Handedness and *situs inversus* in primary ciliary dyskinesia

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> . . . The limbs on the right side are stronger. [The] cause may be . . . [that] . . . motion, and abilities of moving, are somewhat holpen from the liver, which lieth on the right side.
>
> (Sir Francis Bacon, *Sylva sylvarum* (1627).)

Fifty per cent of people with primary ciliary dyskinesia (PCD) (also known as immotile cilia syndrome or Siewert–Kartagener syndrome) have *situs inversus*, which is thought to result from absent nodal ciliary rotation and failure of normal symmetry breaking. In a study of 88 people with PCD, only 15.2% of 46 individuals with *situs inversus*, and 14.3% of 42 individuals with *situs solitus*, were left handed. Because cerebral lateralization is therefore still present, the nodal cilia cannot be the primary mechanism responsible for symmetry breaking in the vertebrate body. Intriguingly, one behavioural lateralization, wearing a wrist-watch on the right wrist, did correlate with *situs inversus*.

1. INTRODUCTION

Humans, like other vertebrates, mostly have their heart on the left side, and there is a secondary asymmetry of other organs such as lungs, liver, spleen, testicles and bowel, the configuration known as *situs solitus*. Over the past few years, as a result of the important work by Hirokawa and Nonaka in mice (Nonaka *et al.* 1998, 2002; Okada *et al.* 1999), the orthodox view, shown in figure 1*a*, has been that visceral asymmetry in vertebrates results from symmetry breaking, as a result of the rotation of 9+0 monocilia in the nodal region for a short period during development (Brueckner 2002), which is then followed by a cascade of biochemical asymmetries determining visceral situs (Raya *et al.* 2004). Defective ciliary rotation in the *kif3b* mouse and *iv* mouse results in a 50 : 50 mixture of *situs solitus* and *situs inversus* (heart on the right, liver on the left, etc.; see figure 1*b*) (Capdevila *et al.* 2000; Mercola & Levin 2001; Brueckner 2002; Essner *et al.* 2002), and *situs inversus* can be induced in the mouse experimentally by reversing the usual nodal flow (Nonaka *et al.* (2002), although see Tabin & Vogan (2003)). Despite noting the 'intellectually satisfying' nature of this model, and while acknowledging that 'some aspect of the cilia model is almost surely right (at least in mice)', Levin (2003) has detailed a range of problems with the ciliary model, both in timing and in functional generalization to species other than the mouse.

Unlike other vertebrates, humans also show functional cerebral lateralization, most people being right handed, and in addition, most people also having left-sided cerebral dominance for language (Knecht *et al.* 2000), although the correlation of handedness and language dominance is far

from perfect but nevertheless can be explained by a straightforward genetic model (McManus 1985, 1999; Annett & Alexander 1996). The complex functional asymmetries of the human brain should not be confused with the anatomical asymmetries found in the diencephalon of fishes and vertebrates (von Woellwarth 1950; Morgan 1977), which are probably controlled by the same mechanisms as control other aspects of *situs* (Concha *et al.* 2000; Concha & Wilson 2001; Gamse *et al.* 2003; Halpern *et al.* 2003).

Sir Francis Bacon (1561–1626), in his posthumous *Sylva sylvarum* of 1627, suggested that human handedness resulted from visceral asymmetry: 'the limbs on the right side are stronger... [because]... motion, and abilities of moving, are somewhat holpen from the liver, which lieth on the right side'. If this Baconian model were correct, then people with *situs inversus* should mostly be left handed (figure 1*b*). However, several large-scale, but old, studies have found that most individuals with *situs inversus* seem to be right handed for writing (Watson 1836; Cockayne 1938; Torgersen 1950), although those studies do suffer from little information being available on aetiology, and they have very limited assessments of laterality. In the absence of a known pathophysiological mechanism for such cases of human *situs inversus*, it is not clear to what extent they provide a challenge to the concept of the nodal cilia as the primary source of symmetry breaking, and hence of body asymmetry in general.

