

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kibar Z, Torban E, McDearmid JR, et al. Mutations in *VANGL1* associated with neural-tube defects. *N Engl J Med* 2007;356:1432-7.

Supplementary Methods

Interaction between Vangl and Dishevelled proteins

A cDNA segment corresponding to the cytoplasmic domain of the wild type (Accession # BC065272; 975-1829nt) as well as V239I, D259E, R274Q, M328T, and S467N

hVANGL1 protein variants were cloned into the yeast plasmid pBDT7 (pBD-hVANGL1). Individual cDNA fragments overlapping either the amino half of

Dishevelled proteins 1, 2 and 3 [Dvl1(1-404aa), Dvl12(1-418), Dvl3(1-395aa)], or the carboxyl portion of Dvl3 (389-717aa) were inserted in the pGADT7 plasmid.¹⁰

hVANGL1 constructs were introduced into *Saccharomyces cerevisiae* (strain AH109, MAT α), and independent AH109 clones were mated to *S. cerevisiae* clones (strain Y187, MAT α) expressing individual Dvl proteins. GAL4 activity was tested as previously described.¹⁰ In the matchmaker system 3, reconstituted GAL4 activity (interaction between fusions to the GAL4 DNA binding and activation domains) is tested by plating diploid cells on selection media of various stringency: low stringency lacking leucine and tryptophan (-Leu/-Trp), intermediate stringency lacking histidine, leucine and tryptophan (-His/-Leu/-Trp) and high stringency lacking adenine, histidine, leucine, and tryptophan (-Ade/-His/-leu/-Trp).

<i>Supplementary Table 1. Clinical features of familial NTD cases and their affected relatives*</i>		
Patient	Clinical features of patient	Affected relatives and their clinical signs
Patient 19 M, 17 years old	MMC, Chiari II	Brother (dermal sinus)
Patient 32 M, 18 years old	MMC, Chiari II, Hydrocephalus, tethered cord	Maternal grandmother (dermal sinus)
Patient 48 F, 19 years old	MMC (level L5-S1), Hydrocephalus, congenital club feet	Mother and maternal aunt (vertebral schisis)
Patient 49 F, 13 years old	MMC, Chiari II	Sister (MMC, Chiari II)
Patient 55 F, 35 years old	Lipomys, tethered cord	Sister, mother, two nephews (vertebral schisis)
Patient 56 M, 21 years old	MMC, Hydrocephalus, Hydrosyringomyelia, tethered cord	Brother (MMC)
Patient 89 M, 16 years old	CR (type IV [†]), costo- vertebral malformations, kyphosis, ectopic kidney	Paternal aunt, second-degree cousin (closed NTD)
Patient 96 M, 18 years old	CR (type V [†]), ano-rectal malformations	Mother (absence of coccygeal segments)
Patient 103 F, 10 years old	CR (Type IV [†]), lipomys, Ano-rectal malformation, hydromyelia, tethered spinal cord	Brother (dermal sinus)
Patient 140 F, 8 years old	MMC (L5-S1), Chiari II, hydrocephalus, absence of sacrum, single kidney	Grandgrandfather (closed NTD)
Patient 146 F, 14 years old	MMC, Chiari II, hydrocephalus	Maternal first-degree cousin (MMC, Chiari II, hydrocephalus)
Patient 147 F, 11 years old	LipoMMC	Paternal uncle (spinal lipoma)
Patient 155 M, 3 years old	MMC, hydrocephalus	Maternal second-degree cousin (MMC)

*M denotes male, F female, MMC Myelomeningocele, lipoMMC lipomyelomeningocele, lipomys lipomyeloschisis, CR caudal regression

[†]According to Pang's classification.¹²

Supplementary Table 2. Sequence variants detected in the ORF of VANGL1 and their frequency in NTD patients and controls

nt (aa)	Sequence variants in patients and controls				Sequence variants specific to patients						
	A274G (I92V)	C330T (Y110Y)	G346A (A116T)	C780T (G260G)	A323G (K108R)	T435C (C145C)	C523T (R175W)	G715A (V239I)	G821A (R274Q)	T983C (M328T)	G1341A (A447A)
Spina bifida (N=137)	1	1	23	1	1	1	1	1	1	1	1
Cranio- rachischisis (N=7)	0	1	1	0	0	0	0	0	0	0	0
Controls^a	1 (146)	3 (150)	24 (150)	1 (116)	0 (150)	0 (150)	0 (150)	0 (166)	0 (168)	0 (165)	0 (156)

^a The numbers shown in parentheses indicate the number of controls analyzed for each sequence variant (including 85-106 Italians ethnically-matched for the spina bifida cases and 65 CEPH individuals)