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2 **Evaluation of intervertebral disc degeneration in young adult asymptomatic Dachshunds with**
3 **magnetic resonance imaging and radiography**

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21

22 **Abstract**

23 **Background**

24 Dachshunds have a high prevalence of intervertebral disc disease (IVDD) to which they are
25 predisposed due to early intervertebral disc (IVD) degeneration and calcification. Moreover, the
26 recently found 12-*FGF4RG* retrogene is associated with calcified discs visible on radiographs

27 (CDVR) and IVDD. Earlier studies suggest that all IVDs of one-year-old Dachshunds show signs of
28 degeneration. This prospective, analytical, blinded study aimed to investigate the extent and
29 distribution of IVD degeneration in young adult (24-31 months) asymptomatic Dachshunds (n =
30 21). Another aim was to explore the correlations between IVD degeneration evaluated with
31 magnetic resonance imaging (MRI), the number of CDVR, and the dog's 12-*FGF4*RG retrogene
32 status. The study protocol included grading the CDVR on spinal radiographs, grading the IVD
33 degeneration on T2-weighted sagittal and transverse high-field MR images of all IVDs (n = 546),
34 and 12-*FGF4*RG variant genotyping.

35 **Results:**

36 Of all IVDs evaluated, 2% (n=11) were normal based on MRI grading. Despite the study population
37 having moderately degenerated IVDs (median MRI grade 3), there was also variation in the degree
38 of IVD degeneration between individuals and in the distribution of IVD degeneration between
39 different vertebral regions. The number of CDVR correlated significantly with the magnitude of
40 IVD degeneration based on MRI evaluation and with the 12-*FGF4*RG genotype. The odds for being
41 12-*FGF4*RG homozygous were higher for Dachshunds with CDVR. However, the 12-*FGF4*RG
42 variant did not alone explain the phenotypic variation in IVD degeneration.

43 **Conclusions:**

44 The number of CDVR is a valid indicator of overall IVD degeneration, as it correlates with MRI-
45 based IVD grading. Also, as the extent of IVD degeneration varies between individual Dachshunds,
46 selective breeding against IVDD using radiographic screening and 12-*FGF4*RG variant genotyping
47 is possible.

48

49 **Key words:** Chondrodystrophic breed; *FGF4* retrogene; IVD; IVDD; MRI

50

51 **Background**

52 Dachshunds have a high risk for intervertebral disc (IVD) degeneration and intervertebral disc
53 disease (IVDD) at an early age ((1–4). The high IVDD prevalence of 15-30% in the breed makes it
54 a major animal welfare problem causing pain, suffering, and increased mortality rate (3–6).
55 Pathogenesis of IVD degeneration in Dachshunds involves chondroid metaplasia, including
56 replacement of notochordal cells within the nucleus pulposus by chondrocytes and fibrocartilage
57 formation. In addition, nucleus pulposus loses proteoglycans and becomes dehydrated and finally

58 potentially calcified (7–11). Clinically, the degree of IVD degeneration and the number of calcified
59 discs visible on radiographs (CDVR) affect the risk for IVDD (2,4,6,11,12) Studies also show that
60 many CDVR in the thoracolumbar area or presence of completely degenerated IVDs on MRI
61 clearly increase the risk of IVDD recurrence (13,14).

62 The breed-typical disproportionate dwarfism, elicited by *FGF4* retrogene insertions in different
63 genomic locations, is associated with premature IVD degeneration (15). Dachshunds have two
64 distinct *FGF4* retrogene insertions: on chromosome 12 (12-*FGF4*RG, chondrodystrophy) and on
65 chromosome 18 (18-*FGF4*RG, chondrodysplasia) (16,17). All Dachshunds are homozygous for 18-
66 *FGF4*RG (16) which defines their short-limbed morphology, but does not seem to significantly
67 affect the odds of CDVR (18). However, 12-*FGF4*RG, which is nearly fixed in Dachshunds, is
68 associated with CDVR and IVDD in multiple breeds (17,18).

69 Mineralized IVDs are seen on radiographs, and this has been utilized in the screening programmes
70 of Dachshunds, as the heritability of CDVR is high in the breed (19,20). Selective breeding utilizing
71 radiographic screening at the age of 24-48 months is a proven method for reducing IVDD risk in
72 Dachshunds (2,4,6,21).

