



**Fluctuating asymmetry does not consistently reflect severe developmental disorders in human fetuses.**

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6 developmental disorders in human fetuses.  
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## Abstract

Developmental instability (DI) as measured by fluctuating asymmetry (FA), may reflect fitness and facilitate the expression of morphological variation. Insights in the underlying mechanisms and magnitude of DI during early development are required to better understand its role in evolutionary biology. We studied associations between FA and congenital abnormalities of different origins and functional systems in deceased human fetuses. Major congenital abnormalities corresponded to severe, often lethal developmental disorders disrupting normal development from early organogenesis onward, but only moderately increased FA. Lower FA with age also supported the hypothesis that more severe abnormalities, leading to an earlier death, increased DI. Although FA related to measures of fitness or health, effects were anticipated to be stronger given the detrimental health effects many fetuses experienced. Furthermore, elevated FA was only observed in 4 out of 17 disorders (left-right patterning, limb defects, problems of bronchopulmonary and uro-genital system). Fetuses experiencing major abnormalities other than these 4 types, did not show increased FA. This suggests that the functional importance of symmetry in limbs has resulted in strong selection for symmetry and reduced its sensitivity to stress. Finally, the observed patterns suggest that specific developmental pathways have a stronger effect on DI than others.

*Keywords:* fluctuating asymmetry, developmental instability, prenatal development, human fetuses, congenital abnormalities, still births, phenodeviance

## Introduction

A wealth of data has accumulated on the role of developmental instability (DI, the sensitivity of a developing trait to random perturbations) as measured by fluctuating asymmetry (FA, small directionally random deviations from perfect symmetry) in evolutionary biology. DI and FA have received a lot of attention, because they might serve as a fitness proxy and signal of individual (genetic) 'quality' in studies of sexual selection and the evolution of sexual behavior (e.g. Van Dongen et al., 2009). Despite over half a century of research the consensus is that the evolutionary patterns in DI are heterogeneous and that very little is known about the underlying causes of the heterogeneity (e.g. Klingenberg, 2003; Van Dongen 2006; Polak & Taylor, 2007). There is a large variation in observed associations between asymmetry and components of fitness (e.g., Clarke 2003), and the genetic basis of DI is also controversial (Fuller and Houle, 2003; Leamy and Klingenberg, 2005; Van Dongen, 2006; 2007). A complicating factor that plays a role is variation in levels of DI between traits with different functionality, which may result in diversity in sensitivity to stress. Furthermore, developmental buffering has been proposed as an important factor in evolutionary processes, because it can maintain adaptive phenotypic traits in the presence of genetic and environmental variation and can conceal genetic variation from selection (e.g., Breuker et al., 2006). Patterns in variation are important because they can help understand how developmental systems produce variation and how they can interact with evolutionary processes (Hallgrímsson et al., 2002). Increased levels of environmental stress may result in a breakdown of the buffering capacity, increase morphological variation and even affect macro-evolutionary changes (Badyaev & Foresman, 2000). The effects of environmental and/or genetic stress have been suggested to be trait and stress-specific (e.g., Van Dongen 2006) complicating relationships between stress, buffering capacity, morphological variability, DI and fitness. To better understand the use of FA as a measure of developmental buffering and

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2  
3 its relationship to quality and stress, more insights in the underlying processes that map  
4 variation in DI into the observable degree of asymmetry are urgently needed (Klingenberg,  
5 2003). More specifically, insights in the roles that different types of developmental  
6 disturbances play in determining DI are scarce (Breuker et al., 2006). To better understand  
7 these underlying mechanisms, we have investigated the relationship between quantifiable  
8 disturbances of development of different types and DI as measured by FA in humans. We  
9 anticipate that DI and FA are sensitive indicators of health and fitness, as associations appear  
10 very general (see e.g. Møller 2006 for a recent review in humans).  
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22 We investigate FA in deceased human fetuses and infants, in many of which the  
23 development was severely disturbed by problems of maternal origin, fetal origin or both. We  
24 expect FA to be relatively high overall, because it has been suggested that selection against  
25 asymmetric individuals with high DI would occur early in development (e.g. Møller, 1997).  
26 The serious abnormalities could be classified in a number of relatively distinct groups  
27 referring to different functional systems or developmental events (although often several  
28 abnormalities occur together). In the majority of cases the earliest disturbances had already  
29 started at the early organogenesis stage. This can be deduced from the frequent association of  
30 these serious abnormalities with the occurrence of cervical ribs. These are ribs on the seventh  
31 cervical vertebra originating from a homeotic transformation of this cervical vertebra into a  
32 rib-bearing thoracic vertebra and are induced during early organogenesis (Galis et al. 2006).  
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48 To investigate whether the early disturbances during prenatal development lead to  
49 asymmetric development, we tested for relations between FA of limb bones and various  
50 maternal and fetal problems with deleterious effects. This approach is novel and allows  
51 comparing effects of different types of disturbances within a well defined population. In  
52 contrast, most other studies focus on either single particular abnormalities or general fitness  
53 components (Møller 2006). Formal comparison with a control group is hampered by the  
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3 absence of data from healthy fetuses due to the impossibility to carry out these measurements  
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5 in utero. Nevertheless, this does not preclude us to compare FA among groups of individuals  
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7 with abnormalities of different severity or congenital abnormalities associated to different  
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9 developmental systems. Both the environment (i.e., the mother) and the condition of the  
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11 individual affect chance of death and thus fitness. Therefore, we were able to formulate three  
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13 hypotheses that allow evaluating the importance of maternal and fetal condition on DI. First,  
14  
15 besides effects of the maternal condition, fetuses experiencing congenital abnormalities of  
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17 different severity can be categorized in 3 broad groups on the basis of their degree of  
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19 phenotypic abnormalities or degree of phenodeviance. We opted for a classification of the  
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21 fetal congenital abnormalities in no/minor/major as it reflects our expectation on the strength  
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23 of the abnormalities on the levels of FA and DI (Galis et al., 2006). The presence of  
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25 phenodeviants has been proposed as a measure of DI as well and after thorough medical  
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27 examination, some fetuses show no detectable morphological abnormalities, suggesting that  
28  
29 their development followed a normal trajectory and that they died because of acute medical  
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31 conditions (e.g. chronic hypoxia because the fetus got strangled by the umbilical cord during  
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33 birth, Fig 1A). In a second group of fetuses, only minor (i.e., as such not drastically  
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35 compromising the normal functioning of an individual) phenotypic abnormalities were  
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37 detected. These minor abnormalities include hypertrophy of the heart, spleen, liver or lungs;  
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39 supernumerary phalanges; club foot; hydrops fetalis (edema of the whole body); ventricular or  
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41 atrial septal defect, abnormal hair growth (Fig. 1B) and slight facial dysmorphologies (Fig.  
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43 1C)(Galis et al., 2006). As a third category, several fetuses exhibit major congenital  
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45 abnormalities (i.e., compromising the normal functioning and sometimes potentially lethal),  
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47 indicating major disturbances of their development starting during the very early  
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49 organogenesis. These major abnormalities include absence of one or both kidneys, skeletal  
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51 dysplasias (most commonly thanatophoric dysplasia which is lethal and characterized by a  
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3 severe shortening of the limbs, narrow trunk, macrocephaly and normal trunk length, Fig.  
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5 1D), macrocephaly (Fig. 1E), extreme scoliosis (Fig. 1E), anencephaly (Fig. 1F), cleft  
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7 lip/palate (Fig. 1F), Cyclops, dextroposition of the heart, monoventricular or monoarterial  
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9 heart; atresia of the aorta; chromosomal abnormalities (e.g. Turner syndrome, Fig. 1G),  
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11 amnion disruption syndrome (Fig. 1H), chondrodysplasia and sirenomalia (see also Galis et  
12  
13 al., 2006). In many cases, several (major) congenital abnormalities co-occur, and in a few  
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15 exceptional fetuses it is not always possible to identify abnormalities very precisely (e.g., Fig.  
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17 1I). It is important to note that the presence of these major abnormalities cannot be missed  
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19 during medical examinations. As such, we are certain that this latter group of fetuses differs  
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21 strongly from the other in the severity of the developmental disorders. Since even minor  
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23 morphological deviations or phenodeviants have been reported to associate with increased DI  
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25 (e.g. Polak and Taylor, 2007), we predict increased limb bone asymmetry in both fetuses with  
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27 minor and with major congenital abnormalities. The fetuses without minor or major  
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29 abnormalities died at a very young age and may therefore have elevated FA as well. However,  
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31 the severity and profoundness of the major congenital abnormalities renders this latter group  
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33 to correspond to fetuses suffering from the worst imaginable developmental disorders. If any,  
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35 an association between developmental disorders and asymmetry can be expected to emerge in  
36  
37 this group. Secondly, and after studying FA in these broadly defined groups on the basis of  
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39 severity, the different congenital abnormalities will be categorized into distinct groups  
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41 associated with different functional systems, developmental origins and/or ontogeny. The  
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43 effects of these different categories on the levels of asymmetry will be compared. This will  
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45 allow us to specifically identify specific developing events or functional systems that are  
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47 associated with increased FA and DI. Finally, because age at death can be regarded as a  
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49 general measure of the severity of the abnormalities on the fetal development, the relationship  
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3 between asymmetry and fetal age was also examined. We predict a negative association  
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5 between asymmetry and age if DI would be associated with more a general measure of health.  
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8 In conclusion, we study a population where variation in health problems was relatively  
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10 large in spite of the fact these fetuses deceased at a very young age. We will be able to  
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12 determine if FA is associated with severe developmental disturbances that specifically affect  
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14 particular developmental events and/or with more general measures of developmental  
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16 disturbance. We discuss our results in light of different mechanisms that do or do not affect  
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18 DI of limb bones and the use of FA as a measure of stress and individual quality in  
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20 evolutionary biology in general.  
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## 27 **Materials and methods**

