Characterization of Nodal/TGF-Lefty Signaling Pathway Gene Variants for Possible Roles in Congenital Heart Diseases



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Abstract

Background: Nodal/TGF-Lefty signaling pathway has important effects at early stages of differentiation of human embryonic stem cells in directing them to differentiate into different embryonic lineages. LEFTY, one of transforming growth factors in the Nodal/TGF-Lefty signaling pathway, plays an important role in the development of heart. The aim of this work was to find evidence on whether *Lefty* variations are associated with congenital heart diseases (CHD).

Methods: We sequenced the *Lefty* gene for 230 Chinese Han CHD patients and evaluated SNPs rs2295418, rs360057 and g.G169A, which are located within the translated regions of the genes. The statistical analyses were conducted using Chi-Square Tests as implemented in SPSS (version 13.0). The Hardy-Weinberg equilibrium test of the population was carried out using online software OEGE, and multiple-sequence alignments of LEFTY proteins were carried out using the Vector NTI software.

Results: Two heterozygous variants in *Lefty1* gene, g.G169A and g.A1035C, and one heterozygous variant in *Lefty2* gene, g.C925A, were identified. Statistical analyses showed that the rs2295418 (g.C925A) variant in *Lefty2* gene was obviously associated with the risk of CHD (P value = 0.016 < 0.05). The genotype frequency of rs360057 (g.A1035C) variant in *Lefty1* gene was associated with the risk of CHD (P value = 0.007 < 0.05), but the allele frequency was not (P value = 0.317 > 0.05).

Conclusions: The SNP rs2295418 in the Lefty2 gene is associated with CHD in Chinese Han populations.

Citation: Deng X, Zhou J, Li F-F, Yan P, Zhao E-Y, et al. (2014) Characterization of Nodal/TGF-Lefty Signaling Pathway Gene Variants for Possible Roles in Congenital Heart Diseases. PLoS ONE 9(8): e104535. doi:10.1371/journal.pone.0104535

Editor: Reiner Albert Veitia, Institut Jacques Monod, France

Received April 16, 2014; Accepted July 9, 2014; Published August 11, 2014

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper. We deposited our data in the NIH Short Read Archive dataset, with the accession number SRP043439.

Funding: This work was supported by a grant from Heilongjiang Innovation Research Foundation for Graduate Studies (YJSCX2012-199HLJ) to XD; a grant from Heilongjiang Province (ZD200917) to KJY; a grant from Pharmacy College of Harbin Medical University to FFL for training undergraduate students; and grants of National Natural Science Foundation of China (NSFC81271786, 81110378, 30970119, 81030029) to SLL. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Congenital heart diseases (CHD) are a group of common and complex illnesses with high morbidity and mortality. Despite the enormous advances in surgical treatments over the past decades, the genetic etiology is still largely unknown [1]. The incidence of moderate and severe forms of CHD is about 6/1,000 of live births. If tiny muscular ventricular septal defects and other trivial lesions are included, the total incidence is about 75/1,000 of live births [2]. For the CHD patients, about one percent would require intervention [3] and about thirteen percent show recognizable chromosomal variants [4]. Most adult CHD patients are predisposed to cardiac complications, such as coronary heart diseases, arrhythmias or heart failure [5]. Although extensive genetic studies and high-resolution technologies have revealed the genetic defects in many familiar and sporadic CHD cases [6,7], the genetic abnormalities in the majority of CHD patients remain largely unknown.

In the embryonic development, heart is the first formed organ, strictly controlled by gene regulatory networks, involving transcription factors, signaling pathways, epigenetic factors, and miRNAs [8,9]. During the last few decades, a variety of CHD-causing gene mutations have been identified, such as those in *CITED2* [10], *CFC1* [11], *GATA4* [12] and *TBX1* [13]. These genes play critical roles in cardiac development; mutations in these genes lead to cardiovascular malformations and contribute to

CHD [14]. Human embryonic stem (HES) cells may differentiate to various cell types and develop to different embryonic lineages, including those of ectoderm (neurons and epidermal cells), endoderm (hepatocytes and pancreatic cells), and mesoderm (muscle cells and cardiomyocytes cells) under the control of certain factors[15]. LEFTY negatively regulates the Nodal/TGF-Lefty signaling pathway [16] and inhibits cellular proliferation and differentiation [17,18]. It has been shown that when the Nodal/ TGF-Lefty pathway goes wrong, serious malignant transformation may occur. In malignant melanoma cells, for example, LEFTY inhibits the malignant properties of melanoma cells [19] [20,21]. During the early differentiation of HES cells, LEFTY is expressed in a subset of cells, playing an important role in mesodermal cell differentiation [22]. The Nodal/TGF-Lefty signaling pathway also has an important effect in early stages of HES cell differentiation, directing specific cells into different embryonic lineages. LEFTY, as one of the important transforming growth factors in the Nodal/ TGF-Lefty signaling pathway, inhibits the signaling of NODAL, which may play an important role in the development of heart.