73 Unlike radiographs, MRI allows detection of non-mineralized stages of disc degeneration (22,23). It
74 is the most accurate imaging modality for evaluating the degree of IVD degeneration, and MRI
75 grading of IVD degeneration correlates with the degree of histologically visible IVD degeneration
76 (23–25). Pfirrmann grading using T2-weighted MRI is well established for evaluating IVD
77 degeneration in humans. It is based on MRI signal intensity, disc structure, distinction between
78 nucleus pulposus and anulus fibrosus, and disc height (26). Furthermore, it has been proven suitable
79 for grading canine IVD degeneration using T2-weighted sagittal plane low-field MRI (27) and more
80 recently high-field transverse plane MRI (28).

81 For the last decades, since Hansen's studies on canine IVD degeneration pathology (7), the general
82 belief in veterinary medicine has been that all IVDs in Dachshunds degenerate at a young age.
83 However, no prospective MRI studies classifying IVDs of young Dachshunds exist. The main aim
84 of this study was to investigate the extent and distribution of IVD degeneration in young
85 Dachshunds with high-field MRI. This was accomplished through modified Pfirrmann grading
86 combining sagittal and transverse MR images of IVDs. A second aim was to explore the
87 correlations between the number of CDVR, the IVD degeneration evaluated with MRI, and the

88 dog's 12-*FGF4*RG retrogene status. We hypothesized that not all IVDs of two-year-old
89 Dachshunds are degenerated and that the degeneration status in MRI correlates with the number of
90 CDVR and the 12-*FGF4*RG retrogene status.

91

92 **Methods**

93 *Animals*

94 Client-owned Dachshunds aged 24-31 months were recruited to this prospective, analytical, blinded
95 study. We aimed to increase the possibility of finding Dachshunds with relatively less degenerated
96 IVDs and consequently more 12-*FGF4*RG genotypic variation. Thus, we included dogs with either
97 their own screening result with a maximum of two CDVR or their parents' screening results with a
98 maximum of two CDVR. Exclusion criteria were any signs of systemic or neurologic illness.
99 The study protocol was approved by the Project Authorization Board of the Regional State
100 Administrative Agency of Southern Finland (ESAVI/ 29986/ 2020). Participation was voluntary
101 and all examinations were performed between January 2021 and January 2022 at the Veterinary
102 Teaching Hospital, University of Helsinki, with owners' informed and written consent. Recruitment
103 was performed by social media advertisement during the year 2021.

104 *Clinical evaluation of dogs*

105 To ensure absence of clinical signs, a patient history was obtained from the owner and a physical
106 examination was performed by one of the authors (VR). Next, a neurological examination was
107 performed by a board-certified veterinary neurologist (TSJ). This included evaluation of mental
108 status, posture, gait, postural reactions, spinal reflexes, cranial nerves, and possible spinal pain on
109 palpation.

110 *Anaesthesia and diagnostic imaging*

111 Radiographs were taken and MRI was conducted under general anaesthesia for all dogs.
112 Haematocrit and serum protein and creatinine concentration were analysed before anaesthesia. The
113 anaesthesia protocol was planned individually by the presiding hospital anaesthesiologist. Dogs
114 were discharged after full recovery from general anaesthesia.

115 Spinal radiographs were taken with undirect (CPI Indico 100 RAD 150kV 2006, Fujifilm FCR XG-
116 1) or direct (Arcoma Intuition DR 125 kV 630 mA, Canon CXDI-401C) digital imaging technique.
117 The Finnish Kennel Club spinal imaging protocol was utilized; thus, the set consisted of
118 laterolateral radiographs of the cervical spine, cervicothoracic junction, thoracic spine,
119 thoracolumbar junction, and lumbar spine including sacrum (29). This same protocol is utilized
120 internationally and has been described previously (19,21,30).

121 All MRI examinations were conducted in dorsal recumbency with a 1.5 Tesla scanner (Phillips,
122 Ingenia 1.5T S, Philips Medical System) using head and spine coils. The MRI protocol was a
123 combination of two previously reported protocols for imaging and grading IVD degeneration
124 (27,28). Turbo-Spin-Echo T2-weighted sagittal and transverse plane MRI was performed for the
125 cervical, thoracic, and lumbar spine. Imaging parameters (TR for sagittal imaging 3020 ms, TR for
126 transverse imaging 3648 ms, TE 100 ms, flip angle 90°, slice thickness 2.5 mm) were chosen
127 according to the requirements for evaluation of IVDs.

