Estimation, version 6.0.2 Groeneveld *et al.* 2010), following the restricted (residual) maximum likelihood (REML) approach.

RESULTS

Demographic and radiographic data

Inclusion criteria for this study were met by 27,579 GSDs born between 2006 and 2015. Mean age of examined dogs was 16.0 months. Of the included dogs, 12,962 were male (47.0%) and 14,617 were female (53.0%). Normal anatomic lumbosacral transition (LTV type 0) was found in 21,017 dogs (76.2%). LTV type I, characterised solely by a separation of the spinous processes between the two most cranial sacral vertebrae, was detected in 4,145 of the dogs (15.0%). The prevalences of the symmetrical LTV type II and the asymmetrical LTV type III were very similar with 1,198 (4.3%) for type II and 1,219 (4.4%) for type III.

Prevalence data

The graphic presentation of frequencies of the different LTV types for GSDs born between the years 2006 and 2015 illustrates the phenotypic trends within the breeding population of the SV (Fig 1). A significant decrease of the frequency of normal morphologic lumbosacral junctions (LTV type 0) by 11.0% could be depicted since 2007 (87.0% (n=47 of 54) in year 2007; 76.0% (n=2,170 of 2,857) in year 2015; (P < 0.001)). This trend was paralleled by a significant increase of 6.9% (P < 0.001) of the number of GSDs with LTV type I with values of 9.3% (n = 5 of 54) and 16.2% (n = 462 of 2,857) in the respective years. The frequencies of LTV types II and III showed only minor fluctuations with maxima around 3% [minimum of 2.8% (n = 5 of 176) in year 2008; maximum of 5.9% (n = 39 of 665) in year 2009] and 5% [minimum of 0.0% (n = 0 of 54) in year 2007; maximum of 5.2% (n = 249 of 4,812) in year 2014].

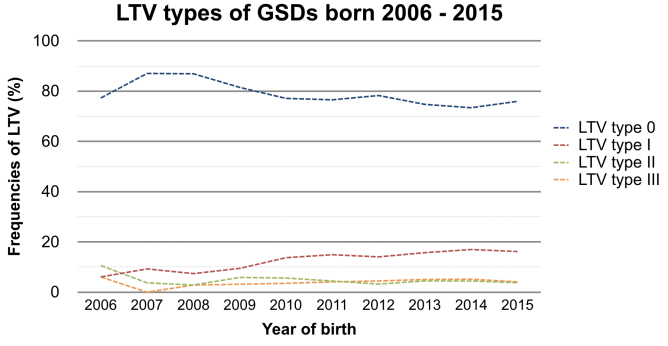

FIG 1. Phenotypic trends of different LTV types in GSDs born between 2006 and 2015

Influences on LTV type expression

Results of multivariate analysis of variance showed a significant influence of sex on the phenotypic expression of LTV types, with female dogs tend to develop pathologic LTV more commonly (F-value = 23.6; P < 0.0001). Age was found significant for LTV types 0 (F-value = 13.2; P < 0.0001) and I (F-value = 15.7; P < 0.0001), but not for the others. Year of radiologic examination consistently showed P-values <0.0001 for all traits (F-value = [6.5; 14.0]). The F- and P-values of the fixed effects are given in Table 2.

Results of genetic analyses

For the different LTV types, univariately estimated heritabilities ranged between $h^2 = 0.08$ and $h^2 = 0.27$ (SE = 0.01–0.02), with the highest estimate obtained using the comprehensive categorical trait definition of nLTV. The heritability estimate for normal anatomic lumbosacral junctions (LTV type 0) was $h^2 = 0.27$ (SE = 0.02). LTV types I, II and III had low heritability estimates, which ranged between 0.08 and 0.12 (SE = 0.01). Multivariate estimations of variance components revealed values of $r_g > 0.89$ (SE = 0.04) between LTV types II and III and values of $r_g = 0.53$–0.63 (SE = 0.06–0.07) of LTV type I to both LTV types II and III. Estimates of heritabilities and additive genetic correlations with their related standard errors are shown in Table 3.

