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ORIGINAL ARTICLE

Analytical view of the simultaneous occurrence of sacralisation and congenital anomalies

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SUMMARY

The modulated mutations of the HOX11and PAX1/9 families cause defective sacralisation with associated congenital anomalies in organs such as the hindlimb, forelimb, thymus, parathyroid etc in mice. Thus, an attempt has been made to extend the study of the sacralisation of dry human sacra with associated anomalies in human beings analogous to mice by conceiving a hypothesis to be established by future research. The objective of the present study is to facilitate for clinicians the management and treatment of sacralisation-related diseases along with associated anomalies extracted from mice and extended to humans. Sixty-six classified sacralised dry human sacra were examined at the osteology laboratory of CSM Medical University Lucknow, UP, India. These sacra were genetically analysed to establish the relationship between sacralisation and associated anomalies analogous to those found in mice.

The combined effect of mutation of HOX11and PAX1/9 gene expression causes several types of sacralisation with associated anomalies in human beings that are similar to those in mice. Sacralisation in humans may be accompanied by particular associated anomaly alerting clinicians. The study may revolutionize clinical practice and diagnosis.

Key words: Sacralisation – Genes – Coccygeal vertebra – Sacrum

INTRODUCTION

The normal sacrum is formed by the fusion of five sacral vertebrae. The HOX11 group is essential for the genesis of the sacral and caudal vertebrae (Wellik and Capecchi, 2003) and the overexpression of HOX11 genes is expected to produce signs of sacralisation or caudalisation at other levels of the axial skeleton. This overexpression in varying degrees may create variants of the sacrum containing six vertebrae with complete and incomplete fusion of the various components of vertebrae. Sacra with six vertebrae may be due to fusion of the fifth lumbar vertebra with the first sacral vertebra at the cranial end or the first coccygeal vertebra with apex of the sacrum at the caudal end. This process is known as sacralisation of the lumbar vertebra in the former case and sacralisation of the coccygeal vertebra in the latter case.

Apart from this, in triple Hox11 (Hoxa11, Hoxc11 and Hoxd11) mutant mice knee

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joints were severely misshapen - (Koyama et al., 2009) - and partially fused. The epiphysis of the fibula was markedly enlarged and articulated with the female epiphysis. The abnormal development and morphology of fibula was correlated with specific paralogous Hox11 gene mutant combinations by Koyama et al. in their oral presentation.

Further, experiments on mice that were deficient in Pax1/ Pax9 paralogous genes revealed several malformations such as fused vertebrae, mainly involving the lumbar and caudal regions, split vertebrae, as well as ossified fusion between vertebrae and neural arches (Peters et al., 1999), and in mutant mice a reduction in or the absence of transverse process and pedicles were also seen in the caudal vertebrae (Wallin et al., 1994). During mouse embryogenesis, the Pax1 and Pax9 genes are expressed in similar patterns in the 3rd and 4th pharyngeal pouches, the ultimobranchial body and in developing limbs (Neubuser et al., 1995). Hence, Pax1/Pax9 genes are essential for the organogenesis of thymus, the parathyroid, the ultimobranchial body and the limbs of mice. Mice homozygous for Pax1/Pax9 deletion lack a thymus, a parathyroid, and ultimobranchial bodies. Teeth are also absent. The secondary palate is cleft. Supernumerary digits are formed and the flexor of hind limb is missing (Peters et al., 1998).

These two sets of genes are also seen in other vertebrate including human beings, and functions of these genes are expected in human beings, also similar to those in mice. Therefore, dry-bone, classified (unpublished data, Singh R) sacralised sacra have been examined and analysed for their genetic origin and the detailed function of genes have been correlated from mice to humans. The findings prompted the author to conceive of the following hypothesis.

Hypothesis:- Varying degrees of expression of the HOX11 family of genes together with absence of pair of PAX1 and PAX9, or a mutation of homozygosity or heterozygosity, or both of this pair of genes to varying degree, may cause sacralisation, fused and split vertebrae, ossified fusions between vertebrae and neural arches, reduced or absence of transverse process and pedicles, underdevelopment or deficiency of thymus, parathyroid, ultimobranchial bodies, teeth, cleft secondary palate, supernumerary digits along with flexor/malformation of hind/fore limbs and joints in human beings analogous to those in mice.