128 *Image analyses and genetic analyses*

129 Blinded radiographic evaluation of IVDs was performed and recorded in a random order on the sets
130 of radiographs by an experienced radiologist (AKL). Every IVD space was graded as calcified or
131 not (30,31). Briefly, a normal, totally radiolucent IVD with a normal width of the IVD space was
132 graded as zero (0 = normal). An IVD with a mineralization, regardless of the size and opacity of the
133 mineralization was graded as one (1 = calcified). Thereafter, the number of CDVR was calculated
134 for every dog and for each IVD space.

135 For MRI grading of the IVDs, the authors (TSJ and VR) developed a combined two image plane
136 classification scheme previously validated separately for transverse and sagittal images (26–28)
137 (Table 1), including model images of each grade (Fig. 1).

138 Before the actual blinded classification of the anonymized MR images, the two authors evaluated
139 together seven non-anonymized MR image sets (182 discs) to practise and synchronize the grading.
140 Thereafter, the evaluators, blinded to the signalment and radiographic results, independently graded
141 every IVD of anonymized sets of MR images in random order. Immediately afterwards, the grade
142 for each disc was determined by the consensus of the two evaluators. The MRI grades of all 26
143 IVDs were summed for each dog. This figure (MRI total sum) describes the degree of overall IVD
144 degeneration of an individual dog.

145 Three millilitres of whole blood sample (EDTA) was collected from all dogs while they were
146 sedated. The blood samples were stored at -18°C. After all samples were collected, they were sent
147 for 12-*FGF4*RG and 18-*FGF4*RG variant genotyping to a commercial laboratory (Laboklin GmbH
148 & Co. KG, Bad Kissingen, Germany).

149 ***Statistical methods***

150 To investigate the association between the overall IVD degeneration and the number of CDVR, a
151 linear regression model was fitted for the association between the number of CDVR and the MRI
152 total sum, having the MRI total sum as the response variable. In addition, as the IVD calcification
153 indicates a more advanced stage of IVD degeneration (7), we investigated the association between
154 the severely degenerated MRI grade 4 IVDs and the number of CDVR. It was analysed utilizing
155 Poisson regression models, having the number of MRI grade 4 IVDs as the response variable. The
156 associations between the 12-*FGF4*RG variant and the presence of CDVR (with or without CDVR),
157 MRI total sum, and the number of MRI grade 4 IVDs were each assessed with logistic regression
158 applying Firth's penalized likelihood. Odds ratios (OR) with 95% confidence interval (CI) were
159 calculated from the logistic regression models.

160 To investigate the distribution of IVD generation within different vertebral regions, the proportions
161 of normal or only mildly degenerated MRI grade 1-2 IVDs were compared between the three
162 vertebral regions (cervical, thoracic, lumbar) using a logistic regression model.

163 The effects of demographic variables (age, sex, weight) on the number of CDVR were assessed
164 simultaneously with a Poisson regression model, and the effects of the demographic variables on
165 the MRI total sum were evaluated with a multivariate ANOVA model.

166 All statistical analyses were performed by a professional statistician (JJ) using SAS System for
167 Windows, version 9.4 (SAS Institute Inc., NC USA). P-values < 0.05 were considered significant.

168

169 **Results**

170 Altogether 21 clinically normal Dachshunds (546 IVDs) were included (seven Standard Smooth-
171 haired, five Standard Long-haired, four Standard Wire-haired, three Miniature Long-haired, one
172 Miniature Wire-haired, and one Miniature Smooth-haired). Their mean age was 27 months (SD 2.4

173 months, range 24-31 months) and mean weight 7.9 kg (SD 1.8 kg, range 4.2-10.9 kg). Of the
174 Dachshunds, 12 were females and nine males (two neutered). All of the dogs had normal physical
175 and neurological examinations. Eight dogs were eligible according to their own previous CDVR
176 grading result and ten dogs according to parents' CDVR grading results. Additionally, three
177 Dachshunds without their own or parents' previous CDVR grading results were included in the
178 study.

179 The CDVR distribution is presented in Fig. 2. The median number of CDVR per dog was two
180 (range 0-13). Of all 546 IVD spaces, CDVR were observed in 67 (12%). Most often CDVR were
181 detected in IVD spaces C7-T1 and T5-T6 (both in five dogs), whereas no CDVR were observed in
182 L6-L7 and L7-S1.