DISCUSSION

The prevalence of LTV was high (23.7%) in the examined population of GSDs and equalled prior reports (Fialová *et al.* 2014). Fialová had found a prevalence of 25.9% in 205 GSDs using a similar LTV classification scheme. In our study, LTV type I was the most common anomalous form and was present in 15.0% of the examined GSDs. LTV types II and III were found in 8.7% of dogs. This figure implies reasonable motivation to search for possible disease management strategies, because the latter types of LTV are considered risk factors for the development of CES (Morgan *et al.* 1993, Flückiger *et al.* 2006, Ledecký *et al.* 2007) and CHD (Larsen 1977, Ziegler 1989, Morgan 1999, Komsta *et al.* 2015, Flückiger *et al.* 2017). Our statistical analyses indicated a predisposition for LTV in bitches, which is in contrast to the results of different authors (Larsen 1977, Morgan 1999, Flückiger *et al.* 2006, Julier-Franz 2006). Different sampling and varying sample sizes may be responsible for the inconsistent results regarding the effect of sex. Investigation of the phenotypic trends showed that the frequency of LTV type I has increased significantly over the last decade (2006 to 2015), whereas the frequency of normal lumbosacral anatomy has declined progres-

Table 2. Results of multivariate analysis of variance (SAS procedure GLM) with F-statistics and significances (P-value) for considered fixed effects (sex, group of age at time of radiologic examination, year of radiologic examination, season of birth)

Trait	Trait values	Definition of trait	Sex		Group of age		Year		Season	
			F	P	F	P	F	P	F	P
nLTV	0-3	LTV-classification (types)	23.6	<0.0001	5.8	0.0006	11.5	<0.0001	5.3	0.0012
LTV0	0-1	0=LTV types I-III, 1=LTV type 0	12.8	0.0004	13.2	<0.0001	14.0	<0.0001	6.0	0.0005
LTV1	0-1	0=not LTV type I, 1=LTV type I	0.3	0.6189	15.7	<0.0001	9.4	<0.0001	2.8	0.0384
LTV2	0-1	0=not LTV type II, 1=LTV type II	5.7	0.0168	1.0	0.3841	9.6	<0.0001	0.7	0.5732
LTV3	0-1	0=not LTV type III, 1=LTV type III	17.3	<0.0001	0.7	0.5485	6.5	<0.0001	1.9	0.1298
LTV23	0-1	0=not LTV type II/III, 1=LTV type II or III	22.5	<0.0001	1.5	0.2214	7.1	<0.0001	2.5	0.0618

Table 3. Heritabilities and additive genetic correlations with related standard errors for lumbosacral transitional vertebrae estimated in uni- and multivariate analyses

Trait	Trait values	Mode of analysis	Mean	Std	h^2	SE_h^2	r_g	SE_r_g
nLTV	0-3	u	0.369	0.765	0.274	0.014		
LTV0	0-1	u	0.762	0.426	0.271	0.015		
LTV1	0-1	u	0.150	0.357	0.121	0.011		
		b/LTV2			0.122	0.010	0.633	0.065
		b/LTV3			0.120	0.010	0.529	0.064
		b/LTV23			0.121	0.010	0.592	0.052
		t/LTV2			0.122	0.010	0.618	0.058
		t/LTV3					0.539	0.056
LTV2	0-1	u	0.044	0.204	0.075	0.009		
		b/LTV1			0.075	0.008	0.633	0.065
		b/LTV3			0.079	0.008	0.900	0.043
		t/LTV1			0.08	0.008	0.618	0.058
		t/LTV3					0.899	0.042
LTV3	0-1	u	0.044	0.205	0.111	0.010		
		b/LTV1			0.109	0.010	0.529	0.064
		b/LTV2			0.116	0.009	0.900	0.043
		t/LTV1			0.115		0.618	0.058
		t/LTV2					0.899	0.042
LTV23	0-1	u	0.088	0.283	0.192	0.013		
		b/LTV1			0.192	0.013	0.592	0.052

b Bivariate, t Trivariate, u Univariate

sively. From a clinical point of view, this process is not necessarily alarming yet, because LTV type I is considered to be of no or only minor clinical relevance for the individual dog. The observed trend may, in the absence of targeted breeding measures, give reason to expect drastic changes in the future distribution of different LTV types in the population of GSDs. Given the highest and further increasing frequency of LTV type I, its genetic role is of special interest and great importance. A shared genetic basis of LTV type I - III and a continued use of affected dogs in breeding may give reason for relative shifting of frequencies from the LTV type 0 to also LTV types II and III, *i.e.* increasing frequencies of dogs with more remarkable anatomical deviations. And this in turn may increase the prevalences of potentially devastating secondary locomotor disabilities like CES and CHD.

