Mosaic Expression of Med12 in Female Mice Leads to Exencephaly, Spina Bifida, and Craniorachischisis

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BACKGROUND: A precise temporal and spatial regulation of gene expression is necessary to achieve neural tube closure. Med12, a subunit of the mediator complex, can bind transcription factors and modulate expression of their target genes. Med12 is essential during early mouse development and is important for neural tube closure. **METHODS:** We have made use of a mouse line carrying a conditional null allele of the X-linked *Med12* gene to generate heterozygous female embryos that express *Med12* in a mosaic fashion thus allowing the study of the role of *Med12* during neural tube closure. **RESULTS:** Mosaic expression of *Med12* causes a wide variety of embryonic phenotypes. Some embryos were unable to complete turning and were found with arrested development at embryonic day (ED) 9.5. Others were able to pass ED 12.5 and displayed defects in neural tube closure. These defects included exencephaly, spina bifida, craniorachischisis, split face, and curly tail. Histologic and skeletal analyses of these mutant females show that the neural plate is unable to elevate and is completely flat in the regions of the body axis where neural tube closure fails. **CONCLUSIONS:** We report examples of all known neural tube defects implying *Med12* in the full process of neural tube closure along the complete body axis. Our work points to *Med12* being an essential coregulator of transcription factors controlling neural tube closure. *Birth Defects Research (Part A) 88:626–632, 2010.* © 2010 Wiley-Liss, Inc.

Key words: neural tube defects; mediator complex; Med12; exencephaly; spina bifida; craniorachischisis; X-chromosome inactivation

INTRODUCTION

Fine control of gene expression is a crucial step for correct organism development and homeostasis (Taatjes et al., 2004). This control, among other mechanisms, is achieved by transcription factors, which are proteins that bind to specific regulatory DNA sequences. Transcription factor binding and subsequent RNA polymerase II activation leads to transcription of target genes (Orphanides and Reinberg, 2002).

The mediator complex is instrumental in the regulatory process orchestrated by transcription factors. The mediator is composed of 30 subunits that assemble in a modular fashion. This highly conserved structure, present in all eukaryotes, functions as a molecular bridge connecting transcription factors and the polymerase II machinery (Bourbon et al., 2004; Conaway et al., 2005; Malik and Roeder, 2005). Some of the mediator subunits are essential for transcription of practically all genes, because either they directly interact with the transcription machinery (e.g. Med11) or they act as scaffold for the complex as a whole (e.g. Med17; Tudor et al., 1999; Takagi and Kornberg, 2006; Esnault et al., 2008). Other subunits however, are responsible for interactions with specific transcription factors, mediating in this way their capacity for activating transcription of the target genes (Ge et al., 2002; Wang et al., 2005; van Essen et al., 2009).

Recent studies identified two missense mutations in the human *MED12* gene associated with two X-linked mental retardation syndromes, Opitz-Kaveggia and Lujan Syndrome (Risheg et al., 2007; Schwartz et al., 2007),

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leading to macrocephaly, hypotonia and cranial abnormalities.

We have previously shown that Med12, one of the largest subunits of the complex, is essential for specific processes during mouse development leading to early embryonic death in hypomorphic mutants before embryonic day (ED) 10.5 (Rocha et al., submitted). Moreover we showed that Med12 hypomorphic mutants had neural tube defects (NTDs). Mice with reduced Med12 levels had a complete penetrance of NTDs, and some presented a total lack of closure points.

Neural tube closure in mice occurs at ED 9 and is initiated at three different closure points. It consists of several processes that need to be tightly regulated, including cell division, changes in cell morphology, cell-cell interactions, and tissue patterning (Copp et al., 2003; Copp and Greene, 2010). Therefore, it is not a surprise that mutations in genes involved in transcriptional regulation represent one of the largest classes of mutants among more than 150 genes known to cause NTDs in mice (Harris and Juriloff, 2007). This class includes not only transcription factors (e.g., Gli3) but also genes coding for proteins that act as transcriptional coregulators (e.g., p300). Such coregulators can influence transcription by either modifying chromatin or remodeling it, thus facilitating gene transcription (Roeder, 2005).