### 28 **Asymmetry measurements**

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30 Since 1980 deceased fetuses which arrive for examination at the VU Medical Centre in  
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32 Amsterdam are standard radiographed ventrally and laterally (23mA, 70-90 kV, 4-12 sec,  
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34 Agfa Gevaert D7DW Structurix films). This research is carried out on the ventrally taken  
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36 radiographs of 643 deceased fetuses taken between 1992 and 1999. Some fetuses were  
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38 excluded for measurements, because the bones were not in a suitable position or the  
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40 radiographs had insufficient resolution. In total, 310 male and 273 female (aged 14 – 42  
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42 weeks, mean 27.8 weeks,  $\pm$  9.5 weeks) were examined for the size of the left and right ulna,  
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44 radius, femur, tibia, fibula, digit 2, and digit 4. Measurements were made from the midpoint  
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46 of the proximal end of the bones to the midpoint of the distal end with a transparent ruler with  
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48 a resolution of 0.01 cm. Digits were measured from the proximal end of the proximal phalanx  
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50 to the distal end of the distal phalanx (Fig. 2). Measurements were carried out by one of two  
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52 investigators (CTB and FG) without prior knowledge of the autopsy reports (however, several  
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54 congenital anomalies can be seen in radiographs). To compare the accuracy of the  
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3 measurements of the two investigators, 38 fetuses were independently measured by each  
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5 investigator. In addition the entire procedure of positioning a fetus and making a radiograph  
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7 was repeated for 32 fetuses. Degree of measurement error and directional asymmetry were  
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9 analyzed using a mixed regression approach (Van Dongen et al., 1999). As 32 fetuses were  
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11 remounted and photographed a second time independently, measurement error could be  
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13 determined at two levels. At the level of individual measurements, ME was determined on the  
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15 basis of repeated measurements on the same photograph, and a comparison of asymmetry  
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17 levels between photographs of the same bone and fetus allowed to evaluate the error resulting  
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19 from mounting the fetus. Directional asymmetry was tested for by F-tests (Van Dongen et al.,  
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21 1999). Results are summarized in Table 1 showing that for each trait, levels of ME were  
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23 smaller than levels of asymmetry and there were no indications of directional asymmetry  
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25 (except for radius, but the p-value of 0.05 was non-significant after Bonferroni correction).  
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27 Thus, our measurements allowed to measure asymmetry relatively accurately and there was  
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29 no need to correct for directional asymmetry which could potentially decouple the presumed  
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31 association between asymmetry and developmental instability (Klingenberg, 2003; Van  
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33 Dongen 2006).

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35 Distributions of the signed FA (left minus right measurement) were all leptokurtic (kurtosis  
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37 ranging between 5.6 and 68.9), as is often observed in studies of FA. Most likely, this  
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39 leptokurtosis is a reflection of between-individual variation in the underlying DI (e.g. Van  
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41 Dongen, 2006). This is a prerequisite to be able to detect any associations between DI and  
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43 other covariates through patterns in asymmetry (i.e., observable associations with the  
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45 unsigned FA, the absolute value of the signed FA). The high levels of kurtosis suggest a  
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47 relatively strong association between single trait asymmetry and the underlying levels of DI.  
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49 Especially after averaging across the 7 traits, individual average asymmetry can be expected  
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51 to reliably reflect DI (Van Dongen, 2006).  
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## Statistical analyses

Because age and thus size of fetuses differed markedly, levels of the unsigned asymmetry were corrected for individual-specific trait size. Indeed, uncorrected unsigned FA correlated significantly positively with trait size for femur ( $r=0.19$ ,  $N=500$ ,  $p<0.0001$ ), Digit 2 ( $r=0.26$ ,  $N=265$ ,  $p<0.0001$ ), Digit 4 ( $r=0.24$ ,  $N=249$ ,  $p<0.0001$ ), radius ( $r=0.22$ ,  $N=516$ ,  $p<0.0001$ ), tibia ( $r=0.26$ ,  $N=501$ ,  $p<0.0001$ ) and ulna ( $r=0.11$ ,  $N=519$ ,  $p=0.01$ ). A positive trend was observed for fibula ( $r=0.09$ ,  $N=365$ ,  $p=0.08$ ). We divided each unsigned FA value by the trait size (averaged over sides) and multiplied by 100. In this way, all parameter estimates in our statistical models reflect differences in percentages of asymmetry relative to trait size. By applying this correction, however, spuriously negative correlations between the size-corrected FA and size may emerge when ME is relatively large (because ME can be assumed to be an additive factor independent of size). One way to avoid this is to take several repeated measurements to minimize the degree of ME. However, since we do not have repeated X-rays for most fetuses, we could not take this source of ME into account. In spite of the fact that ME was small (on average about 10%, Table 1), we explored the possible effect of ME as confounding factor by comparing associations between size-corrected FA and age (and thus size, see below) among traits with different degrees of measurement error. On the basis of our estimates we would predict that if ME caused spurious correlations with size, associations between size-corrected FA and age would be strongest for femur, radius and digit 4 (highest ME) and weakest for tibia and fibula (ME nearly equal to 0, Table 1).

