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1 **Severe and progressive neuronal loss in myelomeningocele begins before 16**
2 **weeks of pregnancy.**

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54 **Condensation:**

55 In utero repair of myelomeningocele before 16 weeks may improve motor function by rescuing

56 motor neurons within the myelomeningocele and preventing cranial extension of neuronal loss.

57

58

59 **Short title:** Timing of spinal injury in myelomeningocele

60

61

62

63 **AJOG at a GLANCE**

64

65 **Why was the study conducted?**

- 66 • Midtrimester prenatal surgery is not a cure for myelomeningocele : residual intracranial
67 and motor deficits leading to lifelong handicap question the timing of prenatal surgery.
- 68 • We sought to determine the kinetics of spinal cord injury in utero, in a case-control
69 study based on a large post-mortem population.

70

71 **What are the key findings?**

- 72 • Significant neuronal loss is already present ≤ 16 weeks in the exposed cord and
73 progressively extends cranially : before 16 weeks, the exposed cord display 17% of
74 normal motor neurons, whereas this rate falls to $\leq 2\%$ after 16 weeks.

75 **What does this study add to what is already known?**

- 76 • Current surgical strategies might benefit from earlier repair to rescue motor function
77 within the exposed cord.
- 78 • The development of new therapeutic strategies for early in utero repair could
79 significantly improve the neurologic and motor prognosis of these children.

80

81 **KEY WORDS:** Fetal surgery, Myelomeninogocle, Spina bifida, Motor Function, Motor
82 neurons loss, Natural history, Pathophysiology, Timing of spinal cord injury, Autopsies

83

84 **ABSTRACT**

85

86 **Background:**

87 Despite undisputable benefits, midtrimester prenatal surgery (20-24 weeks) is not a cure for
88 myelomeningocele (MMC): residual intracranial and motor deficits leading to lifelong
89 handicap question the timing of prenatal surgery.

90 Indeed, the timing and intensity of intrauterine spinal cord injury remains ill-defined.

91 Based on postmortem pathology, we describe the natural history of neuronal loss in utero.

92

93 **Methods:** Pathology findings were analyzed in 186 cases of isolated MMC with a lesion level
94 between S1 and T1. Using a case-control cross-sectional design we investigated the timewise
95 progression and topographical extension of neuronal loss between 13 and 39 weeks. Motor
96 neurons were counted on histology at several spinal levels in 54 cases meeting quality criteria
97 for cell counting. These were expressed as observed/expected (O/E) ratios, after matching for
98 gestational age and spinal level with 41 controls.

99

100 **Results:** Chiari II malformation increased from 30.7% to 91.6% after 16 weeks. The exposed
101 spinal cord displayed early, severe and progressive neuronal loss: the O/E count dropped from
102 17% to $\leq 2\%$ after 16 weeks. The neuronal loss extended beyond the lesion to the upper levels:
103 in cases <16 weeks, the O/E motor neuron count was 60% in the adjacent spinal cord,
104 decreasing at a rate of 16% per week. Progressive loss was also found in the upper thoracic
105 cord, although in much smaller proportions. The O/E proportion of motor neurons was not
106 correlated with the level of the MMC.

107

108 **Conclusions:** Significant neuronal loss is present ≤ 16 weeks in the exposed cord and
109 progressively extends cranially. Earlier prenatal repair (< 16 weeks) could avoid Chiari II
110 malformation in 69.3% of cases, rescue the 17% remaining motor neurons in the exposed cord
111 and avoid the extension to the upper spinal cord.

112

113

114 INTRODUCTION

115

116 Myelomeningocele (MMC) is a form of neural tube defect characterized by the protrusion of
117 the meninges and spinal cord through open vertebral arches. Leakage of cerebrospinal fluid
118 through the defect is thought to be responsible of a decreased hydrostatic pressure leading to
119 the descent of cerebellar tonsils through the foramen magnum which define Chiari II
120 malformation. Hydrocephalus is associated with Chiari II malformation in 85% of cases¹.
121 Spinal cord injury and intracranial anomalies result in lifelong paralysis, incontinence, and
122 cognitive impairment. Risk factors such as maternal folate metabolism anomalies and obesity
123 have been associated with open neural tube defects, however the pathophysiology of MMC
124 remains poorly understood . Despite efforts to reduce the incidence of MMC using folic acid
125 fortification, it remains around 1/1000 live births ². The diagnosis is usually made following
126 routine mid-trimester ultrasound, although MMCs can be diagnosed as early as in the first
127 trimester ³. Given the burden of disabilities associated with the condition, prenatal diagnosis
128 results in termination of pregnancy in >60% of cases in Europe ⁴.
129 Prenatal mid-trimester intrauterine repair of the defect improves the outcome, thus confirming
130 the hypothesis of progressive spinal cord injury during gestation. However, mid-trimester
131 repair is not a cure as most infants treated in utero remain severely disabled. In the era of
132 developing prenatal therapies, the timing of the spinal cord injury is instrumental for
133 optimizing such strategies and identifying new therapeutic targets.
134 Based on a large collection of post-mortem cases following termination of pregnancy, we
135 sought to describe the natural history of neuronal loss associated with MMC. Using a case-
136 control cross-sectional design, we investigated both the timewise progression and topographical
137 extension of the neuronal loss along the spinal cord.

138

139 MATERIAL AND METHODS

140

141 **IRB:** CERAPHP.5 #00011928 (24/05/2019)

142

143 **Population: cases and controls**

144 We retrospectively collected all post-mortem cases of spinal defects following termination of
145 pregnancy (TOP) and confirmed by pathological examination from 1998 to 2018. A signed
146 consent for fetal autopsy was available for all cases.

