Os odontoideum in identical twins: Comparative gene expression analysis

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Abstract

Background: Os odontoideum is a well identified anomaly of the craniovertebral junction. Since its initial description, there has been a continuous debate regarding the nature of its etiology: Whether congenital or traumatic. We sought to compare the gene expression profiles in patients with congenital os odontoideum, those with traumatic os odontoideum and controls.

Methods: We have evaluated a pair of identical twins both with os odontoideum. We identified two additional patients with and four subjects without os odontoideum. We analyzed the gene expression profiles in these patients using a custom TaqMan microarray and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). The relative gene expression profiles in the two identical twins, the two nontwin patients with os odontoideum and the controls were assessed.

Results: A total of 213 genes with significantly different expression between the twin os odontoideum patients and the subjects without os odontoideum were detected. CACNG6, PHEX, CACNAD3, IL2, FAS, TUFT1, KIT, TGFBR2, and IGF2 were expressed at levels greater than 100-fold more in the twins. There were six genes with significantly different expression profiles in the twins as compared with the nontwin os odontoideum patients: CMK4, ATF1, PLCG1, TAB1, E2F3, and ATF4. There were no statistically significant differences in gene expression in the four patients with os odontoideum and the subjects without. Trends, however, were noted in MMP8, KIT, HIF1A, CREB3, PWHAZ, TGFBR1, NFKB2, FGFR1, IPO8, STAT1, COL1A1, and BMP3.

Conclusions: Os odontoideum has multiple etiologies, both traumatic and congenital and perhaps some represent a combination of the two. This work has identified a number of genes that show increased expression in a pair of twins with congenital os odontoideum and also demonstrates trends in gene expression profiles between a larger group of os odontoideum patients and non-os patients. A number of these genes are related to bone morphogenesis and maintenance.

Key Words: Anomaly, cervical spine, craniovertebral junction, identical twins, os odontoideum
INTRODUCTION

Os odontoideum is a well-defined anatomic anomaly consisting of a smooth ossicle of bone separated from a shortened odontoid process. The incidence and prevalence of os odontoideum is unknown. This entity was first described in 1886 by Giacomini, which has been followed by numerous publications debating its etiology. Two theories—congenital and traumatic—dominate the discussion. A recent review by Arvin et al. provides an excellent discussion of the regional embryology and anatomy as related to the pathogenesis of os odontoideum. The congenital hypothesis is founded on the principle that the os odontoideum is a segmental defect, which represents a failed fusion of the odontoid and C2 vertebral body. Conversely, the traumatic hypothesis considers the os odontoideum an acquired pathology resulting from avascular necrosis following an odontoid fracture. The congenital hypothesis is supported by reports of identical twins both with os odontoideum and two separate communications of families with an autosomal dominant inheritance pattern of os odontoideum. Support of the traumatic hypothesis comes from the observation of a pair of identical twins one with os and one without and from 13 patients reported in the literature who had radiographic documentation of a normal odontoid and subsequently acquired os odontoideum after a trauma. In this manuscript we describe the genetic features of two identical male twins both with os odontoideum, their family members, other patients with traumatic os odontoideum, and control patients without an os odontoideum.

MATERIALS AND METHODS

Patients and study design
IRB approval was obtained from Rush University Medical Center and informed consent was obtained from participants.

Two 20-year-old identical male twins were evaluated in the neurosurgery clinic at Rush University Medical Center. Neither twin had a significant history of trauma and both were active collegiate water-polo players. The clinical presentation was related to neck pain in one of the twins. Imaging revealed an os odontoideum. Flexion-extension imaging revealed an orthotopic os odontoideum with gross instability. Given this finding his brother underwent similar evaluation with comparable results. Preoperative cervical spine films are shown in Figures 1 and 2.

Two additional, unrelated patients with an os odontoideum were subsequently treated. The first was a 49-year-old female who had a prior history of occipitocervical fusion at an outside hospital. She initially presented with hardware failure, pseudoarthrosis, and a wound infection. She did have a remote history of significant head trauma. Imaging revealed an os odontoideum, a C2-3 Klippel–Feil anomaly and a kyphotic deformity [Figure 3]. She ultimately underwent revision of her occipitocervical fusion. The second nontwin os patient was a 53-year-old female with neck pain...
pain and myelopathy. Imaging studies revealed an unstable os odontoideum with cord compression at C1 [Figure 4]. She was treated with a C1-laminectomy and an instrumented atlanto-axial fusion.

Four subjects with prior cervical spine radiography that clearly demonstrated normal craniovertebral junction (CVJ) anatomy—absence of os odontoideum or other congenital anomaly—were identified to serve as controls. One of these subjects was the biological mother of the identical twins with os odontoideum. This control group included a 52-year-old female, an 87-year-old female, a 49-year-old male, and a 59-year-old female.

Comparisons were utilized in a case-control model and included relative gene expression levels between the identical twins with os odontoideum and the controls without os odontoideum; between the identical twins and the unrelated patients with os odontoideum; and finally, between all patients with os odontoideum and all controls without os odontoideum.

Sample collection and RNA preparation
Blood samples were collected via venipuncture from all patients into 5 ml collection tubes containing EDTA and immediately placed on ice. Total RNA from the blood cell pellet of each sample and isolated mRNA for gene expression profiling using the miRNA isolation kit (Ambion, Grand Island, NY).

mRNA qRT-PCR array assays and data analysis
A total of 1 µg of RNA of each sample was used for gene expression profiling. Gene expression profiling was performed on the HT7900 real-time PCR system (Applied Biosystems, Foster City, CA) using custom TaqMan array cards (Applied Biosystems, Supplement 1) on all RNA samples. Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer’s protocol. Amplification using the custom Taqman

Figure 4: Preoperative sagittal T2-weighted cervical spine MRI of non-twin Os-2
array cards was performed using the Taqman Gene Expression Master mix (Applied Biosystem) according to manufacturer’s protocol on a 7900HT Real-time PCR system at the miRNA and Gene Expression Core, Rush University Medical Center. All card amplification reactions were successful. Amplification data were acquired using the SDS2.3/RQ Manager (Applied Biosystem) and DataAssist 3.0 software (Applied Biosystems). The relative expression level for each mRNA was represented as cycle threshold (Ct). mRNAs with Ct < 40 were accepted as “detected”. Eukaryotic 18s rRNA (18S-Hs99999901_s1) was identified as the most stably expressed endogenous control was used as the normalization control. Normalized expression level was calculated as Delta CT (DCt) or ΔCt = Ct (test RNA) -Ct (18s RNA). Differential expression between patients was calculated as DDCt or Δ(ΔCt) = Ave DCT (test) - Ave DCT (control). Fold change or relative quantification (RQ) was calculated as $RQ = 2^{-\Delta\Delta C_{t}}$. The Benjamini–Hochberg method was applied to minimize the false discovery rate. An adjusted $P < 0.05$ was set as the criterion for significance of differential expression. FiRe Macro was used in Microsoft Excel (Microsoft Corporation, Redmond, WA) to generate heatmaps of the normalized RNA expression levels.