The size-corrected unsigned FA of each trait was then used as response variable in a linear model. Because different traits were measured on the same individuals, asymmetry levels were correlated among traits (also because of developmental integration, data not shown). To correct for this dependency, a repeated measures analysis was performed where correlations

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3 among asymmetry values were explicitly modeled assuming an unstructured variance-  
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5 covariance matrix. Degrees of freedom of F-tests and estimation of standard errors of  
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7 parameter estimates were obtained using Kenward and Rogers method in SAS (version 9,  
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9 procedure mixed). In a first analysis, FA was compared between fetuses that were grouped in  
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11 4 classes corresponding to i) those showing no detectable abnormalities (NONE); ii) a group  
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13 showing only minor congenital abnormalities (MINOR); iii) fetuses showing at least one  
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15 major abnormality (MAJOR) and iv) cases with major abnormalities including changes in  
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17 chromosomal numbers (for instance Down syndrome and other trisomies) (CHROM-  
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19 MAJOR). The identification of minor and major abnormalities were mainly based on the  
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21 model by Merks et al. (2003) and Lancaster and Pedisich (1995). We refer to Galis et al.  
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23 (2006) and figure 1 for further details. Next to these factors of fetal abnormalities, also  
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25 mother-specific abnormalities were included when sample sizes were sufficiently large. Age  
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27 (log-transformed) and gender were also added and relevant two-way interactions were tested.  
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29 This first analysis will allow investigating which broad types of abnormalities of different  
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31 severity lead to increased FA and to compare patterns among traits. In a second analysis, a  
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33 more detailed comparison of average asymmetry between congenital abnormalities related to  
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35 different functional units or developmental events was performed. Average asymmetry was  
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37 calculated for all individuals on the basis of the available traits. For about half of the fetuses,  
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39 digits could not be measured correctly and data for those two traits were missing. Thus,  
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41 average asymmetry was based on all 7 traits for nearly half of the fetuses, and based on 5  
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43 traits (without digits 2 and 4) for all others. In order to achieve this, the effect of the presence  
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45 or absence of a total of 17 types of disorders or phenodeviants on FA (within the group of  
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47 fetuses with at least one major congenital abnormality) was investigated relative to the control  
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49 group without any major congenital abnormalities. For a total of 277 fetuses with major  
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51 abnormalities, the following minor and major abnormalities were observed: cervical ribs  
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(61%), cardiovascular abnormalities (39%), limb defects (excluding poly-, olico- syn-, and  
 adactyly (23%)), urogenital abnormalities (23%), bleeding disorders (22%), abnormalities  
 related to midline patterning (20%), chromosomal number changes (19%), digestive system  
 abnormalities (19%), bronchopulmonary abnormalities (18%), dysmaturity (18%), neural  
 crest related abnormalities (14%), nervous system abnormalities (13%), prematurity (13%),  
 infections (10%), abnormalities related to left-right patterning (7%), major craniofacial  
 abnormalities (6%) and dactyly problems (poly-, oligo-, syn- and adactyly) (5%). In 78% of  
 the fetuses, multiple congenital abnormalities of the above types were observed, and the  
 occurrence of multiple abnormalities was also treated as a factor. The fact that many  
 abnormalities co-occur and some are more likely to co-occur than others renders the analysis  
 of the effect of these abnormalities on FA difficult (i.e., multicollinearity problem). First, to  
 evaluate the severity of the multicollinearity, we descriptively analyze the co-occurrence of  
 abnormalities using multiple correspondence analyses (MCA, Manly, 2005). Next, in order to  
 select a set of abnormalities that showed increased (or decreased) fetal FA, a model selection  
 procedure needed to be implemented. Because there were a total of 18 factors in the model  
 there is the problem of multiple testing and the fact that there are  $2^{18}=262144$  possible  
 models. In such situations a Bayesian approach, Stochastic Search Variable Selection (i.e.,  
 SSVS, George and McCulloch, 1993), has been put forward as an adequate approach. SSVS  
 assumes a linear model where the 18 estimates of the effects of each factor, the  $\beta_i$ , are  
 represented by a scaled mixture of two normal distributions:

$$\beta_i \sim (1 - \gamma_i)N(0, \sigma_1^2) + \gamma_i N(0, \sigma_2^2)$$

The first distribution corresponds to  $\beta$ 's which are small (i.e., no biologically relevant effect)  
 and the second corresponds to  $\beta$ 's which are relatively large and indicate an effect of the

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3 factor on the level of FA. The indicator variable  $\gamma_i$  can take on either value 0 or 1 for each of  
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5 the 18 factors in the model ( $i=1 \dots 18$ ). When the data support  $\gamma_i=0$  over  $\gamma_i=1$ , the  $\beta_i$  is  
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7 probably small enough so that factor  $i$  will not be needed in the model. If  $\gamma_i$  is more likely to  
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9 equal 1, the  $\beta_i$  probably indicates an effect of biological significance. This can be achieved by  
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11 setting  $\sigma^2_1$  small such that  $N(0, \sigma^2_1)$  is concentrated around zero and  $\sigma^2_2$  large enough such  
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13 that  $N(0, \sigma^2_2)$  is diffuse. The choice of the values of  $\sigma^2_1$  and  $\sigma^2_2$  can be based on  
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15 considerations of 'practical significance' and in particular for this study will rely on effects  
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17 observed in the first analysis. By performing an MCMC simulation using the Gibbs sampler,  
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19 the joint distribution of all  $\gamma_i$  can be approximated. The set of  $\gamma_i$  that simultaneously equal 1  
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21 most frequently have the highest posterior support and most likely present the model  
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23 containing all  $\beta_i$  that are required. Weak priors were assigned to all model parameters.  
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25 Analyses were performed in OPENBUGS (<http://mathstat.helsinki.fi/openbugs/>) where all  $\beta_i$   
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27 were given a normal prior with zero mean and variance equal to  $10^6$ . The  $\gamma_i$  were assumed to  
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29 follow a Bernoulli distribution with probability of success (i.e., variable selected in model)  
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31 equal to  $P$ . The parameter  $P$  was assigned a beta prior distribution with parameters 1 and 5. In  
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33 this way a slightly higher prior probability for simple models was imposed, but more complex  
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35 models were still allowed a priori. The analysis was based on 5 independent MCMC chains of  
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37 length 10000 after a burn in of 5000 iterations. Of the models receiving most posterior  
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39 support, parameter estimates were obtained to estimate the effects of these disorders on the  
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41 asymmetry. The major advantage of this type of analysis is that all possible models are a  
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43 priori being considered to be equally likely. When analyzing the data, an objective measure  
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45 that allows distinguishing between models that have different degree of support is obtained in  
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47 the form of the posterior distributions of the model parameters. This approach goes beyond a  
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49 simple comparison of significance levels among different factors, which is prone to type I and  
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51 type II errors. If a particular set of factors shows increased FA, and the others do not, we  
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3 expect that these factors will be present in a relative large proportion of the MCMC  
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5 simulations. Thus, this type of analysis will enable us to decide if the effects of the different  
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7 abnormalities are all of a comparable size, or if the effects rather behave as a mixture where  
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9 some have an effect on FA and others have not. By exploring the posterior distributions of  
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11 parameters  $\beta_i$  and  $p_i$ , we will further evaluate the effects of the different congenital  
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13 abnormalities on FA.  
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## 20 Results