147 Within this collection of spinal defects, we selected the cases meeting the following definition
148 of MMC: open vertebral arch, open arachnoidal sheet, an open ependymal canal. Furthermore,
149 to match the surgical criteria of most trials of prenatal surgery, we selected cases with a normal
150 karyotype or array-CGH, and an upper level of the lesion located between the first thoracic
151 vertebra (T1) and the first sacral vertebra (S1).

152 The controls were fetuses resulting from miscarriage or termination of pregnancy performed
153 for defects not involving the central nervous system with a normal karyotype or array-CGH,
154 and for which a consent to post-mortem research had been signed prior to TOP (Supplementary,
155 Table 1). Cases and controls were classified into 4 groups of gestational age: $\leq 16+0$ weeks,
156 $16+1$ to $20+0$ weeks, $20+1$ to $24+0$ weeks, >24 weeks.

157

158 **Study design**

159 We aimed to compare the number of alpha motor neurons on spinal cord of MMC fetuses with
160 controls (Figure 1).

161 In cases with MMC, spinal cords were examined at three levels:

- 162 • within the exposed cord

163 • at the level adjacent above the MMC, defined as the last level where the spinal cord is
164 surrounded by a closed vertebra above the defect.

165 • at a distant, upper thoracic level (T1-T2). This level was considered only in MMCs
166 below T10

167 In controls, spinal cords were examined at five levels:

168 • upper thoracic (T1-T2),

169 • lower thoracic (T11-T12),

170 • upper lumbar (L1-L2),

171 • L3 (until 24 weeks)

172 • below L3 (until 20 weeks)

173

174 We isolated L3 as a specific level because it corresponds to the lumbar enlargement of the
175 spinal cord (i.e. the lumbo-sacral nervous plexus) with a high density of motor neurons at that
176 specific level ⁵.

177 Because of differential growth of the spinal cord in relation with the vertebral column, spinal
178 cord is found below L3 only up until 20 weeks and at L3 up until 24 weeks in normal fetuses.

179 Indeed, the vertebral column grows more rapidly than the spinal cord resulting in a relative
180 ascension of the conus medullaris with gestation from S2 at 12 weeks to L2-L3 at birth ⁶⁻⁸.

181

182 **Procedures**

183 **Termination of pregnancy:** By law, in France, TOP is possible at any gestational age for
184 severe fetal conditions such as MMC. Vaginal delivery is obtained following cervical ripening
185 by mifepristone and overnight cervical dilators prior to induction; intravaginal prostaglandins
186 are given following the placement of an epidural catheter; in cases >21+0 weeks, fetocide is
187 performed by cordocentesis or intracardiac injection of lidocaine.

188 **Macroscopic examination:** Fetal pathological examination was performed according to the
189 French national guidelines⁹. All cases had a full pathological examination and photographs of
190 all macroscopic findings including the MMC before dissection, X-rays and visceral sampling.
191 Cases with signs of advanced lysis of the brain or birth trauma were not considered for
192 neuropathological examination. Chiari malformation was noted when the brainstem, fourth
193 ventricle and caudal tip of cerebellar tonsils crossed the foramen magnum, both at craniotomy
194 and on the shape of these structures post formaldehyde preservation.

195 Cases with a descent of cerebral structures but that did not yet cross the foramen magnum were
196 not considered with Chiari malformation.

197 Hydrocephalus/ventriculomegaly was defined as an enlargement of the cerebral ventricles >16
198 weeks. Before 16 weeks, hydrocephalus is not evaluated given the physiologically large
199 ventricles of the brain in early gestation. Foot deformities and calf muscle hypotrophy were
200 also recorded. All macroscopic recordings followed a triple assessment of postmortem pictures
201 by three different observers. Discrepancies between observers were excluded.

202

203 **Spinal sampling:** Within the exposed cord, five tiered samples were taken on average (Figure
204 1). All specimens were immersed in a 4% zinc formalin solution (pH=7.4) for preservation, for
205 1 to 5 weeks depending on gestational age. Samples >18 weeks were decalcified by 7%
206 hydrochloric acid (Surgipath Decalcifier II, Leica biosystem) for 30 min to 180 min depending
207 on gestational age. All samples were cut transversally and included in paraffin for histology.

208

209 **Motor neuron count:** For histological analysis, 7 µm thick sections of the spinal cord were
210 stained by hematoxylin & eosin (HE). For cell count, we selected the cases among these MMC
211 with levels of spinal cord sampling meeting quality criteria related to sampling on histological
212 analysis: samples strictly cut transversally, with a continuous open ependyma, a thick ventral

213 horn and an interrupted funiculus (behind the ventral horns). Cases lacking on of these quality
214 criteria were excluded for cell count. The selected HE slides were scanned (Hamamatsu slide
215 scanner) at X40 magnification and visualized using NDP view 2 software (Hamamatsu
216 photonics).For each case, within the exposed cord, the slide with the largest amount of ventral
217 horn was chosen for counting. All counts were conducted on HE staining and only alpha motor
218 neurons were counted. A morphologically normal alpha motor neuron was defined as a large
219 cell showing either a large pale differentiated euchromatic nucleus with a prominent nucleolus
220 surrounded by a light cytoplasm, or a stellate shape with light cytoplasm holding regularly
221 scattered intra-cytoplasmic basophilic Nissl bodies ^{5,10-12}. All motor neurons showing nuclear
222 and/or cytoplasmic characteristics of injury were excluded from cell count. Such features
223 included nuclear shrinkage and increased nuclear basophilia without distinguishable nucleolus
224 (pyknosis), cytoplasmic eosinophilia without Nissl bodies (chromatolysis) (Figures 1, 3).