183 On MRI evaluation, six dogs (29%) had MRI grade 1 IVDs (1-4 per dog), 12 dogs (57%) MRI
184 grade 2 IVDs (1-6 per dog), 21 dogs (100%) MRI grade 3 IVDs (12-26 per dog), and 18 dogs
185 (86%) MRI grade 4 IVDs (1-13 per dog). None of the dogs had MRI grade 5 IVDs. The median
186 MRI grade for all examined IVDs was 3. The median MRI total sum, calculated for each dog
187 separately, was 81 (range 66-90) (Fig. 3). A strong association emerged between the number of
188 CDVR and the MRI total sum ($P = 0.002$, estimate 1.28, CI 0.52-2.03) and between the number of
189 CDVR and the number of MRI grade 4 discs ($P < 0.0001$, estimate 1.13, CI 1.08-1.19) (Fig. 4).
190 In all 546 IVD spaces of examined dogs, the distribution of MRI grades was as follows: 11 MRI
191 grade 1 (2%), 40 MRI grade 2 (7%), 395 MRI grade 3 (72%), and 100 MRI grade 4 (18%) (Fig. 5).
192 A significant difference in IVD degeneration existed between vertebral regions. The probability for
193 IVDs to be graded as normal or mildly degenerated (MRI grade 1 or 2) was significantly higher in
194 the cervical region than in the thoracic or lumbar region ($P < 0.0001$ and $P = 0.0004$), but no
195 significant difference emerged between thoracic and lumbar regions (Table 2).

196 The demographic variables (age, sex, or weight) did not have a significant connection with the MRI
197 total sum ($P = 0.323$, $P = 0.577$, and $P = 0.249$) or the number of MRI grade 4 discs ($P = 0.552$, $P =$
198 0.726 , and $P = 0.459$). Sex and weight also did not have a significant association with the number of
199 CDVR ($P = 0.767$ and $P = 0.692$). Moreover, when one extreme outlier, the youngest dog with 13
200 CDVR (highest of the range) was removed from the analysis, age ceased to have a significant
201 association ($P = 0.169$).

202 Three Dachshunds were heterozygous and 18 were homozygous for the 12-*FGF4*RG variant. The
203 heterozygous dogs were all standard-size Dachshunds and the only ones without CDVR. In
204 addition, they represented all coat types (wire-, smooth-, and long-haired). The probability of being
205 homozygous for the 12-*FGF4*RG variant was clearly higher for dogs with CDVR than for dogs
206 without CDVR but with a broad confidence interval, due to the low number of observations (OR
207 259, CI 2.9-22802). As expected, all Dachshunds were homozygous for the 18-*FGF4*RG variant.
208 The median MRI total sum, indicating the degree of the dog's overall IVD degeneration, was lower
209 (71, range 66-75) for 12-*FGF4*RG heterozygous dogs than for homozygous dogs (82, range 69-90).
210 Still, a statistical connection between MRI total sum and the 12-*FGF4*RG variant could not be
211 shown with the current data (OR 1.30, CI 0.99-1.71 for MRI total sum).

212

213 **Discussion**

214 We investigated the extent and distribution of IVD degeneration in clinically healthy young adult
215 Dachshunds with high-field MRI in this prospective, analytical, blinded study. To the authors'
216 knowledge, this is the first prospective study using high-field MRI for canine IVD degeneration.
217 We observed variation in IVD degeneration including some non-degenerated IVDs in our study
218 population.

219 Our results contradict the generally accepted conclusion in veterinary medicine that all IVDs of
220 young adult Dachshunds are degenerated, as six dogs had altogether 11 non-degenerated IVDs
221 based on MRI evaluation. Since IVD degeneration and the number of CDVR are highly heritable
222 (6,17–20), we expected to gain a study population of Dachshunds with relatively less degenerated
223 IVDs and more phenotypic and genetic variation by including dogs according their own or parents'
224 radiographic results. Still, only 2% of all IVDs were non-degenerated and our MRI grade median of
225 3 was consistent with moderate overall IVD degeneration. By contrast, Kranenburg et al. reported a
226 Pfirrmann grade median of 4 (severe IVD degeneration) for older chondrodystrophic dogs with
227 symptomatic IVDD and a median of 3 for older non-chondrodystrophic dogs with symptomatic
228 IVDD (25). The discrepancy is explained with our inclusion criteria. However, it is notable that the
229 IVD degeneration of these young clinically healthy Dachshunds is equivalent to older non-
230 chondrodystrophic dogs with IVDD, consequently suggesting a similar risk for IVDD in these two
231 groups.