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22 In a first analysis, FA was compared among 4 groups of congenital abnormalities: none  
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24 (N=92), minor (N=105), major (N=224), chromosomal-major (N=55). Age (log-transformed)  
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26 and gender were also added to the model. For mother-specific disorders, only few were  
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28 present in sufficient – albeit low – numbers, namely whether an infection was present or not  
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30 (4%) and whether there was an utero-placental insufficiency (6%). These mother-related  
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32 disorders were not related to the degree of fetal asymmetry (infection:  $F_{1,411}=0.07$ ,  $p=0.79$ ;  
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34 utero-placental insufficiency:  $F_{1,445}=0.15$ ,  $p=0.70$ ). Furthermore, there were no significant  
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36 differences between male and female fetuses ( $F_{1,435}=0.07$ ,  $p=0.49$ ). Asymmetry differed  
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38 significantly among the four congenital abnormality groups ( $F_{3,448}=3.05$ ,  $p=0.03$ ) and  
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40 decreased with age ( $F_{1,465}=6.11$ ,  $p=0.01$ ). As indicated above, the negative associations  
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42 between size-corrected asymmetry and age might have emerged as a by-product of our  
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44 correction for trait size and ME being independent of trait size. However, this negative  
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46 association was strongest when analyzing only tibia and fibula (slope=-0.61,  $p=0.005$ ) while  
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48 these traits showed very small degrees of ME (Table 1). For ulna and digit 2 (traits with  
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50 moderate ME) and femur, radius and digit 4 (traits with largest degrees of ME) only negative  
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52 trends were observed (slope=-0.4,  $p=0.07$  and slope=-0.3,  $p=0.08$ ). This result indicates that  
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3 increased levels of ME did not artificially strengthen associations between size-corrected-FA  
4 and age.  
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8 Asymmetry differed significantly among traits ( $F_{6,335}=20.3$ ,  $p<0.0001$ ) and Tukey post-hoc  
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10 pairwise comparisons revealed three groups of traits. Asymmetry was lowest in the radius  
11 (0.74%), intermediate in the femur (0.90%) and ulna (1.03%) and largest in the fibula  
12 (1.48%), digits 2 (1.47%) and 4 (1.67%) and tibia (1.53%). None of the two-way interactions  
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14 between trait and any of the other factors was significant (all  $p>0.15$ ). Thus, observed patterns  
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16 were consistent among traits.  
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22 When performing pairwise comparisons of asymmetry among the four congenital abnormality  
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24 groups, there was no difference between the none- and minor-group and between the major-  
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26 and chromosomal number changes-group. The none- and minor-group on the one hand  
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28 appeared to show a lower degree of asymmetry compared to the major- and chromosomal  
29  
30 number changes-major group (Fig. 3). On the average, the degree of asymmetry was 0.22%  
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32 higher in fetuses with major (chromosomal number changes or not) congenital abnormalities,  
33  
34 compared to the fetuses with no or minor abnormalities and this difference was highly  
35  
36 significant ( $F_{1,439}=8.47$ ,  $p=0.004$ ). In order to explore the association between asymmetry and  
37  
38 age graphically, average asymmetries across all traits was plotted against age for fetuses with  
39  
40 and without major congenital abnormalities. The difference in FA between these two groups  
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42 was relatively small compared to between-fetus variation in asymmetry (Fig. 4), which could  
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44 suggest that not all abnormalities affected FA to the same extent.  
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53 The co-occurrences of different types of abnormalities in fetuses with major abnormalities  
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55 were weak as the first two components of the MCA explained only 24% of the total variation.  
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57 A graphical representation of these two components (Fig. 5) allowed visualizing which types  
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59 of abnormalities co-occurred more frequently (when they are located in closer proximity on  
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3 the graph). Craniofacial and dactyly problems appeared to co-occur often, as well as  
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5 premature birth and bleeding disorders. To a lesser extent, problems related to the neural  
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7 crest, and patterning along the midline and the left-right axis were observed together more  
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9 often as well (Fig. 5). Nevertheless, problems of multicollinearity were probably small.

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11 Based on the Bayesian SSVS analysis of average asymmetry, the posterior support, i.e.,  
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13 proportion of MCMC simulations in which each possible model was sampled, for the 20 most  
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15 likely models is provided in figure 6. The model with most support occurred in approximately  
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17 14% of all MCMC simulations. All others obtained half or less support. This 'best' model  
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19 contained the following factors (parameter estimate (SE), reflecting the 'effect' of this  
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21 abnormality on the average level of FA as a percentage): problems in left-right patterning  
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23 (0.63% ( $\pm 0.19\%$ )), limb defects (excluding dactyly problems) (0.40% ( $\pm 0.10\%$ )),  
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25 bronchopulmonary disorders (0.34% ( $\pm 0.11\%$ )) and uro-genital disorders (0.40% ( $\pm 0.11\%$ ))  
26  
27 and disorders in the digestive system (-0.55% ( $\pm 0.12\%$ )). In models 2 and 3, which had  
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29 between 7 and 8% support, these 5 factors were also present, and additionally also dactyly  
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31 and prematurity respectively. However, 95% credibility intervals of the difference in FA  
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33 values contained zero such that they were not statistically significant in a frequentist  
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35 interpretation. The presence of a limited number of models which get relatively high support  
36  
37 over the many other possible models as observed here (Fig. 6), confirmed that we could  
38  
39 distinguish between two types of congenital abnormalities, namely those that were associated  
40  
41 with increased FA and those that were not. Indeed, an examination of the posterior  
42  
43 distributions of the effects of each factor further supported that the 'best' model based on this  
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45 SSVS analysis contained all factors which showed a very strong support for an effect on  
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47 levels of FA, while most other factors received little support (Fig. 7). One exception could be  
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49 premature birth, which might be associated with lower FA (Fig. 7). We thus conclude that our  
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51 data provide evidence that FA was larger in fetuses with problems in left-right patterning,  
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3 limb defects and bronchopulmonary and uro-genital disorders. Somewhat unexpectedly, FA  
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5 was lower in fetuses with problems of the digestive system. The latter counterintuitive result,  
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7 however, might be attributed to the problem of multicollinearity because problems of the  
8  
9 digestive system appear to co-occur frequently with bronchopulmonary and uro-genital  
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11 problems and problems in left-right patterning (Fig. 5). This interpretation was further  
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13 supported by the fact that any of the 5 factors in the above 'best' model is statistically  
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15 significant in simple t-tests ( $p < 0.001$ ), except for the digestive system problems ( $p = 0.2$ ).  
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19 Although one could correctly argue that the absence of a significant effect of a particular  
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21 abnormality does not prove that the null hypothesis is correct, a further exploration of the  
22  
23 average asymmetries supported that only a subset of the abnormalities increased FA.  
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25 Apparently, the above identified types of congenital abnormalities that increased FA  
26  
27 significantly explain the higher FA in fetuses with major abnormalities entirely. A linear  
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29 model including a factor indicating the presence of at least one of these 4 abnormalities  
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31 eliminated the significance of the effect of the presence of other major congenital abnormality  
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33 ( $F_{1,379} = 0.26$ ,  $p = 0.61$ ). The association with age, however, remained statistically significant  
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35 ( $F_{1,379} = 10.1$ ,  $p = 0.002$ ). The average degree of asymmetry in fetuses without major congenital  
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37 abnormalities (mean asymmetry = 1.11% of trait size based on 141 fetuses) did not differ  
38  
39 significantly from that of fetuses with major abnormalities excluding problems of left-right  
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41 patterning, limb defects, bronchopulmonary and uro-genital problems (mean  
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43 asymmetry = 1.01% of trait size based on 120 fetuses) (difference in average asymmetry =  
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45 -0.10%, 95% confidence interval: -0.28 – 0.09%,  $t_{379} = 0.92$ ,  $p = 0.36$ ). In fact, the latter group of  
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47 fetuses showed a lower – albeit not significantly so – average asymmetry in spite of having  
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49 experienced major developmental disturbances other than the four identified above. The upper  
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51 limit of the 95% confidence interval indicates that the maximal increase in FA due to the  
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53 other major congenital abnormalities is equal to 0.09%. Asymmetry of fetuses with major  
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3 congenital abnormalities related to problems of left-right patterning, limb defects, the  
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5 bronchopulmonary or uro-genital system showed significantly higher asymmetry (mean  
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7 asymmetry=1.49% of trait size based on 125 fetuses) compared to the fetuses without major  
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9 abnormalities (difference in average asymmetry=0.38%, 95% confidence interval: 0.20 –  
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11 0.58%,  $t_{379}=3.81$ ,  $p=0.0002$ ) and those with other major abnormalities (difference in average  
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13 asymmetry=0.48%, 95% confidence interval: 0.28 – 0.67%,  $t_{379}=4.55$ ,  $p<0.0001$ ).  
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## 19 Discussion

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24 *Associations between fluctuating asymmetry, congenital abnormalities, maternal effects and*  
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26 *age*  
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31 It is unknown to what extent different disorders affect developmental instability in the  
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33 bilaterally symmetric traits such as arms, hands and legs in humans. Such insights are crucial  
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35 to be able to evaluate the usefulness of fluctuating asymmetry as a measure of stress and  
36  
37 fitness, and to evaluate to what extent developmental perturbations can destabilize  
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39 developmental buffering mechanisms. When developmental buffering systems are  
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41 destabilized, phenotypic variation is increased and the expression of genetic variability may  
42  
43 be released, potentially affecting evolutionary changes (Badyaev & Foresman, 2000; Breuker  
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45 et al., 2006). In our population of deceased human fetuses, maternal problems (infection and  
46  
47 utero-placental insufficiency) did not affect FA, but asymmetry increased with the severity  
48  
49 of the fetal disorder (major vs. no or minor) and decreased with age. These results indicate  
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51 that at least some aspects of fetal death and malformation affect the stability of limb  
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53 development.  
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3 Associations between asymmetry and many other measures of health and fitness reported in  
4 the literature consist of health and fitness proxies that were not as detrimental as those  
5 observed in our population (e.g., Møller 2006). In this study, fetuses with major congenital  
6 abnormalities can be regarded as individuals in which development was extremely severely  
7 disturbed in nearly the worst possible way. Therefore, we had hypothesized that the effects of  
8 these major congenital abnormalities on FA would be among the strongest effects observed  
9 compared with previous studies. To compare our results to effect sizes routinely observed on  
10 human FA, we analyzed 27 studies that allowed to calculate effect sizes and/or proportional  
11 differences in FA (Appendix 1). On the basis of these studies, we combined a total 71 effect  
12 sizes and 24 proportional differences in boxplots and contrasted effect sizes from this study  
13 with those more general results (Fig. 8). Unexpectedly, the effect of major abnormalities and  
14 age in our deceased fetuses were of the same magnitude as the average effect sizes found in  
15 the literature. All other effect sizes in this study (minor vs. no abnormalities, maternal  
16 infection and utero-placental insufficiency) were nearly zero, while mostly positive ones have  
17 been published in the literature.