**Functional analysis**

To better understand the functions of the differentially expressed mRNAs, we performed a NCBI Gene search using the test RNA as a key word to compile known functions of this mRNA. We report the NCBI Gene Summary of selected genes in Tables 1, 3, and 4.

**RESULTS**

A comparison between the identical twins with os odontoideum (Twins) to the four control subjects (non-Twins) revealed 213 statistically significant differences in gene expression. The top 30 genes and a brief summary of their function are provided in Table 1. Nine genes were expressed in the Twins at levels greater-than-100 times that of the non-Twins group. CACNG6 showed a 565-fold increase in expression in the Twins ($P < 0.05$). This mRNA is derived from a gene located on chromosome 19p13.4 and encodes the gamma-6 subunit of the voltage dependant calcium channel. PHEX expression was 447-fold higher in the Twins ($P < 0.005$). The PHEX mRNA encodes a transmembrane zinc-dependent endopeptidase involved in bone and dentin mineralization and renal phosphate reabsorption. CACNA2D3 showed a 125-fold increase in the Twins ($P < 0.005$). This gene is located on chromosome 3p21.2 and encodes the alpha-2/delta-3 subunit in the voltage-dependent calcium channel. Interleukin 2 (IL2) was increased 116-fold in the Twins ($P < 0.0005$); this gene is located on 4q26-q27 and encodes an inflammatory cytokine that is important for T- and B-cell proliferation. FAS was increased 113-fold in the Twins ($P < 0.05$); this gene is located on chromosome 10q24.1 and encodes a transmembrane protein important in mediating the extrinsic pathway of apoptosis. It is also involved in transducing proliferative signals from normal fibroblasts and T-cells. TUFT1 was increased 109-fold ($P < 0.005$) in the Twins; this gene is located on chromosome 1q21 and is involved in the mineralization and structural organization of enamel. KIT was increased 107-fold in the Twins ($P < 0.0005$); this gene is located on 4q11-q12 and encodes a transmembrane receptor for mast cell growth factor. It is a proto-oncogene and mutations are associated with malignancies (e.g. gastrointestinal stromal tumor and acute myelogenous leukemia). TGFBR2 was increased 102-fold in the twins ($P < 0.05$). This gene is located on chromosome 5p22 and encodes a transmembrane Ser/Thr protein kinase related to cell proliferation. Mutations have been associated with Marfan syndrome, Loeys–Dietz Aortic Aneurysm Syndrome and various malignancies. IGF2 was increased 101-fold in the Twins ($P < 0.05$); this gene is located on 11p15.5 and encodes a member of the insulin-like growth factors that is a major fetal growth factor. Complete results of all 213 genes with significant differences in expression between the Twins and non-Twins groups are shown in Table 2.

Comparing the Twins with the two unrelated patients with os odontoideum (non-Twin os) revealed six genes with statistically significant differences in expression. These genes and a brief summary of their functions are presented in Table 3. CAMK4 was increased 31-fold in the Twins as compared with the non-Twin os patients ($P < 0.05$). This gene is located on chromosome 5q21.3 and encodes a calcium-calmodulin dependent Ser/Thr kinase. ATF1 was increased 23-fold in the Twins ($P < 0.05$); this gene is located on chromosome 1q213 and encodes an activating transcription factor and fusion products have been implicated in angiomatoid fibrous histiocytoma and clear cell sarcoma. Expression of PLCG1 was increased 21-fold in the Twins ($P < 0.05$). This gene encodes gamma-1 phospholipase-C and is located on chromosome 20q12-q13.1. TAB1 was increased 21-fold ($P < 0.05$); this gene is located on chromosome 22q13.1 and encodes a protein involved in the regulation of the MAP kinase pathway. E2F3 was increased 19-fold ($P < 0.05$). This gene is located on chromosome 6p22 and encodes a protein regulator of the cell cycle. ATF4 was found to be elevated 14-fold in the Twins ($P < 0.05$); this gene is located on chromosome 22q13.1 and encodes a transcription factor that modulates DNA transcription in response to cAMP.

Comparing both the Twins and non-Twin os (Os) to the non-os group revealed no statistically significant differences in gene expression. We report the 10 genes with the greatest fold-change and an unadjusted $P < 0.1$ [Table 4].
Table 1: Top 30 gene expression differences between Twins and non-os

