The molecular genetics of hand preference revisited

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Abstract

Hand preference is a prominent behavioural trait linked to human brain asymmetry. A handful of genetic variants have been reported to associate with hand preference or quantitative measures related to it. Most of these reports were on the basis of limited sample sizes, by current standards for genetic analysis of complex traits. Here we performed a genome-wide association analysis of hand preference in the large, population-based UK Biobank cohort (N=331,037). We used gene-set enrichment analysis to investigate whether genes involved in visceral asymmetry are particularly relevant to hand preference, following one previous report. We found no evidence implicating any specific candidate variants previously reported. We also found no evidence that genes involved in visceral laterality play a role in hand preference. It remains possible that some of the previously reported genes or pathways are relevant to hand preference as assessed in other ways, or else are relevant within specific disorder populations. However, some or all of the earlier findings are likely to be false positives, and none of them appear relevant to hand preference as defined categorically in the general population. Within the UK Biobank itself, a significant association implicates the gene *MAP2* in handedness.

Introduction

Hand preference is a conspicuous behavioural trait, with about 10% of people preferring to use their left hand for many tasks, about 1% having no preference, and the large majority preferring to use the right hand [1]. Hand preference is probably initiated during prenatal phases, and further established in early infancy [2-5]. Gene expression analysis has revealed left-right differences in the human central nervous system as early as four weeks post conception [6,7], which indicate that laterality is an innate and pervasive property of the central nervous system. Across different regions of the world and throughout history, the behavioural variation has persisted, albeit that cultural suppression of left-handedness has sometimes occurred [8-11].

There has been much interest in possible causes of hand preference, as well as its associations with cognitive variation and disorders [12-15]. The existence of a genetic component for hand preference has been investigated in large twin studies, consisting of thousands of individuals. These studies found additive heritabilities of around 25% for left-hand preference [16-19]. In terms of individual genetic loci, monogenic models for hand preference were originally conceived, such as the dextral/chance model [20]. The model proposed that there are two alleles, D (dextral) and C (chance), with the homozygous DD genotype producing right-handers (directional asymmetry), the homozygous CC genotype producing random asymmetry (50% right-handers and 50% left-handers) due to a hypothesized loss

of direction-setting in brain development, and the heterozygote, DC, being intermediate and producing 25% left-handers and 75% right-handers. Another related idea, conceived with respect to continuous measures of left-versus-right hand motor skill, was the 'right-shift' model, which proposed a major gene whose alleles cause either rightward mean asymmetry, or no directional bias [21].

However, genetic linkage analyses in families with elevated numbers of left-handed members have not found convincing evidence for single major genes [22-24]. Genome-wide association scan (GWAS) analysis has also not found any genomic locus with a large enough effect on hand preference to be compatible with monogenic models, unless there would be extensive genetic heterogeneity (i.e. many different Mendelian effects involved in the population)[25]. Meanwhile, as analyses based on twins have indicated 25% heritability, there is clearly ample room for environmental influences, although these remain mostly unknown. Early life variables such as birthweight and twinning can influence handedness, but not to an extent which is remotely predictive at the individual level [26,27].

Various molecular genetic studies have identified individual genes as potential contributing factors to handedness, with small effects, which would be consistent with a polygenic architecture underlying the heritable component to this trait. We review these genes briefly here:

- A sib-pair analysis (N=89 sibships with reading disability) found suggestive linkage of left versus right relative hand skill, a continuous measure based on the pegboard task [28], to a broad region on chromosomes 2 [29]. A follow-up study suggested the signal came from paternal inheritance [30]. Further analysis suggested *LRRTM1* (leucine rich repeat transmembrane neuronal 1) as the most likely gene, as it showed evidence for paternal-specific association with relative hand skill of a particular haplotype, as well as imprinted expression in cell lines [31].
- A CAG-repeat polymorphism in the gene *AR* (androgen receptor), located on chromosome X, has been studied in relation to hand preference, due to a longstanding theory that brain laterality is influenced by sex hormones [32]. One study found association effects which were opposite in males and females for the CAG-repeat [33]. A different study found that right-handedness was associated with shorter CAG repeats in both sexes [34], while another, which only included males, found that mixed-handedness was associated with longer CAG repeats [35].
- In a set of families affected by bipolar disorder, an association was found of a variant in *COMT* (catechol-O-methyltransferase) with relative hand skill, although this did not survive multiple testing correction [36]. *COMT* was studied because it was considered a candidate gene for bipolar disorder [37].