We have generated heterozygous female embryos that express Med12 in a mosaic fashion. In these embryos, some cells express wild-type Med12, and other cells are incapable of Med12 expression. This condition allowed the bypass of the early mortality of Med12 hypomorphic embryos and permitted the study of later phenotypes, as well as Med12 involvement in the process of neural tube closure. Because the amount and distribution of Med12-null cells in the mosaic females is caused by the random process of X-chromosome inactivation, these embryos present a graded phenotypic severity of NTDs. These results implicate the X-linked Med12 in closure of the neural tube along the whole body axis. Several processes seem affected, which leads to the hypothesis that more than one transcription factor responsible for neural tube closure uses Med12 for interaction with the polymerase II machinery.

MATERIALS AND METHODS

Mice Husbandry and Breeding

CMV-Cre mice on a C57Bl6 background (Schwenk et al., 1995) and Med12flox on a 129Sv-C57BL6 F1 hybrid background (Rocha et al., in press) have been described. Mice were kept under normal dietary conditions (Complete Feed V1124-3, Sniff, Soest, Germany), with a 12-hour light-dark cycle, and were mated overnight. Hemizygous *Med12^{flox}* male mice were mated with *CMV-Cre* females, and the day of plug finding was designated as ED 0.5. Unless stated otherwise, embryos were dissected in phosphate-buffered saline and fixed in 10% formalin while the yolk sacs were used for DNA genotyping.

Embryo Genotyping

DNA was prepared from yolk sacs by overnight lysis in digestion buffer (200 mM NaCl, 100 mM Tris-HCl (pH 8.3), 5 mM EDTA, 0.2% SDS, and 100 μg/ml proteinase K (Roche, Mannheim, Germany)) at 56 °C and then precipitated using isopropanol (Laird et al., 1991). Genotypes were determined by PCR using the primers P1 (GTTTCCGGCAGTAATCGA GAGTTTC), P2 (TACATTCAAAGCCGTCAGTTCATCC), P3 (AGGCACCGAGTACCTGTTCAAGAAT) and P4 (AT CATTCCTGATCCCCATCTTCCT). Primers for gender genotyping have been described previously (Mroz et al., 1999).

Protein Analysis

Protein extracts were prepared from freshly dissected ED 9.5 mouse embryos using a Nuclear/Cytosol Fractionation Kit (Biovision, Palo Alto, USA) and resolved in pre-cast 4-12% sodium dodecyl sulfate (SDS)-polyacrylamide gels (Invitrogen, Darmstadt, Germany) and subsequently blotted onto PVDF membranes (Millipore, Schwalbach, Germany). The antibodies used were: anti-Med12 (1:1000 dilution; Novus Biologicals, Cambridge, UK-NB100-2357) and anti-Histone H3 (1:10,000 dilution; Abcam, Cambridge, UK-1791).

Preparation for Scanning Electron Microscopy

Embryos were fixed in 0.1 M sodium cacodylate-buffered 2.5% glutaraldehyde (pH 7.4) for 3 days at 4 °C. After rinsing several times in cacodylate buffer, the specimens were postfixed for 4.5 hr in 1% osmium tetroxide at 4 °C and washed again in cacodylate buffer. Dehydration through a graded series of ethanol solutions was followed by critical point drying with liquid carbon dioxide using the CPD 030 (BAL-TEC, Leica, Wetzlar, Germany). Specimens were then mounted on stubs for scanning electron microscopy, sputtered with gold (sputter coater SCD 005, BAL-TEC), and examined with a LEO 1430 scanning electron microscope.

Skeletal Stainings of ED 17.5 Embryos

Embryos were dissected, eviscerated, and kept in water for 2 hr at room temperature. Embryos were then immersed in water at 65 °C for 1 min to facilitate skin removal and fixed over night in 100% ethanol. Cartilage staining was done overnight in Alcian Blue staining solution (150 mg/L Alcian Blue 8GX (Sigma, München, Germany) in 20% acetic acid and 80% ethanol) at room temperature, followed by ethanol fixation overnight and 2 hr of tissue clearing using 2% KOH. Alizarin Red (Sigma) staining (50 mg/L in 2% KOH) was then used for membranous bone staining, and a final clearing overnight step was done in 2% KOH (Mallo and Brandlin, 1997).