| Gene    | R/Q  | BH     | Location | NCBI Gene summary |
|---------|------|--------|----------|-------------------|
| CACNG6  | 565.44 | P<0.05 | 19q13.4  | Voltage-dependent calcium channels are composed of five subunits. The protein encoded by this gene represents one of these subunits, gamma, and is one of two known gamma subunit proteins. This particular gamma subunit is an integral membrane protein that is thought to stabilize the calcium channel in an inactive (closed) state. This gene is a part of a functionally diverse eight-member protein subfamily of the PMP-22/EMP/MCP family and is located in a cluster with two family members that function as transmembrane AMPA receptor regulatory proteins (TARPs). Alternative splicing results in multiple transcript variants. Variants in this gene have been associated with aspirin-intolerant asthma |
| PHEX    | 446.70 | P<0.005 | Xp22.2-p22.1 | The protein encoded by this gene is a transmembrane endopeptidase that belongs to the type II integral membrane zinc-dependent endopeptidase family. The protein is thought to be involved in bone and dentin mineralization and renal phosphate reabsorption. Mutations in this gene cause X-linked hypophosphatemic rickets |
| CACNA2D3 | 124.92 | P<0.005 | 3p21.1   | This gene encodes a member of the alpha-2/delta subunit family, a protein in the voltage-dependent calcium channel complex. Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization and consist of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio. Various versions of each of these subunits exist, either expressed from similar genes or the result of alternative splicing. Research on a highly similar protein in rabbit suggests the protein described in this record is cleaved into alpha-2 and delta subunits. Alternate transcriptional splice variants of this gene have been observed but have not been thoroughly characterized |
| IL2     | 116.28 | P<0.0005 | 4q26-q27 | The protein encoded by this gene is a secreted cytokine that is important for the proliferation of T and B lymphocytes. The receptor of this cytokine is a heterotrimeric protein whose gamma chain is also shared by interleukin 4 (IL4) and IL7. The expression of this gene in mature thymocytes is monoallelic, which represents an unusual regulatory mode for controlling the precise expression of a single gene. The targeted disruption of a similar gene in mice leads to ulcerative colitis-like disease, which suggests an essential role of this gene in the immune response to antigenic stimuli |
| FAS     | 113.27 | P<0.05  | 10q24.1  | The protein encoded by this gene is a member of the TNF-receptor superfamily. This receptor contains a death domain. It has been shown to play a central role in the physiological regulation of programmed cell death, and has been implicated in the pathogenesis of various malignancies and diseases of the immune system. The interaction of this receptor with its ligand allows the formation of a death-inducing signaling complex that includes Fas-associated death domain protein (FADD), caspase 8, and caspase 10. The autoproteolytic processing of the caspases in the complex triggers a downstream caspase cascade, and leads to apoptosis. This receptor has been also shown to activate NF-kB, MAPK3/ERK1, and MAPK8/JNK, and is found to be involved in transducing the proliferating signals in normal diploid fibroblast and T cells. Several alternatively spliced transcript variants have been described, some of which are candidates for nonsense-mediated mRNA decay (NMD). The isoforms lacking the transmembrane domain may negatively regulate the apoptosis mediated by the full length isoform |
| TUFT1   | 108.81 | P<0.005 | 1q21     | Involved in the mineralization and structural organization of enamel |
| KIT     | 107.28 | P<0.0005 | 4q11-q12 | This gene encodes the human homolog of the proto-oncogene c-kit. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. This protein is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. Multiple transcript variants encoding different isoforms have been found for this gene |
| TGFBR2  | 102.40 | P<0.05  | 3p22     | This gene encodes a member of the Ser/Thr protein kinase family and the TGFβ receptor subfamily. The encoded protein is a transmembrane protein that has a protein kinase domain, forms a heterodimeric complex with another receptor protein, and binds TGF-beta. This receptor/ligand complex phosphorylates proteins, which then enter the nucleus and regulate the transcription of a subset of genes related to cell proliferation. Mutations in this gene have been associated with Marfan Syndrome, Loey-Dietz Aortic Aneurysm Syndrome, and the development of various types of tumors. Alternatively spliced transcript variants encoding different isoforms have been characterized |

(Contd...)
| Gene    | R/Q  | BH   | Location | NCBI Gene summary[^2]                                                                 |
|---------|------|------|----------|-------------------------------------------------------------------------------------|
| IGF2    | 101.36 | P<0.05 | 11p15.5 | This gene encodes a member of the insulin family of polypeptide growth factors, which are involved in development and growth. It is an imprinted gene, expressed only from the paternal allele, and epigenetic changes at this locus are associated with Wilms tumor, Beckwith–Wiedemann syndrome, rhabdomyosarcoma, and Silver–Russell syndrome. A read-through INS-IGF2 gene exists, whose 5' region overlaps the INS gene and the 3' region overlaps this gene. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. |
| PLCG2   | 99.79 | P<0.005 | 16q24.1  | The protein encoded by this gene is a transmembrane signaling enzyme that catalyzes the conversion of 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate to 1D-myo-inositol 1,4,5-trisphosphate (IP3) and DAG, using calcium as a cofactor. IP3 and DAG are second messenger molecules important for transmitting signals from growth factor receptors and immune system receptors across the cell membrane. |
| CACNA1H | 97.34 | P<0.05 | 16p13.3  | This gene encodes a T-type member of the alpha-1 subunit family, a protein in the voltage-dependent calcium channel complex. Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization and consist of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio. The alpha-1 subunit has 24 transmembrane segments and forms the pore through which ions pass into the cell. There are multiple isoforms of each of the proteins in the complex, either encoded by different genes or the result of alternative splicing of transcripts. Alternate transcriptional splice variants, encoding different isoforms, have been characterized for the gene described here. Studies suggest certain mutations in this gene lead to childhood absence epilepsy (CAE). |
| TGFBI   | 91.58 | P<0.05 | 5q31     | This gene encodes an RGD-containing protein that binds to type I, II and IV collagens. The RGD motif is found in many extracellular matrix proteins modulating cell adhesion and serves as a ligand recognition sequence for several integrins. This protein plays a role in cell–collagen interactions and may be involved in endochondral bone formation in cartilage. The protein is induced by transforming growth factor-beta and acts to inhibit cell adhesion. Mutations in this gene are associated with multiple types of corneal dystrophy. |
| MMP8    | 90.29 | P<0.0001 | 11q22.3 | Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMPs are secreted as inactive proproteins, which are activated when cleaved by extracellular proteinases. However, the enzyme encoded by this gene is stored in secondary granules within neutrophils and is activated by autolytic cleavage. Its function is degradation of type I, II and III collagens. The gene is part of a cluster of MMP genes, which localize to chromosome 11q22.3. |
| CACNA2D2 | 86.02 | P<0.05 | 3p21.3   | This gene encodes a member of the alpha-2/delta subunit family, a protein in the voltage-dependent calcium channel complex. Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization and consist of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio. Various versions of each of these subunits exist, either expressed from similar genes or the result of alternative splicing. Research on a highly similar protein in rabbit suggests the protein described in this record is cleaved into alpha-2 and delta subunits. Alternate transcriptional splice variants of this gene, encoding different isoforms, have been characterized. |
| SMAD7   | 83.99 | P<0.05 | 18q21.1  | The protein encoded by this gene is a nuclear protein that binds the E3 ubiquitin ligase SMURF2. Upon binding, this complex translocates to the cytoplasm, where it interacts with TGF-beta receptor type-1 (TGFBR1), leading to the degradation of both the encoded protein and TGFBR1. Expression of this gene is induced by TGFBR1. Variations in this gene are a cause of susceptibility to colorectal cancer type 3 (CRC3). Several transcript variants encoding different isoforms have been found for this gene. |
| TBP     | 77.39 | P<0.005 | 6q27     | Initiation of transcription by RNA polymerase II requires the activities of more than 70 polypeptides. The protein that coordinates these activities is transcription factor IID, which binds to the core promoter to position the polymerase properly, serves as the scaffold for assembly of the remainder of the transcription complex, and acts as a channel for regulatory signals. TFII D is composed of the TATA-binding protein and a group of evolutionarily conserved proteins known as TBP-associated factors or TAFs. TAFs may participate in basal transcription, serve as coactivators, function in promoter recognition or modify general transcription factors (GTFs) to...