This may not only provide the new dimension to diagnose sacralisation-related diseases in the clinical practice, but also prompt the clinicians to look for other complications simultaneously occurring in patients in the light of this work. This encouraged the author to carry out this study.

Material and Methods

Sixty-six adult human sacra were examined for different types of sacralisation depending on varying degrees of ossification, fusion and other variations in parts of the vertebrae.

Sacralised sacra were classified (unpublished data of this author) as follows:

Type-I: This is a class of sacra which have complete fusion of the fifth lumbar vertebra with the first sacral vertebra (Fig. 1).

Type-II: These sacra have incomplete fusion of the fifth lumbar vertebra with the first sacral vertebra (Fig. 2).

Type-III: Sacra that display complete fusion of the first coccygeal vertebra with the apex of the sacrum, the coccygeal cornua to the sacral cornua, and the transverse process to the inferior lateral angle of sacrum (Fig. 3).

Type-IV: This class covers those sacra with a fusion of the body of the first coccygeal vertebra with the apex of sacrum and transverse process of the coccygeal vertebra with the inferior lateral angle of sacrum but no fusion of the coccygeal cornua of the first coccygeal vertebra with the sacral cornua (Fig. 4).

Type-V: Sacra with fusion of the body of the first coccygeal vertebra with the apex of the sacrum and the coccygeal cornua to the sacral cornua but no fusion of the transverse process of the first coccygeal vertebra with the inferior lateral angle of sacrum, as shown in (Fig. 5).

Genetic studies on mice carried out by various authors pertaining to sacralisation and simultaneously occurring anomalies have been extended analogously to human beings, as described in the above-formulated hypothesis.

Results

Of 66 sacra, 46 (68.75%) were found to be normal, having five sacral vertebrae or four pairs of sacral foramina and 20 remaining

(31.25%) with anatomical anomalies (sacralised sacra) as in the subject under study. Sacralisation of the lumbar vertebra was found in eleven cases (16.6%). Of these eleven sacra, six (9.09%) were classified as Type-I (Fig. 1) and five sacra (7.57 %) were grouped as Type-II (Fig. 2). Further, the sacralisation of the coccygeal vertebra was detected in nine cases (13.6%), in which Type-III (Fig. 3) was observed in five (7.57%), Type-IV (Fig. 4) in two (3.03%) and Type-V (Fig. 5) in the remaining two (3.03%) sacra.

In Type-I sacralisation, the mean width of body is 5 cm and that of ala is 3 cm. In Type-II sacralisation, width of body is 4.6 cm and that of ala is 4.6 cm. On ventral aspect there is gap between fused part of fifth lumbar vertebra and first sacral vertebra. The length and width of this gap are 1.8 cm and 3 mm respectively. In Type-III, width of body 4.5 cm and that of ala is 3.2 cm. In the same type, length of coccygeal cornua and transverse process on two sides are 1.0 cm, 1.2 cm and 0.9 cm, 1.0 cm respectively. In Type-IV, width of body is 5.2 cm and that of ala is 3.1 cm. In the same type, length of coccygeal cornua on right and left sides are 0.84 cm and 0.8 cm respectively and transverse process are 1.1 cm on both sides while in Type-V, average length of coccygeal cornua on both right and left sides are 1.5 cm and transverse process are as stump. Width of body and ala in Type-V are 4.4 cm and 3 cm respectively.