Alcian Blue Staining in Paraffin Sections

Formalin-fixed embryos were washed in phosphate-buffered saline, dehydrated in ethanol, and embedded in paraffin after a xylene incubation step. Embedded embryos were cut in 4-μm transverse sections. Paraffin was then removed using xylene, and sections were rehydrated through an ethanol series. Sections were then immersed in Alcian Blue solution (1% Alcian Blue 8GX [Sigma] in 0.1 N HCl, pH 1.0) for 15 min and after a quick wash in water counterstained with a Neutral Red stain solution (1% Neutral Red [Sigma], 0.1% glacial acetic acid) for 1 min. Afterward, ethanol dehydration sections were mounted and photographed.

RESULTS

Generation of Med12 Heterozygous Female Embryos

We have previously generated a mouse line carrying a modified version of the 45 exon spanning X-linked *Med12* gene with exons 1 to 7 flanked by *loxP* sites and therefore called $Med12^{flox}$. Mice hemizygous and homozygous for the $Med12^{flox}$ allele are viable and fertile, and they are indistinguishable from wild-type siblings. *loxP* sites can be recognized by Cre recombinase and if positioned in the same orientation, recombination leads to the deletion of the DNA sequences flanked by them. In the case of the $Med12^{flox}$ line, Cre-mediated excision results in the $Med12^{\Delta 1\text{-}7}$ allele with the first seven exons, including the translational start codon, deleted from the allele (Fig. 1A).

For the studies reported here, hemizygous $Med12^{flox}$ male mice ($Med12^{flox/Y}$) were mated with homozygous *CMV-Cre* females that express Cre-recombinase ubiquitously at high levels in all tissues including the germ line. Our breeding scheme is depicted in Figure 1B and generated embryos with only two genotypes. All males received a wild-type X chromosome from their mother. Although male embryos carried the *CMV-Cre* transgene, they expressed Med12 normally and will be referred to as wild-type control embryos. Female embryos, however, were heterozygous for *Med12*, carrying one $Med12^{flox}$ allele and one wild-type allele. Because of *CMV-Cre* transgene expression, excision occurred between the two *loxP* sites of the $Med12^{flox}$ allele, leading to the $Med12^{\Delta 1\text{-}7}$ allele. These female embryos will be hereafter named $Med12^{\Delta 1\text{-}7/wt}$, which reflects their heterozygosity.

We verified these theoretical predictions by PCR-genotyping. Using the mouse X (*Smcx*) and Y (*Smcy*) chromosome-specific genes, (Fig. 1C, bottom) we identified the sex of the embryos (Mroz et al., 1999) and confirmed that all males carried only a wild-type copy of *Med12* (Fig. 1C, top, lane 2). However, female embryos carried both the wild-type and the excision allele $Med12^{\Delta 1\text{-}7}$ (Fig. 1C, top, lane 1). In some females, the nonexcised $Med12^{flox}$ allele could be detected (Fig. 1D, lane 2) reflecting a known chimerism in the action of Cre, where some cells fail to perform excision. As predicted, heterozygosity of *Med12* caused a reduction of Med12 protein levels. Analysis of protein lysates of whole ED 9.5 embryos revealed that $Med12^{\Delta 1\text{-}7/wt}$ heterozygous females had an approximately 50% reduction of Med12 (Fig. 1E).

Heterozygous $Med12^{\Delta 1\text{-}7/wt}$ Females Die Prenatally

The average litter size obtained from our matings was only 2.5 pups. They all appeared healthy, but at the time of weaning we identified only one female (containing an unexcised $Med12^{flox}$ allele; data not shown) among 15 males (Table 1), which is a clearly compromised sex ratio. These data suggested that heterozygous $Med12^{\Delta 1\text{-}7/wt}$ female embryos had died in utero. DNA analyses at ED 13.5 demonstrated the presence of a slightly abnormal sex ratio (1:1.27, [28 males and 22 females], instead of 1:1). In addition, we detected five embryos undergoing embryonic resorption (one male, four females) and nine full resorptions of unknown sex (Table 1). Already at ED 11.5, heterozygous female $Med12^{\Delta 1\text{-}7/wt}$ embryos showed