To elucidate possible associations of *Lefty* genes with CHD, we analyzed the transcribed region and splicing sites of the *Lefty1* and *Lefty2* genes and compared the *Lefty* gene sequences between 230 Chinese Han CHD patients and 263 controls. We found that the rs2295418 (g.C925A) variant in the *Lefty2* gene was closely associated with the risk of CHD.

Materials and Methods

The study population

For this study, a total of 230 CHD patients and 263 control subjects with no reported cardiac phenotypes were recruited from Linyi People's Hospital and the Second Affiliated Hospital of Harbin Medical University, Harbin, China (Table 1). The 263 control subjects were enrolled at the Medical Examination Center of the Second Affiliated Hospital of Harbin Medical University. All these subjects had physical and electrocardiogram examinations and ultrasonic echocardiogram examination, and none of them showed any defects in the heart or other parts of the body. A written informed consent was obtained from each participant, and this work had been approved by the Ethics Committee of Harbin Medical University, consistent with the 1975 Declaration of Helsinki. Detailed records on their medical history, physical examination and chest X-ray examination, electrocardiogram, and ultrasonic echocardiogram were obtained. We deposited our data in the NIH Short Read Archive dataset, with the accession number SRP043439.

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. The human *Lefty1* and *Lefty2* genes are located on 1q42.1 and are encoded by four exons. The four exons and the splicing sites of the two genes were amplified by

Table 1. Clinical characteristics of study population.

Parameter	CHD	Control
Sample (n)	230	263
Male/Female (n)	142/88	171/92
Age (years)	16.18±10.22	8.36±9.98

Data are shown as mean \pm SD.

doi:10.1371/journal.pone.0104535.t001

polymerase chain reaction (PCR) with the primers shown in Table 2. PCR products were sequenced using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and the ABI 3130XL (Applied Biosystems) sequencer for mutational analysis.

Rs2295418, Rs360057 and g.G169A *Lefty* SNP genotyping analysis and Statistical methods

Genotypes of the rs2295418 and rs360057, g.G169A SNPs, within the *Lefty2* or *Lefty1* genes (Figure 1), were determined using two stage methods. We amplified rs2295418, rs360057 and g.G169A (Table 2, Lefty2exon4; Lefty1exon4 and exon1) and sequenced the PCR products to determine the genotype. The statistical analyses were conducted using Chi-Square Tests to calculate odds ratios and P value as implemented in SPSS (version 13.0). We also used online software OEGE to conduct the Hardy-Weinberg equilibrium test of the CHD and control population.

Multiple sequence alignments

From the NCBI website (http://www.ncbi.nlm.nih.gov/), the LEFTY protein sequences of various species were obtained, and using the Vector NTI software, multiple-sequence alignments of LEFTY proteins were carried out.

Results

Patients

Clinical diagnosis of the recruited patients was confirmed in Linyi People's Hospital and The Second Affiliated Hospital of Harbin Medical University. There was no history of other systemic abnormalities in these CHD patients, and their mothers did not have a history of taking medicines or attracting infections during pregnancy. The 230 CHD patients contained 12 pulmonary stenosis, 14 tetralogy of Fallot, 14 patent ductus arteriosus, 22 mitral valve insufficiency, 41 atrial septal defect, 95 ventricular septal defect and 32 other complex congenital heart diseases.

Lefty gene analysis

We sequenced *Lefty* to test the hypothesis that germline common genetic variants in *Lefty* may confer susceptibility to CHD. We first compared the transcribed region and splicing sites of *Lefty* and found two variations in the *Lefty1* gene [g.G169A (p.Arg33Gln) and g.A1035C-rs360057 (p.Asg322Ala)] and one variation in the *Lefty2* gene [g.C925A-rs2295418 (p.Pro286Leu)] in the CHD cases (Figure 1). These variations were located within the translated region of the genes, and the g.A1035C-rs360057 and g.C925A-rs2295418 variations were located within the transforming growth factor- β -like domain of LEFTY protein (Figure 2).

Rs2295418, Rs360057 and g.G169A Lefty SNP genotyping and Statistical analysis

To further test any possible associations between *Lefty* and CHD, we conducted SNP analyses and found that the rs2295418 (g.C925A) variant in *Lefty2* gene was obviously associated with the risk of CHD; the genotype frequency of the rs360057 (g.A1035C) variant in *Lefty1* gene was associated with the risk of CHD, but there was no statistical significance in the allele frequency. The g.G169A variant in *Lefty1* gene was not associated with the risk of CHD in the Chinese Han population (Tables 3, 4). We also conducted the Hardy-Weinberg equilibrium test for the CHD patients and controls and our results were in line with the Hardy-Weinberg equilibrium.