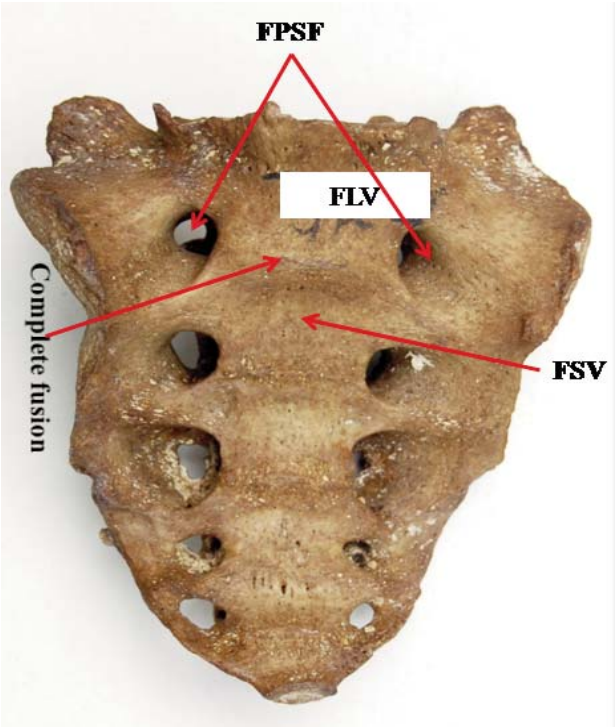

Fig.1. Type-I sacralisation. S-1= First sacral vertebra, S-5= Fifth sacral vertebra.

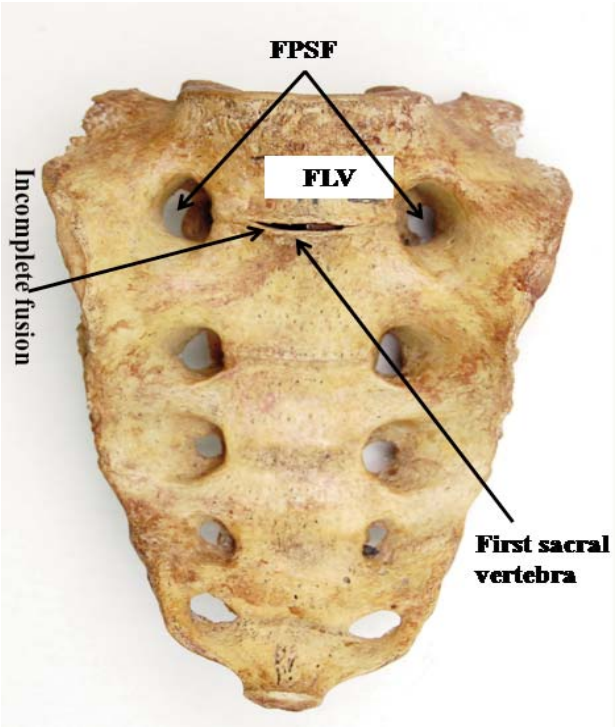

Fig. 2.- Type-II sacralisation.

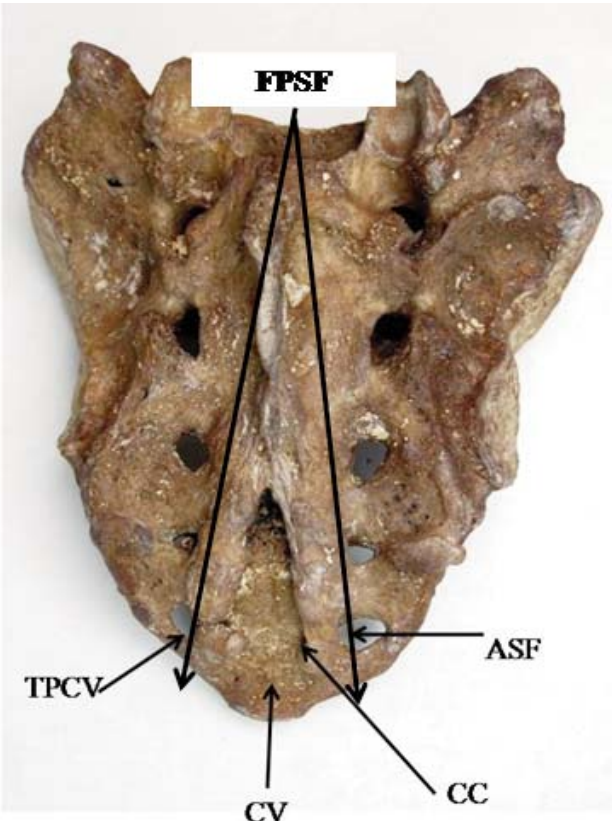

Fig. 3.- Type-III sacralisation. TP= Transverse process, Co-1= First coccygeal vertebra.