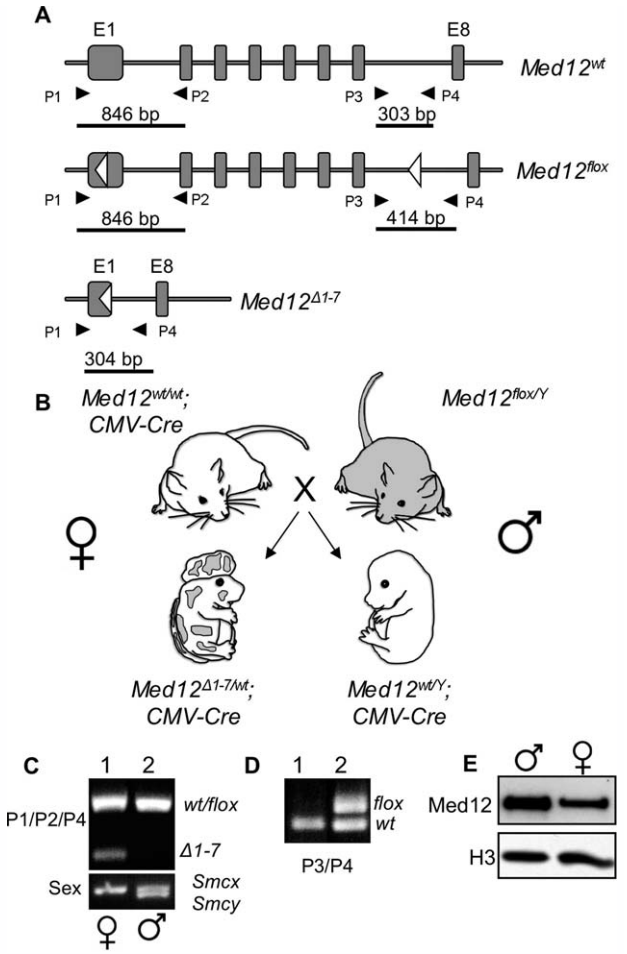

Figure 1. Generation of Med12 heterozygous female embryos. (**A**) Schematic diagram showing from top to bottom the wild-type locus of *Med12*, the conditional ($Med12^{flox}$), and the Med12 excision allele ($Med12^{\Delta 1\text{-}7}$). In the conditional allele, exons 1 (E1) to 7 are flanked by *loxP* sites (*white arrowheads*). Cre recombinase-mediated excision of exons 1 to 7 results in the $Med12^{\Delta 1\text{-}7}$ allele. *P1*, *P2*, *P3*, and *P4* indicate the location of primers used for PCR genotyping on genomic DNA. (**B**) Schematic diagram showing the cross of a hemizygous $Med12^{flox}$ male with a homozygous *CMV-Cre* female, resulting in wild-type males ($Med12^{wt/Y}$;*CMV-cre*) and Med12 heterozygous females ($Med12^{\Delta 1\text{-}7/wt}$;*CMV-Cre*). (**C**) Embryos were genotyped for *Med12* by PCR amplification on genomic DNA. (*Top*) Primers P1, P2, and P4 distinguished between wild-type (*wt*) or *Med12* conditional (*flox*) and null alleles (Δ*1-7*). (*Bottom*) PCR amplification of the X- and Y-linked genes, *Smcx* and *Smcy*, yielded differently sized products and was used to identify the sex of embryos and fetuses. *1*, $Med12^{\Delta 1\text{-}7/wt}$ heterozygous female; 2, $Med12^{wt/Y}$ wild-type control male. (**D**) PCR amplification using primers P3 and P4 identified $Med12^{flox}$ and wild-type alleles. *1*, $Med12^{flox/wt}$ heterozygous female showing complete excision of the $Med12^{flox}$ allele. *2*, $Med12^{flox/wt}$ heterozygous female with incomplete excision of the $Med12^{flox}$ allele. (**E**) $Med12^{flox/wt}$ heterozygous females show an approximately 50% reduction in Med12 levels. Nuclear protein lysates from ED 9.5 embryos were analyzed by Western blotting using an anti-Med12 antibody (*top*) and an anti-histone H3 antibody as loading control (*bottom*)—wild-type male (*left*) and $Med12^{\Delta 1\text{-}7)/wt}$ heterozygous female (*right*). Experiments were performed in duplicates; quantification was performed using ImageJ software.

