The molecular mechanisms directing vertebrate heart asymmetry have been subject to many reviews (*1-5, 7, 6, 9, 13-15, 18, 20, 21*). Many developmental biologists emphasize conserved elements of the nodal cascade (*3, 5, 7-9, 11, 12, 16, 18, 19, 22*) while others emphasize the differences (*2, 4, 10, 14, 17, 20, 21*).

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Table S1. Inheritance of direction of asymmetry in plants and animals exhibiting different types of asymmetry variation.

Organism and trait	direction of asymmetry inherited?	Source
a) antisymmetry		
Plants		
Direction of phyllotaxy in palm tree trunks	no	(*12, 51*)
Direction of phyllotaxy in tobacco stems	no	(*1*)
Direction of phyllotaxy in Egyptian cotton stems	no	(*36*)
Direction of phyllotaxy in wild teasel stems	no	(*13*)
Direction of twining in morning-glory stems	no	(*26*)
Seedling handedness in two-rowed barley	no	(*8*)
Seedling handedness in Triticale	no	(*45*)
Seedling handedness in pigeon pea	no	(*46*)
Direction of leaf rolling in cocoyams	no	(*53*)
Direction of leaf rolling in *Begonia*	no	(*49*)
Direction of leaf torsion in *Plantago major*	no	(*25*)
Direction of leaf spiral in *Corchorus capsularis*	no	(*31*)
Frond handedness in duckweed	no	(*28*)
Direction of floral spiral in *Spiranthes*	no	(*29*)
Direction of style bend in monomorphic enantiostyly	no	(*2*)
Direction of style bend in *Heteranthera* flowers	yes, Mendelian	(*27*)
Animals		
Side of operculum in serpulid polychaetes	no	(*56*)
Side of red spots on elytra of female *Bruchus* beetles	no	(*5*)
Side of major claw in American lobsters	no	(*17*)
Side of major claw in snapping shrimp	no	(*11*)
Side of major claw in male fiddler crabs	no	(*54*)
Top wing when folded in fruit flies	no	(*44*)
Direction of bill crossing in crossbill finches	no	(*15*)
Direction of upper-beak crossing in cross-beak chickens	no[a]	(*32*)
Preferred paw (right or left) in mice	no	(*4*)
Visceral situs in *iv* mutant mouse line	no	(*34*)
Direction of crossing of optic chiasma in trout	no	(*33*)
Direction of crossing of optic chiasma in cod	no	(*33*)
Side of mouth opening in scale-eating cichlids	yes, Mendelian?[b]	(*24*)
b) both wild-type and mutant exhibit directional asymmetry(*) or biased antisymmetry(†)		
Plants		
Direction of phyllotaxy in *Medicago**	yes, Mendelian	(*35*)
Direction of helical growth in roots, hypocotyls, petioles and petals in mutant *Arabidopsis**	yes, Mendelian	(*21*)

Animals

Fiber chirality in the cuticle of mutant nematodes*	yes, Mendelian	(*3*)
Shell coiling direction in the snail *Lymnaea*†	yes, Mendelian	(*16*)
Shell coiling direction in the snail *Partula*†	yes, Mendelian	(*39*)
Shell coiling direction in the snail *Laciniaria*†	yes, Mendelian	(*14*)
Eye side in polymorphic starry flounder†	yes, polygenic?	(*20, 42*)
Preferred hand (right or left) in humans†	yes, weak	(*37*)
Direction of heart asymmetry in *inv* mutant mice[c]*	yes, Mendelian	(*55*)

c) wild-type exhibits directional asymmetry, mutant exhibits antisymmetry

Animals

randomized heart asymmetry in mutant mice:
- *ActRIIb*, *Cryptic (EGF-CFC), Foxj1, Gdf1, iv(lrd), Kif3a,b, Mgat1, Nodal*[d]*, Polaris, Sil & Smad5* genes; No Turning — reviewed in ref (*19*)
- *Delta-like1* (*Dll1*) — (*30*)
- *Fused toes (ft)* — (*22*)
- *Pkd2* — (*41*)
- *RBPjk* — (*47*)

randomized heart asymmetry in mutant zebrafish:
- *Cyclops (nodal-related2)* — (*48*)
- *No tail (Brachyury), Floating head (Xnot)* — (*10*)
- *Notch* — (*47*)
- *Inversin (Inv)* knockdown — (*40*)