[^2]: Contd...
Table 1: Continued

| Gene  | R/Q   | BH    | Location | NCBI Gene summary[28] |
|-------|-------|-------|----------|------------------------|
| UBC   | 77.01 | P<0.05| 12q24.3  | The gene represents an ubiquitin gene, ubiquitin C. The encoded protein is a polyubiquitin precursor. Conjugation of ubiquitin monomers or polymers can lead to various effects within a cell, depending on the residues to which ubiquitin is conjugated. Ubiquitination has been associated with protein degradation, DNA repair, cell cycle regulation, kinase modification, endocytosis, and regulation of other cell signaling pathways |
| NFKB1 | 76.42 | P<0.05| 4q24     | This gene encodes a 105 kDa protein, which can undergo cotranslational processing by the 26S proteasome to produce a 50 kDa protein. The 105 kDa protein is a Rel protein-specific transcriptional inhibitor and the 50 kDa protein is a DNA binding subunit of the NF-kappa-B (NFKB) protein complex. NFKB is a transcription regulator that is activated by various intra- and extra-cellular stimuli such as cytokines, oxidant-free radicals, ultraviolet irradiation, and bacterial or viral products. Activated NFKB translocates into the nucleus and stimulates the expression of genes involved in a wide variety of biological functions. Inappropriate activation of NFKB has been associated with a number of inflammatory diseases while persistent inhibition of NFKB leads to inappropriate immune cell development or delayed cell growth. Two transcript variants encoding different isoforms have been found for this gene |
| PLCB2 | 74.99 | P<0.0005| 15q15   | The protein encoded by this gene is a transmembrane signaling enzyme that catalyzes the conversion of 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate to 1D-myo-inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG), using calcium as a cofactor. IP3 and DAG are second messenger molecules important for transmitting signals from growth factor receptors and immune system receptors across the cell membrane |
| GATA2 | 72.84 | P<0.005| 3q21.3   | This gene encodes a member of the GATA family of zinc-finger transcription factors that are named for the consensus nucleotide sequence they bind in the promoter regions of target genes. The encoded protein plays an essential role in regulating transcription of genes involved in the development and proliferation of hematopoietic and endocrine cell lineages. Alternative splicing results in multiple transcript variants |
| SIRT6 | 72.37 | P<0.005| 19p13.3  | This gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class IV of the sirtuin family. Alternative splicing results in multiple transcript variants |
| TBP   | 71.91 | P<0.005| 6q27     | Initiation of transcription by RNA polymerase II requires the activities of more than 70 polypeptides. The protein that coordinates these activities is TFIIID, which binds to the core promoter to position the polymerase properly, serves as the scaffold for assembly of the remainder of the transcription complex, and acts as a channel for regulatory signals. TFIIID is composed of the TBP and a group of evolutionarily conserved proteins known as TBP-associated factors or TAFs. TAFs may participate in basal transcription, serve as coactivators, function in promoter recognition or modify GTFs to facilitate complex assembly and transcription initiation. This gene encodes TBP. A distinctive feature of TBP is a long string of glutamines in the N-terminus. This region of the protein modulates the DNA binding activity of the C terminus, and modulation of DNA binding affects the rate of transcription complex formation and initiation of transcription. The number of CAG repeats encoding the polyglutamine tract is usually 32-39, and expansion of the number of repeats increases the length of the polyglutamine string and is associated with spinocerebellar ataxia 17, a neurodegenerative disorder classified as a polyglutamine disease. Two transcript variants encoding different isoforms have been found for this gene |

(Contd...)
This gene is a member of the RUNX family of transcription factors and encodes a nuclear protein.

The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses. The E2F proteins contain several evolutionarily conserved domains found in most members of the family. These domains include a DNA binding domain, a dimerization domain, which determines interaction with the differentiation regulated transcription factor proteins (DP), a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain, which is embedded within the transactivation domain. This protein and another 2 members, E2F1 and E2F2, have an additional cyclin binding domain. This protein binds specifically to retinoblastoma protein pRb in a cell-cycle dependent manner. Two transcript variants encoding different isoforms have been found for this gene.