225 Inter- and intra-observer reproducibilities of cell count based on these criteria were assessed by
226 2 blinded observers with excellent agreement (Intraclass correlation coefficient: ICC=0.995
227 [0.989-0.998] and ICC=0.998 [0.994-0.999] for the inter and intra observer assessment
228 respectively). In a subset of fifteen slides, the motor neuron count was compared to
229 immunohistochemistry using a Neu-N antibody (1/500, clone A60, MAB377, Millipore),
230 showing excellent agreement (intraclass correlation coefficient = 0.992 [0.976-0.997]).

231

232 **Analysis**

233 Every case count was divided by the mean motor neuron count on control samples matched for
234 level and gestational age: this ratio represents an observed over expected (O/E) ratio and is
235 presented as a percentage. Mean O/E ratios are plotted with error bars of 1 standard error of the
236 mean. Control counts per gestational age group and per spinal level are presented in the

237 supplementary materials (Supplementary, Table 2). All analyses were conducted using R (R
238 Foundation for Statistical Computing, Vienna, Austria).

239

240 **Data Availability Statement**

241 Data sharing is not applicable to this article as no new data were created in this study.

242

243

244

245 **RESULTS**

246

247 **Description of the study population**

248 Following the exclusion of cases with defects >T1 or <S1, closed defects and cytogenetic
249 anomalies, we collected 186 cases (Figure 2). Gestational age at TOP ranged from 13 to 39.5
250 weeks. Whereas most of the cases were terminated at >24 weeks following mid-trimester
251 ultrasound diagnosis, a significant proportion of cases were terminated at an early gestational
252 age with 21 (11.3 %) and 36 (19.4%) cases at ≤ 16 weeks and 16-20 weeks respectively (Table
253 1). Apart from the expected CNS, spinal and limb anomalies related to MMC, 15/186 (8.06%)
254 cases presented associated anomalies including caudal dysgenesis (N=4), multiple
255 malformations (N=4), and cleft lip and palate (N=3).

256 The first open vertebra was L3 or L4 in the majority of cases, the others being L1-L2, L5-S1
257 and thoracic level defects (Table 1). Although the proportion of thoracic defects was higher in
258 the earlier gestational age groups, the distribution of levels did not significantly change across
259 gestational age (Fisher exact test, $P=0.5$).

260 Because of advanced lysis, CNS examination could not be performed in twenty four cases. In
261 the remaining one hundred sixty two cases, the proportion of cases with Chiari malformation
262 (defined by cerebellar tonsils crossing the foramen magnum on craniotomy) increased from
263 30.7% to 91.6% between ≤ 16 weeks and 16-20 weeks (Table 1).
264 Hydrocephalus/ventriculomegaly was found in 88.5% of cases, consistently from 16 weeks
265 onwards. However, postmortem pathological examination does not allow to assess the intensity
266 of hydrocephalus/ventriculomegaly: therefore, we could not investigate the relationship
267 between the severity of ventriculomegaly and gestational age.

268 The overall rate of talipes was 39.1% and increased from 14.2% to >40% between ≤ 16 weeks
269 and >20 weeks. Calf muscle hypotrophy was found in forty-three (23.5%) of all cases.

270

271 Progression of motor neuron loss as a function of gestational age

272 Motor neuron counts in MMC cases (N=10, N=14, N=15, N=15 at ≤ 16 weeks, 16+1-20+0
273 weeks, 20+1-24+0 weeks and >24 weeks respectively) were matched for gestational age and
274 spinal level with N=41 controls (N=7, N=8, N=9, and N=17 controls at ≤ 16 weeks, 16+1-20+0
275 weeks, 20+1-24+0 weeks and >24 weeks respectively), yielding a total of one hundred and nine
276 samples (Supplementary, Tables 1 & 2).

277 Figure 4A shows the observed/expected (O/E) proportion of morphologically normal alpha
278 motor neuron as a function of gestational age and at three spinal levels: within the exposed
279 cord, in the adjacent spinal cord above the defect (i.e. the first level with a closed vertebra and
280 a round spinal cord above the defect), and in the upper thoracic spinal cord (T1-T2). Within the
281 exposed cord, cell count showed an early, severe and progressive loss in motor neurons: at ≤ 16
282 weeks, the O/E proportion of morphologically normal motor neurons was only 17% and
283 decreased rapidly to $\leq 2\%$ after 16 weeks. However, in 2/10 cases at 14 weeks (T11-S3 defect)
284 and 15 weeks (L2-S2 defect), the motor neuron count was $>80\%$. Only one of these two cases
285 was a saccular form (15 weeks) and we could not identify specific features in these two cases.
286 The adjacent level showed the same behavior across gestational age, but with a lesser loss of
287 motor neurons: at 16 weeks, the O/E was 60% and decreased linearly to reach 8% at >24 weeks:
288 the average loss at that level was 16% per week of gestation (linear regression, $P < 0.001$). At a
289 distance from the defect, although the O/E was close to 100% throughout gestational age
290 groups, a similar trend is also visible, on the upper thoracic spinal cord, with an average loss of
291 2% per week of gestation (linear regression, $P = 0.01$).

292

293 Extension of the motor neuron loss along the spinal cord

294 Figure 4B shows the O/E proportions of motor neurons as function of spinal cord level in the
295 four groups of advancing gestational age. A steep increase in O/E proportions is seen as the
296 count recedes from the lesion from <20% within the exposed cord, to 80-100% in the upper
297 thoracic cord, in all gestational age groups.