Gene	Exon	Forward primer	Reverse primer	Size	Tm
LEFTY1	1	TGCCTGAGACCCTCCTGC	CCCTCACTCAGCCTCCCA	436	59.9
	2	TTTGCCCCAGAAATAGAACAGG	GACCCAGCGCCGCTTGAG	499	62.1
	3	CAACCGCACCTCCTCAT	CATTCATTCCCACAGCACTC	513	59.2
	4	TAAATCTCCATCCCAGACGC	ACCCTCGAACACTTCAGAAACA	499	57.9
LEFTY2	1	СТСССТСТТССССС	ACAGCCTCCCACAGAGTCCC	511	60.5
	2	GCCTGGCTGCCAGCTCAG	GACCCAGCGCCGCTTGAG	462	62.7
	3	CAACCGCACCTCCTCATC	GCAATCGCTGGCATCCTG	570	61.7
	4	CCTCCCAGGTGCCCACTA	GGGATGGAGTAACTTGCTAA	549	56.5

Table 2. PCR primers used for Lefty sequence analysis.

doi:10.1371/journal.pone.0104535.t002

Conservation of the protein in evolution

Comparison of the LEFTY1 and LEFTY2 protein sequences from species including birds, fishes and mammals by multiplesequence alignment analysis showed that the 286Pro residue in LEFTY2 was highly conserved among the mammals but the 33Arg and 322Asp residues in LEFTY1 were just conserved in Chimpanzee and Humans (Figure 3).



Figure 1. DNA sequence chromatograms of the *Lefty-1* and *Lefty -2* genes. A: g.G169A (p.Arg33Gln); B: g.A1035C-rs360057 (p.Asp322Ala); C: g.C925A-rs2295418 (p.Pro286Leu). doi:10.1371/journal.pone.0104535.g001



Figure 2. Schematic diagrams of rs2295418 and rs360057 locations within the translated region of *Lefty-2* and *Lefty -1* genes and transforming growth factor-β-like domain of the proteins. A: Lefty-1; B: Lefty-2. doi:10.1371/journal.pone.0104535.q002

Discussion

In this study, we analyzed the transcribed regions and splicing sites of the *Lefty* genes in large cohorts of CHD patients and controls and found that two variants, rs2295418 (g.C925A) and rs360057 (g.A1035C), were associated with the risk of CHD in the Chinese Han population, demonstrating the involvement of the *Lefty* genes in the CHD etiology.

The formation of the human heart starts on day 18 or 19 in the mesoderm after fertilization and involves strict temporal, spatial, and sequential gene expressions. Nodal/TGF-Lefty signaling pathway acts upon gastrulation, which develops to progenitor cells of the mesoderm and endoderm [22]. In mice, the formation of mesendoderm was affected by the expression level of Nodal/TGF-Lefty signaling pathway [23], and mutations in the Nodal gene can affect the formation of primitive streak, which is formed by mesendoderm progenitor cells. The vascular systems of the mouse arise from extraembryonic mesoderm that migrate through the primitive streak to the presumptive yolk sac [24]. At later stages of embryonic development, Nodal expression initiates a series of signal transduction and induces its own and *Lefty* gene expression, and the LEFTY negatively regulates the Nodal/TGF-Lefty signaling pathway [16,22].

We analyzed genes of the Nodal/TGF-Lefty signaling pathway, which has been demonstrated to play vital roles in mouse mesoderm differentiation and heart formation; such genes are also temporally expressed in the differentiation of the HES cells [22]. We here demonstrated that the rs2295418 (g.C925A) and the rs360057 (g.A1035C) variants in *Lefty2* and *Lefty1* genes were associated with the risk of CHD in the Chinese Han population. These nucleotides were conserved only between Chimpanzee and man among the species compared. SNP-rs1904589 within the Nodal gene, which we also analyzed in the study, was not found to be significantly associated with the risk of CHD in the population (data not show).

Of great interest, although the translated regions of the two genes are 97.18% similar in nucleotide sequence, *Lefty2* plays a more central role in the mesoderm differentiation [25], which may at least partly explain why the rs2295418 variants in *Lefty2* gene were so closely associated with the risk of CHD. In contrast to *Lefty2*, *Lefty1* seems to be less involved: although the genotype frequency of the rs360057 variant in *Lefty1* gene was apparently associated with the risk of CHD, its allele frequency was not. Further work will be needed on the Nodal/TGF-Lefty signaling pathway for their involvement in the pathogenesis of CHD at the molecular level.