Table 1
Progeny of Intercrosses of *Med12*$^{wt/wt}$;*CMV-Cre* and *Med12*$^{flox/Y}$

Stage	Males	Females	Total	Resorptions[a]	Resorbing[b] Males	Resorbing[b] Females
ED 13.5	28	22	50	9	1	4
At weaning	15	1	16	–	–	–

[a]At ED 13.5, nine implantation sites were found in *CMV-Cre* uteri, but embryo gender genotyping was not possible because embryos were fully resorbed.
[b]Five embryos were found in the uteri of *CMV-Cre* females in process of embryonic resorption. Gender genotyping was still possible in these cases. ED, embryonic day.

NTDs (Fig. 2B, C) whereas no males had obvious malformations (Fig. 2A).

Heterozygous *Med12*$^{\Delta 1\text{-}7/wt}$ Females Have a Variety of NTDs

Because *Med12* is X linked, heterozygous *Med12*$^{\Delta 1\text{-}7/wt}$ female mice and embryos are expected to exhibit somatic cell mosaicism with respect to *Med12* expression as a result of random X-chromosome inactivation, potentially leading to various degrees of deviation from normal phenotypes. To verify this finding, we analyzed embryos at various stages of development. At ED 9.5, some *Med12*$^{\Delta 1\text{-}7/wt}$ embryos showed arrested development, being unable to complete turning, and were smaller than their littermates (Fig. 3C). Others showed NTDs with variable severity. Mutant embryos that passed ED 12.5 displayed NTDs such as exencephaly, craniorachischisis, split face, and curled tail (Fig. 3D–I). Only one of 27 female embryos at stages later than ED 12.5 did not show NTDs (Table 2). Sixty-three percent showed exencephaly and spina bifida (Fig. 3H), 30% showed craniorachischisis (Fig. 3D–F), and 4% had only exencephaly. Moreover, 30% of the embryos showed a split face (Fig. 3I) and 18 embryos had a curled tail (Fig. 3D, E, G–I). Skeletal preparation of fetuses at ED 17.5 showed abnormalities in the vertebral column and the skull of *Med12*$^{\Delta 1\text{-}7/wt}$ females compared with male littermates. As it is common in mouse mutants with NTDs in the cranial region, *Med12*$^{\Delta 1\text{-}7/wt}$ fetuses missed some of the calvarial bones like the frontal, parietal and temporal bones (Fig. 4A–D). Along the mutant vertebral column, the spinal processes are splayed and the vertebrae lack vertebral arch formation (Fig. 4A, B, E, F). Transverse sections through the thoracic region of an ED 13.5 embryo showed that spina bifida in *Med12*$^{\Delta 1\text{-}7/wt}$ mutant females was caused by a primary failure of neural tube closure. The neural plate appeared to fold at the median hinge point, but the neural tissue was flat (Fig. 4G, H). These results show that Med12 is essential for neural tube closure along the complete body axis and suggest that the variety of defects in *Med12*$^{\Delta 1\text{-}7/wt}$ heterozygous females is caused by the mosaic expression of Med12 in these embryos.

DISCUSSION

In the present study, we have identified the X-linked *Med12* as a novel gene whose mosaic expression leads to severe NTDs. We made use of an Med12 conditional mouse line (*Med12*^{flox}), whose hemizygous and homozygous mice are phenotypically normal and fertile (Rocha et al., in press). Taking advantage of the general Cre-deleter mouse line *CMV-Cre*, we were able to generate *Med12*$^{\Delta 1\text{-}7/wt}$ females with a mosaic expression of *Med12* owing to random X-chromosome inactivation. This strategy allowed the bypass of the early mortality of Med12 hypomorphic embryos (Rocha et al., in press) and facilitated the study of the fate of different somatic cells, as well as Med12 involvement in the process of neural tube closure.

At ED 9.5, the genotypes of the offspring were in Mendelian ratio in both sexes (two litters: five males, five females). However, because female embryos looked abnormal at ED 9.5 and possessed NTDs that had also been observed in *Med12* hypomorphic mutants (Rocha