- A study of the genes *SETDB1* (SET domain bifurcated 1) and *SETDB2* (SET domain bifurcated 2) found that the single nucleotide polymorphism (SNP) rs4942830 in S*ETDB2* was significantly associated with hand preference as measured as a quantitative trait on the Edinburgh scale [38] (N=950 healthy people), although for the binary trait of left versus right-handedness, the p-value did not survive multiple testing correction [39]. This result was replicated, including the stronger effect for the quantitative trait (right=732, left=75, healthy people)[40]. *SETDB2* was chosen as a candidate for study as it has been shown to regulate structural left-right asymmetry in the zebrafish central nervous system [41].
- A GWAS study of relative hand skill, in a sample of people with reading problems (3-stage design, N=728), found a genome-wide significant association in the gene *PCSK6* (proprotein convertase subtilisin/kexin type 6) [42]. The same research group performed a second GWAS in a cohort of individuals from the general population (N=2666), and found no genome-wide significant associations, and no indication of involvement of *PCSK6* [43]. The GWAS results also yielded evidence that genes influencing visceral asymmetry might be involved in relative hand skill in both the dyslexic group and the general population group. For this analysis, the authors tested sets of genes for an enrichment of association signals in the GWAS data, where the sets were defined based on mutations in mice that influence the organization of the visceral organs on the left-right axis [43].
- In a GWAS study of 263 left vs 2092 right-handers, and 173 left vs 1412 right-handers (this was a study of healthy twins, and the GWAS analysis was divided to achieve non-relatedness of subjects), no genome-wide significant results for self-reported hand preference were found [25]. The most significant associations were near the neighbouring genes *SCN11A/TTC21A/WDR48* (sodium voltage-gated channel alpha subunit 11/ tetratricopeptide repeat domain 21A/ WD repeat domain 48), and *AK3/RCL1* (adenylate kinase 3/ RNA terminal phosphate cyclase like 1) with p<1E-6, i.e. a suggestive level of association which does not survive statistical correction for multiple testing over the whole genome.
- A GWAS of 4268 individuals from the general population, using a questionnaire-based definition of handedness, found no association p-values below 5E-06 in the genome [44]. Possibly other negative results remained unpublished.

It has become increasingly clear during the last decade that psychiatric, cognitive and behavioural traits are usually influenced by many genetic factors with very small effects of each individual locus, so that tens of thousands of subjects are needed to detect effects reliably [45]. None of the studies listed above approached that level of sample size or statistical power. Low power reduces the likelihood that a statistically significant result reflects a true effect [46]. Most of the studies summarized

above made secondary use of datasets which were collected for different purposes, so that left-handedness was present at only roughly 10% within the datasets. This meant that statistical power was even less than might have been achieved in such sample sizes, for example through selection schemes balanced for handedness. Given the statistical issues affecting the earlier studies, it seems likely, from a contemporary perspective on statistical genetics, that most or all of the findings were false positives.

Some of the genetic associations summarized above were identified in groups with specific disorders, including children with reading disability [29,42], and families with bipolar disorder [36]. Therefore, even if the genetic associations were real, they may not extrapolate to the general population. Finally, a variety of different measures related to handedness were used in these studies, including binary traits based on simple questions such as 'which hand do you write with', to quantitative indices based on the peg board test.

An opportunity arose recently to perform a high-powered GWAS of hand preference in a very large, adult population sample from the UK, known as the UK Biobank dataset [47]. This dataset comprises more than 300,000 subjects with handedness information, assessed by questionnaire (see below). SNP-based heritability analysis, based on variation over all of the autosomes (i.e. all chromosomes minus the sex chromosomes) arrived at an explained variance (SNP-heritability) for left-handedness of only 1.8% in the UK Biobank (p=6.4e-03), which is markedly lower than previous indications from twin studies (see above), while ambidexterity had an estimated SNP-heritability of 6.8% (p=8.7e-04)[48].