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19 The negative association that we found between FA and age confirmed our a priori  
20 expectations. However, although we were unable to generate sufficient repeats and  
21 measurement error (ME) was low, our analyses showed no indications that the negative  
22 correlation was a result of our size correction. In agreement, a negative association between  
23 FA and gestational age was also observed in a large study of surviving newborn infants by  
24 Kobylansky et al. (1990). There are however two biological explanations for this negative  
25 association. Possibly, age of birth or death may be related to health problems such that more  
26 severely disturbed developments lead to earlier birth or death. In turn, these health problems  
27 may increase FA such that the negative association between age and FA indicate that FA  
28 signals health status. Alternatively, the higher FA in younger fetuses and preterm newborns  
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3 may also be the result of the higher growth rates during earlier development, as found in mice  
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5 (Hallgrímsson et al., 2003). Thus, further study on the prenatal evolution of FA in a healthy  
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7 population will be required to evaluate the causation of this association between FA and age.  
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12 *Congenital abnormalities of different developmental origins or functional systems*  
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14 *heterogeneously affect fluctuating asymmetry*  
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20 Many types of congenital abnormalities did not appear to significantly increase asymmetry.  
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22 This heterogeneity was not due to differences in occurrence of the disorders (i.e., no power  
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24 problem). For example, cervical ribs occurred in 61% of the cases but were not associated  
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26 with increased asymmetries in spite of its severe fitness consequences (Galís et al., 2006). On  
27  
28 the other hand, problems in left-right patterning were significantly associated with asymmetry  
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30 but constituted only a small fraction (7%). The disorders that were significantly associated  
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32 with the level of asymmetry were left-right patterning disorders, limb defects (excluding  
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34 dactily problems), bronchopulmonary and uro-genital disorders. The higher asymmetry in  
35  
36 fetuses with major congenital abnormalities was entirely due to the effects of these four  
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38 groups. Although on the average, fetuses with at least one of these 4 abnormalities showed an  
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40 increased FA by 38% compared to fetuses without major abnormalities, the effect size was  
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42 also moderate compared to patterns in the literature (Fig. 8).  
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49 It makes intuitive sense that a disturbance of the left-right patterning may disturb the left-right  
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51 symmetry. A large part of the bronchopulmonary disorders were due to lung hypoplasia and  
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53 this is often indicative of asymmetric pressure within the thorax, either due to  
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55 oligohydramnion (too little amniotic fluid) or because of a diaphragmatic hernia, an abnormal  
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57 opening in the diaphragm through which the intestines protrude into the thorax. Limb defects  
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59 (excluding dactily problems) were dominated by club foot (pes equinovares), rocker bottom  
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3 foot, crossed fingers and (other) abnormal positions of fingers, hand or feet. Whenever the  
4 position of a trait was dubious to be measured, it was not included in the analyses, therefore,  
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6 the higher FA was not an artefact of the abnormalities directly. However, it could be an  
7  
8 indirect effect, because limb defects are often unilateral and possibly the disturbed  
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10 development that lead to the congenital limb defects also influenced growth rate on the same  
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12 side as the defect. Thus, both problems of the bronchopulmonary system and limbs defects  
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14 could have increased asymmetry indirectly. However, since all four types of abnormalities  
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16 were associated with a very comparable increase in FA, a direct effect on the mechanisms of  
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18 developmental instability appears more likely. The increased FA in fetuses with urogenital  
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20 problems may at first glance be unexpected. However, it could hint towards an involvement  
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22 of homologous developmental pathways in limbs and the urogenital system including 5'  
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24 members of the Hoxa and Hoxd clusters (Hoxa9-13 and Hoxd9-13) or other genes involved in  
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26 the formation of both limbs and genitalia (Du and Taylor, 2004; Cohn, 2004). Changes in the  
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28 number of digits were, however, not associated with FA levels, but this may be due to the  
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30 small number of cases (5%).

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38 It is surprising that many other congenital abnormalities appear not to affect DI and FA in our  
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40 population. Previous studies have found increased dental FA in people with Down syndrome  
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42 (Thownsend 1983, 1987), yet, in our study abnormalities in chromosomal numbers did not  
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44 lead to higher FA. The higher asymmetry in the group of fetuses with chromosomal  
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46 abnormalities in figure 2 (chrom-major) was not supported by the formal comparison of the  
47  
48 different types of congenital abnormalities using the Bayesian SSVS model. The main  
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50 difference between both approaches is that the latter explicitly corrects for the presence of  
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52 other congenital abnormalities. The higher FA in fetuses with chromosomal abnormalities is  
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54 therefore likely to be the result of the co-occurrence of other abnormalities in this group. It  
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56 turned out that 60% of the fetuses with chromosomal abnormalities also showed limb defects  
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3 which did increase FA. Developmental delay (slower growth) has also been found to increase  
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5 FA (Naugler & Ludman, 1996; Møller, 1999), yet we did not find this in our study as  
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which did increase FA. Developmental delay (slower growth) has also been found to increase FA (Naugler & Ludman, 1996; Møller, 1999), yet we did not find this in our study as dysmaturity and utero-placental insufficiency did not coincide with increased FA here either. The presence of cervical ribs, as indicator of medical risks (Steigenga et al., 2006; Galis et al., 2006), could have been expected to increase FA as well. However, previous studies have shown that the presence of cervical ribs as such probably have little direct fitness consequences, but rather the pleiotropic effects appear to be detrimental. Nevertheless, the absence of higher FA with cervical ribs suggests that the developmental modification at the level of the vertebrae has no effect on the stability of limb development. In conclusion, the comparison of effects of congenital abnormalities that were associated with different developmental origins or functional systems supports the hypothesis that only some of the severe developmental perturbations affect the stability of the development of limb bones.

*Is there evidence for early elimination of highly asymmetric individuals?*

It has been hypothesized that during early development, individuals with high levels of DI and FA would be eliminated before they enter the surviving population (Møller, 1997). This hypothesis has only rarely been tested. On average, asymmetry in our population of deceased fetuses and a few infants, equaled about 1-1.5% of the trait size. It is, however, difficult to compare this level to that of the healthy population, because we cannot perform these measurements on healthy fetuses in utero. Comparisons of our estimates to those of limb asymmetry in adults are complicated by the potential effect of mechanical loading which may either increase asymmetry due to lateralization of behavior (Cuk et al., 2001; Auerbach and Ruff, 2006; Van Dongen et al., 2009) or decrease asymmetry when limbs are used more symmetrically (Pettersson et al., 2000). Although it has been suggested that behavioral

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3 lateralization may already affect asymmetry in fetal limbs, results are sometimes based on  
4 small samples and/or appear to show contradictory patterns (Schultz, 1926; Pande & Singh,  
5 1971; Bagnall et al., 1982; Gawlikowska et al., 2007). In this study population no indications  
6 of directional asymmetry were found (above and Van Dongen et al., submitted for more  
7 elaborate analyses).