Table 1: Continued

| Gene     | R/Q | BH     | Location          | NCBI Gene summary                                                                 |
|----------|-----|--------|-------------------|-----------------------------------------------------------------------------------|
| PRKCZ    | 71.11 | P < 0.05 | 1p36.33-p36.2     | Protein kinase C (PKC) zeta is a member of the PKC family of serine/threonine kinases, which are involved in a variety of cellular processes such as proliferation, differentiation and secretion. Unlike the classical PKC isoenzymes, which are calcium-dependent, PKC zeta exhibits a kinase activity, which is independent of calcium and diacylglycerol but not of phosphatidylserine. Furthermore, it is insensitive to typical PKC inhibitors and cannot be activated by phorbol ester. Unlike the classical PKC isoenzymes, it has only a single zinc finger module. These structural and biochemical properties indicate that the zeta subspecies is related to, but distinct from other isoenzymes of PKC. Alternative splicing results in multiple transcript variants encoding different isoforms. |
| CREB3    | 69.57 | P < 0.005 | 9p13.3            | This gene encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds to the cAMP-response element and regulates cell proliferation. The protein interacts with host cell factor C1, which also associates with the herpes simplex virus (HSV) protein VP16 that induces transcription of HSV immediate-early genes. This protein and VP16 both bind to the same site on host cell factor C1. It is thought that the interaction between this protein and host cell factor C1 plays a role in the establishment of latency during HSV infection. This protein also plays a role in leukocyte migration, tumor suppression, and endoplasmic reticulum stress-associated protein degradation. Additional transcript variants have been identified, but their biological validity has not been determined. |
| CAMK4    | 67.76 | P < 0.0001 | 5q21.3           | The product of this gene belongs to the serine/threonine protein kinase family, and to the Ca (2+) /calmodulin-dependent protein kinase subfamily. This enzyme is a multifunctional serine/threonine protein kinase with limited tissue distribution, which has been implicated in transcriptional regulation in lymphocytes, neurons, and male germ cells. |
| CASP4    | 67.28 | P < 0.0005 | 11q22.2-q22.3    | This gene encodes a protein that is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes composed of a prodomain and a large and small protease subunit. Activation of caspases requires proteolytic processing at conserved internal aspartic residues to generate a heterodimeric enzyme consisting of the large and small subunits. This caspase is able to cleave and activate its own precursor protein, as well as caspase 1 precursor. When overexpressed, this gene induces cell apoptosis. Alternative splicing results in transcript variants encoding distinct isoforms. |
| YWHAZ    | 67.13 | P < 0.005 | 8q23.1            | This gene product belongs to the 14-3-3 family of proteins, which mediate signal transduction by binding to phosphoserine-containing proteins. This highly conserved protein family is found in both plants and mammals, and this protein is 99% identical to the mouse, rat and sheep orthologs. The encoded protein interacts with IRS1 protein, suggesting a role in regulating insulin sensitivity. Several transcript variants that differ in the 5' UTR but that encode the same protein have been identified for this gene. |
| POLR2C   | 66.64 | P < 0.05 | 16q13-q21         | This gene encodes the third largest subunit of RNA polymerase II, the polymerase responsible for synthesizing messenger RNA in eukaryotes. The product of this gene contains a cysteine rich region and exists as a heterodimer with another polymerase subunit, POLR2J. These two subunits form a core subassembly unit of the polymerase. A pseudogene has been identified on chromosome 21. |
| RUNX2    | 66.00 | P < 0.005 | 6p21              | This gene is a member of the RUNX family of transcription factors and encodes a nuclear protein with a Runt DNA-binding domain. This protein is essential for osteoblastic differentiation and skeletal morphogenesis and acts as a scaffold for nucleic acids and regulatory factors involved in skeletal gene expression. The protein can bind DNA both as a monomer or, with more affinity, as a subunit of a heterodimeric complex. Mutations in this gene have been associated with the bone development disorder cleidocranial dysplasia. Transcript variants that encode different protein isoforms result from the use of alternate promoters as well as alternate splicing. |
| E2F3     | 65.11 | P < 0.0001 | 6p22             | The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and includes a DNA binding domain, a dimerization domain, which determines interaction with the differentiation regulated transcription factor proteins (DP), a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain, which is embedded within the transactivation domain. This protein and another 2 members, E2F1 and E2F2, have an additional cyclin binding domain. This protein binds specifically to retinoblastoma protein pRb in a cell-cycle dependent manner. Two transcript variants encoding different isoforms have been found for this gene. |
Table 2: Complete results of os vs. non-os relative gene expression

| Gene                   | R/Q  | BH (56%) |
|------------------------|------|----------|
| CACNG6-Hs00230428_m1   | 0.563| >0.05    |
| PHEx-Hs01011692_m1     | 0.446| <0.005   |
| CACNA2D3-Hs00145030_m1 | 1.249| >0.05    |
| IL2-Hs00174114_m1      | 1.116| <0.0005  |
| FAS-Hs00531110_m1      | 1.113| <0.005   |
| TUF1-Hs00360629_m1     | 1.080| <0.0001  |
| KIT-Hs00174029_m1      | 1.072| <0.0005  |
| TGFBR2-Hs00559661_m1   | 1.024| <0.005   |
| IGF2-Hs00171254_m1     | 1.016| <0.005   |
| PLCG2-Hs00182192_m1    | 0.998| <0.005   |
| CACNA1H-Hs00103523_m1  | 0.974| <0.005   |
| TGFBI-Hs00165908_m1    | 0.917| <0.005   |
| MMP8-Hs00233972_m1     | 0.905| <0.0001  |
| CACNA2D2-Hs00121049_m1 | 0.864| <0.005   |
| SMAD7-Hs00178696_m1    | 0.839| <0.005   |
| TBP-Hs99999910_m1      | 0.773| <0.005   |
| UBC-Hs00624723_m1      | 0.770| <0.005   |
| NFKB1-Hs00765730_m1    | 0.764| <0.005   |
| PLCB2-Hs00190117_m1    | 0.749| <0.005   |
| GATA2-Hs00231119_m1    | 0.723| <0.005   |
| SIRT6-Hs00213036_m1    | 0.724| <0.005   |
| TBP-Hs00427621_m1      | 0.710| <0.005   |
| PRKZC-Hs00177051_m1    | 0.710| <0.005   |
| CREB3-Hs00197255_m1    | 0.697| <0.005   |
| CAMK4-Hs00174318_m1    | 0.674| <0.0001  |
| CASP4-Hs00139147_m1    | 0.671| <0.005   |
| YWHAZ-Hs00237047_m1    | 0.667| <0.005   |
| POLR2C-Hs00160308_m1   | 0.663| <0.005   |
| RUNX2-Hs00231692_m1    | 0.655| <0.005   |
| E2F3-Hs00605457_m1     | 0.652| <0.0001  |
| SMAD5-Hs00195437_m1    | 0.619| <0.005   |
| ELF4-Hs01086125_m1     | 0.619| <0.005   |
| GNAI3-Hs00197803_m1    | 0.612| <0.005   |
| PSMAM3-Hs00541095_m1   | 0.590| <0.005   |
| RELA-Hs00153294_m1     | 0.580| <0.005   |
| CSN1D-Hs001017895_m1   | 0.571| <0.005   |
| GNAI2-Hs00164686_m1    | 0.568| <0.005   |
| TGFBR1-Hs00610319_m1   | 0.561| <0.005   |
| GNG2-Hs00828232_m1     | 0.561| <0.005   |
| POLR2B-Hs00265558_m1   | 0.560| <0.005   |
| PK3CD3-Hs00192399_m1   | 0.551| <0.005   |
| GNAQ-Hs00387073_m1     | 0.532| <0.005   |
| SIRT5-Hs00202043_m1    | 0.536| <0.005   |
| EIF5-Hs00231092_m1     | 0.525| <0.005   |
| GADD45B-Hs00169587_m1  | 0.520| <0.005   |
| CASP7-Hs00169152_m1    | 0.518| <0.005   |
| MRPL19-Hs00608519_m1   | 0.509| <0.0005  |
| TCF4-Hs00162613_m1     | 0.502| <0.005   |