298

299 The O/E proportion of motor neurons was not correlated with the level of the MMC (i.e. the
300 level of the first open vertebra), neither in the exposed spinal cord, nor in the adjacent level: in
301 the exposed spinal cord, adjusting for gestational age, thoracic lesions were counted with 1%
302 less motor neurons than lumbar/sacral lesions ($P=0.79$, linear regression); at the adjacent level,
303 thoracic lesions were counted with 5% more than lumbar/sacral lesions ($P=0.63$).

304

305 **COMMENT**

306

307 In this study, we have shown that the exposed spinal cord is significantly damaged early in
308 pregnancy. We have also shown that the spinal injury extends cranially with a significant
309 reduction in motor neurons in the adjacent cord right above the defect, which worsens with
310 advancing gestation at a rate of 16% per week.

311 A two-hit mechanism has been hypothesized for the neurological damage in MMC: it assumes
312 that the functionally normal, yet dysraphic (first “hit”) exposed spinal cord is progressively
313 destroyed during intrauterine life by trauma and toxic effect of the amniotic fluid (second “hit”).
314 Several observations support this hypothesis: the gross architecture of the spine is intact very
315 early in gestation ^{13,14} and animal studies have demonstrated the toxic effect of the amniotic
316 fluid on the exposed spinal cord ^{15,16}. The kinetics of neuronal death we describe do not favor
317 primary anomalies or progressive secondary damage. However, the magnitude of early
318 neuronal loss suggests that these mechanisms occur largely <16 weeks, possibly before
319 amniotic fluid irritants come into play.

320 Prenatal in utero surgical closure of MMC aims to stop CSF leakage and to protect the neural
321 placode from the intra uterine environment. A randomized trial (MOMS) has showed that
322 midgestational fetal surgery for MMC at 19-26 weeks reduces hindbrain herniation and
323 hydrocephalus ¹⁷. However, given that motor neuron regeneration is improbable, our results
324 suggest that midgestational prenatal surgery is unlikely to improve the spinal damage observed
325 in the open spinal cord. Nonetheless, in the 30 months follow-up of the MOMS trial, prenatal
326 surgery has been associated with an improvement in motor function of at least one anatomic
327 level in 37.9%, compared 19.3% with postnatal surgery, together with a significant
328 improvement in walking status ¹⁸. Based on our results, we can hypothesize that this
329 improvement is not related to the protection of the open neural placode where the damage is

330 probably irreversible, but rather to the prevention of the cranial extension of neuronal damage
331 over time. As shown in Figure 4A, the neuronal loss rate is much slower at the adjacent upper
332 level, some of which may be prevented by mid-gestation interventions, despite overall
333 significantly lower counts compared to controls. This possible benefit can be seen from <16
334 weeks to >24 weeks. Moreover, the fact that motor neuron loss within the defect is similar in
335 low and high-level lesions does not contradict the prognostic value of lesion level : the intensity
336 of loss within the open lesion is comparable to a cord section, and is therefore in line with
337 clinical observations regarding the prognostic value of lesion level. Indeed, prenatal surgery
338 has no effect on bladder function and anal sphincter incontinence ^{19,20}.

339 Interestingly, two of the younger cases showed a normal count of motor neurons, which suggest
340 that progressive functional loss does not occur in all fetuses with MMC. More developments
341 based on imaging or biomarkers are needed to identify this subset of patients who may not
342 benefit from fetal repair of MMC and who otherwise would be exposed to unnecessary maternal
343 and fetal morbidity. However, early therapy (before 16 weeks) would possibly cure the disease
344 and prevent all associated handicap. An early intervention could also prevent the development
345 of the Chiari malformation, found in only 30.7% of cases before 16 weeks. In addition, earlier
346 intervention could save the remaining 17% motor neurons at the level of the MMC and 60% on
347 the adjacent upper spinal cord. Although the proportion of morphologically normal motor
348 neurons required to produce limb function is unknown, a small number could be sufficient ²¹⁻
349 ²³. This hypothesis is supported by a strikingly high rate (19%) of ambulating children following
350 prenatal surgery in lesions above L2 in the long term follow-up of the MOMS trial ¹⁸.

351 Furthermore, our results suggest that the cranial gradient of neuronal loss may extend beyond
352 the directly adjacent level, possibly up to the upper thoracic levels, even in lumbar defects.
353 Indeed, we found a decreasing trend in the proportion of normal motor neurons in the upper

354 thoracic cord with advancing gestation. This finding is supported by studies of physical testing
355 in older children with a MMC \leq L5, reporting a reduction in upper limb strength²⁴.

356 Several aspects make this study unique: compared to previous reports examining MMCs at the
357 cellular level^{22,25,26}, we used a consistent methodology for cell count, using age- and level-
358 matched controls for comparisons, in the largest collection of human fetuses with MMC
359 especially regarding early cases. The robustness of our results is ensured by several key steps:
360 stringent quality criteria were required for cell counting, although this led to analyzing only
361 54/186 cases from the initial population; reproducibility studies and confrontation to
362 immunohistochemistry ensured consistent counts; comparison with matched controls allowed
363 to qualitatively and quantitatively assess motor neurons.

364 Nonetheless, our study population does not include cases <13 weeks, which would be valuable
365 to further investigate the timing of spinal cord injury. Strictly matching each case for gestational
366 age of controls (e.g. per week of gestational age) was not possible given the limited number of
367 available controls. We acknowledge that ultrasound data regarding lower limb movement and
368 MRI in utero or post-mortem prior to autopsy regarding the degree of hindbrain herniation
369 were not available, nor were reliable measurements of ventricle width (which could not be
370 reliably measured on pathology because of fluid leakage during sampling, but was defined by
371 the concordance of three independent observers). Studying the spinal cord below the lesion
372 could have been informative, but since motor function is provided by vertical transmission
373 along the spinal cord, which imply not only a loss of function at the level of the lesion but also
374 in all distal segments of the spinal cord, the presence of potentially functional motor neurons
375 below the defect is of little interest in the context of therapeutic approaches²⁷.