232 We observed that some Dachshunds in our study had variation in IVD degeneration and some did
233 not. In other words, individual dogs had 0-15% of non-degenerated IVDs and all MRI grades were
234 found in some individuals, but some had only MRI grade 3 and 4 IVDs. This variation between
235 young asymptomatic adult Dachshunds has not been investigated before with MRI grading.
236 Moreover, it is an important finding because without variation selective breeding against IVDD
237 would be impossible.

238 Our study shows, based on MRI grading, an interesting and statistically significant difference in
239 IVD degeneration between the vertebral regions. We observed much less IVD degeneration in the
240 cervical spine than in the thoracic and lumbar spine. These findings are parallel with the role of
241 premature IVD degeneration in IVDD of chondrodystrophic breeds, as the localization of their
242 IVDD is most often in the thoracolumbar region (7,12,14,32). The current study is the first
243 prospective study utilizing MRI-based grading of IVD degeneration for young adult dogs. The
244 uneven distribution of IVD degeneration in our data differs from the study of Hansen (7), which had
245 suggested that early IVD degeneration occurs simultaneously in all intervertebral spaces of
246 chondrodystrophic dogs.

247 Of all 21 Dachshunds, three did not have any CDVR. Furthermore, when calculating the MRI total
248 sum for each dog to describe their overall IVD degeneration, we noticed that the median of MRI
249 total sums of these three dogs were about ten units below the overall median. In addition, a strong
250 statistical association existed between the number of CDVR and the number of severely
251 degenerated (MRI grade 4) discs and between the number of CDVR and the MRI total sum,
252 indicating dogs' overall IVD degeneration. Our findings suggest that in young adult Dachshunds
253 the number of CDVR reflects the frequency of severely degenerated IVDs and that the radiographic
254 grading and the number of CDVR are reliable indicators of overall IVD degeneration. Similar
255 conclusions have been reported earlier about the association between CDVR and clinical IVDD
256 (2,4,6,11). IVD degeneration appears to be somewhat higher based on MRI grading than based on
257 CDVR which is in accordance with previous reports (12,33). Computed tomography has also been
258 shown to be a more effective imaging method than radiography in detecting calcified IVDs (11,34).

259 Our study population included three 12-*FGF4*RG heterozygous dogs, which allowed us to
260 investigate the possible correlation of the number of alleles (heterozygous versus homozygous) with
261 IVD degeneration. These heterozygous individuals were also the only ones without CDVR and with
262 lower overall degeneration level based on MRI total sum. We detected a statistically significant

263 association between the 12-*FGF4RG* allele number and the dog's CDVR status, which somewhat
264 contradicts an earlier report (6). They found a perfect sensitivity (1.0) but very low specificity
265 (0.14) in wire-haired Dachshunds and no association in smooth- or long-haired Dachshunds.
266 However, the setup was different, as they compared dogs with less than five CDVR and dogs with
267 more than five CDVR, while we compared dogs with or without CDVR. This different setup can
268 explain the partial discrepancy between the two studies.

269 We detected variation in IVD degeneration between young adult asymptomatic Dachshunds, which
270 cannot completely be explained by homozygosity or heterozygosity of the 12-*FGF4RG* variant.
271 Three 12-*FGF4RG* homozygous Dachshunds had MRI grade 1 IVDs and lower MRI total sum than
272 one of the 12-*FGF4RG* heterozygous Dachshunds. The findings cannot be explained by
273 demographic variables, as age, sex, or weight did not have a significant association with MRI total
274 sum. Moreover, this variation cannot be explained by the 18-*FGF4RG* variant either, as all
275 Dachshunds in our study were homozygous for this variant. Our results reinforce the previous
276 conclusion of Bruun et al.(6) that the two currently recognized *FGF4RG* variants do not explain all
277 phenotypic variation in IVD degeneration and IVDD in Dachshunds. In addition, other complicated
278 genetic or environmental factors might exist (18,35). Also earlier studies state that IVDD is a
279 multifactorial disease (2,15,36).