Here we performed GWAS for hand preference in this large and well powered dataset, to identify novel loci associated with this trait, and to investigate whether any of the previously reported loci, summarized above, show evidence for association with binary trait handedness in the general adult population. We also applied different methods of gene-set enrichment analysis to the GWAS results, in order to test the relevance of gene-sets that are involved in laterality of the visceral organs.

Methods

Data

We used the data collected in the UK Biobank [47] and provided to Clyde Francks as part of research application 16066. Signed informed consent was obtained from all participants, and the UK Biobank has ethical approval from the UK Biobank Research Ethics Committee under number 16/NW/0274. All methods were performed in accordance with the relevant guidelines and regulations. For descriptions of all traits in the UK Biobank cohort, see http://biobank.ctsu.ox.ac.uk/crystal/search.cgi.

144 The phenotype 1707 'Handedness'' was based on a touchscreen question "Are you right or left
145 handed?", with four answering options: 'right-handed', 'left-handed', 'use both right and left hands
146 equally', and 'prefer not to say'. We merged 'right-handed' and 'use both right and left hands
147 equally' into the category 'non-left-handed' to create a dichotomous phenotype. 'Prefer not to say'
148 was treated as missing data.

149 In a previous study we identified various early life factors which affect handedness in the UK Biobank
150 dataset[49]. For the purposes of the present study, some of these were relevant to use as covariate
151 effects when performing genetic association analysis: sex (trait 31), year of birth (trait 34), country of
152 birth (trait 1647), and being part of a multiple birth (trait 1777). The traits 'birthweight' (trait 200022)
153 and 'being breastfed' (trait 1677) also showed association with hand preference[49], but we did not
154 include them here as covariates, as they have significant SNP-heritabilities of their own in the UK
155 Biobank dataset (https://nealelab.github.io/UKBB_ldsc/). In this case, some genetic loci may
156 conceivably influence both handedness and one of these traits, while we wished to detect any
157 genetic influences on hand preference in our GWAS without correcting for shared genetic effects. In
158 addition, as covariates we used the first 20 principal components which describe genome-wide
159 diversity within the dataset, as provided by the UK Biobank[50], in order to control for population
160 stratification. We restricted the analysis to participants with British ancestry, as reported before[50].
161 Individuals from this restricted dataset were selected for the present study when they had data for
162 the handedness phenotype and covariates used. Within this subset, we excluded randomly one
163 individual from each pair of participants whose genetic relatedness was inferred to be 3rd degree or
164 closer, on the basis of genome-wide genotype data, as previously calculated [50], individuals were
165 removed when there was a mismatch of their reported and genetically inferred sex, putative
166 aneuploidy of the sex chromosomes, excessively high genome-wide heterozygosity (> 0.19) or
167 genotype missingness (missing rate >0.05). The total number of participants remaining was N=
168 331,037: left-handed= 31,856, non-left-handed = 299,181, the latter comprised of right-handed =
169 293,857 and ambidextrous = 5,324. The GWAS for left-handed versus non-left-handed was our
170 primary analysis, the results of which we used for downstream analyses at the whole gene level and
171 gene-set level (below) However, in addition, we ran GWAS for the trait right-handed vs non-right-
172 handed and ambidextrous vs non-ambidextrous, for look-ups of previously reported individual SNPs
173 (see Introduction).

174 Imputed genotype data [50] were available as dosage data for ~97 million SNPs per individual. QCtool
175 (version: 2.0.1, revision 872b463, http://www.well.ox.ac.uk/~gav/qctool_v2/index.html) was used to
176 assess SNP quality, and SNPs were retained if in the selected subsample the imputation 'info' score
177 was > 0.7, the minor allele frequency (MAF) was > 0.001, and the P value for the Hardy-Weinberg

178 equilibrium (HWE) test was > 1E-07. This left ~15 million SNPs. GWAS analysis was run using linear
179 regression assuming an additive genetic model, with BGENIE v1.2 [50].

180 When we ran GWAS for 'ambidextrous' versus 'non-ambidextrous', due to the much smaller size of
181 the ambidextrous group than the other handedness groups, we raised the MAF threshold to 0.01.