randomized heart jogging in majority of mutant zebrafish embryos: (Table 4 of ref. *6*)
- *Curly up, Dino, Locke, Schmalhans, tj2a, tm243b, tm317b* & *tw29b*

randomized diencephalic asymmetry in zebrafish *oep* mutant (*9*)

randomized parapineal asymmetry in mutant zebrafish:
- *Cas, Cyc=Ndr2, Flh, Ntl, Oep (EGF-CFC), Sqt, Sur* — reviewed in ref (*18*)

randomized liver and pancreas asymmetry in zebrafish *Chordino* mutant (*50*)

randomized rotation of P11/P12 nerve cell homologues in *C. elegans*
- *unc-40* & *dpy-19* mutants — (*23*)

d) wild-type exhibits directional asymmetry, mutant exhibits symmetry

Animals

symmetrical heart tube in mutant mice:
- *Fgf8, Foxa2 (Hnf3-□), Furin, Lefty1*[d], & *Lefty2*[d] — reviewed in ref (*19*)

symmetrical lungs in mutant mice:
- *ActRIIb*, *Gdf1, Pitx2*[d]; Smoothened — reviewed in ref (*19*)
- *Lefty1*[d] — (*38*)
- *Pkd2* — (*41*)
- *Shh* — (*52*)
- *Zic3* — (*43*)

no heart jogging in mutant zebrafish embryos: (Table 4 of ref. *6*)
- *Floating head, Lost-a-fin, No tail, Snailhouse, Spadetail*

symmetrical heart tube in mutant zebrafish:
- *Lost-a-fin (Laf)* — (*7*)

[a] upper beaks crossed to the right are weakly but consistently more common than left-crossed ones, but the proportions of dextral and sinistral offspring did not vary among parents of different orientation.

[b] data open to alternate interpretations as breeding was not controlled. Interpretation was based on field-collected offspring from broods guarded by a pair of parents of known asymmetry (*24*). Unfortunately, individuals of this species "have the unusual habit of farming out their fry to other breeding pairs" (p. 217), so controlled laboratory crosses are still needed.

[c] approx. 10 % of *Inv* homozygotes still exhibit normal visceral situs (*situs solitus*).

[d] genes known to be expressed asymmetrically in wild-type individuals.

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Table S2. References and notes to accompany Fig. 3. Genes (and a few proteins) that express or function asymmetrically during early development of one or more deuterostome species and occurrence of selected anatomical asymmetries in these taxa. Each color (column) applies to only one taxon. Each row applies to a single gene or trait. Colored entries indicate direction of asymmetry as indicated in column 3. Non-colored entries indicate some pattern other than that indicated in column 3. Gene order from bottom to top roughly parallels temporal order of expression (see footnotes for exceptions). LPM- lateral plate mesoderm, sym- symmetrical expression, no expr.- no expression, ?- no data or not known, n/a- not applicable, L- left, R- right, perinodal/paraxial- on the periphery of, or adjacent to the node (birds and mammals) or node equivalent in amphibians and teleost fish (Spemann's Organizer or shield, respectively) or similar physical location in lower deuterostomes, CNS- central nervous system, ss- somite stage, hpf- hours post fertilization, HH- Hamburger-Hamilton stage, E- days post coitus.