376

377

378 **CONCLUSION**

379 Current surgical strategies might benefit from earlier repair to rescue motor neurons within the
380 exposed cord. However, these procedures possibly prevent further cranial extension of neuronal
381 loss.

382 The development of new therapeutic strategies for early in utero repair of MMC could
383 significantly improve the neurologic and motor prognosis of these children: early prenatal
384 repair (≤ 16 weeks) could avoid -better than reverse - Chiari II malformation in 69.3% of cases,
385 rescue the 17% remaining motor neurons in the exposed cord and prevent the extension to the
386 upper spinal cord.

387

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 436 [rt_elaboration_cd_20140528_vd.pdf](https://www.has-sante.fr/portail/upload/docs/application/pdf/201406/protocole_examen_foetal_neonatal_rapport_elaboration_cd_20140528_vd.pdf). Accessed December 26, 2018.

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- 502 27. Wrathall JR, Lytle JM. Stem cells in spinal cord injury. *Dis Markers*. 2008;24(4-
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504

505 **Table 1.** Macroscopic finding in MMC cases across gestational age.
 506

	≤16+0 wg N=21	16+1–20+0 wg N=36	20+1–24+0 wg N=60	>24 wg N=69	All N=186
Chiari (no/total no (%))	4/13 (30.7%)	33/36 (91.6%)	50/52 (96.1%)	60/61 (98%)	147/162 (90.7%)
Hydrocephalus (no/total no (%))	–	31/36 (86.1%)	46/52 (88.4%)	55/61 (90.1%)	132/149 (88.5%)
Talipes (no/total no (%))	3/21 (14.2%)	13/36 (36.1%)	26/58 (44.8%)	30/69 (43.4%)	72/184 (39.1%)
Upper lesion					
Thoracic (no/total no (%))	5/21 (23.8%)	8/36 (22.2%)	8/60 (13.3%)	8/69 (11.5%)	29/186 (15.6%)
L1 – L2 (no/total no (%))	9/21 (42.8%)	13/36 (36.1%)	16/60 (26.6%)	23/69 (33.3%)	61/186 (32.8%)
L3 – L4 (no/total no (%))	4/21 (19%)	11/36 (30.5%)	25/60 (41.6%)	27/69 (39.1%)	67/186 (36%)
L5 – S1 (no/total no (%))	3/21 (14.2%)	4/36 (11.1%)	11/60 (18.3%)	11/69 (15.9%)	29/186 (15.6%)
N open vertebrae	5 [3-7]	6 [4-8]	5 [4-6]	5 [4-6]	5 [4-7]

507

508

509

510

511

- a. “wg” are weeks of gestation
- b. Percentages may not total 100 because of rounding
- c. Hydrocephalus was not evaluated in the group <16 weeks as it is physiological
- d. N open vertebrae (number of open vertebrae) is median [1st quartile-3rd quartile]

512 LEGENDS TO TABLES AND FIGURES

513

514 **Figure 1.** Study design: comparison between the spinal cord of a fetus with a L2-L5 MMC and
515 a control matched for gestational age.

516 Three levels were examined in each MMC case:

- 517 • the distant spinal cord is located in T1-T2 (upper thoracic)
- 518 • the adjacent level is L1 (first closed vertebral level above the defect)
- 519 • the exposed cord: the L3 slide is chosen here, based on quality criteria

520 An altered alpha motor neuron (histology HE staining, left) is compared to a normal motor
521 neuron (histology HE staining, right)

522

523 **Figure 2.** Flow chart of the study population.

524 Within this collection of spinal defects, we selected the cases meeting the following definition
525 of MMC: open vertebral arch, open arachnoidal sheet, an open ependymal canal. Furthermore,
526 to match the surgical criteria of most trials of prenatal surgery, we selected cases with a normal
527 karyotype or array-CGH, and an upper level of the lesion located between the first thoracic
528 vertebra (T1) and the first sacral vertebra (S1) (N=186).

529 For cell count, we selected the cases among these 186 MMC with levels of spinal cord sampling
530 clearly filled and meeting quality criteria related to sampling on histological analysis (N=54):
531 samples strictly cut transversally, with a continuous open ependyma, a thick ventral horn and
532 an interrupted funiculus (behind the ventral horns). Cases lacking on of these quality criteria
533 were excluded for cell count.

534

535 **Figure 3.** Histopathology of the anterior horn at three spinal levels in two L2-L5 MMC cases
536 at 16 (A,B,C) and 23 weeks (G,H,I) and two age-matched controls (D, E, F and J, K, L
537 respectively). The 3 levels presented are the exposed cord, the adjacent spinal cord (L1) and
538 the distant spinal cord (T1-T2). Arrows show cells with normal morphology (large cell showing
539 either a large pale differentiated euchromatic nucleus with a prominent nucleolus surrounded
540 by a light cytoplasm, or a stellate shape with light cytoplasm holding regularly scattered intra-
541 cytoplasmic basophilic Nissl bodies) and Arrowhead show injured cells (nuclear shrinkage and
542 increased nuclear basophilia without distinguishable nucleolus (pyknosis), cytoplasmic
543 eosinophilia without Nissl bodies (chromatolysis)). H&E staining with X40 magnification.