182

183 Previously implicated SNPs and genes

184 We checked the GWAS catalog (http://www.ebi.ac.uk/gwas/search, 3 July 2018) for the terms
185 'handedness' and 'hand' to find SNPs and genes previously implicated in traits related to hand
186 preference. In addition, we checked the literature through Pubmed (July 2018) for (('hand
187 preference' OR 'hand skill' OR 'handedness') AND genetic) to identify SNPs or genes found in non-
188 GWAS studies (such as candidate gene studies). We also checked references in the papers we found.
189 The results of this search are summarized in the Introduction.

190 In the results of our GWAS for hand preference in the UK Biobank, we first checked the p-values for
191 association, for each of the specific SNPs reported in the previous papers (Table 1). We also
192 generated association scores for each individual gene implicated in the previous papers, by use of the
193 Magma program as implemented in the FUMA package v1.3.3c (see Supp. Table 1 for specific
194 settings) [51](November 2018). Each gene-based score summarizes the overall level of association at all
195 SNPs spanning a given gene, into one single score and generates a corresponding P value for that
196 entire gene.

197

198 Gene-sets involved in visceral laterality

199 To test for the involvement of genes associated with visceral asymmetry in relative hand skill,
200 Brandler et al.[43] had selected a number of mouse phenotypes related to visceral asymmetry (see
201 Introduction), such as 'dextrocardia' and 'situs inversus', from all those available in the Mouse
202 Genome Informatics (MGI) resource (http://www.informatics.jax.org/). In this database, mutations in
203 specific genes have been linked to resulting phenotypes, such that each phenotype has a set of
204 individual genes annotated to it. The exact criteria by which Brandler et al. selected their specific
205 phenotypes for analysis were not described. In addition, new relevant mouse laterality phenotypes,
206 and their associated genes, may have been added since 2013. We therefore made an up-to-date
207 selection of phenotypes related to visceral asymmetry in the MGI, in the following way: In the
208 hierarchical tree for mammalian phenotypes (http://www.informatics.jax.org/vocab/mp_ontology,
209 MGI 6.12, last updated 06/19/2018), we searched for 'heterotaxy', 'situs inversus', 'isomerism' and

210 'dextrocardia', and also included all child terms if they also related to asymmetry phenotypes. We
211 only included terms with at least 15 genes. This approach resulted in a list of 11 mouse phenotypes
212 (See Results). The mouse genes were then mapped to corresponding human genes, using
213 homologene.data (v68, May 2014) from ftp://ftp.ncbi.nih.gov/pub/HomoloGene/current, by
214 matching mouse genes with human genes that had the same HID (HomoloGene group identifier). The
215 list of phenotypes and the associated human genes can be found in Supplementary Table 2.

216 In the Online Mendelian Inheritance in Man database OMIM (https://omim.org/, last updated on 26
217 Jun 2018), we also identified all genes in the human phenotypic series 'Primary Ciliary Dyskinesia'
218 (MIM #244400), because half of the patients with this disorder present with situs inversus. These
219 genes were used to form one additional gene-set, thus in this study we tested a total of twelve sets,
220 eleven from the mouse database and one based directly on human genetics (See Results).

221 Seven of our mouse phenotypes were among those previously analysed by Brandler et al. (Supp.
222 Table 3). We also repeated our analyses using the exact same list of mouse phenotypes used by
223 Brandler et al. (Supp. Table 3). However, the gene lists associated with each mouse phenotype may
224 have changed since Brandler et al. performed their study five years ago, as new information on genes
225 affecting visceral asymmetry can have been incorporated.

226 Gene-set enrichment analysis

227 We ran three different gene-set enrichment algorithms that have been developed for application to
228 GWAS results. Brandler et al., in their investigation of gene-sets involved in visceral laterality, had
229 used MAGENTA [52]. For consistency we also used MAGENTA (v2, 21 July 2011), plus two newer
230 algorithms called Magma ([53], v1.06) and Pascal ([54], no version number). The three enrichment
231 algorithms work in different ways, but essentially they first assign a single association score to each
232 gene based on the GWAS data, and then test whether the genes in a given set show, on average,
233 more evidence for association with the trait in question than the rest of the genes in the genome for
234 which scores could be calculated (hence these are sometimes called 'competitive' analyses [55]), while
235 accounting for non-independence of SNPs due to linkage disequilibrium. For Magma and Pascal, we
236 used default settings, while for MAGENTA we chose the settings as used by Brandler *et al.*
237 (parameter settings for all analyses can be found in Supp. Table 1). Pascal and Magma offered the
238 option to calculate gene-scores based on the single most associated SNP within a gene (the 'max' or
239 'top' option), or on a combination of SNPs within the gene (the 'sum' option), and we ran both
240 approaches with both softwares, for a robust assessment of whether the previously implicated gene-
241 sets in the literature might affect handedness in the UK Biobank.