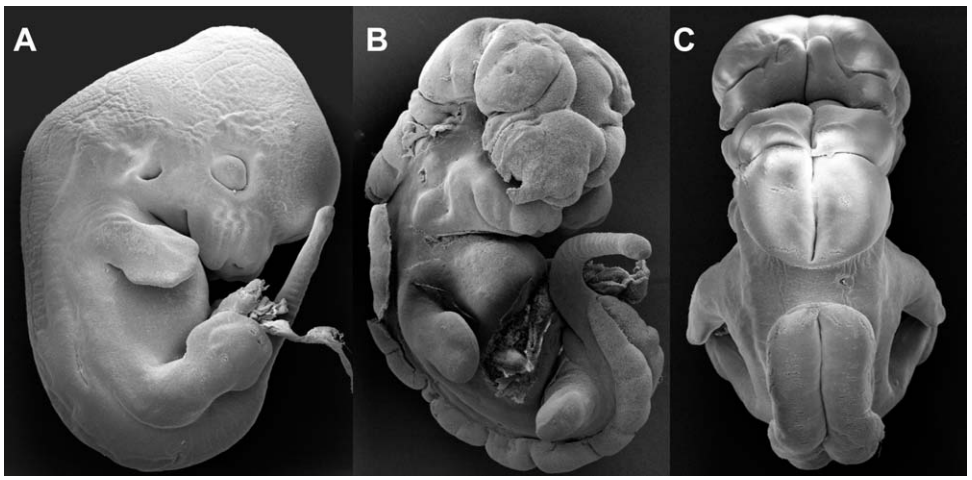

Figure 2. NTDs of Med12 heterozygous females at ED 11.5. Scanning electron micrographs showing a wild-type male (**A**, lateral) and two heterozygous *Med12*$^{\Delta 1\text{-}7/wt}$ mutant females (**B** [lateral] and **C** [dorsal]) at ED 11.5. Mutant embryos show NTDs such as exencephaly, craniorachischisis, and curled tail. The embryo in **B** shows a mild hernia; additional damage during specimen processing cannot be ruled out.

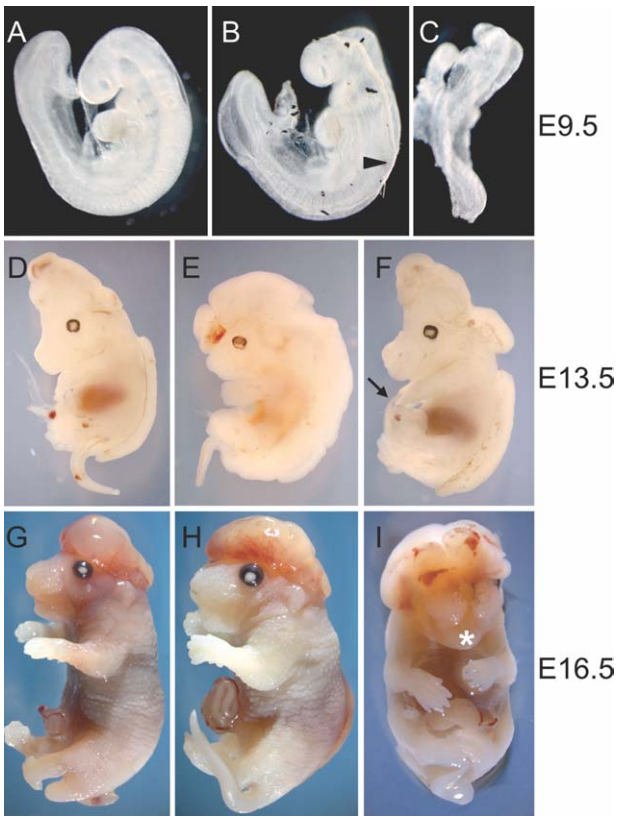

Figure 3. Med12 heterozygous females have a variety of NTDs. *Med12*$^{\Delta 1\text{-}7/wt}$ mutant females show various degrees of NTDs from ED 9.5 onward. Wild-type ED 9.5 (**A**), heterozygous *Med12* mutants at ED 9.5 (**B**, **C**), ED 13.5 (**D**–**F**), ED 16.5 (**G**–**I**). (**A**–**H**) Lateral view. (**I**) Frontal view. (**A**–**C**) ED 9.5 embryos. Some *Med12*$^{\Delta 1\text{-}7/wt}$ females presented a severe impairment in neural tube closure, being able to achieve fusion of the neural folds only in a small portion of the spinal region (*arrowhead* in **B**). Others showed a more severe phenotype with arrested development and being unable to complete embryonic turning (**C**). (**D**–**F**) ED 13.5 embryos. The embryo in **D** achieves neural tube closure in a fashion similar to the embryo shown in **B**. **E** is an example of an embryo with craniorachischisis, and **F** shows an embryo with exencephaly and spina bifida, but having a correctly developed tail (*arrow* in **F**). (**G**–**I**) ED 16.5 embryos. The embryo in **G** shows exencephaly and curled tail, but no NTD in the spinal region. The embryo in **H** shows exencephaly and spina bifida. *Asterisk* in **I** highlights the split-face phenotype in addition to exencephaly.