Acknowledgments

The authors thank the patients and family members for their cooperation and participation in this study.

Patient consent. Obtained.

Ethics approval. Ethics Committee of Harbin Medical University.

Table 3. The genotype	e and allele frequency c	of SNP rs229	15418, rs360057 and g.G1	69A in 230 Chinese Ha	n CHD patients and	d 263 non-CHD controls.	·
SNP	Group		Genotype frequency (%)			Allele frequency (%)	
rs2295418	Genotype		G/G	G/A	A/A	ß	Α
	CHD	230	173(75.2)	45(19.6)	12(5.2)	391 (85.0)	69(15.0)
	Controls	263	223(84.8)	35(13.3)	5(1.9)	481 (91.4)	45(8.6)
rs360057	Genotype		Т/Т	T/G	G/G	Т	U
	CHD	230	148(64.3)	62(27.0)	20(8.7)	358(77.8)	102(22.2)
	Controls	263	167(63.5)	89(33.8)	7(2.7)	423(80.4)	103(19.6)
g.G169A	Genotype		G/G	G/A	A/A	U	A
	CHD	230	179(77.8)	47(20.4)	4(1.7)	405(88.0)	55(12.0)
	Controls	263	203(77.2)	56(21.3)	4(1.5)	462(87.8)	64(12.2)
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Genotyped SNP	Associated gene		Pearson Chi-squ	are			Pearson's R			
			Value	Min count ^a	df	Asymp. Sig. (2- sided)	Value	Asymp. Std. error ^t	° Approx. T ^c	Approx. Sig
rs2295418	LEFTY2	Genotype	8.274	7.93	2	0.016	-0.129	0.044	-2.893	0.004 ^d
		Allele	9.968	53.18	-	0.002	-0.101	0.032	-3.170	0.002 ^d
rs360057	LEFTY1	Genotype	10.069	12.60	2	0.007	-0.044	0.045	-0.966	0.334 ^d
		Allele	1.001	95.64	-	0.317	-0.032	0.032	- 1.000	0.318 ^d
g.G169A	LEFTY1	Genotype	0.086	3.73	2	0.958	0.005	0.045	0.100	0.920 ^d
		Allele	0.010	55.52	-	0.919	0.003	0.032	0.101	0.919 ^d
a: The minimum expe b: Not assuming the n c: Using the asymptot.	cted count; null hypothesis; ic standard error assum	ing the null hypothesis;								