280 We used high-field T2-weighted MRI and a combined two image plane MRI grading to evaluate
281 IVD degeneration as accurately as possible. When evaluating IVDs of small dogs, MRI resolution
282 may hinder the evaluation and the difference between two Pfirrmann grades might not be clear in
283 every IVD (26,27). With two image plane evaluation, our purpose was to achieve higher subjective
284 certainty when detecting discriminating features as homogeneous versus inhomogeneous and
285 differentiating nucleus pulposus and anulus fibrosus.

286 The main limitation in this study is the small sample size of only 21 Dachshunds when the 12-
287 *FGF4RG* allele frequency is extremely high in the breed. Investigating the correlation between 12-
288 *FGF4RG* and IVD degeneration was not the main objective of this study. However, we achieved a
289 statistically significant result for the association between the 12-*FGF4RG* variant and the presence
290 or absence of CDVR, but the precision was quite low. Moreover, the statistical connection of the
291 MRI total sum with the 12-*FGF4RG* variant remains unclear due to our limited data, warranting
292 future studies with larger study populations. An additional limitation in our study is the absence of
293 intra- or interrater analysis for our IVD grading based on two plane MRI evaluation. However,

294 these methods have been previously separately validated for grading canine IVD degeneration (26–
295 28). As we did not specifically address this issue in our study, further studies of sensitivity and
296 specificity of combined two image plane grading are warranted.

297 **Conclusions**

298 This study confirms that although IVD degeneration of young adult Dachshunds without
299 neurological signs is substantial these dogs have non-degenerated IVDs as well. Moreover, there is
300 variation in the extent of IVD degeneration between individual Dachshunds and in the distribution
301 of IVD degeneration between different vertebral regions. The number of CDVR can be used as an
302 indicator for magnitude of MRI-graded IVD degeneration. Despite the correlation with the number
303 of CDVR, the 12-*FGF4*RG variant alone does not explain the phenotypic variation in IVD
304 degeneration in Dachshunds. IVDD is a significant animal welfare issue, especially for many
305 chondrodystrophic breeds. Therefore, for effective breeding against premature IVD degeneration
306 and IVDD, the full potential of radiographic screening and genetic testing should be used.

307

308 **Abbreviations**

309 CDVR: calcified discs visible on radiographs

310 IDD: intervertebral disc disease

311 IVDD: intervertebral disc disease

312 MRI: magnetic resonance imaging

313 12-*FGF4*RG: *FGF4* retrogene insertion on chromosome 12, chondrodystrophy

314 18-*FGF4*RG: *FGF4* retrogene insertion on chromosome 18, chondrodysplasia

315

316 **Declarations**

317 **Authors' contributions**

318 VR, TJ, MH, and AL planned the study and VJ, TJ, MH, JJ, and AL collected, analysed, and
319 interpreted the data. VR drafted the manuscript, and all authors reviewed and edited it. All authors
320 read and approved the final manuscript.

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323 Johanna Salomäki for their technical assistance.

324 **Competing interests**

325 The authors declare that they have no competing interests.

326 **Availability of data and materials**

327 The data will not be made openly available to third parties or outside the original research team for
328 patient confidentiality reasons.

329 **Consent for publication**

330 Not applicable.

331 **Ethics approval**

332 The study protocol was approved by the Project Authorization Board of the Regional State
333 Administrative Agency of Southern Finland (ESAVI/ 29986/ 2020). All owners signed an informed
334 written consent to participate in the study.

335 **Prior publication**

336 Data have not been published previously.

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456 **TABLES**

457 **Table 1 Two image plane MRI classification scheme for IVD degeneration**

458

MRI grade	IVD structure	Distinction of nucleus and anulus	IVD signal intensity	IVD Width	Grade of IVD degeneration
1	Homogeneous, bright white. Vertical band is accepted in sagittal plane if transverse plane is homogeneous and bright white.	Clear	Hyperintense or isointense to cerebrospinal fluid	Normal	Normal / healthy IVD
2	Inhomogeneous with or without vertical bands. Maximum one-third of the nucleus pulposus is hypointense.	Clear	Hyperintense or isointense to cerebrospinal fluid	Normal	Mild degeneration