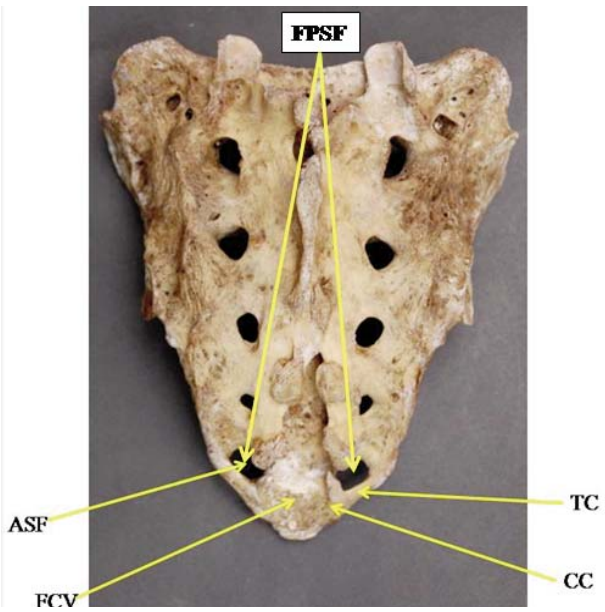

Fig. 4.- Type-IV sacralisation. SC- Sacral cornua, TP= Transverse process, Co-1= First coccygeal vertebra, CC-= Coccygeal cornua.

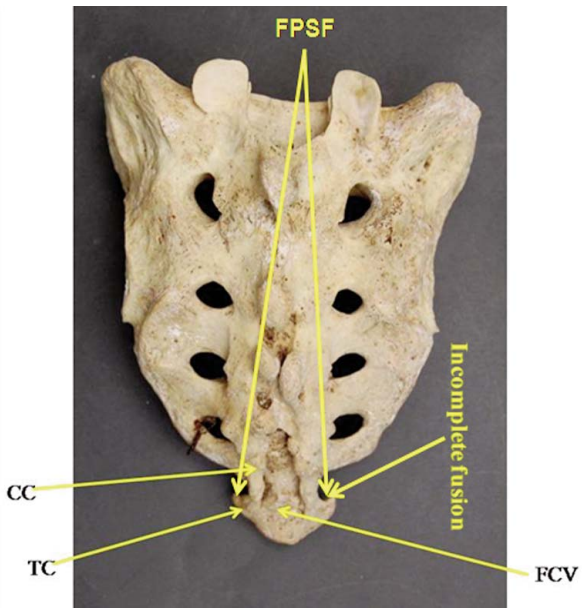

Fig. 5.- Type-IV sacralisation. CC= Coccygeal cornua, TP= Transverse process, Co-1= First coccygeal vertebra.

From above it is clear that in type-III the coccygeal cornua are short and in type-IV they are shorter than in Type-III. Similar observations were recorded for type V, where the coccygeal cornua were fused but the transverse processes remained hypoplastic, appearing merely as a stump. It is interesting to note that sample sacra presented as figures 1-5 in this article with various types of sacralisation, to the best of my knowledge and experience, were pertaining to Indian males.

Discussion

Vertebral column develops from the sclerotome of somites. Each sclerotome consists of loosely arranged cells cranially and densely packed cells caudally. Some densely packed cells migrate cranially opposite the centre of myotome where they form intervertebral disc. The remaining densely packed cells fuse with loosely arranged cells of the immediately caudal sclerotome to form the mesenchymal centrum, which develops into the vertebral body (Moore and Persaud, 2005). Medial derivatives of sclerotome are vertebral body and intervertebral disc, while neural arches derive from the lateral regions of sclerotome.