242 # Results

243 None of the SNPs previously reported in the literature, in datasets other than the UK Biobank itself
244 (see Introduction), had a significant effect on left-handedness in the UK Biobank (Table 1). Nor was
245 there any effect at the whole-gene-level, for any of the previously reported genes (Table 1). Also for
246 the trait definitions of 'ambidextrous vs non-ambidextrous and 'right-handed vs non-right-handed',
247 none of the previously claimed SNPs from other datasets showed significant association in the UK
248 Biobank (Table 1).

249

250 ***Table 1.*** *Significance of association with hand preference measures in the UK Biobank, for SNPs and*
251 *genes previously implicated in the literature.*

GENE	CHR	START	END	SNP	P-GENE[a]	P-SNP LEFT[a]	P-SNP RIGHT[b]	P-SNP AMBI[c]	REF
LRRTM1	2	80515483	80531874	rs1007371	0.42	0.81	0.74	0.80	[31]
PCSK6	15	101840818	102065405	rs7182874	0.22	0.97	0.90	0.69	[43]
COMT	22	19929130	19957498	rs4680	0.78	0.95	0.89	0.64	[36]
SETDB2	13	50018429	50069138	rs4942830	0.52	0.45	0.94	0.11	[39]
SCN11A	3	38887260	38992052	rs883565	0.80	0.14	0.09	0.47	[25]
AK3	9	4711155	4742043	rs296859	0.77	0.98	0.92	0.76	[25]
AR	X	66764465	66950461	micro-satellites[d]	0.76	-	-	-	[33]

252 ***Notes:*** *Gene-level association is shown for the left-handed versus non-left-handed phenotype, and*
253 *SNP level association for all three hand preference contrasts. START and END are according to the*
254 *reference genome build 37 (see methods).[a] Using GWAS of left-handed vs non-left-handed. [b] Using*
255 *GWAS of right-handed vs non-right-handed. [c] Using GWAS of ambidextrous vs non-ambidextrous. [d]*
256 *The original study made use of a microsatellite repeat polymorphism which was not genotyped or*
257 *imputed in the UK Biobank dataset.*

258

259 The gene-set enrichment analyses did not convincingly support the proposition that sets related to
260 visceral asymmetry are particularly associated with left-handedness (Table 2, Supp. Table 3). There
261 was one potential association involving the gene-set 'right-sided isomerism' in the analysis with
262 MAGENTA, but this was not supported by any of the other methods.

263

264 ***Table 2**. Gene-set enrichment results for visceral asymmetry-related gene-sets, based on the UK*
265 *Biobank GWAS for left-handedness versus non-left-handedness*

mouse ID	Description	Pascal sum P	Pascal max P	Magma sum P	Magma max P	Magenta P
MP:0000508	right-sided isomerism	0.65	0.83	0.98	0.46	0.006[a]
MP:0000531	right pulmonary isomerism	0.64	0.68	0.97	0.76	0.06
MP:0000542	left-sided isomerism	0.77	0.59	0.52	0.73	0.10
MP:0000644	Dextrocardia	0.68	0.87	0.85	0.84	0.23
MP:0001706	abnormal left-right axis patterning	0.93	0.84	0.67	0.93	0.41
MP:0002766	situs inversus	0.40	0.51	0.70	1.00	0.49
MP:0003178	left pulmonary isomerism	0.70	0.52	0.55	0.90	0.13
MP:0004133	Heterotaxia	0.80	0.89	0.16	0.99	0.35
MP:0010808	right-sided stomach	0.34	0.72	0.86	0.95	0.17
MP:0011250	abdominal situs ambiguus	0.08	0.62	0.70	0.94	0.31
MP:0011252	situs inversus totalis	0.23	0.67	0.71	0.99	0.44
PCD	human primary ciliary dyskinesia	0.33	0.31	0.84	0.70	0.30