3	Inhomogeneous, grey. Minimal black spot is accepted if it is only in one image plane.	Unclear, but visible at least in one image plane	Intermediate: isointense or hypointense to cerebrospinal fluid	Normal to slightly decreased	Moderate degeneration
4	Inhomogeneous, grey to black.	Lost	Intermediate or hypointense to cerebrospinal fluid	Normal to moderately decreased	Severe degeneration
5	Inhomogeneous, black.	Lost	Hypointense to cerebrospinal fluid	Collapsed IVD space	Severe degeneration and IVD herniation

459 MRI, magnetic resonance imaging; IVD, intervertebral disc.

460 Modified according to previous literature (26–28).

461

462 **Table 2 The probability of MRI grades 1 and 2 versus MRI grades 3 and 4 IVDs according to**
463 **the vertebral region**

464

Vertebral region	MRI grade 1+2, n (%)	MRI grade 3+4 n (%)	Comparison between vertebral regions	*Odds ratio	95% CI	P-value
Cervical spine	45 (35.7%)	81 (64.3%)	Lumbar versus cervical spine	0.006	0.0004 – 0.101	0.0004
Thoracic spine	6 (2.2%)	267 (97.8%)	Thoracic versus cervical spine	0.044	0.018 – 0.103	< 0.001
Lumbar spine	0 (0%)	147 (100%)	Thoracic versus lumbar spine	7.168	0.397 – 129.360	0.1821

465 MRI, magnetic resonance imaging; IVD; intervertebral disc; MRI grade 1 = non-degenerated IVD;

466 MRI grade 2 = mildly degenerated IVD; MRI grade 3 = moderately degenerated IVD; MRI grade 4

467 = severely degenerated IVD; n= number of intervertebral discs.

468 *Logistic regression Odds ratios for the probability of MRI grades 1 and 2; 95% CI, 95%

469 confidence interval.

470

471 **FIGURE LEGENDS**

472 **Fig. 1 Model images of each MRI grade in the two image plane classification scheme of IVD** 473 **degeneration**

474 Legend: MRI = magnetic resonance imaging; IVD = intervertebral disc.

475 1A sagittal and 1B: transverse plane image of non-degenerated MRI grade 1 intervertebral disc
476 (IVD). 2A sagittal and 2B: transverse plane image of mildly degenerated MRI grade 2 IVD. 3A
477 sagittal and 3B: transverse plane image of moderately degenerated MRI grade 3 IVD. 4A sagittal
478 and 4B: transverse plane image of severely degenerated MRI grade 4 IVD.

479 **Fig. 2 Distribution of calcified discs visible on radiographs (CDVR) per dog**

480 Legend: CDVR = calcified discs visible on radiographs.

481 **Fig. 3 Distribution of different MRI grades (1-4) and overall IVD degeneration per dog**

482 Legend: MRI = magnetic resonance imaging; IVD = intervertebral disc; MRI grade 1 = non-
483 degenerated IVD; MRI grade 2 = mildly degenerated IVD; MRI grade 3 = moderately degenerated
484 IVD; MRI grade 4 = severely degenerated IVD.

485 X-axis represents the 21 Dachshunds. The MRI grading results of each dog are at the bottom of the
486 Y-axis and the MRI total sum is at top of the Y-axis. The MRI total sum indicates the degree of
487 overall IVD degeneration in each individual and is calculated by summing MRI grades of all 26
488 discs. The higher the column, the more advanced the overall IVD degeneration.

489 **Fig. 4 Correlation between the number of calcified discs visible on radiographs and MRI** 490 **grade 4 discs**

491 Legend: MRI = magnetic resonance imaging; IVD = intervertebral disc; MRI grade 4 = severely
492 degenerated IVD.

493 Scatter plot visualizing the correlation between the number of calcified discs visible on radiographs
494 (CDVR) and the number of IVDs with MRI grade 4 degeneration. A strong association emerged
495 between the number of CDVR and the number of MRI grade 4 discs based on Poisson regression
496 analysis (estimate 1.132, 95% CI 1.080-1.187, $P < 0.0001$).

497 **Fig. 5 Distribution of MRI grades between intervertebral disc (IVD) spaces**

498 Legend: MRI = magnetic resonance imaging; IVD = intervertebral disc; MRI grade 1 = non-
499 degenerated IVD; MRI grade 2 = mildly degenerated IVD; MRI grade 3 = moderately degenerated
500 IVD; MRI grade 4 = severely degenerated IVD.