In primary ciliary dyskinesia (PCD) (also known as Siewert–Kartagener syndrome or immotile cilia syndrome) a motility defect of 9+2 cilia results in bronchiectasis, chronic sinusitis, and male infertility (Bush *et al.* 1998). In addition visceral *situs* is randomized, 50% of cases having complete *situs inversus* (with the heart on the right, liver on

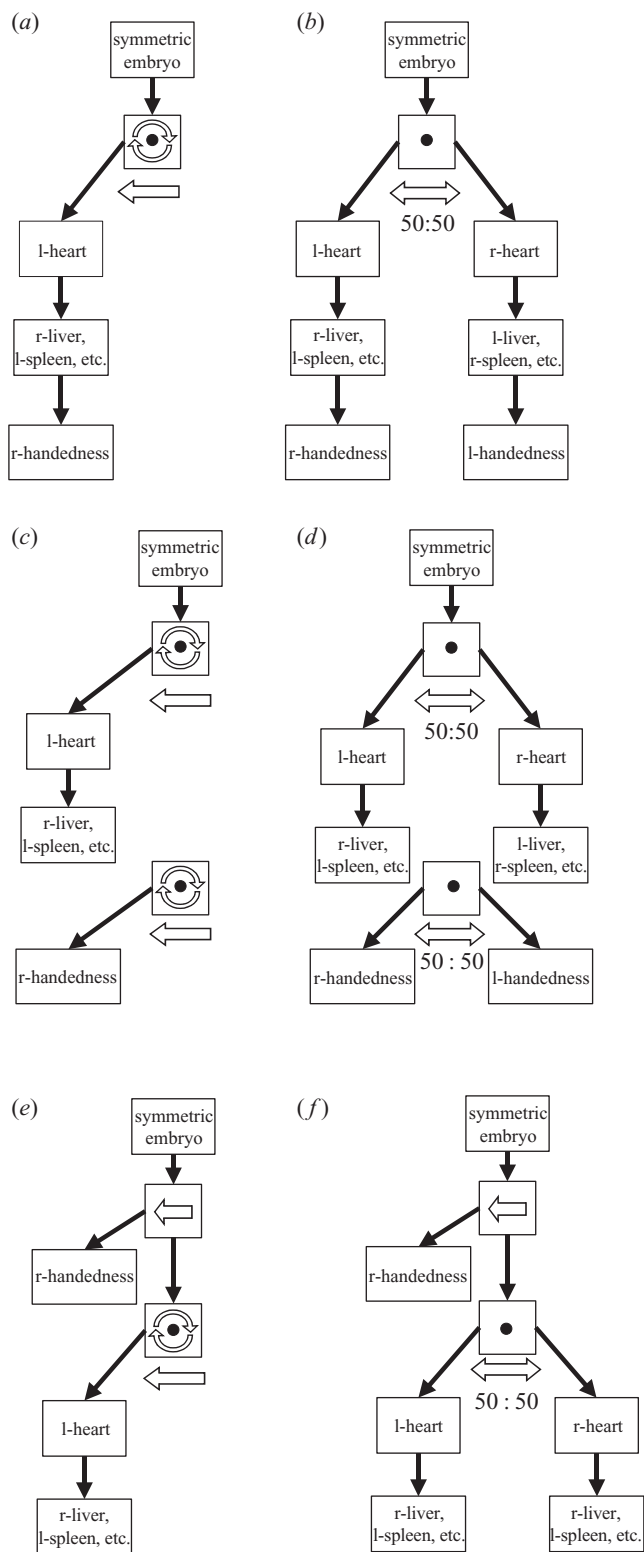[*] Author for correspondence (i.mcmanus@ucl.ac.uk).

