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Use of Synteny Conversion in Identification of Candidate Genes for Somitogenesis in Humans

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ABSTRACT

Understanding the genetic component of scoliosis in humans has relied on the assumption that spine development is conserved across species. Since evolutionary conserved genes tend to lie within synteny blocks (HSBs) and genes which are not conserved lie within evolutionary breakpoint regions (EBRs), HSB analysis may be used to determine if spine development is conserved across species. We hypothesized that vertebral patterning genes are conserved in amniotes and their location is within stable or "syntenic" regions of chromosomes. Seventy seven patterning genes involved in Fgf, Wnt and Notch signaling pathways were analyzed to determine their location within HSBs or EBRs in the genomes of several amniotic species. The human genome was divided into 1 Mbp intervals and a comparison was made to determine whether these genes were preferentially localized within HSBs or EBRs associated with rapid evolution. The results indicate that genes associated with somite development in humans are preferentially located away from the EBRs: 0.014 genes in EBRs on genome average vs. 0.030 on average in other parts of the genome (p-value = 0.01). The concentration of vertebral patterning genes in HSBs, provides evidence that developmental pathways involved in vertebral morphogenesis are likely conserved across amniotes, consistent with their known function. These data support prior observations indicating that gene networks associated with major developmental processes such as neuronal, central nervous system, bone and blood vessel development, some mediated by Wnt and Notch signaling pathways, were less likely to be localized at EBRs.

Keywords: Synteny; Candidate Genes; Vertebral Patterning Genes; Congenital Scoliosis; Idiopathic Scoliosis

1. Introduction

Congenital and idiopathic scoliosis constitute two major categories of spinal curvature. Idiopathic scoliosis, as defined by the Scoliosis Research Society refers to a lateral curvature of 10˚ or greater on plane radiographs, is not associated with any underlying cause [1]. Congenital scoliosis is a spinal curvature that results from developmental abnormalities in vertebral bodies, which are referred to as congenital vertebral malformations (CVM). These abnormalities may further be divided into disorders of formation (wedge vertebrae, hemivertebrae) or disorders of segmentation (vertebral bar). Both congenital and idiopathic scoliosis are clinically and etiologically heterogeneous. Although the genetic mechanisms responsible for both conditions are not well understood, there is an observed prevalence of 17.3% of congenital scoliosis in families with idiopathic scoliosis, suggesting similar underlying pathogenic mechanisms [2].

Mutations in genes associated with somitogenesis represent ideal candidates for scoliosis. Mesodermally derived somites are paired structures that give rise to the vertebral bodies, ribs, spinal and rib associated muscles and tendons. Somitogenesis occurs by an intricate interplay of patterning genes which are expressed in the presomitic mesoderm. Much of our understanding of the somitogenesis process has been derived from experimental work in mice, chicken and Xenopus species. The Notch, Fgf and Wnt signaling pathways regulated by a molecular oscillator in which the Notch and Fgf genes oscillate in opposite phase to the Wnt genes [3]. Since a large number of human disorders are characterized by aberrant spine development including congenital scoliosis, spondylocostal dysostosis, spondylothoracic dysostosis, Klippel-Feil syndrome (fusion of cervical vertebrae, short neck), hemifacial microsomia (ear tags, microtia, cardiac abnormalities, vertebral abnormalities) and VACTERL syndrome (vertebral anomalies, anal atresia, cardiac abnormalities, tracheo-esophageal fistula, renal abnormalities, limb abnormalities) the clinical relevance for understanding the genetic basis of somitogenesis becomes important. Several prior studies have incorporated a candidate gene approach based on the assumption that human

genes that are syntenic to mouse genes are associated with spine development [3-9].

There are inherent difficulties in the identification of genes contributing to scoliosis in humans. Most cases of congenital vertebral malformations (CVMs) represent sporadic occurrences within a single family, thus making traditional linkage approaches difficult to utilize. The large number of potential candidate genes to choose from, compounded by a clinical heterogeneity of CVM phenoltypes, makes this a difficult area to provide genetic diagnosis and counseling for families.