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10 Nonetheless, we attempted to compare our measurements to the available asymmetries in  
11 bone lengths of the same traits as ours and based on either direct measurements or  
12 radiographs. Average FA equaled 1.26% in our study and 1.24% across all estimates we  
13 retrieved from the literature (Table 2). Arguably, the average degrees of asymmetry in adults,  
14 young children and fetuses in other studies are of the same magnitude as our estimates (Table  
15 2). Hence, we conclude that at present there is no convincing evidence that limb asymmetry is  
16 exceptionally high in our population of fetuses with severe developmental disorders.  
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### 34 *Concluding remarks*

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38 In spite of studying a population with severe abnormalities and zero fitness, FA of limb bone  
39 increased only moderately. This suggests strong buffering of limb development, which is  
40 presumably due to stabilizing selection for symmetrical limbs (Garland and Freeman, 2005).  
41 This also implies that the breakdown of developmental buffering and the expected associated  
42 increase in morphological variation and presumed release of expression of genetic variation,  
43 is limited in traits with high functional importance like the vertebrate limbs. This  
44 interpretation is in agreement with the observation that in shrew mandibles stability of  
45 development of functionally integrated parts of the mandible appear more strongly buffered  
46 against stress compared to the non-integrated part. In turn, these differences in sensitivity to  
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3 stress appeared to play an important role in species divergence (Badyaev and Foresman,  
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5 2000).

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8 Furthermore, although more research is required, our results failed to support the hypothesis  
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10 that individuals with high DI and FA would be eliminated during early development (Møller,  
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12 1997; Van Dongen 2006). While the severity of congenital abnormalities increased FA,  
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14 within the group of fetuses with major congenital abnormalities, only few types of  
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16 abnormalities appeared to significantly increase FA. This could again be attributable to a  
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18 presumed strong selection for symmetry in limbs, and suggests that particular abnormalities  
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20 increase asymmetry in later developmental stages only. Nevertheless, we clearly  
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22 demonstrated that depending on the functional system and/or developmental event, congenital  
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24 abnormalities have varying effects on the developmental instability in fetuses. Fluctuating  
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26 asymmetry measures in limbs cannot be regarded as a general measure of the effects of  
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28 developmental disorders because many congenital abnormalities with important fitness effects  
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30 did not increase FA.  
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36 In sum, the observed patterns in this paper clearly show that specific developmental pathways  
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38 and/or defects in particular functional systems have stronger effects on DI than others and this  
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40 heterogeneity will form the basis for further research into the developmental and/or genetic  
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42 basis of developmental instability.  
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Table 1. Overview of levels of measurement error as a result of mounting the fetus and taking a photograph on the one hand (ME-photogr.) and of measuring on a single photograph (ME-meas.) relative to real FA, percentage of variation due to ME (%ME) and levels of directional asymmetry (DA) in different bones of deceased fetuses. Variance components were multiplied by 1000.

Trait	real FA	ME-photogr.	ME-meas.	%ME	DA
Femur	4.37	0.45	0.19	13	$F_{1,427}=0.56$ ( $p=0.45$ )
Tibia	8.47	0.00	0.16	2	$F_{1,431}=0.14$ ( $p=0.70$ )
Fibula	14.2	0.00	0.28	2	$F_{1,303}=0.08$ ( $p=0.77$ )
Radius	2.32	1.18	0.48	42	$F_{1,442}=3.96$ ( $p=0.05$ )
Ulna	12.6	0.63	0.18	6	$F_{1,444}=0.32$ ( $p=0.57$ )
Digit 2	1.90	0.00	0.15	7	$F_{1,229}=0.00$ ( $p=0.99$ )
Digit 4	2.41	1.65	0.12	42	$F_{1,210}=1.43$ ( $p=0.23$ )

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Table 2: Overview of estimates of size-corrected asymmetries (in %) in lengths of limb bones in this study and in the literature. The last column presents the average across all available traits.

femur	tibia	fibula	radius	ulna	digit 2	digit 3	digit 4	digit 5	humerus	hand	average	ref.
0.90	1.53	1.48	0.74	1.03	1.47	-	1.67	-	-	-	1.26	1
-	-	-	-	-	-	-	-	-	1.30	-	1.30	2
-	-	-	-	-	-	-	-	-	1.50	-	1.50	3
0.78	0.55	-	1.20	-	-	-	-	-	1.50	-	1.00	4
-	-	-	-	-	-	-	-	-	1.20	-	1.20	5
0.52	0.93	-	1.02	-	-	-	-	-	0.83	-	0.82	6
0.64	0.60	-	1.00	-	-	-	-	-	1.00	-	1.00	7
-	-	-	-	-	0.90	1.10	1.00	1.15	-	-	1.04	8
-	-	-	-	-	-	-	-	-	-	1.90	1.90	9
0.00	1.72	-	-	2.46	-	-	-	-	2.08	-	1.57	10



- 1: this study
- 2: Schultz (1926): direct measurements in deceased fetuses
- 3: Schultz (1926): direct measurements in deceased adults
- 4: Auerbach and Ruff (2006): direct measurements in adults
- 5: Auerbach and Raxter (2008): direct measurements in adults
- 6: Hallgrimsson (1999): direct measurements in juveniles and subadults (raw data kindly provided by Prof. Hallgrimsson)
- 6: Hallgrimsson (1999): direct measurements in adults (raw data kindly provided by Prof. Hallgrimsson)
- 8: Livshits et al. (1998): X-rays in adults, means obtained from figure 3
- 9: McLeod and Coupland (1992): X-rays in adults, average across 19 hand bones
- 10: Kanchan et al. (2007): direct measurements on a single recent skeletal remain

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3 Figure 1: Examples of minor and major congenital abnormalities in our population of  
4 deceased fetuses  
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8 A) Female fetus without notable congenital abnormalities of 40 weeks gestation which died  
9 due to complications during birth; B) Male fetus of 28 weeks gestation with excessive hair  
10 growth (minor abnormality); C) an example of low-set ears which are considered to be minor  
11 and cervical ribs in a female fetus of 22 weeks gestation; D) Thanatophoric dysplasia in a  
12 male fetus of 20 weeks gestation; E) Macrocephaly, scoliosis and wavy ribs in a male fetus of  
13 18 weeks gestation. F) A full term female fetus of 42 weeks gestation with anencephaly (the  
14 rostral part of the neural tube fails to close and there is no development of the brain) and cleft  
15 palate; G) A female fetus of 18 weeks gestation showing mosaic Turner syndrome and  
16 cervical ribs; H) Female fetus of 25 weeks gestation with amnion disruption syndrome (a  
17 congenital disorder caused by entrapment of fetal parts (usually a limb or digits) in fibrous  
18 amniotic bands while in utero); and I) Female fetus of 20 weeks showing multiple major  
19 congenital abnormalities, which could not be identified easily due to a strong hydrops foetalis  
20 (excessive extravascular fluid accumulation).  
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41 Figure 2: Left: Radiograph of an entire fetus. Lines indicate measurements of limb bones  
42 from the midpoint of the proximal end of the bones to the midpoint of the distal end; Right:  
43 Radiograph of right hand. Lines indicate length of the midpoint of the proximal end of the  
44 proximal phalanx to the midpoint of the distal end of the distal phalanx.  
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53 Figure 3: Average size corrected asymmetries (percentage of trait size) in human fetuses for  
54 the 4 groups of congenital abnormalities. Based on pairwise comparisons two significantly  
55 different groups (white vs. black bars) were identified (see text for details).  
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3 Figure 4: Relationship between mean size-corrected asymmetry (percentage of trait size) and  
4 age (log transformed) for human fetuses without any major congenital abnormalities (gray)  
5 and those with at least one major congenital abnormality including major chromosomal  
6 abnormalities (black).  
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15 Figure 5. Plot of the two first components of the multiple correspondence analysis on the  
16 occurrence of the different types of congenital abnormalities in human fetuses.  
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22 Figure 6: Posterior support (i.e., proportion of iterations that contained a particular set of  
23 factors) for the 20 most likely models as based on the Bayesian SSVS analysis (see text for  
24 details).  
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31 Figure 7: Posterior distributions of estimates of the effect of each category of congenital  
32 abnormalities on fluctuating asymmetry (FA) where positive values indicate an increase of  
33 FA. Percentages within brackets indicate the proportion of MCMC simulations in which the  
34 different factors were included. Thus, high proportions indicate strong support for an effect of  
35 that particular factor.  
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45 Fig 8: boxplots of effect sizes (left) and percentage difference in FA (right)  
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For Review Only

Appendix (possibly to be added as online material?)