d: Based on normal approximation. doi:10.1371/journal.pone.0104535.t004

Δ		p.Arg33GIn
Bos taurus #2	1	MOPLULCWALUVLPLAGPGAALTEER <mark>I</mark> LDSLLOOLHLSEVPIVDKATVEGLVIPAHVRAQVVALLORGH
Capra hircus #2	1	MRPLWLCWALWVLPLAGPGAALTEER <mark>I</mark> LDSPLOOAOGNCD <mark>P</mark> KAP
Pantholops hodgsonii #2	1	MWPLWLCWALWVLPLAGPGAALTEEW <mark>I</mark> LDSLLCQLHLSEVPIVDKAA <mark>V</mark> EGLVIPAHVRAQYVALLQRGH
Canis lupus familiaris #1 Canis lupus familiaris #2		MTRANE CHALLED TO DE LA CEDITI COLLODI HI SONDEL DE COME AL MTRANE A CEDITI DE LA C
Danio rerio #1	1	-MTSVRAACLLCAALFAMARGFTHEDNKDALLKKLGLNEIPOTHKRDLENLVIPTNVKNKYISMLKLHH
Danio rerio #2	1	MALFIQLFILTTAISLTQGFQHEDIKQALLOKLGLTEPPRIOKRDLENLVVPAHIKSKYLSMLKLHH
Gallus gallus #2	1	-MEVSFTRMLYVLCLVMMACAFTQEGFKEVMLKQLGLSEVPKLHKRDLVDLVIPEHVKNKYISMLKRHR
Mesocricetus auratus #1	1	<mark>Mulcualualsladpgaaltgeqilg</mark> sll <mark>q</mark> lqlseppvldkadveglvipshvraqvvsllqhsh
Mus musculus #1		MESLALCARLAVESLVGFGRREIGEQVERSEIGEQUESSERUPUEBRUVEGERTPSHVREQIVALLOHSH
Rattus norvegicus #1	1	MRFLULCWALWALFQVSPGEALTGEQVLGSLLQQLRLDRPPVLDKADVEGNVIPSHVRAQVVALLQHSH
Microtus ochrogaster #2	1	MQSLWLCWVLGVLPLAGHGVA <mark>VTEEQV</mark> LVRLLQQLKLSQAPILD <mark>R</mark> VD <mark>VEG</mark> MAIPAHVRAQVVTLLQRSH
Mus musculus #2	1	MKSLULCUALUVLPLAGPGAAMTEEQVLSSLLQQLQLSQAPTLDSADVEEMAIPTHVRSQYVALLQCSH
Hattus norvegicus #2	- 1	MODINI CHALINAL DI ASDCANI TEEQVISSIIKOLOLVEVETI DEADWERI MIDTHVSSQVVALLQGSH
Pan troglodytes #1	1	MOPLULCUTLUVLPLASPGAALTGEOLLGSLLRDLOLREVPTLDRADMEELVIPTHVRAOVVALLORSH
Homo sapiens #2	1	NUPLULCUALUVLPLAGPGAALTEEQLLGSLLR <mark>OLQLSEVPVLDRADMEKLVIPAHVRAQVVVLLRRSH</mark>
Pan troglodytes #2	1	NUPLULCUALUVLPLAGPGAALTEEQ <mark>LLG</mark> SLL <mark>R</mark> QLQLSEV <mark>PV</mark> LD <mark>R</mark> AD <mark>MEKLVIPAHVRAQVVVLLR</mark> RSH
Macaca fascicularis #1	1	MRPLULCUALUVLPLAGPGTALTGEQLLGSLLQDLQLSEAPVLDRADMEELVIPAHVRAQVVTLLQRSH
Macaca mulatta #2	4	MRPLWLCWALWULPLAGPGIRLIGEQLLGSLLQQLQLSERPVLDRADMEFLUIPAHVRAQIVILLDQRSH
	1	M PLWLCWALWULPLAGPGAALT FOILGSLUODLOLSE PULDWADWE LUIDAHWDAOWWALLOPSH
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P		► p.Asp322Ala
D Bos taurus #2	286	TRANSPORTECTOR TRANSPORTER AND A CONTRACT
Capra hircus #2	103	PFGFLATECVGTCOOPPESLTFKWPFLGFROCTASETTELPHIVSTCEGGGCLOPOVVSLPNMRVOTCSC
Pantholops hodgsonii #2	169	PPGFLAYECVGTCQQPPESLTFKWPFLGPRQCTASETTPLPLIVHIQAGGRLQPQVVSLPNMRVQTCSC
Canis lupus familiaris #1	219	PPGFLAYECVGTCQQPPRPLPFEWPLLGPRQCVPSETTSLPMIITVKEGGRPRPPVVSLPNMRVQKCSC
Canis lupus familiaris #2	286	PPGFLAYECVGTCQQPPRPLPFEWPLLGPRQCWPSETTSLPMIVTVKEGGRPRPRVVSLPNMRVQKCSC
Danio rerio #1 Danio rerio #2	287	PSGYQAFRCKGGCRQPKRNYGYGERKCAVVESAPLPMMYLWKKGDYTEIEVAEFPNMIVEKCGC
Gallus gallus #2	284	PAGYOAYSCRGGCLOLPG-PLOLWGGR-ERACAVAESSPLPINYLVRRGNHTEIEATEFPNNITEKCSC
Mesocricetus auratus #1	189	PPGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASENTSLPVIVSNKEGGRTRPQVVSLPNMRVQTCSC
Microtus ochrogaster #1	287	PPGFLIYECVGSCLQLPESLTIKWPFLGPRQCVASENTSLPMIVSMKEGDRTRTQVVSLPMMRVQKCSC
Mus musculus #1	287	PPGFLTYECVGSCLQLPESLTSRWPFLGPRQCVASEMTSLPMIVSVKEGGRTRPQVVSLPNMRVQTCSC
Microtus ochronaster #2	287	PPGFLTYECVGSCLOPPESLTTRWPFLGPROCWASEMIBLPLIVSIKEDGRTRPOVVSLPNMRVORCSC
Mus musculus #2	287	PPGFLTYECVGSCLQLPESLTIGWPFLGPRQCWASENTSLPMIVSWKEGGRTRPQVVSLPMMRVQTCSC
Rattus norvegicus #2	285	PPGFLIYECVGSCRQLPESLTIGWPFLGPRQCWASENTSLPMIVSIKEDGKTRPQVVSLPMMRVQTCSC
Homo sapiens #1	285	PPGFLAYECVGTCROPPEALAFKWPFLGPROCIASETDSLPMIVSIKEGGRTRPOVVSLPMMRVQKCSC
Homo sapiens #2	285	PPGFLAVECVGTCCOPPERLAF KUPFLGPROCTASETUBLPHIVSTKEGGRTRPOVVSLPNMRVOKCSC
Pan troglodytes #2	285	PPGFLAYECVGTCQQPPEALAFNWPFLGPRQCTASETASLPMIVSIKEGGRTRPQVVSLPMMRVQKCSC
Macaca fascicularis #1	OF AL	
	251	PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC
Macaca mulatta #1	251	PPGFLAYECVGTCQOPPEALAFKWPFLGPRQCIASETASLPMIVSIKEGGRTRPQVVSLPMMRVQKCSC PPGFLAYECVGTCQOPPEALAFKWPFLGPRQCIASETASLPMIVSIKEGGRTRPQVVSLPMMRVQKCSC