Multiple factors may contribute to the development of idiopathic scoliosis including muscle imbalance and changes in the connective tissue matrix. Linkage and association studies have identified a number of genetic regions associated with idiopathic scoliosis [10-13]. Polymorphisms in *CHD*7, a chromeodomain helicase which is associated with CHARGE syndrome (Coloboma, Atresia Choanae, Retarded Growth, Ear Anomalies), have been associated with idiopathic scoliosis [11]. *CHD*7 is associated with embryonic axial development in mice, providing additional evidence that congenital and idiopathic scoliosis may have a unifying pathogenetic mechanism [14].

Prior studies have demonstrated that considerable evolutionary activity exists at the evolutionary breakpoint regions (EBRs) which are located between homologous synteny blocks (HSBs) including reuse, increased gene density, segmental duplication accumulation and the emergence of centromeres and telomeres [15]. EBR is defined as an interval between two adjacent HSBs that is demarcated by the end-sequence coordinates of those HSBs on each side. Because the process of spinal column development is similar among amniotes, we hypothesized that genes associated with scoliosis are conserved in amniotes and their location is within the regions of conserved synteny of chromosomes in different mammals.

2. Methods

Ninety seven patterning genes including genes from the Wnt, Fgf, and Notch signaling pathways in addition to other patterning genes operative in mice somitogenesis and associated with scoliosis phenotypes, were initially identified for synteny block analyses [3]. The analysis was performed in order to determine whether these genes involved in somitogenesis and scoliosis are preferentially located in the regions of mammalian chromosomes that are stable in evolution, or whether they are located in the regions that correspond to positions of EBRs in the genomes of several amniotic species (human, chimp, macaque, mouse, rat, dog, pig, cattle, opossum, chicken).

Mouse gene coordinates corresponding to the human chromosome coordinates for the 77 genes from **Table 1** (20 of the original 97 patterning genes did not have corresponding mouse coordinates) were obtained by using Ensemble homology tables [16]. The human genome was divided into 2980, 1 Mbp intervals; the number of the genes from **Table 1** was counted in each of those intervals. A determination was made as to which bins are overlapping with positions of the HSBs or EBRs. Student's t-test analysis with unequal variances, as described previously, was performed to determine whether the somite patterning genes are preferentially located in the EBRs or HSBs [17,18].

Table 1. Mouse genes and coordinates studied with corresponding human syntenic gene region.

Name	Mouse Chromosome	Mouse Start	Mouse End	Mouse Ref Seq Gene	Human Gene	Human Chromosome	Reference
NOTCH							**[Dequeant *et al.* 2006 [3]]**
*Hey*2	chr10	30521775	30532199	*Hey*2	*HEY*2	6	
*Psen*1	chr12	84577950	84624947	*Psen*1	*PSEN*1	14	
*HES*1	chr16	29985104	29987543	*Hes*1	*HES*1	3	
*Dll*1	chr17	15072317	15081835	*Dll*1	*DLL*1	6	
*Jag*1	chr2	136772897	136808085	*Jag*1	*JAG*1	20	
lfng	chr5	140859815	140868017	*lfng*	*LFNG*	7	
*Dll*3	chr7	28002472	28010998	*Dll*3	*DLL*3	19	
*Mesp*1	chr7	79665755	79667301	*Mesp*1	*MESP*1	15	
*Mesp*2	chr7	79684240	79686946	*Mesp*2	*MESP*2	15	
*Nkd*1	chr8	91411459	91483156	*Nkd*1	*NKD*1	16	
*Rtf*1	chr2	1193666509	119426848	*Rtf*1	*RTF*1	15	