Disease/health/quality Measure	Effect size	Percentage difference	Number of traits	Sample size	Year	remarks	reference
Lower back pain	0.11	9	9	95	2004		1
Intelligence	0.21	-	9	112	1997	study 1	2
	0.24	-	9	128	1997	study 2	2
Atypical prenatal hormones (digit ratio)	0.07	-	7	116	2008	males	3
	0.10	-	7	172	2008	females	3
Hormonal stress (sexual orientation)	0.36	36	7	116	2008	males	3
	0.52	50	7	172	2008	females	3
Breast cancer	0.24	47	1	500	1997		4
Depression	0.29	-	6	52	1999	males	5
	0.05	-	6	50	1999	females	5
Number of children	0.30	36	1	172	1995	Spain	6
	0.34	97	1	50	1995	New Mexico	6
Number of sperm	0.31	-	3	50	2003		7
Sperm motility	0.28	-	3	50	2003		7
Sperm head length	0.30	-	3	50	2003		7
Sperm tail length	0.10	-	3	50	2003		7
Number of sperm	0.28	-	4	53	1998		8
Average sperm speed	0.33	-	4	53	1998		8
Sperm migration test	0.20	-	4	53	1998		8
Azoospermia	0.24	33	4	53	1998		8
Body Mass Index	0.09	-	6	965	2003		9
Waist/hip ratio	0.03	-	6	965	2003		9
Systolic blood pressure	0.00	-	6	965	2003		9
Cholesterol	0.01	-	6	965	2003		9
Fitness	0.03	-	6	965	2003		9
Fetal alcohol syndrome	0.40	106	1	53	1993		10
Fetal alcohol effect	0.20	43	1	40	1993		10

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4	Maternal smoking	-	8	6	215	1997	11
5	Maternal obesity	-	141	6	225	1997	11
6	Maternal smoking and obesity	0.19	215	6	222	1997	11
7	Psychiatric patients vs. employees	-	25	10	71	1999	12
8	Maternal immunoreactivity (number of older brothers)	0.30	-	10	71	1999	12
9							
10	Maternal alcohol and paternal smoking	0.26	-	8	651	2007	boys
11							
12		0.17	-	8	585	2007	girls
13	Heterozygosity	-0.02	-	26	200	1993	
14	Extremely preterm vs. control	-	28	8	126	1988	Children
15	Preterm vs. control	-	12	8	196	1988	Children
16	Extremely preterm vs. control	-	23	8	126	1988	Mother
17	Preterm vs. control	-	6	8	196	1988	Mother
18	Extremely preterm vs. control	-	21	8	126	1988	Father
19	Preterm vs. control	-	6	8	196	1988	Father
20	Low birth weight	0.02	4	4	436	2004	
21	Preterm vs. control	0.31	93	8	572	2000	
22	Maternal alcohol	-	99	16	232	1992	
23	Hormonal stress (sexual orientation)	0.21	18	5	99	2008	males
24							
25		0.03	6	5	68	2008	females
26							
27							
28							
29	Respiratory infections:						
30	Number of infections	0.07	-	7/9	203	2006	Males-body
31	Number of days infected	0.08	-	7/9	203	2006	Males-body
32	Number of infections	0.16	-	10	203	2006	Males-facial
33	Number of days infected	0.15	-	10	203	2006	Males-facial
34	Number of infections	0.11	-	7/9	203	2006	Females-body
35	Number of days infected	-0.04	-	7/9	203	2006	Females-body
36	Number of infections	0.08	-	10	203	2006	Females-facial
37	Number of days infected	0.13	-	10	203	2006	Females-facial
38	Stomach and intestine infections:						
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Number of infections	0.01	-	7/9	203	2006	Males-body	20
Number of days infected	-0.02	-	7/9	203	2006	Males-body	20
Number of infections	-0.03	-	10	203	2006	Males-facial	20
Number of days infected	-0.14	-	10	203	2006	Males-facial	20
Number of infections	0.14	-	7/9	203	2006	Females-body	20
Number of days infected	0.09	-	7/9	203	2006	Females-body	20
Number of infections	-0.05	-	10	203	2006	Females-facial	20
Number of days infected	0.06	-	10	203	2006	Females-facial	20
Antibiotics use	0.05	-	7/9	203	2006	Males-body	20
	-0.01	-	7/9	203	2006	Females-body	20
	0.13	-	10	203	2006	Males-facial	20
	0.08	-	10	203	2006	Females-facial	20
ADHD	-0.07	-	5	61	2006	Male-body	21
	-0.05	-	2	61	2006	Male-ridge	21
	0.06	-	4	61	2006	Male-facial	21
	0.24	-	5	161	2006	female-body	21
	0.07	-	2	161	2006	female-ridge	21
	-0.06	-	4	161	2006	female-facial	21
Birth order	0.23	-	10	205	2004		22
Season of birth	0.29	-	10	205	2004		22
Number of partners	0.30	-	7	122	1994		23
Age of first sexual contact	0.40	-	7	122	1994		23
Number of partners	0.27	-	8	100	2009		24
Age of first sexual contact	0.33	-	8	100	2009		24
Likelihood of sexual contact outside stable relationship	0.22	-	8	100	2009		24
Number of partners	0.27	-	9	271	2001		25
Intelligence	0.25	-	6	81	2006		26
Hormonal stress (sexual orientation)	0.11	-	2	410	2008		27