266 ***Notes:** Different softwares and settings were used. See Supplementary Table 1 for the settings. The*
267 *uncorrected P values for each gene-set and enrichment analysis are shown.* [a] *FDR=0.035.*

268

269 In the GWAS for left-handedness vs non-left-handedness in the UK Biobank dataset (Figure 1), the
270 following significant associations were found with P values below the threshold 5E-08 for correcting
271 for multiple testing over the whole genome: on chromosome 2q34 the SNP rs142367408 (p=3.8E-
272 08), located 28 kilobases upstream of the gene *MAP2* (Microtubule associated protein 2); on
273 chromosome 17q21 the SNP rs144216645 (p=1.2E-09) within a large inversion haploblock spanning
274 the genes *STH, KANSL1, ARL17B, ARL17A, LRRC37A, LRRC37A2* and *NSF*; on chromosome 13q22 the
275 SNP rs11454570 (p=4.7E-08) within an intron of the non-coding RNA-gene LINC00381.

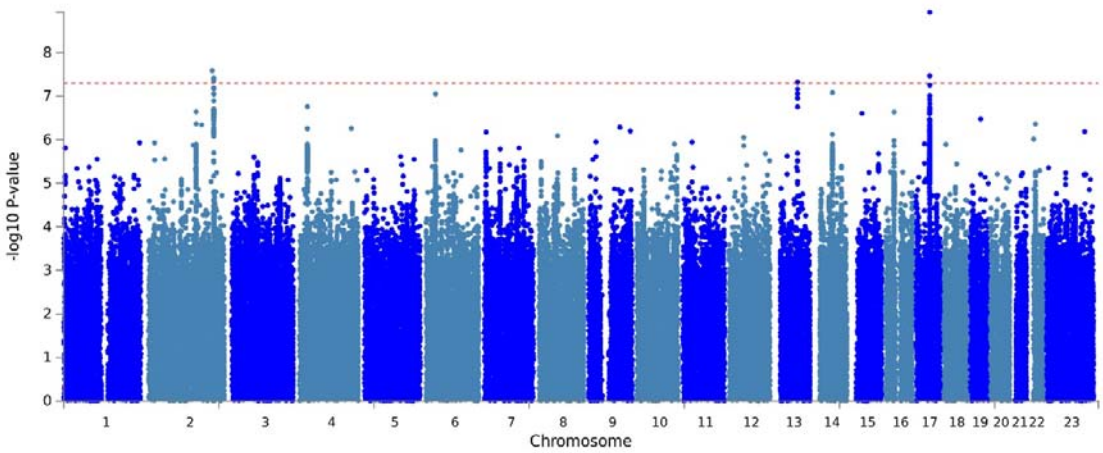

Figure 1. Manhattan plot of GWAS results for left-handed vs non-left-handed people in the UK Biobank sample. The red dashed line marks the genome-wide significance threshold of p=5.0E-08. (Plot produced with FUMA[51]).

The results for right-handed vs non-right-handed people were similar to those for left-handed versus non-left-handed (Supp. Figure S1), which was expected as only the relatively small group of ambidextrous people had moved between groups. For right-handed versus non-right-handed, the region around *MAP2* on chromosome 2 did not reach genome-wide significance. Instead, the major histocompatibility complex region on chromosome 6p21 showed significant associations. The most associated SNP was rs9366770 (p=2.2E-09), located in an exon of the non-coding RNA *HCG27*. The inversion region on chromosome 17 was also associated with the right-handed versus non-right-handed phenotype, again most significantly with the SNP rs144216645 (p=2.3E-08). An additional association on chromosome Xq25 was detected with the SNP rs767669906 (p=1.0E-08), located 44 kilobases downstream of the gene *DCAF12L1*. However, with a minor allele frequency of 0.0035, this signal may not be reliable.

The GWAS for 'ambidextrous' vs 'non-ambidextrous' showed no significant associations.

Zoomed in figures showing genetic association within the significantly associated regions can be found in the supplement (Figures S2-S5)[56].