A) Asymmetrical expression or effect during larval development

Gene or trait	*Gene family or function*[3]	*Side of expression or function*	*Location*	(larval) Ascidian	Lancelet	Zebrafish	Xenopus	Quail	Chick	Mouse	Rabbit
BMP4	*TGFβ* family growth factor	left	heart tube	?	sym[42a]	yes 22ss[11]	yes stg 32[8]	?	sym[10]†	sym[10]†	?
Nkx3.2	homeobox gene	left	LPM	?	?	?	yes[10]†	?	yes[10]†	right[10]†	?
Pitx2	bicoid-type homeobox transcription factor	left	LPM	yes[5b]	yes[66c]	yes 19-20ss[10]†	yes[10]†	yes[69]	yes[10]†	yes E8.25[64]	yes[20]
Snail/SnR	zinc finger transcription factor	right	LPM	sym[17]	sym[28]	sym[17]†	sym[31]	yes[69]	yes[10]†	yes[10]†	?
Lefty2	*TGFβ* extracellular protein signal	left	LPM	?	?	yes 19-20ss[54]	yes[7d]	?	yes[10]†	yes E8.0[56]	yes[20]
Nodal	*TGFβ* extracellular protein signal	left	LPM	yes[39e]	yes[68]	yes 19-20ss[10]†	yes[10]†	yes[69]	yes[10]†	yes e8.0[56]	yes[20]
Caronte/ Cerberus	multifunctional extracellular protein	left	paraxial/ LPM	?	?	sym[24f]	sym[65g]	yes[69]	yes HH6[10]†	right[44h]	?
Lefty1/ Antivin	*TGFβ* extracellular protein signal	left	perinodal/ paraxial	?	?	yes 19-20ss[61]	yes[12i]	yes[69]	yes[10]†	yes E8.0[64]	yes[20]
Nodal	*TGFβ* extracellular protein signal	left	perinodal/ paraxial	?	yes[68]	yes 10-12ss[33j]	no expr[10]†	yes[69]	yes[10]†	yes E7.5[64]	yes[20]
Ciliary 'nodal flow'	leftward flow across node required	n/a	node/ organizer	?	?	?	no[26]	?	no[15,59]	yes[10]†	?
Node monocilia	present on node cell surface	midline	node/ organizer	?	?	yes 10ss[18]	yes stg 14[18k]	?	yes HH4[18l]	yes E7.5[18]	?
Lunatic fringe	Notch pathway modulator	left	perinodal/ paraxial	?	sym[35]	sym[45]	sym[63]	?	yes HH6[49]	sym E7.5-E8[48]	?
Cryptic/CFC1/ oep	*EGF-CFC* membrane associated protein	left	perinodal/ paraxial	?	?	sym[9]	sym[67m]	?	yes HH5-7[55]	sym[9]	?
Fgf8	fibroblast growth factor, multifunctional	right	perinodal/ paraxial	sym[14n]	?	sym[25]	sym[10]†	yes[69]	yes HH5[10]†	sym[62]	sym[20]

BMP4	*TGF□* family growth factors	right	perinodal/ paraxial	sym[60]	sym[43p]	sym[22]	yes[46q]	?	yes HH5[38]	sym E7.75[2]	sym[20]
Shh	intercellular signalling protein	left	perinodal/ paraxial	sym[60]	yes[58r]	sym[61]	sym[10]†	yes[69]	yes HH5[10]†	sym E7.75[2]	sym[20]
Delta-like1	Notch ligand	left	perinodal/ paraxial	?	?	sym 12hpf[48]	?	?	yes HH5[49]	sym E7.5-E8[48]	?
Chordin	antagonist of BMP signal molecules	left	perinodal/ paraxial	sym[14s]	?	sym gastrula[37]	sym gastrula[37]	?	yes HH5[38]	sym E7.75[2]	?
ActRIIA	*TGF□* receptor	right	perinodal/ paraxial	?	?	sym[61]	?	yes[69]	yes HH5-[10]†	sym[10]†	sym[9]
Fgf18	fibroblast growth factor, multifunctional	right	perinodal/ paraxial	?	?	?	?	?	yes HH4[41]	sym[34]	?
Hnf3□	HNF3/forkhead transcription factor	left	perinodal/ paraxial	sym[39]	right[66t]	sym[61]	?	?	yes HH4-[29]	sym[40]	sym[20]
Noggin	antagonist of BMP signal molecules	right	perinodal/ paraxial	?	?	sym[22u]	sym[22]†	?	yes HH4-[19]	sym E7.75[2]	?
Fgf4	fibroblast growth factor, multifunctional	right	perinodal/ paraxial	?	?	no expr[25]	sym[10]†	?	yes[10]†	sym[62]	?
Activin□b	*TGF□* extracellular signal	right	perinodal/ paraxial	?	?	sym[61]	sym[32]	?	yes HH3/4[10]†	sym[10]†	sym[20]
N-cadherin	Epithelial structural proteins	posterior L, anterior R	primitive streak	?	?	?	?	?	yes HH3+[23]	?	?
phosphorylated Syndecan-2	transmembrane proteoglycan	right	side of gastrula	sym[53v]	?	?	yes stg 11[26]	?	?	?	?
Vg1[w] or *GDF1*	*TGF□* extracellular signal	left	side of blastula	?	?	no expr[16]	yes 16 cell[10]†	?	sym HH5[57]	sym[47,52x]	?
H+/K+ ATPase		right	2-cell stage	?	?	?	yes[30]	?	yes[30,36 y]	no[30z]	?
calcium signaling		side of polar body	2-cell stage	yes[1]	?	?	?	?	?	?	?