Macaca mulatta #1 Macaca mulatta #2	251 287 285	PPGFLAYECVGTQQPPEALAFKUPFLGPRQCIASETASLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTQQPPEALAFKUPFLGPRQCIASETASLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTQQPPEALAVKUPFLGPRQCIASETASLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC
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Macaca mulatta #1 Macaca mulatta #2 Consensus	251 287 285 299	PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTC QPPESL FKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC L p.Pro286Leu
Macaca mulatta #1 Macaca mulatta #2 Consensus	251 287 285 299	PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAVKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTC QPPESL FKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC ↓ p.Pro286Leu
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Canra bircus #2	251 287 285 299 254 71	PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC W PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2	251 287 285 299 254 71 137	PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC W p.Pro286Leu PKAPVTEGTRCCRQEMYIDLQGMKWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASEA PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1	251 287 285 299 254 71 137 187	PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASET PLFPGFLAYECVGTCQPPEALAVKWPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLQGMKWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2	251 287 285 299 254 71 137 187 254	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAVKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC w p.Pro286Leu PKAPVTEGTRCCRQEMYIDLCGMKWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCVPSET
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #1 Danio rerio #1	251 287 285 299 254 71 137 187 254 258 251	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNNRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNNRVQKCSC PPGFLAYECVGTCQQPPEALAVKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNNRVQKCSC PPGFLAYECVGTCQPPEALFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNNRVQKCSC PPGFLAYECVGTCQPPEALFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNNRVQKCSC PPGFLAYECVGTCQPPEALFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNNRVQKCSC PKAPVTEGTRCCRQENYIDLQGNKWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLRGNRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQENYIDLRGNRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGNRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGNRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGNRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGNRWAENWVLEPFGFLAYECVGTCQQPPRLFFEUPLLGPRQCVPSET NNKDREMCCRQVYIDLRGNKWAENWVLEPFGFLAYECVGTCQQPPRLPFEUPLLGPRQCVPSET
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #1 Danio rerio #2 Gallus gallus #2	251 287 285 299 254 71 137 187 254 258 261 253	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPRLPFEUPLLGPRQCVPSET NNKDREMCCRQVYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPRLPFEUPLLGPRQCVPSET NNKDREMCCRQVYIDLRGMRWAENUVLEPFGFLAYECVGTCQQPPRLPFEUPLLGPRQCVPSET DCV-046KTCCRQVFINFRATUPFTSWTQYWTEFAGYQAFRCKGGCRQPKRNVGVGCCRKCAVVES SSPNSKCCREEMFINFRETSWTQYWTEFAGYQAFRCKGGCRQPKRPLOLUGGRERKCAVVES
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #1 Danio rerio #1 Canis rerio #1 Banio rerio #1 Mesocricetus auratus #1	251 287 285 299 254 71 137 187 254 258 261 253 163	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAVKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLQGMKWAENWVLEFFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET PUPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKWPFLGPRQCIASET NNKDREMCCRQVFINFRALTWTQYWIIFFGSVQAFRCKGGCRQPKRNVGVGERKCAVVES SSPNSSKCREEHFINFRELTWTQYWIIFFGSVQAFRCKGGCRQFKR-FNGVGGERKCAVVES SSPNSSTCCRQKHVINFRELSWTQYWIIFFASVQAFRCKGGCRQFKR-FNGVGGERKCAVVES S