544

545 **Figure 4.** Observed over expected (O/E) ratios of motor neurons.

546 A: O/E ratios of motor neurons as a function of gestational age at the level of the MMC, at the
547 adjacent spinal cord and at upper thoracic level.

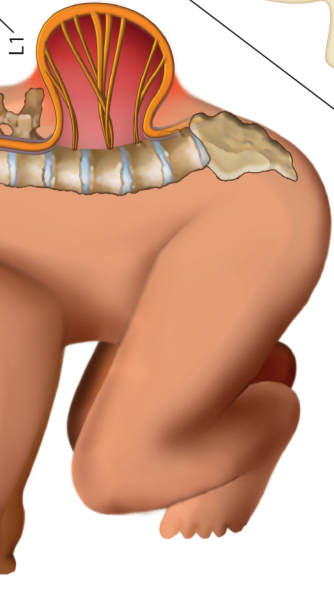
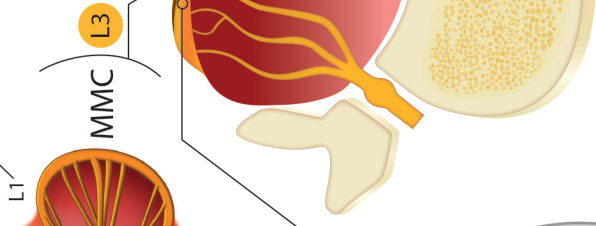
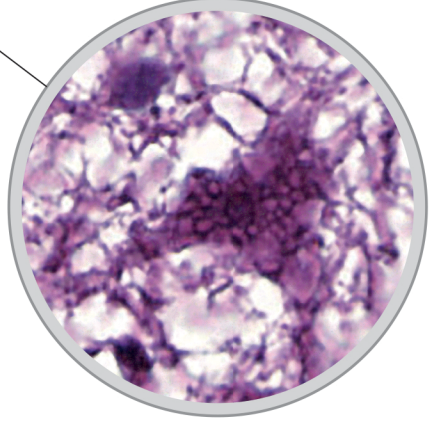
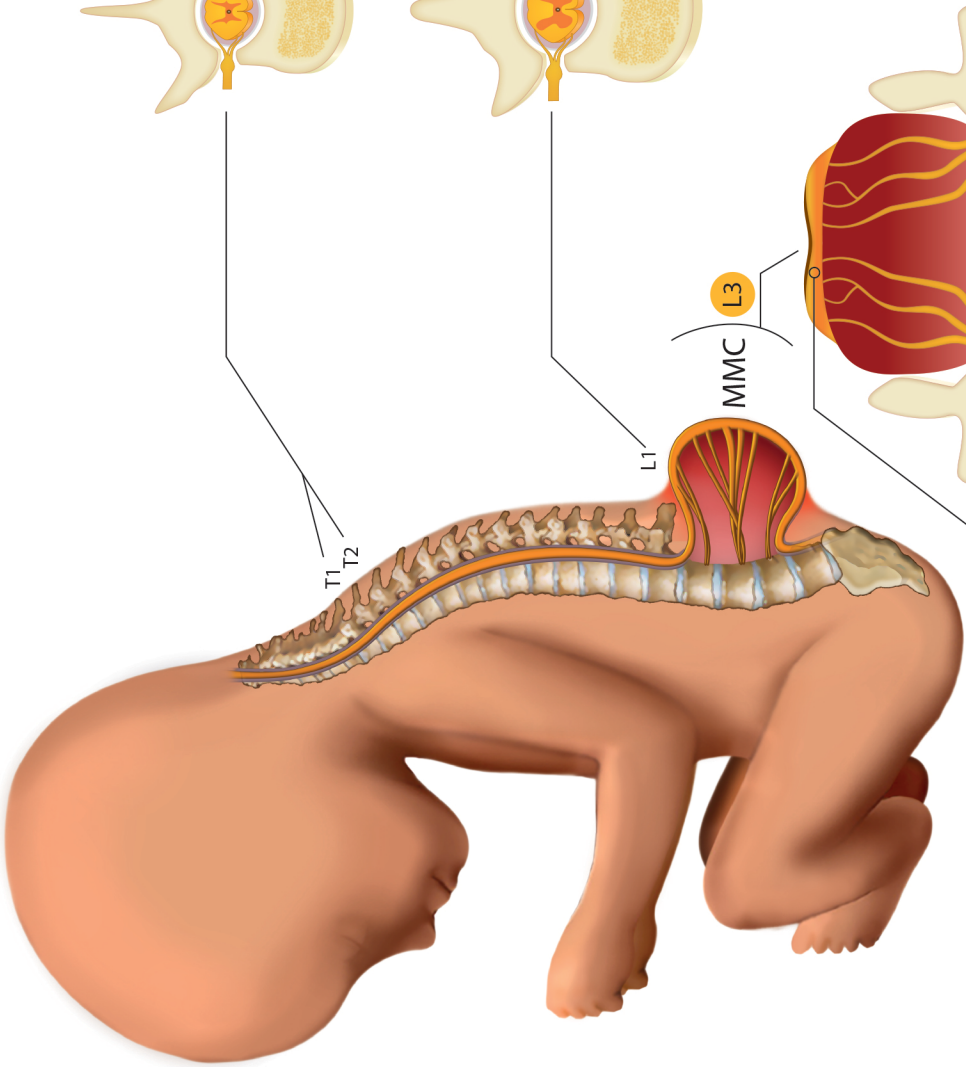
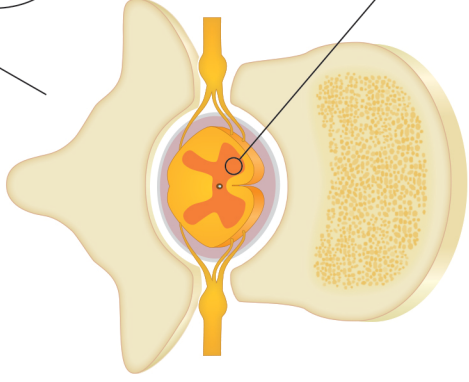
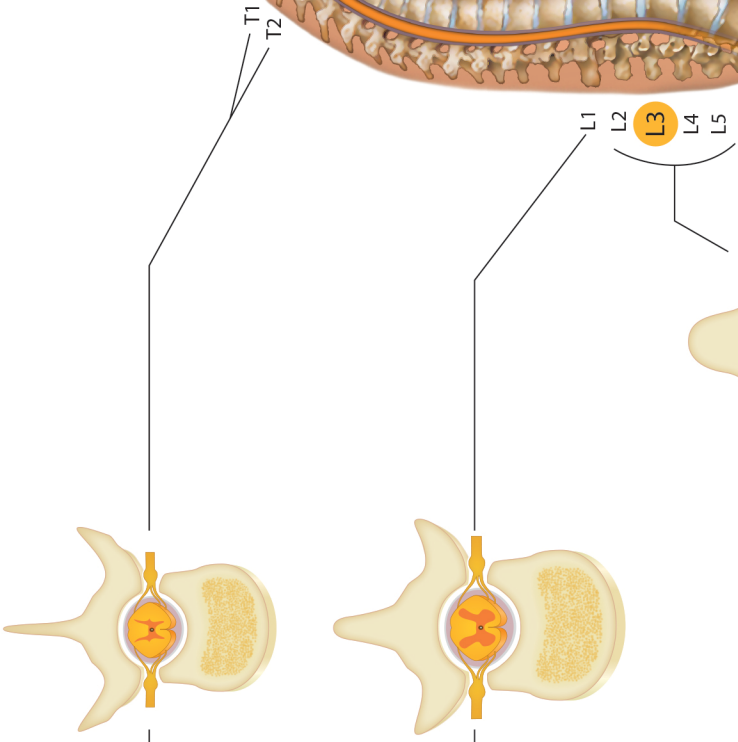
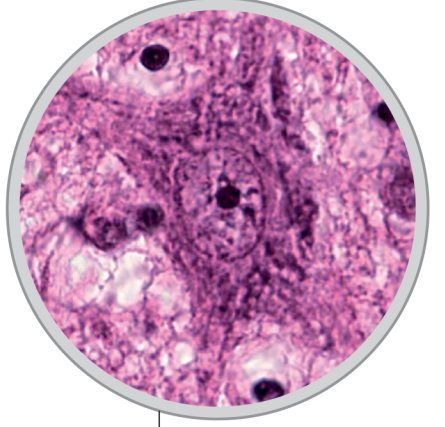
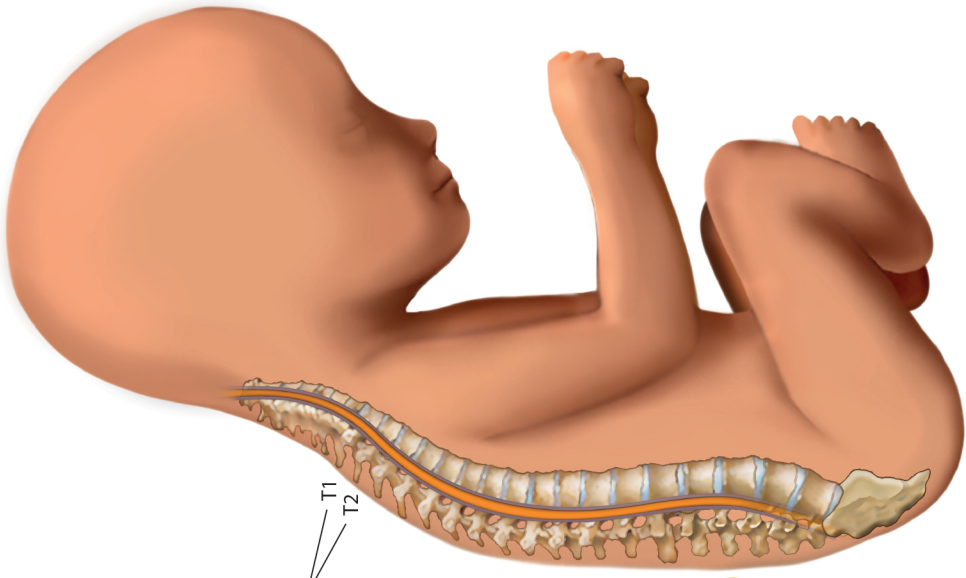
548 B: O/E ratios of motor neurons at each spinal level: exposed cord, adjacent spinal cord and
549 upper thoracic levels according to gestational age.