B) Anatomical asymmetries

Trait	Ascidians	Lancelet	Fish	Amphibians	Chick	Mouse
gut branched, coiled or looped to one side	yes?[6aa]	yes[6]	yes[50]	yes[50]	yes[50]	yes[50]
heart looped or displaced to left of midline	no?[4]	no[21,51bb]	yes[21]	yes[21]	yes[21]	yes[21]
brain / anterior CNS	yes[13]	yes[27]	yes-L[13cc]	yes-L[13dd]	yes-L[13ee]	yes-R[13ff]

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Notes:

† data are from other papers cited by this source.

[a] expressed in late neurula.

[b] expressed in epidermis of early tailbud stage; also, only one *Pitx* gene is known in ascidians so cannot say if it is *Pitx2* or not (Boorman & Shimeld 2002).

[c] expressed in all three germ layers, asymmetric expression starts in late gastrula and persists through late larval stage; also, only one *Pitx* in lancelets so cannot say if it is *Pitx2* or not (Boorman & Shimeld 2002).

[d] as *Xlefty*.

[e] expressed in epidermis of tailbud stage.

[f] as *Charon*; first detected at 2-3 somite stages in hypoblast of tailbud region; at 10-somite stage expression was observed as a horseshoe-shaped zone (Hashimoto et al. 2004).

[g] expressed only at early gastrula stage in anterior mesendoderm.

[h] as *Dante*.

[i] as *Xlefty*.

[j] as *Spaw* in paraxial mesoderm.

[k] expressed well before left-sided nodal expression in the lateral plate mesoderm at stage 17.

[l] expressed well before left-sided perinodal expression of *Nodal* at HH6.

[m] as *FRL-1*.

[n] at 24-cell stage.

[o] as *BMP2/4*; restricted to dorsal and ventral ectodermal midlines.

[p] as *BMP2/4* at late gastrula; expression continues throughout hypoblast; *author's note*: the apparent right side expression in this study is clearly an artifact of a right-deflected midline at the late neurula stage.

[q] right-side expression is much earlier in development (blastula stage) and only implied via mis-expression studies.

[r] first appears in pharyngeal endoderm at mid-neurula stage.

[s] as *HrChordin*; expressed in notochord precursor cells at 44-cell stage.

[t] extends further anterior on right side of coelom margins of late gastrula, neurula; asymmetric expression begins after initial asymmetric expression of *Pitx*.

[u] as *Nog1*; in paraxial hypoblast, late gastrulation just before somitogenesis.

[v] maternal expression throughout embryo until gastrulation.

[w] only the mature Vg1 protein (not mRNA) ends up asymmetical, after post-transcriptional processing.

[x] as *GDF1*; both studies show stronger left-sided expression but much later in development (8-8.5 days post-coitus).

[y] expression is symmetrical, but function is asymmetrical at the primitive streak stage (Mercola 2003).

[z] transgenic mutants lacking this gene exhibit no *situs* reversal.

[aa] no asymmetry in ascidian larvae except nervous system (Burighel & Cloney 1997), however larvaceans — which resemble larval ascidians in form and are believed to be the sister taxon to the remaining Urochordata — do a have a conspicuously asymmetrical gut, which suggests gut asymmetry may have been an ancient chordate trait (Boorman & Shimeld 2002).

[bb] a distinct heart is generally considered absent; blood is circulated by contraction of some ventral and some gill-bar vessels; the anterior-most aortic arch is unpaired and found only on the right side (Ruppert 1997).

[cc] left habenula is larger in *Danio*; however, either the right or left habenula may be larger in other fish species.

[dd] left habenula is larger in all anurans examined, no data on *Xenopus*.

[ee] habenular anatomical asymmetry is subtle; only data available are for chick.

[ff] habenular anatomical asymmetry is subtle in albino mice; left habenular nucleus is larger in albino rats.

Table S3. References and notes to accompany Fig. 4. Incidence of reversed heart asymmetry or spontaneous *situs inversus* (SI) in vertebrates.