(Contd...)
| Table 2: Continued |
|-------------------|
| **Twin os (n=2) vs non-os (n=4)** | **Gene** | **R/Q** | **BH (56%)** |
| GADD45G-Hs00198672_m1 | 34.26597171 | P<0.05 |
| TAB1-Hs00196143_m1 | 34.16783805 | P<0.0005 |
| PIK3CA-Hs00180679_m1 | 33.87123977 | P<0.005 |
| ATR-Hs00354807_m1 | 33.82496736 | P<0.05 |
| EP300-Hs00230938_m1 | 33.80868932 | P<0.05 |
| ACRV2B-Hs00069603_m1 | 33.4621255 | P<0.05 |
| CASP2-Hs00234982_m1 | 32.85577281 | P<0.05 |
| PTEN-Hs01920652_s1 | 32.81195837 | P<0.05 |
| PRKACB-Hs00176944_m1 | 32.81096995 | P<0.0005 |
| PRKCE-Hs00178455_m1 | 32.62503402 | P<0.0005 |
| CDK4-Hs00179535_m1 | 32.55518355 | P<0.05 |
| DVL2-Hs00182901_m1 | 32.43891581 | P<0.005 |
| CAMK2D-Hs00241833_m1 | 32.35867661 | P<0.05 |
| TAB2-Hs00248373_m1 | 32.35869844 | P<0.05 |
| MAPK1-Hs01046830_m1 | 32.26353183 | P<0.05 |
| PRKDC-Hs00179161_m1 | 32.20590998 | P<0.05 |
| TFRC-Hs99999111_m1 | 32.0255323 | P<0.05 |
| BCL2-Hs99999018_m1 | 31.98634237 | P<0.0005 |
| SMA4D-Hs00929647_m1 | 31.75346606 | P<0.05 |
| EFNB1-Hs00270004_m1 | 31.69950049 | P<0.005 |
| PIAS1-Hs00184008_m1 | 31.59519581 | P<0.05 |
| MAPK24-Hs00387426_m1 | 31.10656912 | P<0.05 |
| ELK1-Hs00428286_g1 | 31.01493418 | P<0.05 |
| PRKCB-Hs00176996_m1 | 30.86544787 | P<0.05 |
| E2F6-Hs00242501_m1 | 30.83945104 | P<0.05 |
| CACNB1-Hs00609501_m1 | 30.59402173 | P<0.05 |
| ATM-Hs01112307_m1 | 30.48818951 | P<0.05 |
| SIRT2-Hs00247263_m1 | 30.43753762 | P<0.05 |
| PSMCS-Hs00267687_m1 | 30.12796121 | P<0.005 |
| HDAC10-Hs00368899_m1 | 29.7331829 | P<0.05 |
| MDM2-Hs99999008_m1 | 29.45323931 | P<0.05 |
| MAPK7-Hs00177373_m1 | 29.38079948 | P<0.05 |
| SMA4D-Hs00232222_m1 | 29.23583914 | P<0.05 |
| CREB1-Hs00231713_m1 | 28.88115729 | P<0.05 |
| PUM1-Hs00264069_m1 | 27.73935787 | P<0.05 |
| HDAC5-Hs00608366_m1 | 27.73987704 | P<0.05 |
| CASP1-Hs00354836_m1 | 26.53819756 | P<0.05 |
| ATF2-Hs00153179_m1 | 26.54531252 | P<0.05 |
| CREB3L4-Hs00307116_m1 | 26.4487662 | P<0.05 |
| CASP10-Hs01017902_m1 | 26.41388429 | P<0.05 |
| TPS5-Hs01034249_m1 | 26.18113004 | P<0.05 |
| PRKAR2A-Hs00177760_m1 | 25.73676717 | P<0.05 |
| EIF2B1-Hs00426752_m1 | 25.4903807 | P<0.05 |
| HRPT1-Hs99999090_m1 | 25.17679034 | P<0.05 |
| HIPK2-Hs00179759_m1 | 25.05025347 | P<0.05 |
| LTBP3-Hs00221445_m1 | 26.83950085 | P<0.05 |
| HDAC2-Hs00231032_m1 | 26.78680935 | P<0.05 |
| PSM2A-Hs00855061_sH | 26.63525188 | P<0.05 |
| BAX-Hs00180269_m1 | 26.15599313 | P<0.05 |