296 # Discussion

297 In a very large dataset comprising hundreds of thousands of adult subjects from the general
298 population, we found no evidence for an involvement in hand preference of any genetic variants,
299 genes, or gene-sets that have been previously claimed in the literature. We consider the most likely
300 explanation to be that the earlier findings were false positives, as the study sample sizes were orders
301 of magnitude smaller than the present analysis (see Introduction). However, as the earlier studies
302 used various different trait measures related to handedness, and some made use of selected
303 disorder populations, then it remains possible that some of the earlier findings were indeed real, but
304 not detectable in the present analysis (see Introduction).

305 There was no evidence for an involvement, in hand preference, of common variants in genes related
306 to visceral left-right patterning. Note that Brandler et al. [43] performed analysis of a quantitative
307 handedness index based on peg moving, and included a sample with reading disability, when they
308 originally reported such a relationship. Our present analysis was not an attempt to directly replicate
309 the study of Brandler *et al.*, but rather it was a related investigation, of genes involved in visceral
310 asymmetry with respect to questionnaire-defined, binary-trait hand preference, in a large dataset
311 from the general population. In the rare genetic disorder Primary Ciliary Dyskinesia (PCD)(MIM #
312 244400), recessive mutations in specific genes involved in ciliary biology result in a 50% chance of
313 mirror-reversed left-right visceral asymmetry, a condition known as *situs inversus totalis (*SIT) [57].
314 When PCD and SIT co-occur (the combination is known as Kartagener syndrome), it appears that the
315 rate of left-handedness is unchanged from the general population [58]. However, there may be an
316 increase of left-handedness in people with SIT when they do not have PCD [59,60], which would suggest
317 the existence of some developmental mechanisms which link visceral asymmetry with brain
318 asymmetry. Nonetheless, a recent genome-wide mutation screening study did not identify any
319 individual genes as likely candidates to cause non-PCD SIT [61], and it is possible that the aetiology is
320 multifactorial or even non-genetic [61,62]. At present, the study by Brandler et al. remains the only one
321 to report tentative evidence for a molecular genetic link between visceral laterality and handedness.

322 We found that two loci, on 2q34 and 17q21, were associated with left-hand preference in the UK
323 Biobank, as well as a third locus on chromosome 13 which had borderline significance. The most
324 likely causative gene within the large chromosome 17q21 inversion cannot be determined on the
325 basis of these data, as the region spans at least twelve genes showing similar levels of association
326 with the trait (Supp. Figure S2). This region shows association with numerous other human traits (see
327 e.g. http://big.stats.ox.ac.uk/variant/17-43659975-T-C). The variant on chromosome 13 resides in an
328 intron of the non-coding RNA-gene LINC00381 (Supp Figure S3). This SNP is part of a short A-repeat,

329 but is in LD with other SNPs (www.ensembl.org). For the locus on chromosome 2q34, the most
330 probable gene appears to be *MAP2*: the association signal extends from upstream into this gene's
331 coding region, and the single most associated SNP is associated with expression differences of *MAP2*
332 in oesophageal tissue, according to the GTeX database (https://www.gtexportal.org/home/ Release
333 V7 (dbGaP Accession phs000424.v7.p2)), and not with any other gene's expression level (Supp.
334 Figure S4). MAP2 protein is a well-known marker for neuronal cells, and specifically the perikarya and
335 dendrites of these cells [63]. MAP2 is involved in neurogenesis [64], and almost exclusively expressed in
336 the brain [65]. The protein plays a role during the development of various brain structures (e.g. [66,67]),
337 and in mice seems to play a role in motor skills [68]. One report suggested that *MAP2* is more highly
338 expressed in right hippocampus than left during development in the rat [69]. How exactly this gene may
339 act in affecting motor laterality in human development remains unknown. Regardless, as this genetic
340 association is based on hundreds of thousands of study participants, *MAP2* must be considered the
341 single most reliably implicated gene in left-handedness yet identified, although replication will be
342 desirable.

343 When pooling ambidextrous people together with left-handers, to create a 'right-handed' versus
344 'non-right-handed' phenotype, an additional locus within the MHC region of chromosome 6p was
345 detected. Again this is a very broad region of linkage disequilibrium spanning many genes. We found
346 that the most associated SNP is an eQTL for the non-coding RNA *HCG27* (HLA complex group 27) in
347 the cerebellar hemisphere and frontal cortex, according to the GTeX portal. Other associated SNPs
348 within the region, rs3130976 (p=2.0E-08) and rs2854008 (p=2.4E-08), are eQTLs for *MICB* (MHC class
349 I polypeptide-related sequence B) and *C4A* (complement C4A) in the cerebellar hemisphere, but for
350 other genes in other tissues. We cannot conclude which may be the most likely causative gene or
351 genes in the region, on the basis of this information.