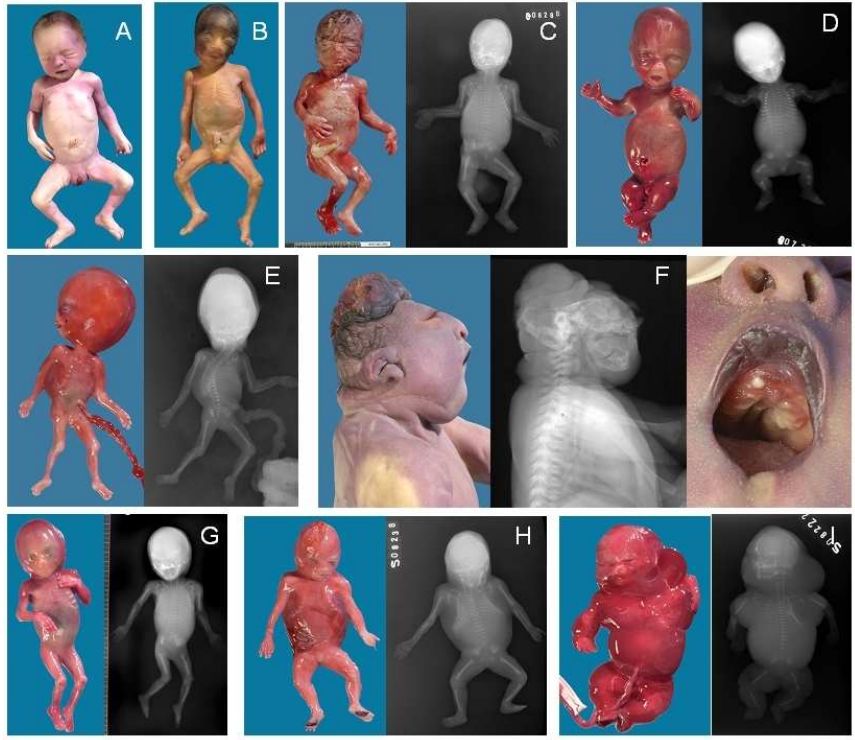
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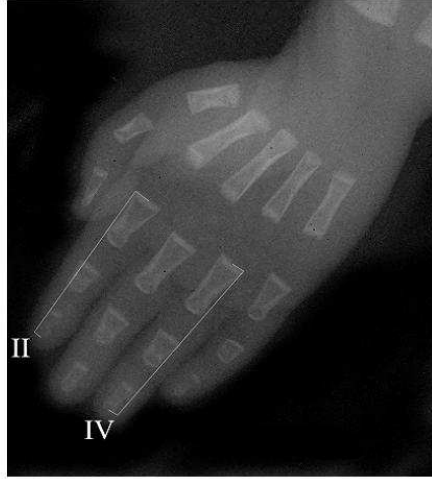
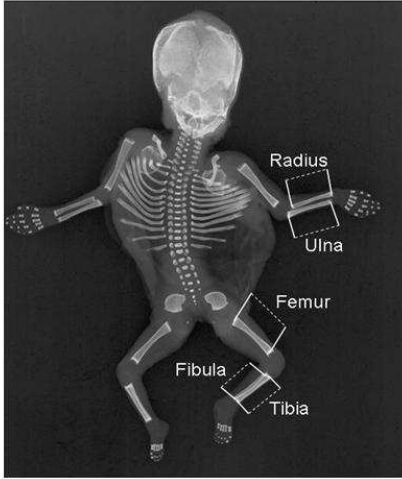
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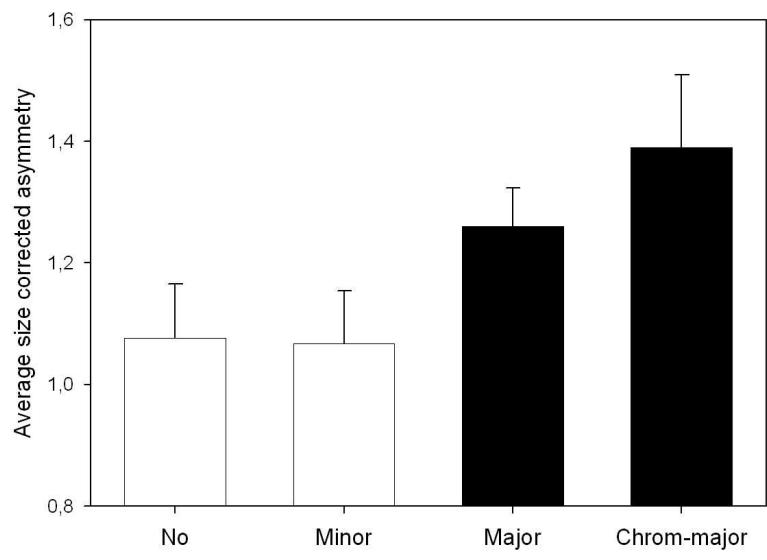
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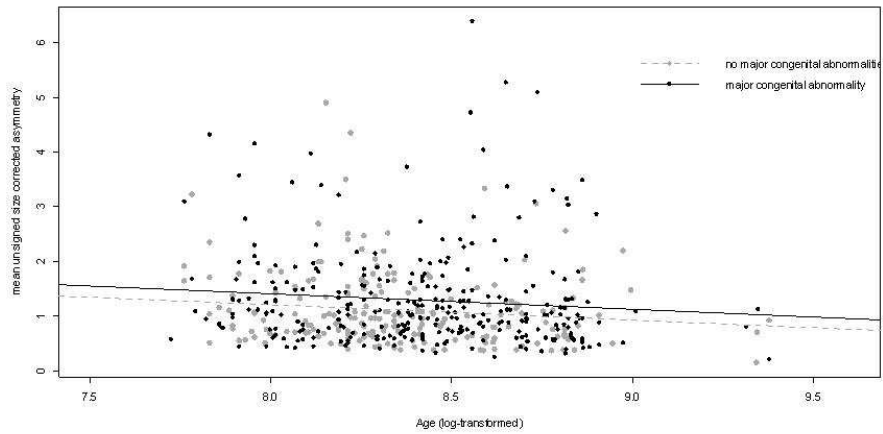
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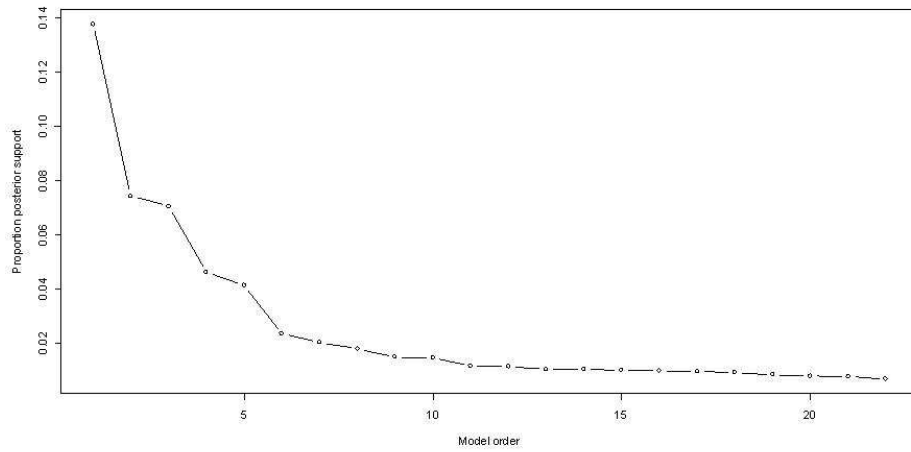


254x190mm (96 x 96 DPI)

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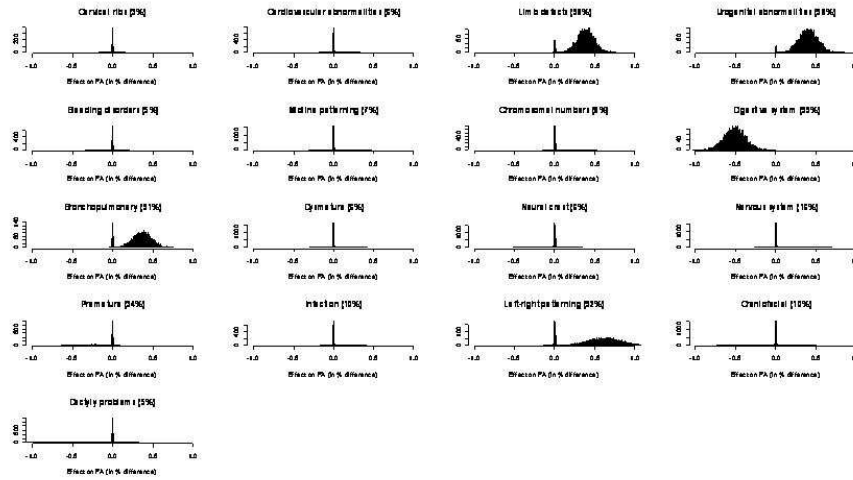
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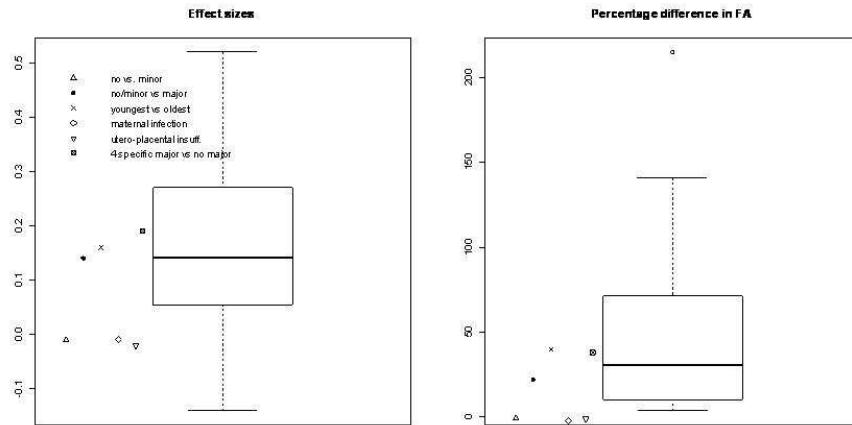


254x190mm (96 x 96 DPI)

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254x190mm (96 x 96 DPI)

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