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #1 Danio rerio #1 Gallus gallus #2 Mesocricetus auratus #1 Microtus ochrogaster #1	251 287 285 299 254 71 137 187 254 258 261 253 163 255	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLQGMKWAENUVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENUVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENUVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEVYIDLRGMRWAENUVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENUVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCVPSET NNKDREMCCRQVFINFRALTUTCYWIIEFSGVQAFRCKGCCRQPRR-NVGVGERKAVVES SSPNSKCCREEHFINFRLTUTCYWIIEFSGVQAFRCKGGCRQFRR-FNGVGGQRTCAVMES
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #1 Danio rerio #1 Danio rerio #1 Musocricetus auratus #1 Microtus ochrogaster #1 Mus musculus #1	251 287 285 299 254 71 137 254 258 261 253 255 255 255	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLQGMKWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEVYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PVVVTQAARCCRQEVYIDLRGMRWAENWVLEPFGFLAYECVGTCQQPPESLTFKUPFLGPRQCVPSET NNKDREMCCRQVFINFRALTUTCYWIIEFSGVQAFRCKGGCRQPKR-NVGVGERKAVVES SSPNSSKCREEHFINFRELTWTQYWIEFAGVQAYSCRGGCLQLPESLTIKUPFLGPRQCVASEM DGV-QAGKSTCCRQEMYIDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKUPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #1 Danio rerio #2 Gallus gallus #2 Mesocricetus auratus #1 Microtus ochrogaster #1 Rattus norvegicus #1	251 287 285 299 254 71 137 187 254 258 261 253 255 255 255 255 255	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLQGMKWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCVPSET NNKDREMCCRQVFINFRALTWTCYWIIFFSGVQAFRCAGGCRQPKR-NVGVGERKAVVES SSPNSSKCREEHFINFRELTWTCYWIIFFSGVQAFRCAGGCRQFKR-FNGVGGERKAVVES SSPNSSKCREEHFINFRELTWTCYWIIFFSGVQAFRCAGGCRQFKR-FNGVGGERKAVVES SRGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLVYECVGSCLQLPESLTIKWPFLGPRQCVASEM
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #1 Danio rerio #2 Gallus gallus #2 Mesocricetus auratus #1 Microtus ochrogaster #1 Rattus norvegicus #1 Microtus ochrogaster #1 Mus musculus #1	251 287 285 299 254 71 137 187 254 258 261 255 255 255 255 255 255	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETIBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLQGMKWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCVPSET NNKDREMCCRQVFINFRALTWTCYWIIFFGVQAFRCAGGCRQPKR-NVGVGERKAVVES SSPNSSKCREEHFINFRELTWTCYWIIFFGSFLAYECVGTCQCPESLTIKWPFLGPRQCVASEM PGAPVTEGTRCCRQEMYIDLQGMKWAENWILEFFGFLYECVGSCLQLESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLYECVGSCLQLPESLTIKWPFLGPRQCVASEM PEAPVTEGTRCCRQEMYLDLQGMKWAENWILEFFGFLYECVGSCLQPESLTIKWPFLGPRQCVASEM
Macaca mulatta #1 Macaca mulatta #2 Consensus C Bos taurus #2 Capra hircus #2 Capra hircus #2 Pantholops hodgsonii #2 Canis lupus familiaris #1 Canis lupus familiaris #1 Canis lupus familiaris #2 Danio rerio #2 Gallus gallus #2 Mesocricetus auratus #1 Microtus ochrogaster #1 Rattus norvegicus #1 Microtus ochrogaster #1 Mus musculus #1 Rattus norvegicus #2 Rattus norvegicus #2	251 287 285 299 254 71 137 187 254 254 255 255 255 255 255 255 255 255	PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETABLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PPGFLAYECVGTCQPPEALAFKUPFLGPRQCIASETBLPMIVSIKEGGRTRPQVVSLPNMRVQKCSC PKAPVTEGTRCCRQEMYIDLQGMKWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEMYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PKAPVTEGTRCCRQEWYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PEVPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPESLTFKUPFLGPRQCIASET PUPVTQAARCCRQEVYIDLRGMRWAENWVLEFFGFLAYECVGTCQQPPRLPFEWPLLGPRQCWPSET NNKDREMCCRQVFINFRALTWTCYWIIFFGVQAFRCAGGCRQPKR-NVGVGERKAVVES SSPNSSKCREEHFINFRELTWTCYWIIFFGSVQAFRCAGGCRQPKR-FNGVGGERKAVVES SSPNSSKCREEHFINFRELTWTCYWIFFASVQAFRCAGGCRQFKR-FNGVGGERKAVVES
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Figure 3. Multiple-sequence alignment of Lefty-1(#1) and -2(#2) from birds, fishes and mammals (including *Homo sapiens, Pan troglodytes, Macaca mulatta* etc.). A: p.Arg33Gln; B: p.Asp322Ala; C: p.Pro286Leu. doi:10.1371/journal.pone.0104535.q003