(Contd...)
MMPS was increased 95-fold in Os patients (P = ns); this gene is located on chromosome 11q22.3 and encodes a matrix metalloproteinase that is stored in neutrophil granules and functions in the degradation of collagen types I, II and III. KIT was elevated 58-fold in the Os patients (P = ns). HIF1A was elevated 43-fold in the Os group. This gene is located on chromosome 17q23.2 and encodes the alpha subunit of hypoxia-inducible factor-1, a transcription factor, which functions in cellular homeostasis in response to hypoxia. CREB3 was elevated 41-fold in the Os group; this gene is located on chromosome 9p13.3 and encodes a transcription factor that regulates cell proliferation. CREB3 is involved in establishing latent herpes simplex virus infections and also plays a role in leukocyte migration, tumor suppression, and endoplasmic reticulum stress-associated protein degradation. YWHAZ was elevated 36-fold in the Os patients (P = ns); this gene is located on chromosome 15q26.1 and encodes a protein that modulates Ran GTPase activity. STAT1 was elevated 24-fold; this gene is located on chromosome 12p12.2 and encodes a protein that serves as a transcription activator in response to cytokine and growth factor signaling. In addition to the 10 genes reported above, COL1A1 was found to be elevated 14.8-fold (P = ns) and BMP3 was elevated 6.6-fold (P = ns) in the Os group. The COL1A1 gene is located on chromosome 17q21.23 and encodes the pro-alpha1 chain of type-I collagen, which is found in most connective tissues and is abundant in bone. Mutations in COL1A1 are associated with osteogenesis imperfect types I-IV, Ehlers-Danlos syndrome type VIIA, Ehlers-Danlos syndrome Classical type, Caffey disease and idiopathic osteoporosis. BMP3 (ostegenin) is a gene that is located on chromosome 4q21 and encodes a member of the TGF-B superfamily that induces bone formation.

### Table 2: Continued

| Twin os (n=2) vs non-os (n=4) | Gene | R/Q | BH (56%) |
|------------------------------|------|-----|----------|
| E1F4E-Hs00908915_g1          | 13.9589797 | P < 0.05 |
| BMP6-Hs00233470_m1           | 13.3569678 | P < 0.005 |
| HHH-Hs00745531_s1            | 13.26092052 | P < 0.05 |
| ACVR1-Hs00153836_m1          | 13.00447825 | P < 0.005 |
| CHEK2-Hs00200485_m1          | 12.92288988 | P < 0.005 |
| CACNB3-Hs00167873_m1         | 12.67721395 | P < 0.005 |
| HDAC6-Hs00195869_m1          | 12.52217699 | P < 0.005 |
| COL4A4-Hs00164150_m1         | 11.96933439 | P < 0.005 |
| GNB2-Hs00929275_g1           | 11.79646015 | P < 0.005 |
| ADCY3-Hs00269618_m1          | 11.54764087 | P < 0.005 |
| KAT2B-Hs00187332_m1          | 10.6524953 | P < 0.005 |
| OA21-Hs00427923_m1           | 10.36895925 | P < 0.005 |
| GNAS-Hs00255603_m1           | 9.843423704 | P < 0.005 |
| PDGFA-Hs00234994_m1          | 9.287349766 | P < 0.005 |
| PIK3R2-Hs00178111_m1         | 8.604032114 | P < 0.005 |
| TNNT1-Hs00162848_m1          | 8.05044173 | P < 0.005 |
| E2F1-Hs00153451_m1           | 7.929855963 | P < 0.005 |
| ATF4-Hs00909568_g1           | 7.697303147 | P < 0.005 |
| PRKAR2B-Hs00176966_m1        | 7.608900397 | P < 0.005 |

BH: Benjamini-Hochberg correction. RQ: Relative quantification value.

### DISCUSSION

The identification of a pair of identical twins without a history of trauma both harboring os odontoidea of nearly identical morphology [Figures 1 and 2] lends further support to the concept of a congenital etiology for this disorder at least for some individuals. While the traumatic hypothesis has been widely supported, it is clear from this and other studies that the development an os odontoideum may result from a different process. Some cases may be clearly traumatic in origin and as such represent odontoid fracture nonunions, others have a congenital etiology and there may be a few that represent a combination of the two.

Numerous case reports and case series attest to the rarity of this anomaly and neither the overall incidence nor the prevalence is well documented. The closest (albeit flawed) estimate is provided by Sankar et al., who noted a 3.1% prevalence of os odontoideum following a review of all abnormal radiographs at the Children’s Hospital of Philadelphia. In this series of 16 patients, only 3 individuals were identified as having a clinical history compatible with a traumatic etiology, while 6 harbored associated cervical spine anomalies. If one accepts the existence of both a traumatic and congenital etiology, the question of whether these two separate etiologies result in distinct clinical disorders, especially with regards to ligamentous competency and overall spinal stability must be addressed as this may impact the natural history and accordingly treatment recommendations.
This gene encodes a transcription factor that was originally identified as a widely expressed mammalian protein kinase with limited tissue distribution, which has been implicated in transcriptional regulation in lymphocytes, neurons and male germ cells

**Table 3: Twins vs non-twin os**

| Gene     | R/Q    | BH   | Location | NCBI Gene summary |
|----------|--------|------|----------|-------------------|
| CAMK4    | 31.22  | P<0.05 | 5q21.3 | The product of this gene belongs to the serine/threonine protein kinase family, and to the Ca (2+) calmodulin-dependent protein kinase subfamily. This enzyme is a multifunctional serine/threonine protein kinase that catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol. |
| ATF1     | 23.04  | P<0.05 | 12q.13 | This gene encodes an activating transcription factor, which belongs to the ATF subfamily and activates transcription of downstream target genes, which are related to growth, survival, and other cellular activities. The encoded protein is phosphorylated at serine 63 in its kinase-inducible domain by serine/threonine kinases, and is involved in transcriptional regulation in lymphocytes, neurons and male germ cells. |
| PLCG1    | 21.22  | P<0.05 | 20q12-q13.1 | The protein encoded by this gene catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol. This reaction uses calcium as a cofactor and plays an important role in the intracellular transduction of receptor-mediated tyrosine kinase activators. For example, when activated by SRC, the encoded protein causes the Ras guanine nucleotide exchange factor RasGRP1 to translocate to the Golgi, where it activates Ras. Also, this protein has been shown to be a major substrate for heparin-binding growth factor 1 (acidic fibroblast growth factor)-activated tyrosine kinase. Two transcript variants encoding different isoforms have been found for this gene. |
| TAB1     | 20.65  | P<0.05 | 22q13.1 | The protein encoded by this gene was identified as a regulator of the MAP kinase kinase kinase MAP3K7/TAK1, which is known to mediate various intracellular signaling pathways, such as those induced by TGFβ, interleukin 1, and WNT-1. This protein interacts and thus activates TAK1 kinase. It has been shown that the C-terminal portion of this protein is sufficient for binding and activation of TAK1, while a portion of the N-terminus acts as a dominant-negative inhibitor of TGF beta, suggesting that this protein may function as a mediator between TGF beta receptors and TAK1. This protein can also interact with and activate the mitogen-activated protein kinase 14 (MAPK14/p38alpha), and thus represents an alternative activation pathway, in addition to the MAPK pathways, which contributes to the biological responses of MAPK14 to various stimuli. Alternatively spliced transcript variants encoding distinct isoforms have been reported. |
| E2F3     | 18.63  | P<0.05 | 6p22   | The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses. The E2F proteins contain several evolutionarily conserved domains found in most members of the family. These domains include a DNA binding domain, a dimerization domain which determines interaction with the differentiation regulated transcription factor proteins (DP), a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain, which is embedded within the transactivation domain. This protein and another 2 members, E2F1 and E2F2, have an additional cyclin binding domain. This protein binds specifically to retinoblastoma protein pRB in a cell-cycle dependent manner. Two transcript variants encoding different isoforms have been found for this gene. |
| ATF4     | 13.93  | P<0.05 | 22q13.1 | This gene encodes a transcription factor that was originally identified as a widely expressed mammalian DNA binding protein that could bind a tax-responsive enhancer element in the LTR of HTLV-1. The encoded protein was also isolated and characterized as the cAMP-response element binding protein 2 (CREB-2). The protein encoded by this gene belongs to a family of DNA binding proteins that includes the AP-1 family of transcription factors, cAMP-response element binding proteins and CREB-like proteins. These transcription factors share a leucine zipper region that is involved in protein–protein interactions, located C-terminal to a stretch of basic amino acids that functions as a DNA binding domain. Two alternative transcripts encoding the same protein have been described. Two pseudogenes are located on the X chromosome at q28 in a region containing a large inverted duplication. |