Organism	trait scored	sample/treatment group	% reversed	N	Source
		Fish			
Zebrafish (*Danio rerio*)	heart looping	wild-type sibs 1-5 pooled	5.84	736	Table 2 of (*2*)
	heart looping	uninjected only	1.33	150	Table 1 of (*22*)
	heart looping	uninjected only	4.37	206	Table 1 of (*3*)
	heart looping	B-gal treatment	4.19	167	Table 1 of (*3*)
	heart looping	wild-type sibs of mutants	4.03	782	Table 4 of (*3*)
	heart looping	wild-type sibs of mutants	8.01	1,223	Table 4 of (*3*)
	heart looping	wild-type sibs of mutants	1.57	415	Table 4 of (*3*)
	heart looping	uninjected only	4.00	144	Table 1 of (*7*)
		pooled	5.30	3,823	
		average	4.17		
Salmon (*Onchorynchus*)	complete SI	'normal singles'	6.5	200	Table 6 of (*1*)
Brook trout (*Salvelinus fontinalis*)	complete SI	'normal singles'	4.71	510	Text p. 5 of (*16*)
		Amphibians			
Frog (*Xenopus laevis*)	heart asymmetry	uninjected only	2.65	151	Table 1 of (*4*)
	heart asymmetry	uninjected only	1.00	500	Table 1 of (*11*)
	heart asymmetry	Expt A except Activin into R3	1.98	353	Table 1 of (*10*)
	heart asymmetry	uninjected only	3.38	148	Text p. 1467 of (*15*)
	heart asymmetry	all injected <10% reversal	1.79	446	Table 1 of (*10*)
	heart asymmetry	uninjected only	2.22	1,127	Table 1 of (*28*)
	heart asymmetry	uninjected only	2.22	1,127	Table 1 of (*28*)
	heart asymmetry	0 ug/ml of Activin	1.23	162	Table 1a of (*27*)
	heart asymmetry	control embryos	0.50	201	Table 1 footnote of (*27*)
	heart asymmetry	activin injected late	1.04	96	Text p. 323 of (*27*)
	heart asymmetry	control sibling embryos	1.72	987	Table 2 of (*27*)
	complete SI [a]	uninjected & vector ONLY	1.54	195	Table 2 of (*21*)

	complete SI	control embryos	1.08	93	Table 3 of (*13*)
	complete SI	control embryos	0.00	132	Table 7A of (*13*)
		pooled	1.82	5,718	
		average	1.60		
Newt (*Triturus vulgatus*) [b]	complete SI		1.75	228	Table 1 of (*17*)
Newt (*Triturus alpestris*) [c]	complete SI		2.14	607	Table 1 of (*17*)
	complete SI	control	0.24	3,801	Text p. 10 of (*30*)
		pooled	0.50	4,408	
		average	1.19		
		<u>Birds</u>			
Chicken (*Gallus domesticus*)	heart looping	control	3.45	29	Fig. 3 of (*8*)
	heart looping	neutral red & DMSO only	3.50	143	Table 1 of (*24*)
	heart situs	control beads	0.00	65	Table 2 of (*14*)
	internal organs	spontaneous reversal	0.10		Text p. 60 of (*14*)
	Shh or *Nodal* expr.	control culture	1.27	158	Table 1 of (*13*)
	complete SI	normal littermates	0.00	38	Text p. 403 of (*20*)
		pooled	1.85	433	
		average	1.39		
		<u>Mammals</u>			
Mice (*Mus domesticus*)	complete SI	offspring of x-irradiated parents	0.0550	1,817	Bagg 1925, cited in (*9*)
	complete SI	NHO group	0.0010	210,000	Text p. 341 of (*25*)
	complete SI	offspring from complex cross	0.0000	11,500	Text p. 29 of (*26*)
		pooled	0.0013	223,317	
		average	0.0187		
Rats (*Rattus norvegicus*)	reversed heart		0.3643	549	(*6*)
	complete SI		0.0365	30,134	cited in (*5*)
		pooled	0.0424	30,683	

			average 0.2004		
Hamster (*Mesocricetus auratus*)	atrial displacement	embryos of control group	0.0289	3,461	Table 1 of (*23*)
	complete SI	embryos of control group	0.2332	1,715	Table 3 of (*23*)
			pooled 0.0966	5,176	
			average 0.1311		
Sheep (*Ovis aries*)	complete SI	detected in slaughterhouse	0.00001	10,000,000[d]	(*12*)
Human (*Homo sapiens*)	complete SI	25 reports pooled	0.0106	3,289,210	Table 5 of (*1*)
	complete SI		0.0125	8,000	abstract of (*19*)
	complete SI	chest clinic & natural pop. pooled	0.0305	137,698	abstract of (*29*)
	complete SI		0.0415	12,050	abstract of (*18*)
			pooled 0.0115	3,446,958	
			average 0.0238		

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Notes.

[a] heterotaxias were noted but were excluded from these counts
[b] reported as *Triton taeniatus* in the original paper
[c] reported as *Triton alpestris* in the original paper
[d] sample size is an anecdotal estimate