Author Contributions

Conceived and designed the experiments: FFL SLL. Performed the experiments: JZ XD PY EYZ. Analyzed the data: FFL KJY SLL.

References

- Verheugt CL, Uiterwaal CS, van der Velde ET, Meijboom FJ, Pieper PG, et al. (2010) Mortality in adult congenital heart disease. Eur Heart J 31: 1220–1229.
- Hoffman JI, Kaplan S (2002) The incidence of congenital heart disease. J Am Coll Cardiol 39: 1890–1900.
 Hoffman JI, Kaplan S, Liberthson RR (2004) Prevalence of congenital heart
- Hoffman JI, Kaplan S, Liberthson RR (2004) Prevalence of congenital heart disease. Am Heart J 147: 425–439.
- 4. Pierpont ME, Basson CT, Benson DW, Jr., Gelb BD, Giglia TM, et al. (2007) Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation 115: 3015–3038.
- van der Bom T, Zomer AC, Zwinderman AH, Meijboom FJ, Bouma BJ, et al. (2011) The changing epidemiology of congenital heart disease. Nat Rev Cardiol 8: 50–60.
- Bruneau BG (2008) The developmental genetics of congenital heart disease. Nature 451: 943–948.
- Richards AA, Garg V (2010) Genetics of congenital heart disease. Curr Cardiol Rev 6: 91–97.
- Buckingham M, Meilhac S, Zaffran S (2005) Building the mammalian heart from two sources of myocardial cells. Nat Rev Genet 6: 826–835.
- van Weerd JH, Koshiba-Takeuchi K, Kwon C, Takeuchi JK (2011) Epigenetic factors and cardiac development. Cardiovasc Res 91: 203–211.
- Sperling S, Grimm CH, Dunkel I, Mebus S, Sperling HP, et al. (2005) Identification and functional analysis of CITED2 mutations in patients with congenital heart defects. Hum Mutat 26: 575–582.
- 11. Wang B, Wang J, Liu S, Han X, Xie X, et al. (2009) CFC1 mutations in Chinese children with congenital heart disease. Int J Cardiol.
- Butler TL, Esposito G, Blue GM, Cole AD, Costa MW, et al. (2010) GATA4 Mutations in 357 Unrelated Patients with Congenital Heart Malformation. Genet Test Mol Biomarkers.
- Wang H, Chen D, Ma L, Meng H, Liu Y, et al. (2012) Genetic analysis of the TBX1 gene promoter in ventricular septal defects. Mol Cell Biochem 370: 53– 58.

Contributed reagents/materials/analysis tools: JZ LH. Contributed to the writing of the manuscript: FFL SLL. Funding: KJY SLL.

- Gong W, Gottlieb S, Collins J, Blescia A, Dietz H, et al. (2001) Mutation analysis of TBX1 in non-deleted patients with features of DGS/VCFS or isolated cardiovascular defects. J Med Genet 38: E45.
- Schuldiner M, Benvenisty N (2003) Factors controlling human embryonic stem cell differentiation. Methods Enzymol 365: 446–461.
- Tabibzadeh S, Hemmati-Brivanlou A (2006) Lefty at the crossroads of "stemness" and differentiative events. Stem Cells 24: 1998–2006.
- Ikushima H, Miyazono K (2010) TGFbeta signalling: a complex web in cancer progression. Nat Rev Cancer 10: 415–424.
- Heldin CH, Landstrom M, Moustakas A (2009) Mechanism of TGF-beta signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition. Curr Opin Cell Biol 21: 166–176.
- Postovit LM, Margaryan NV, Seftor EA, Kirschmann DA, Lipavsky A, et al. (2008) Human embryonic stem cell microenvironment suppresses the tumorigenic phenotype of aggressive cancer cells. Proc Natl Acad Sci U S A 105: 4329–4334.
- Costa FF, Seftor EA, Bischof JM, Kirschmann DA, Strizzi L, et al. (2009) Epigenetically reprogramming metastatic tumor cells with an embryonic microenvironment. Epigenomics 1: 387–398.
- Malchenko S, Galat V, Seftor EA, Vanin EF, Costa FF, et al. (2010) Cancer hallmarks in induced pluripotent cells: new insights. J Cell Physiol 225: 390–393.
- Dvash T, Sharon N, Yanuka O, Benvenisty N (2007) Molecular analysis of LEFTY-expressing cells in early human embryoid bodies. Stem Cells 25: 465– 472.
- Schier AF (2003) Nodal signaling in vertebrate development. Annu Rev Cell Dev Biol 19: 589–621.
- Barroso-delJesus A, Lucena-Aguilar G, Sanchez L, Ligero G, Gutierrez-Aranda I, et al. (2011) The Nodal inhibitor Lefty is negatively modulated by the microRNA miR-302 in human embryonic stem cells. FASEB J 25: 1497–1508.
- Meno C, Gritsman K, Ohishi S, Ohfuji Y, Heckscher E, et al. (1999) Mouse Lefty2 and zebrafish antivin are feedback inhibitors of nodal signaling during vertebrate gastrulation. Mol Cell 4: 287–298.