et al., in press), we presumed that embryonic lethality and smaller litter sizes in mice and embryos older than ED 12.5 was a result of *Med12* mosaicsm (Tables 1 and 2). A change in sex ratio can be detected already at ED 13.5; four of five resorbing embryos have been genotyped as being females, suggesting that reduced Med12 activity is critical in mosaic females. Unfortunately, because of the degree of tissue destruction, we were not able to genotype the gender of nine resorptions identified at ED 13.5, but we speculate that this high number represents mostly mutant females. The compromised sex ratio is most striking at weaning, when only one of 16 mice was female.

From the 27 analyzed *Med12* heterozygous females older than ED 12.5, only one did not show NTDs; all others had NTDs with a variable degree such as exencephaly, craniorachischisis, spina bifida, split face, and curled tail (Table 2). Whereas the majority of NTD mouse mutant strains have either exencephaly or spina bifida aperta (Harris and Juriloff, 2007), 63% of the mutant females in our study showed both defects. In fact, only one of the *Med12* mutant females exhibited exencephaly as its only NTD. The second highest group of NTDs in *Med12* mutants are embryos and fetuses with craniorachischisis, representing approximately 30%. It appears that mosaic *Med12* mutants have a high risk for both cranial and caudal failure. The high number of resorbing and already resorbed embryos at ED 13.5 likely results from major developmental defects other than those from NTDs. This finding reflects the situation that we observed in our *Med12* hypomorphic mutants that die before ED 10.5 (Rocha et al., submitted).

It appears that once X-inactivation has generated mosaicism for the Med12 cellular phenotype, there is a strong divergence in phenotypes between Med12 mosaic females. This divergence might reflect the different Med12 activity in *Med12*$^{\Delta 1\text{-}7/wt}$ cells in the mosaics resulting from random X-inactivation in the cells. Skewed X-inactivation can also be responsible for the variable severity of phenotypes as described in the α-thalassemia X-linked mental retardation (XLMR) syndrome (*Atrx*) mutants, with skewing occurring at specific stages of development and differentiation in different tissues (Muers et al., 2007). We have not yet studied systematically whether or at what stage cell selection takes place; therefore, we cannot rule out that skewed X chromosome inactivation at later stages of development leads to the variation in *Med12* mutant phenotypes.

Thus far, four X-linked NTD mouse models with exencephaly and/or spina bifida have been described (*exma*, *Nap1l2*, *Piga*, and *Zic3*), but none with craniorachischisis (Harris and Juriloff, 2007). The penetrance of all these X-linked NTD mutants is variable. The Bent tail (Bn) mouse that carries a deletion of the *Zic3* gene region shows variable phenotypes, including loss of early embryos from resorptions, to variations in NTDs (Klootwijk et al., 2000), as observed in our Med12 mosaic mutants. These phenotypes can also be partly explained by cell mosaics resulting from random X inactivation.

Table 2
Defects of *Med12*$^{\Delta 1\text{-}7/wt}$ Heterozygous Females at Stages Later than ED 12.5 (n = 27)

No NTD	Exencephaly	Exencephaly and spina bifida	Craniorachischisis	Split face	Tail defects
1 (3.7%)	1 (3.7%)	17 (63%)	8 (29.6%)	8 (29.6%)	18 (66.6%)

Embryos were divided into one of the four classes of NTDs: no NTDs, exencephaly, exencephaly and spina bifida, and craniorachischisis; 29.6% of the analyzed embryos had split face, and 66.6% had defects in the tail.
ED, embryonic day; NTD, neural tube defect.