Hox genes are among the major players in the specification of the morphological identity of the vertebrae (Krumlauf, 1994). In addition, it has recently been described that some of the Hox genes play a global patterning role in vertebral development (Wellik and Capecchi, 2003). When and how Hox genes determine somitic segmental identity is still an unresolved question. It is generally accepted that a specific combination of Hox genes expressed at a particular somitic level determines the axial identity of the resulting structures (Krumlauf, 1994; Kessel and Gruss, 1991). However, an association between Hox somitic expression and mutant phenotypes is not always easy to establish. For instance, axial phenotypes were observed in embryos that recovered appropriate Hox expression domains after retarded activation or transient expression in the presomitic mesoderm (Zakany et al., 1997; Kondo and Duboule, 1999). Hox 11-dependent vertebral sacralisation requires Hoxa11 expression in the presomitic mesoderm, while their caudal differentiation requires that Hoxa11 be expressed in the somites. Therefore, Hox gene patterning activity is different in the somitic and presomitic mesoderm. The Hox11 group is essential for the genesis of the sacral and caudal vertebrae (Wellik and Capecchi, 2003), and thus their over expression is expected to produce signs of sacralization or caudalization at other levels of the axial skeleton. The patterning by the Hox11 group requires a combination of instructions given in the segmental plate and later in the somites. While the formation of sacral structures is apparently instructed by the expression of Hox group 11 genes in the presomitic mesoderm, caudal vertebrae seem

to require the activity of these genes in the somites. Interestingly, both areas are affected when all six Hox group 11 alleles are inactivated (Wellik and Capecchi, 2003).

In mice that are deficient for one functional copy of Pax1, heterozygosity and homozygosity of Pax9 mutations result in vertebral malformations such as fused vertebra, split vertebra, and ossified fusions between the vertebrae and neural arches (Peters et al., 1999).

Moreover, in mutant mice a reduction in or the absence of transverse process and pedicles are also seen in caudal vertebra (Wallin et al., 1994). These anomalies are most commonly observed in the lumbar and caudal region and might be either due to decreased cell proliferation or increased cell death since the Pax1/Pax9 genes are required to maintain high rates of cell proliferation during sclerotome development. Further, an increased apoptosis has been observed in Pax1/ Pax9-deficient mice. These processes are gene dosage-dependent (Peters et al., 1999).

During mouse embryogenesis, Pax1/Pax9 are also expressed in the 3rd and 4th pharyngeal pouches, the ultimobranchial body and the developing limbs (Neubuser et al., 1995). Hence the Pax1/Pax9 genes are essential for organogenesis of the thymus, parathyroid, ultimobranchial body and limbs of mice. Mice homozygous for Pax1/Pax9 deletion lack a thymus, parathyroid, and ultimobranchial bodies. Teeth are also absent. The secondary palate is cleft. Supernumerary digits are formed and the flexor of hind limb is missing (Peters et al., 1998).

Besides producing sacralisation/defective sacralisation or caudalisation/ defective caudalisation in human beings analogous to those seen in mice, a modulated effect of the actions of Hox11 and Pax1/9 mutant genes may generate the above mentioned associated anomalies too simultaneously. Thus formation of normal sacrum requires the normal expression of Hox11 group and Pax1/9 paralogous genes but as our study surrounds the defects of sacralised/caudalised sacra so these defects must have been created by overexpression of Hox11 family of genes together with varying degree of under/over expression of Pax1/ Pax 9 paralogous genes in human sacra analogous to mice from above mentioned inferences of various authors. The defective sacralisation/caudalisation in dry bone sacra have been examined and classified by the author. The classified defective sacralised/caudalised sacra were correlated with the overlapping overexpression of Hox11 and varying degrees of under/over expression of Pax1/9 genes as follows:

Type-I sacra may be caused by the overexpression of Hox11 in the presomite stage and normal expression of pax1/9 paralogous genes. The defect may be associated with abnormalities of the knee joint and fibula.

Type-II sacralisation may be generated by combined mutation of the overexpression of Hox11 along with a mutation of the pax1/9 paralogous gene, resulting in incomplete fusion of the fifth lumbar vertebra and the first sacral vertebra, because the process is dose-dependent. This type of sacralisation may be associated with abnormalities of the knee joint and fibula due to involvement of the Hox11 gene. There may be overdevelopment of the thymus, the thyroid and defects of limbs due to mutation of pax1/9 genes.

Type-III sacralised sacra are produced by the overexpression of Hoxa11 in the somite stage and the overexpression of the Pax1/9 gene when sclerotome cells migrate to surround the neural tube to form the neural arches, as in mice. This type of sacralisation may be associated with abnormalities of the knee joints and fibula, hyperplasia of thymus, thyroid and parathyroid glands, teeth, secondary cleft and defects of limbs.