352 There may have been some particular sources of noise in the UK Biobank definitions of hand
353 preference. An earlier analysis of the UK biobank dataset [26] suggested a reduction in left-hand
354 preference of roughly 20% among the oldest participants (born around 1940), relative to younger
355 participants (born around 1970), which suggests that some of the older left-handed people were
356 forced to switch to right-handedness during childhood. Our GWAS analysis did adjust for year of birth
357 as a covariate effect, but hand switching may have reduced power. In addition, the location of birth
358 within the UK was found to influence the probability of being left-handed [26], likely primarily for
359 cultural rather than biological reasons. Country of birth was therefore included as a covariate in our
360 GWAS. Furthermore, the ambidextrous group was previously found to give a high rate of inconsistent
361 answers between different assessment visits [26]. However, ambidextrous people comprised only a
362 relatively small subset.

363 It is striking that common genetic variants in the UK Biobank dataset have little overall effect on hand
364 preference, as measured by a very low SNP-based heritability (only 2-3%)[48,70], while previous twin-
365 based studies have indicated a heritability of roughly 25% for left-hand preference (see Introduction).
366 It has been noted before that behavioural traits can show a particularly large difference between
367 twin and SNP-based heritability estimates [71]. A commonly given explanation is that much of the
368 heritability may be due to rare SNPs and mutations, which are not well captured by genotyping
369 arrays and imputation protocols in GWAS studies. Rare mutations can be relatively recent in origin,
370 and under negative selection. Whether these possibilities apply to hand preference will need to be
371 investigated in future large-scale genome sequencing studies.

372

373 Conclusion

374 In conclusion, previously reported genetic associations with measures related to handedness, found
375 in datasets other than the UK biobank, were not supported by analysis of this very large cohort. We
376 also found no supportive evidence that common variants within genes involved in visceral left-right
377 patterning contribute to hand preference. We consider the most likely explanation for the
378 discrepancy to be that the previous findings were noise, which arose from limited sample sizes.
379 Differences of trait measurement and population selection might also be involved. Regardless, what
380 is known of the molecular genetic basis of hand preference remains tentative and extremely limited,
381 with the gene *MAP2* providing the most robust lead so far.

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572

573 Acknowledgements

574 This research has been conducted, using the UK Biobank Resource. We thank all participants of the
575 UK Biobank for their contribution. CGFdeK was supported by an Open Programme grant (824.14.005)
576 to CF from the Netherlands Organization for Scientific Research (NWO). C.F. was supported by the
577 Max Planck Society (Germany).

578 Author contributions

579 CF initiated the study and edited the manuscript. CGFdK carried out and interpreted the data
580 analysis, and wrote the manuscript.

581 Additional information

582 The authors declare to have no conflicting interests.

583 Data availability

584 Data were obtained from the UK Biobank cohort, as part of research application 16066, with Clyde
585 Francks as the principal applicant. Summary and description of these data can be found at
586 http://biobank.ctsu.ox.ac.uk/crystal/. For the use of the data, application must be made to
587 http://www.ukbiobank.ac.uk/register-apply/.
588

589 Figure legends

590 **Figure 1.** Manhattan plot of GWAS results for left-handed vs non-left-handed people in the UK
591 Biobank sample. The red dashed line marks the genome-wide significance threshold of p=5.0E-08.
592 (Plot produced with FUMA, Watanabe, K. et al. (2017) *Nat. Commun.* **8**:1826).

593

594 Supplemental Information

595 Supp. Table 1: Software packages and settings for the various gene-set enrichment analyses.

596 Supp. Table 2 (an Excel file): Gene-sets used in the current study in GMT-format. Genes have Entrez
597 identifiers.

598 Supp. Table 3: Results for visceral asymmetry-related gene-sets as used in the previous analysis by
599 Brandler *et al.* (2013)

600 Supp. Figures S1-S5. Further detailed plots of genetic association results.

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