Figure 1. Models of the relationship between visceral and cerebral *situs*. (*a*) This shows the orthodox ciliary model in which rotation of cilia in the nodal region breaks asymmetry, causing the heart to be on the left, and other asymmetries of viscera and brain to develop asymmetries which are secondary to heart asymmetry. (*b*) This shows that with the orthodox model, randomization of nodal flow will cause half of organisms to show *situs inversus*, with a right-sided heart, left-sided liver, etc. and the other half to show the normal pattern of *situs solitus*, with a left-sided heart, right-sided liver, etc. If cerebral asymmetry is secondary to visceral asymmetry then the Baconian model suggests that individuals with *situs inversus* should be left handed and individuals with *situs solitus* should be right handed (but which our data on PCD show is not actually the case). (*c*) This shows an alternative model to (*a*) in which visceral asymmetry and cerebral asymmetry are caused by independent ciliary rotations. (*d*) This shows that disruption of the separate flows should result in *situs* and handedness being random and independent, so that half of those with *situs inversus* and half of those with *situs solitus* should be left handed. The pattern of handedness in PCD in our data is not consistent with (*d*). (*e*) This shows an alternative model in which visceral asymmetry is still determined by ciliary rotation at the node, but cerebral asymmetry is determined upstream to ciliary rotation by a mechanism not involving ciliary rotation. (*f*) This then shows that disruption of ciliary flow, as in PCD, will result in *situs inversus* in half of all individuals, but that individuals with *situs inversus* and *situs solitus* will both show the same, low, rate of left handedness as the rest of the population. (*f*) is compatible with the present data on PCD.
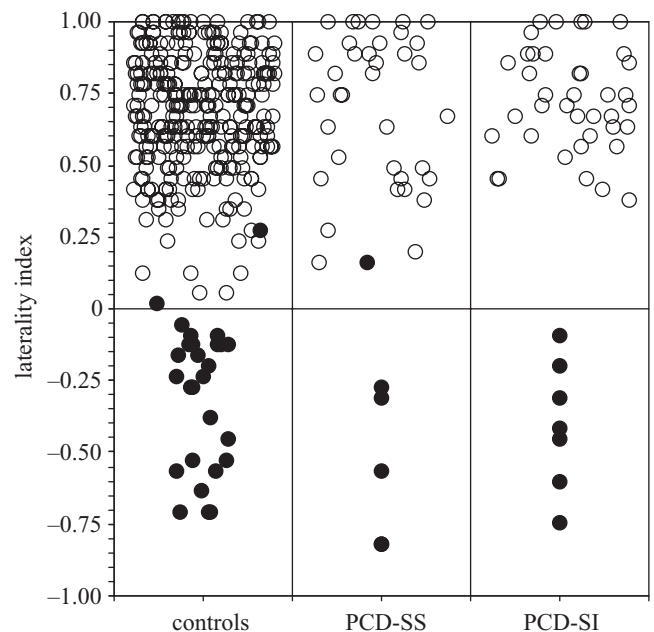

Figure 2. Degree and direction of lateralization of handedness in patients with PCD-SI, PCD-SS, and in controls. Black circles, left writing hand; white circles, right writing hand.

the left, etc.; PCD-SI), and 50% having the normal *situs solitus* (with the heart on the left, liver on the right, etc.; PCD-SS) (Bush *et al.* 1998). The *situs inversus* probably results from a concomitant dysfunction of 9+0 nodal monocilia, as occurs in *Hfh4* null mice (Brody *et al.* 2000), resulting in absent vortical micro-flow and randomization of *situs*, as also occurs in the DNAH5 mutation (Olbrich *et al.* 2002). If vortical flow at the node is the principal cause of symmetry breaking, then its absence in PCD should

either cause left handedness in PCD-SI and right handedness in PCD-SS (if cerebral lateralization is secondary and downstream to *situs*: figure 1*b*), or if the brain and the viscera are randomized independently, a 50% rate of left handedness should occur in both PCD-SI and PCD-SS

Table 1. Percentage of individuals wearing a wrist-watch on the right wrist, in relation to handedness and side of heart.

percentage wearing watch on right wrist	left-sided heart		right-sided heart	
	controls	PCD-SS	PCD-SI	total
right handers	14.0% (43 out of 308)	19.4% (7 out of 36)	35.9% (14 out of 39)	16.7% (64 out of 383)
left handers	37.0% (10 out of 27)	33.3% (2 out of 6)	57.1% (4 out of 7)	40.0% (16 out of 40)
total	15.8% (53 out of 335)	21.4% (9 out of 42)	39.1% (18 out of 46)	18.9% (80 out of 423)

(figure 1*c,d*). The latter pattern is formally equivalent to that found with diencephalic asymmetries in the zebrafish, where heart looping and parapineal asymmetry in $LZoep^{-/-}$ mutants are random and uncorrelated (Concha *et al.* 2000).