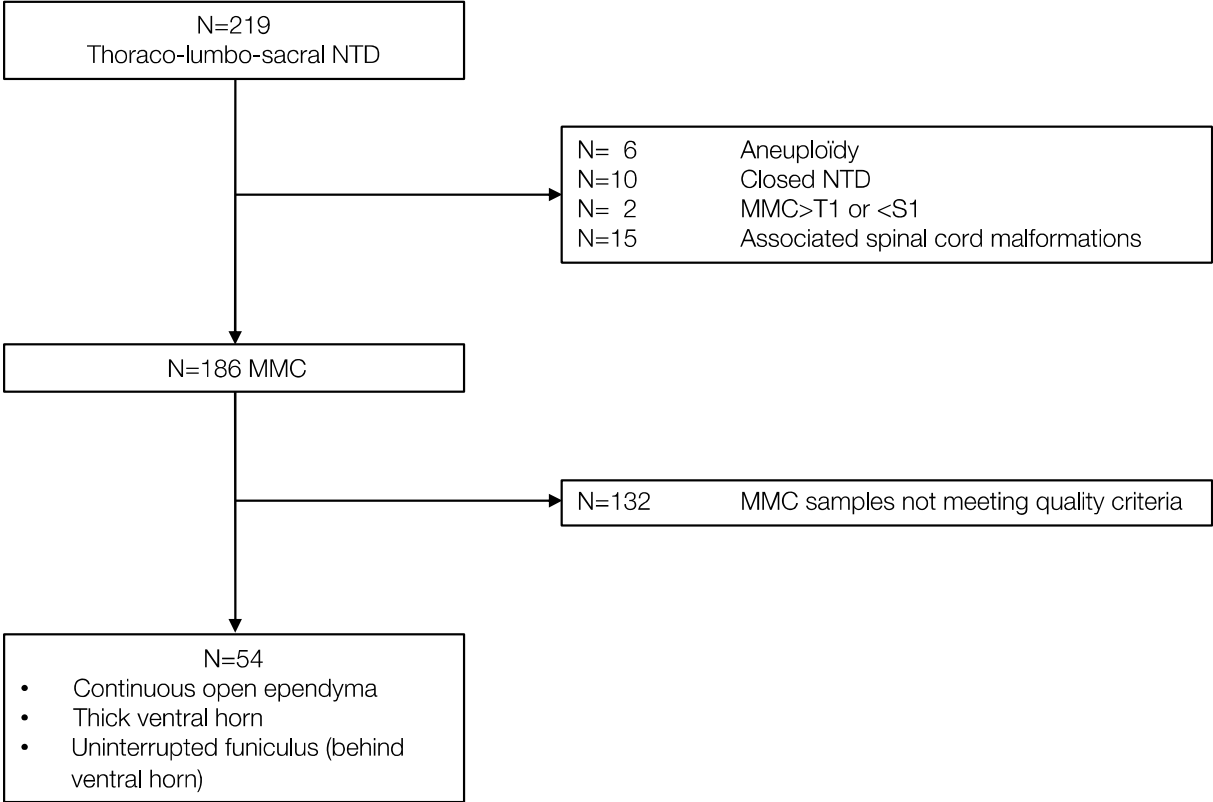
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551