Continued

FGF							[**Dequeant *et al.* 2006 [3]]**
*dusp*6	chr10	98692919	98697172	*Dusp*6	*DUSP*6	12	
*FRS*2	chr10	116474239	110912758	*Frs*2	*FRS*2	12	
*Grb*2	chr11	115460135	115524687	*Grb*2	*GRB* 2	17	
*SOS*1	chr17	80305779	80388265	*Sos*1	*SOS*1	2	
*FGF*8	chr19	45790109	45796226	*Fgf*8	*FGF*8	10	
*Bcl2l*11	chr2	127817479	127853988	*Bcl2l*11	*BCL2L*11	2	
*Efna*1	chr3	89357663	89365568	*Efna*1	*EFNA*1	1	
Erk	chr4	135919615	136108064	*Ephb*3	*EPHB*2	1	
*Hspg*2	chr4	136740845	136842706	*Hspg*2	*HSPG*2	1	
Shh	chr5	28787602	28797888	*shh*	*SHH*	7	
Shp-2	chr5	121391150	121452014	*Ptpn*11	*PTPN*11	12	
*Gab*1	chr8	836601080	83776225	*Gab*1	*GAB*1	4	
WNT							**[Dequeant *et al.* 2006 [3]]**
*Wnt*3*a*	chr11	103590240	103634047	*Wnt*3	*WNT*3	17	
*Axin*2	chr11	108736439	108766873	*Axin*2	*AXIN*2	17	
*Fzd*7	chr1	59426970	59431505	*Fzd*7	*FZD*7	2	
*Fzd*5	chr1	64668689	64672026	*Fzd*5	*FZD*5	2	
*Cdc*73	chr1	145368379	145464902	*Cdc*73	*CDC*73	1	
*Phlda*1	chr10	110910396	110912758	*Phlda*1	*PHLDA*1	12	
*Dvl*2	chr11	69816790	69828496	*Dvl*2	*DVL*2	17	
HDAC	chr12	34663701	35022647	*Hdac*9	*HDAC*9	7	
*Dact*1	chr12	72228589	72237499	*Dact*1	*DACT*1	14	
*Tnfrsf*19	chr14	59918146	60000579	*Tnfrsf*19	*TNFRSF*19	13	
*Fzd*3	chr14	64155136	64216673	*Fzd*3	*FZD*3	8	
*Sprouty*2	chr14	104778114	104782418	*Spry*2	*SPRY*2	13	
*Fzd*6	chr15	38836426	38868268	*Fzd*6	*FZD*6	8	
*Has*2	chr15	56495712	56524587	*Has*2	*HAS*2	8	
c-myc	chr15	61815052	61820027	*Myc*	*MYC*	8	
*Ppp*2*r*1*a*	chr17	20650008	20670613	*Ppp*2*r*1*a*	*PPP*2*R*1*A*	19	
*Fzd*8	chr18	921918	9214975	*Fzd*8	*FZD*8	10	
APC	chr18	34345794	34443382	*Apc*	*APC*	5	
*Smad*4	chr18	73764378	73829149	*Smad*4	*SMAD*4	18	
*LRP*5/6	chr19	3584836	3686546	*Lrp*5	*LRP*5	11	
*Dkk*1	chr19	30611873	30615516	*Dkk*1	*DKK*1	10	
FrzB	chr2	80212809	80248464	*Frzb*	*FRZB*	2	
*Tcf*15	chr2	151835002	151840538	*Tcf*15	*TCF*15	20	
*Fzd*1	chr5	4759879	4764041	*Fzd*1	*FZD*1	7	
*CtBP*1	chr5	33564581	33591839	*Ctbp*1	*CTBP*1	4	
*Fzd*9	chr5	135533565	135535857	*Fzd*9	*FZD*9	7	
*Fzd*4	chr7	89279586	89285277	*Fzd*4	*FZD*4	11	
*Smarca*5	chr8	83595689	83635205	*Smarca*5	*SMARCA*5	4	
*Cer*1	chr4	82352982	82356379	*Cer*1	*CER*1	9	