2. METHODS

Eighty-eight individuals with PCD were studied through the UK PCD Family Support Group, and compared with 334 individuals in a student control group (mean age: PCD, 22.7 years; controls, 20.0 years) (McManus & Drury 2004). Clinical details of these cases are presented elsewhere (McManus *et al.* 2003). Of the cases studied, 47.7% were PCD-SS and 52.3% were PCD-SI. Controls were presumed to have *situs solitus*. A postal questionnaire containing written and photographic questions was used to assess 33 separate behavioural lateralities, including preferred hand for a range of tasks, as well as hand clasping, arm folding, leg crossing, footedness, ear preference, and eye preference (McManus & Drury 2004). Conventional handedness was assessed both in terms of writing hand, and by a standard laterality index, calculated as $100 \times (R - L)/(R + L)$, based on 11 questionnaire items.

3. RESULTS

The rate of left handedness for writing in controls was 8.1% (27 out of 335), PCD-SS, 14.3% (6 out of 42), and PCD-SI, 15.2% (7 out of 46) and this did not differ significantly between the three groups ($\chi^2 = 3.69$, d.f. $= 2$, $p = 0.158$). The laterality index showed clear bimodality (see figure 2), with 91.3% scoring greater than 0, all but three of whom wrote with their right hand, and 8.7% scoring less than zero, all of whom wrote with their left hand. There were 7.4% controls, 11.4% PCD-SS and 14.9% PCD-SI that had a laterality index of less than zero ($\chi^2 = 3.377$, d.f. $= 2$, $p = 0.185$). The absolute laterality index, which assesses degree or strength of handedness, did not differ significantly between the three groups ($F_{(2,419)} = 0.194$, $p = 0.824$).

A systematic comparison was made of left- and right-sided usage for all 33 individual measures of behavioural laterality in those with *situs inversus* (PCD-SI) and those with *situs solitus* (PCD-SS + controls). Because of multiple testing, the Bonferroni correction was used to set alpha at $0.05/33 = 0.0015$. The only significant difference in relation to side of the heart was for the side on which a wrist-watch was worn (see table 1; $\chi^2 = 13.19$, d.f. $= 1$, uncorrected $p = 0.00028$; corrected $p = 0.0107$). Logistic regression predicting right-sided wrist-watch wearing showed independent effects of handedness ($\chi^2 = 10.293$, d.f. $= 1$, $p = 0.0013$) and side of the heart ($\chi^2 = 11.245$, d.f. $= 1$, $p = 0.00080$), with no interaction ($\chi^2 = 0.140$, d.f. $= 1$, $p = 0.709$).

4. DISCUSSION

Our finding of a normal rate of left handedness in PCD-SI is compatible with earlier studies in which individuals with *situs inversus* are mostly right handed for writing (Watson 1836; Cockayne 1938; Torgersen 1950), of whom cases of PCD would have been only a minority (Aylsworth 2001). The present results in PCD, with its well-defined pathophysiology, provide a strong challenge to current understanding of the developmental determination of body lateralization, because despite the absence of symmetry breaking by ciliary rotation, there is still consistent cerebral lateralization. Such a result cannot be explained by the models in figure 1*a,b*, and neither can it be explained by the models in figure 1*c,d* (unless it were the case that despite nodal cilia being non-functional, the cilia determining cerebral asymmetry were still functional). The implication is either that cerebral functional asymmetry results from a separate (and unknown) mechanism of symmetry breaking from that involved in body *situs* (Levin & Mercola 1998; Capdevila *et al.* 2000), or that perhaps the cilia are not the basis of 'step 1' (Levin 2003) in setting up the overall left–right axis of the vertebrate body, so that instead the cilia act to amplify a pre-existing asymmetry (figure 1*e,f*). In either case, functional cerebral asymmetry would remain normal in the presence of random visceral *situs*.

The findings on the side of wearing a wrist-watch were unexpected but statistically robust. There is little research on this common behavioural laterality. Wrist-watches are sophisticated, asymmetric artefacts primarily designed for right handers, particularly when there is a clockwise winder or electronic controls (see www.ac2w.com/en_ac2w.htm). As a result, 'custom helpeth', as Bacon would have put it, to ensure most are worn on the left side. Although left handers are somewhat more likely to wear a watch on the right wrist, nevertheless one in six right handers also wears their watch on the right wrist. Ergonomic factors may partly explain the association with handedness but contribute little to understanding why those with *situs inversus*, who have their heart on the right, are more likely to wear a wrist-watch on the right, irrespective of handedness.

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