Type-IV sacralisation may be caused by the overexpression of a mutant Hox 11 gene. This sacrum was observed to have short coccygeal cornua, which might be due to decreased proliferation or increased apoptosis of cells of the sclerotome destined to form the coccygeal cornua. This may be due to underexpression of Pax1/9 genes since these genes maintain the rates of cell proliferation. This type of defect may be associated with deformities of the knee joint and fibula. There may be hypoplasia of the thymus, thyroid, teeth, secondary palate and limbs of varying degrees.

Type-V, where the coccygeal vertebra is fused with the sacrum and the transverse process has remained hypoplastic. This may also be due to the combined effect of overexpression of Hox11 and mutation of pax1/pax9 genes. This type of sacralisation may be associated with deformities of the knee joint and fibula. Due to the involvement of pax1/9, the defect may also be associated with hypoplasia

of thymus, thyroid and limbs of varying degree.

Though the mutation of expression of Hox11 and Pax1/9 genes in varying degree and their combination may produce very complex results, as concluded from the study of expression of mutation of individual gene family in mice, so will be the case in men. Yet initial work may be started by the clinicians on a simplistic model, as the one described here. In continuation, though the establishment of hypotheses will be a herculean job, and a continuous process as permutation and combination of genes may create a cosmos of variations in the spectrum of diseases, yet progress can be made by taking much simpler cases as a starting point.

Thus the genetic study on mice revealed that mutation of Hox11 and paired box Pax1/Pax9 genes may cause sacralisation along with other anomalies like misshapen joints, over-development of fore and hind limb along with absence or under- or overdevelopment of parathyroid and thymus, teeth, cleft secondary palate, supernumerary digits and missing of flexor of hind limbs. Function of these genes in human beings might be analogous to mice. Therefore sacralisation in men is expected to develop together with associated anomalies.

Clinical Significance

Lumbar sacralisation is observed in 16.6% of the population. A fairly large part of the population is affected by this anomaly. Coccygeal sacralisation is observed in 13.6% of cases. Thus, patients suffering from sacralisation and related diseases may complain for associated anomalies and related diseases in varying degree. This information may alert clinicians to diagnose both types of disease simultaneously for the benefit of the patients. Not only can this information be used as a diagnostic tool, but the revolutionary hypothesis may also be examined, which may be of immense use in clinical practice. In contrast, if one or various combinations of diseases, because of these associated anatomical defects, are diagnosed, the clinician is advised to look for sacralisation since the two defects are genetically determined and associated with each other. The degree of anomaly may vary.

Parathyroid hormone secreted by the parathyroid regulates calcium levels in blood. Calcium is essential for the conduction of nerve impulse, the contraction of muscles and the coagulation of blood, apart from being the main constituent in bone formation. Lack of the parathyroid gland resulting in parathormone deficiency may cause tingling sensations in fingers, cramps in muscles, coagulation disorders and bone malformations.

Thymus gland secrets thymosin hormone, which causes development of T-lymphocytes, and these are responsible for cellular immunity. Aplasia or hypoplasia of thymus gland may lead to lymphopenia, decreased immunity ending in early death due to infection. Due to involvement of ultimobranchial body may result in derangement of parafollicular cell functions. Hind limb flexor may be absent leading to difficulty in walking. Thus, when any of the above symptoms is detected, other associated symptoms may be looked for, and the patient may be screened for sacralisation and vice versa.

Conclusion

The hypothesis regarding the genetic view of sacralisation and associated anomalies is the most important conclusion in this study. This hypothesis, if established by clinicians as advised, will not only revolutionise the entire medical world, but also provide a new dimension to the diagnosis and treatment of sacralisation and associated anomalies-related diseases.

Sacralisation is always be accompanied by anomalies (misshapen knee joints, fore and hind limbs, modified parathyroid, thymus, involvement of ultimobranchial body, the absence of teeth, a cleft secondary palate, supernumerary digits), either in part or in fully, depending on mutation of Hox11 and Pax1/Pax9 genes to varying degrees in human embryos.

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