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***HOX* Related**							
*Hoxc*8	chr15	10281573	102821404	*Hoxc*8	*HOXC*8	12	[Yueh *et al.* 1998 [26]]
Hoxc-4	chr15	102862429	102864631	*Hoxc*4	*HOXC*4	12	[Apiou *et al.* 1996 [27]]
*Hoxd*11	chr2	74480397	74487855	*Hoxd*11	*HOXD*11	2	[Kessel and Gruss 1990 [28]]
*Hoxd*10	chr2	74492730	74495942	*Hoxd*10	*HOXD*10	2	[Kessel and Gruss 1990 [28]]
*Hoxd*3	chr2	74512768	74549113	*Hoxd*3	*HOXD*3	2	[Kessel and Gruss 1990 [28]]
*Hoxa*7	chr6	52144074	52151437	*Hoxa*7	*HOXA*7	7	[Kessel and Gruss 1990 [28]]
*Hoxb*7	chr11	96100653	96106426	*Hoxb*7	*HOXB*7	17	[Kessel and Gruss 1990 [28]]
*Leo*1	chr7	28101775	28108283	*Paf*1	*PAF*1	19	[Apiou *et al.*1996 [27]]
PAX							
*Pax*9	chr12	57613651	57629242	*Pax*9	*PAX*9	14	[Peters *et al.*1999 [29]]
*Pax*1	chr2	147053366	147083649	*Pax*1	*PAX*1	20	[Peters *et al.*1999[29]]
*Pax*7	chr4	139009138	139105044	*Pax*7	*PAX*7	1	[Basch *et al.* 2006; Relaix *et al.* 2005 [30,31]]
TGF*β*							
Nodal	chr10	60813329	60820695	Nodal	*NODAL*	10	[Brennan *et al.* 2001 [32]]
*Tgfbr*2	chr9	115932995	116023987	*Tbfbr*2	*TGFBR*2	3	[Baffi *et al.* 2006 [33]]
Other							
*Rab*23	chr1	33664428	33687110	*Rab*23	*RAB*23	6	[Eggenschwiler *et al.* 2001 [34]]
Ihh	chr1	74878522	74884858	*Ihh*	*IHH*	2	[Vortkamp *et al.* 1996 [35]]
*Plxdc*1	chr11	97739328	97802534	*Plxdc*1	*PLXDC*1	17	[Kanda *et al.* 2007 [36]]
*TWIST*1	chr12	34542918	34545078	*Twist*1	*TWIST*1	7	[Bialek *et al.* 2004 [37]]
*Gli*3	chr13	15254867	15517860	*Gli*3	*GLI*3	7	[Aruga *et al.* 1999 [38]]
FlnB	chr14	6608366	6743464	Flnb	*FLNB*	3	[Krakow *et al.* 2004 [39]]
*Slc*35*a*3	chr3	116662802	116704284	Slc35a3	*SLC*35*A*3	1	[Thomsen *et al.* 2006 [40]]
*Mxd*4	chr5	34492821	34504537	Mxd4	*MXD*4	4	[Yokoyama *et al.* 2009 [41]]
PDGFRA	chr5	75434033	75479895	Pdgfra	*PDGFRA*	4	[Soriano 1997 [42]]
*Tbx*6	chr7	126572631	126576696	Tbx6	*TBX*6	16	[Chapman and others 2003 [43]]
Acd	chr8	108584989	108590214	Acd	*ACD*	16	[Keegan *et al.* 2005 [44]]
*Mid*1	chrX	165029304	165334903	MID1	*MID*1	X	[Quaderi *et al.*1997 [45]]

3. Results

Vertebral patterning and scoliosis associated genes in **Table 1** were found to be preferentially located away from the EBRs, with approximately twice as many genes on average occurring in other parts of the genome as compared to the breakpoint intervals (p-value = 0.011). While this does not appear to be a large difference, if all genes in the genome are counted, the EBRs on average contain ~2 times more genes than the rest of the genome. In general, breakpoint intervals are significantly enriched for genes, and the results of this analysis indicate that they are not enriched for vertebral patterning genes. Examination of large blocks (>3 Mb) of homologous synteny (approximately 7 of these occur in amniote genomes, which are >16.3 Mbp in human coordinates) indicated 0.04 genes from **Table 1** localized in these blocks on genome average, while 0.03 genes localized to the rest of the genome [19]. This result is not statistically significant, probably because of the small number of genes in this comparison.

4. Discussion

Synteny block analysis performed on 77 genes associated with Wnt, Fgf and Notch signaling pathways indicated that these genes are located away from the boundaries of EBRs. The location of vertebral patterning genes away from synteny breakpoints highlights their important and conserved evolutionary function in amniotes. This is the first study to analyze conserved synteny for genes asso-

ciated with somitogenesis in amniotic species and provides additional genetic evidence for similarities in spine development in amniotes.