552 **Table 1.** Macroscopic finding in MMC cases across gestational age.

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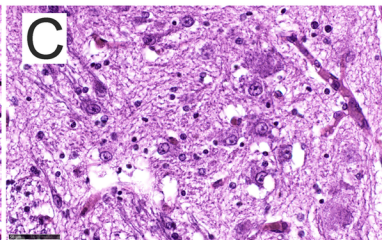
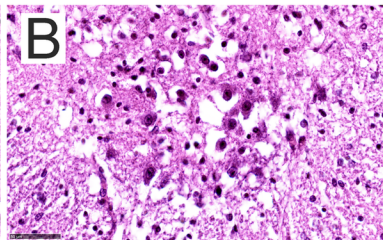
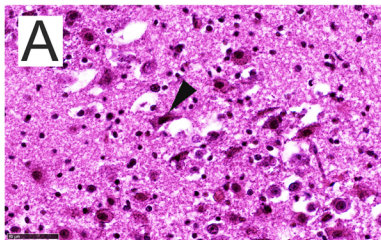
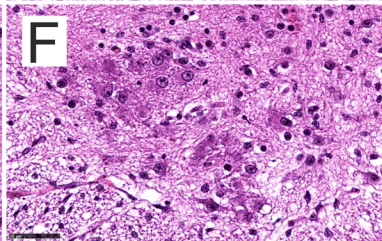
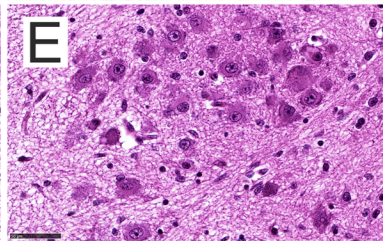
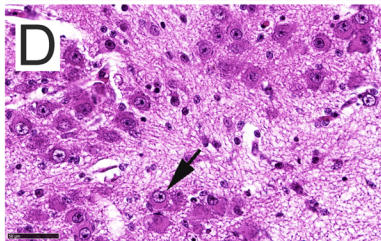
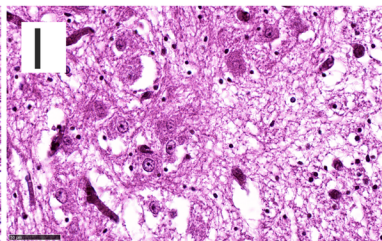
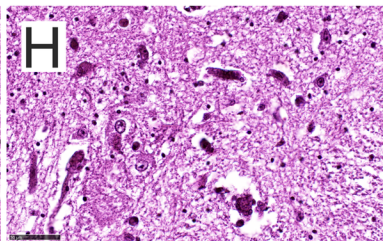
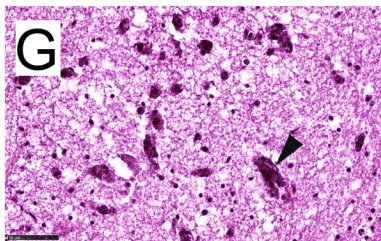




L3

L2

T1

16 w
MMC16 w
CTR23 w
MMC23 w
CTR