This study identified a litany of significant differences in gene expression (213/380 genes in total) between the Twins with the non-os group. Notably, these genes have biological functionality related to bone formation and maintenance. PHEX, a gene involved in bone mineralization, was elevated 447-fold in the Twins. TUFT1, another gene related to mineralization (of enamel), was increased 109-fold. TFGB1, a gene that mediates cell–collagen interactions and is thought to be involved in endochondral bone formation, was elevated 92-fold. MMP8, a neutrophil MMP that breaks down collagen types I, II, and III, was elevated 90-fold.
The protein encoded by this gene is a member of the fibroblast growth factor receptor family, which binds both acidic and basic fibroblast growth factors and is involved in limb induction. Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. Multiple transcript variants encoding different isoforms have been found for this gene.

This gene encodes the human homolog of the proto-oncogene c-kit. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. This protein is a type 3 transmembrane receptor for MGF (mast cell growth factor), also known as stem cell factor. Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. Multiple transcript variants encoding different isoforms have been found for this gene.

This gene encodes the transcription factor complex nuclear factor-κB (NFκB). The NFκB complex can consist of different subunits that form both homo- or heterodimers which bind specific kappa-B elements in target genes. This gene encodes the p100 subunit that is processed into the active p52 subunit. This protein binds to the cAMP-response element and regulates cell proliferation. The protein interacts with host cell factor C1, which also associates with the herpes simplex virus (HSV) protein VP16 that induces transcription of HSV immediate-early genes. This protein and VP16 both bind to the same site on host cell factor C1. It is thought that the interaction between this protein and host cell factor C1 plays a role in the establishment of latency during HSV infection. This protein also plays a role in leukocyte migration, tumor suppression, and endoplasmic reticulum stress-associated protein degradation.

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This gene encodes one of the subunits of the transcription factor complex nuclear factor-kappa-B (NFκB). The NFκB transcription factor complex is expressed in numerous cell types and functions as a central activator of genes involved in inflammation and immune function. The NFκB complex can consist of different subunits that form both homo- or heterodimers which bind specific kappa-B elements in target genes. This gene encodes the p100 subunit that is processed into the active p52 subunit. This protein can function as both a transcriptional activator and repressor, depending on its dimer partner. Alternate splicing results in both coding and noncoding variants.

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This gene encodes the alpha subunit of transcription factor hypoxia-inducible factor-1 (HIF-1), which is a heterodimer composed of an alpha and a beta subunit. HIF-1 functions as a master regulator of cellular and systemic homeostatic response to hypoxia by activating transcription of many genes, including those involved in energy metabolism, angiogenesis, apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. HIF-1 thus plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. Alternatively spliced transcript variants encoding different isoforms have been identified for this gene.

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Additional transcript variants have been identified, but their biological validity has not been determined.

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BMP3 belongs to the TGFB superfamily. Bone morphogenic protein, also known as osteogenin, induces growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein can be activated by various ligands including interferon-alpha, interferon-gamma, EGF, PDGF, and IL6. This protein mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens. Two alternatively spliced variants encoding distinct isoforms have been described. In bone development and maintenance. Further investigations of these genes may aid in further understanding of the cellular and molecular pathogenesis of os odontoideum.

This study is compromised by the small sample size, which may, in part, be responsible for the lack of statistical significance in the trends in gene expression profile that we found between the os and non-os groups. We opted to include the gene expression profile from the twins' mother in order to control for potential hereditary gene expression patterns unrelated to os odontoideum. The comparison group does not contain age-matched cases, adding a potential confounding variable. There is also the limitation of data selection: There were over 200 genes with significant differences in expression profiles between the twins and the non-os controls. The strategy employed to overcome this potential problem was to focus on those genes with the greatest magnitude of change. Though this approach allows for a concise and informative presentation of the results, it may have excluded some relevant gene profiles.

**CONCLUSION**

Os odontoideum has multiple etiologies, both congenital and traumatic and perhaps some cases represent a combination of the two. Further definition of each type and examination of their relative prevalence will be informative. Moreover, investigation of the relevance of this distinction as to the clinical evaluation, natural history, and treatment is appropriate. We have identified a number of genes that show increased expression in a pair of twins with congenital os odontoideum and also demonstrated trends in gene expression profiles between a larger group of os odontoideum patients and non-os patients. A number of these genes are related to bone morphogenesis and maintenance. Further investigations of the molecular biology of these genes may confer a greater understanding of this anomaly.

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