A prior analysis of mouse scoliotic phenotypes using the Mouse Genome Database (MGD), followed by use of the Online Mendelian Inheritance in Man (OMIM), yielded 45 genes with possible scoliosis phenotypes. Twenty eight genes were translated to the human genome coordinates using mouse and human synteny maps [8]. These included *WNT*3*A* and *DLL*3 genes, also members of the cohort of genes in **Table 1**. During this analysis it was not possible to determine whether each vertebral patterning gene was located within EBRs or away from breakpoint intervals. The localization of patterning genes associated with human vertebral development to regions away from synteny breakpoint intervals provides evidence for conservation of the basic vertebral patterning scheme during amniote development.

These data are consistent with prior analyses performed by Larkin *et al.* [19]. Gene networks associated with major developmental processes such as neuronal development, central nervous system, bone and blood vessel development, some of which were mediated by Wnt and Notch signaling pathways, were significantly enriched in HSBs and, therefore, less likely to be localized at EBRs. Gene networks associated with responses to external stimuli such as inflammatory responses and muscle contraction were more likely to be localized to EBRs. Our study focused on 77 genes associated with somitogenesis including 11 NOTCH, 12 FGF, 29 WNT, 8 HOX, 3 PAX, 2 TGFβ pathway and 12 additional genes associated with scoliosis phenotypes and the results demonstrated these patterning genes were significantly overrepresented in the evolutionary conserved regions.

Using a series of bioinformatic approaches including neighbor-joining (NJ) and maximum parsimony (MP), contained within the PHYLIP (PHYLogeny inference package) software package [20], the evolution of Notch family proteins in species from worm to human was analyzed in *C. elegans*, *D. melanogaster*, *C. intestinalis*, and *H. sapiens* using Mapviewer, Geneview, and the BlastP and TBlastN algorithm [21]. The chromosomal distribution of *PBX* (pre-B cell leukemia homeobox), *LHX*3 (Lim homeobox 3), *NRARP* (Notch regulated ankyrin repeat protein), *BRD* (bromodomain) and *CAMSAP*1 (calmodulin regulated spectrin-associated protein 1) was found to follow the distribution of Notch, providing evidence for co-evolution with Notch signaling pathway genes by segmental duplication. The close proximity of these genes may reflect a functional relationship. For instance, in *C. elegans*, PBX appears to be responsible for transcriptional control of Notch signaling [22].

This study has several limitations. While genes in the FGF, WNT and Notch signaling pathways were analyzed, genes in other pathways such as the BMP signaling pathway were not studied. Corresponding mouse coordinates were identified for 77 of the 97 patterning human genes originally identified. Due to the relatively small number of genes studied, it was not possible to determine whether the vertebral patterning genes were preferentially localized to large blocks of homologous synteny.

Besides playing a crucial role in somitogenesis, the Fgf, Wnt and Notch signaling pathways also are involved in the embryogenesis of other organs. Fgf's have important roles in development of the limbs, skin, central nervous system, ear, lungs, liver and have major involvement in the wound healing process [23]. Fibroblast growth factor receptor related disorders in humans include craniosynostosis syndromes such as Apert and Crouzon syndrome, and skeletal dsyplasias, of which achondroplasia is the most common. The Wnt canonical pathway is active in neural tube development [24]. Notch signaling is involved in developmental pathways which affect the vasculature, heart, eye and liver [25]. Both Notch and Wnt pathways are involved in autonomous phenotypes including cellular development and proliferation. It is possible that the conservation of Fgf, Wnt and Notch signaling related genes in HSBs reflects conservation of non-somite signaling functions or conservation of multiple signaling functions. A previous study by Larkin *et al.* focused on association of HSBs with respect to gene networks, while our study was aimed at localization of genes associated with a specific disease process, namely scoliosis with respect to HSBs [19].

In summary we provide further evidence that developmental pathways associated with somitogenesis are conserved across amniotes which is consistent with their known function.

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