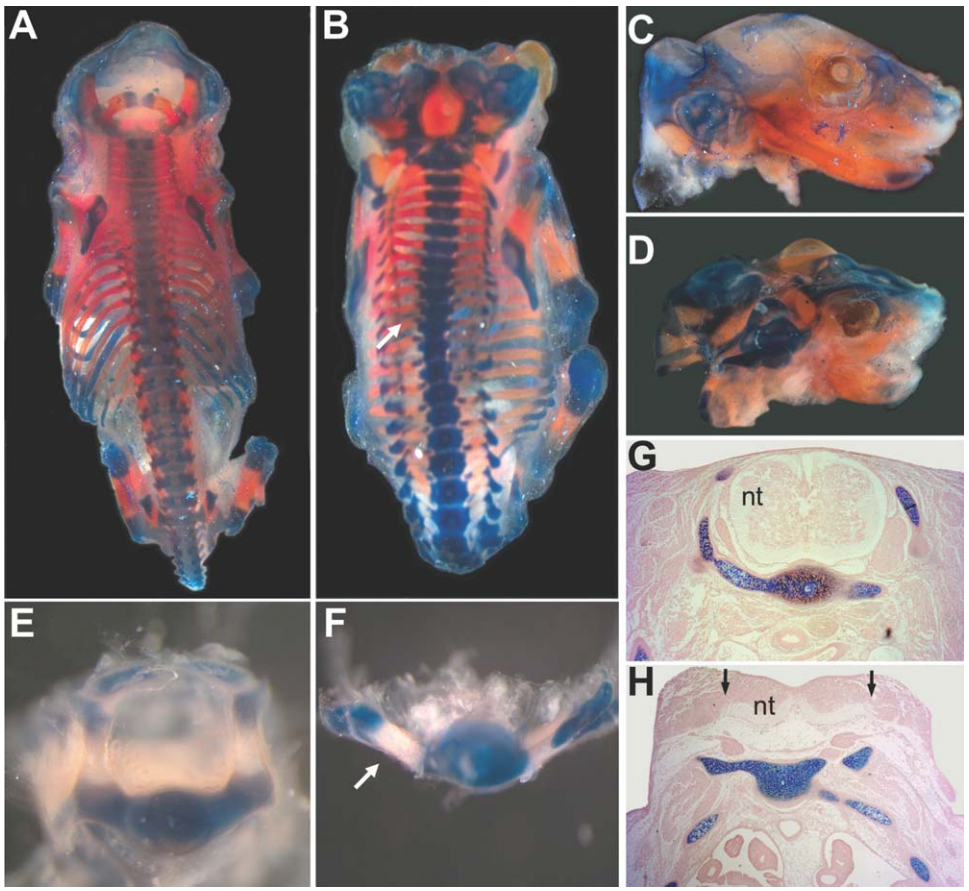

Figure 4. Skeletal defects of Med12 heterozygous females. Neural tube closure defects in *Med12*$^{\Delta 1\text{-}7/wt}$ heterozygous females lead to severe vertebral column and skull defects. (**A**–**F**) Whole-mount skeletal stains of a wild-type male (**A**, **C**, **E**) and *Med12*$^{\Delta 1\text{-}7/wt}$ mutant female (**B**, **D**, **F**) at ED 17.5. Close-ups of the heads (**C**, **D**) and lumbar vertebrae (**E**, **F**) of the fetuses shown in **A** and **B**, respectively. Mutant fetuses show splayed pedicles (*arrows* in **B** and **F**) and cannot form vertebral arches. Alcian Blue staining of cartilage and Neutral Red counterstaining in transverse sections of paraffin-embedded ED 13.5 embryos (**G**, **H**) show the mutant embryo (**H**) with a complete flat neural tube (*nt*; *arrows* in **H**).

Recent studies have identified mutations in the human *MED12* gene that are associated with two XLMR syndromes. Opitz-Kaveggia (Risheg et al., 2007) and Lujan Syndrome (Schwartz et al., 2007) have overlapping but specific manifestations. To date, no NTD has been described for any of these patients, but imperforate anus, seen in Opitz-Kaveggia patients, could be linked to NTDs as cases with OEIS complex develop omphalocele, extrophy of the cloaca, imperforate anus and spinal defects (Carey et al., 1978). In addition, mouse mutants that are associated to the urorectocaudal syndromes (e.g., cloacal exstrophy, anal atresia) also show spina bifida (Gruneberg, 1957).

We have shown that the mouse X-linked *Med12* gene is essential for neural tube closure. The human ortholog also maps to the X chromosome and might be a new candidate for an X-linked NTD gene in humans, justifying a search for mutations in *MED12* that might be associated with sporadic or familial cases of NTDs in humans.

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