The genetic analysis of this study confirmed the moderate heritability of LTV, formerly estimated by Wigger *et al.* (2009), which is a key determinant of the efficiency of breeding selection. To investigate LTV types I to III with respect to an overlapping genetic basis, genetic parameters were estimated multivariately, and the obtained additive genetic correlations among the LTV types were clearly positive with $r_g > 0.89$ (SE = 0.04) between LTV types II and III and $r_g = 0.53$–0.63 (SE = 0.06–0.07) between LTV type I and both LTV types II and III. Therefore, dogs with LTV type I have, compared with dogs with a normal lumbosacral junction, a relatively increased risk to produce progeny with the more severe LTV types II and III. This has major implications on the practical implementation of breeding programmes. Regulation confined of the clinically relevant types II and III is likely to show clear limitations in efficacy and may no longer be seen as adequate. Positive additive genetic correlations between the different types of LTV emphasise the importance of more comprehensive breeding strategies, including all pathologic LTV types (I to III). The high prevalence of LTV and their direct link with potentially detrimental secondary diseases underline the need for reasonable, targeted and efficient breeding strategies against LTV.

Maximum genetic progress should be achieved when selecting against the general genetic disposition of LTV. Even though phenotypic selection against LTV is an applicable breeding strategy and likely showing some effect on the LTV frequencies, if practiced consequently, higher efficacy will be achieved by the use of estimated breeding values (EBVs) and EBV-based selective mating. Until genetic evaluation for LTV in the GSD has been developed and implemented in the routine, such that EBVs for LTV become available, breeding measures should be based on

the comprehensive phenotype indicated as nLTV in this study. Prior experience from breeding strategies against CHD, which has a similar level of heritability (Leppänen *et al*. 2000), can serve as a guideline in the development of breeding strategies against LTV. It is known from CHD that phenotypic breeding may have satisfactory breeding progress in the beginning, but then typically behaves static with trend curves levelling off or even develops a reverse trend. Therefore, methods supporting sustainable progress are considered superior. Estimation of breeding values has been proposed to improve genetic progress in CHD prospectively and progressively (Mäki *et al*. 2000, Malm *et al*. 2008, Lewis *et al*. 2013). According to our results, this would be the method of choice for LTV as well. Moreover, the implementation of any breeding measures against LTV must take their distribution pattern into account. Given the large proportion of GSDs with LTV type I within the SV, rigorous exclusion of all affected dogs from breeding would have tremendous impact on the population. Substantial reduction of genetic diversity and increased inbreeding rates with concurrent negative side effects like reduced overall fitness and high risk of promoting other hereditary diseases could be the result. Furthermore, genetic correlations of LTV with other traits of relevance for dog breeding are currently unknown, but need to be considered in a balanced and sustainable breeding programme. Taking this into account, it is advisable to develop breeding strategies for LTV, which are based on EBVs rather than on LTV phenotypes.

Practitioner contributions to the screening for LTV are very valuable and simply start with ensuring the lumbosacral transition is included and imaged properly on ventrodorsal hip radiographs. The vertebra located cranially to the sacrum should be depicted on the radiographs to the full extent. Scrutineers should be encouraged to record the LTV status of dogs during CHD screening and submit obtained data for further analysis and long-term monitoring of phenotypic and genetic trends. The present data should be used for an evidence-based policy to provide owners of breeding dogs with information about the adjustments of breeding recommendations. Furthermore, kennel clubs should review their current approaches to LTV screening critically based on the genetic correlations of LTV types found in this study.

CONCLUSION

The results of the present study confirm the high prevalence and the genetic basis of LTV in GSDs. The moderate heritability of LTV in GSDs enables an efficient reduction of their frequency and thereby of potentially correlated locomotor diseases through targeted breeding measures. Data collected during the routine radiographic screening on CHD are providing a suitable basis for breeding analyses and selection purposes. Because positive additive genetic correlations between the different types of LTV indicate a shared genetic background, all types of LTV, including LTV type I, need to be considered for future breeding programmes and selection schemes. Although the use of EBVs has clear advantages over phenotypic selection, LTV phenotypes can serve as input for first-line breeding measures until breeding strategies based on EBVs are set. To enable both, LTV recording must become an integral part of the routine screening protocols of dog breeding.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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