symmetry

MDPI

Article

The Genetics of Asymmetry: Whole Exome Sequencing in a Consanguineous Turkish Family with an Overrepresentation of Left-Handedness

Sebastian Ocklenburg [1,*,†], Ceren Barutçuoğlu [1,†], Adile Öniz Özgören [2], Murat Özgören [2], Esra Erdal [3], Dirk Moser [4], Judith Schmitz [1], Robert Kumsta [4] and Onur Güntürkün [1]

1 Institute of Cognitive Neuroscience, Biopsychology, Ruhr-University, 44780 Bochum, Germany; crn.barutcuoglu@gmail.com (C.B.); judith.schmitz@rub.de (J.S.); onur.guentuerkuen@rub.de (O.G.)
2 Department of Biophysics, Faculty of Medicine, Dokuz Eylül University, 35340 Izmir, Turkey; adile.oniz@gmail.com (A.Ö.Ö.); murat.ozgoren@deu.edu.tr (M.Ö.)
3 Department of Medical Biology, Faculty of Medicine, Dokuz Eylül University, 35340 Izmir, Turkey; esra.erdal@deu.edu.tr
4 Genetic Psychology, Faculty of Psychology, Ruhr-University, 44780 Bochum, Germany; Dirk.Moser@rub.de (D.M.); robert.kumsta@rub.de (R.K.)
* Correspondence: sebastian.ocklenburg@rub.de; Tel.: +49-234-322-6804
† These authors contributed equally.

Academic Editor: Lesley Rogers
Received: 9 March 2017; Accepted: 27 April 2017; Published: 1 May 2017

Abstract: Handedness is the most pronounced behavioral asymmetry in humans. Genome-wide association studies have largely failed to identify genetic loci associated with phenotypic variance in handedness, supporting the idea that the trait is determined by a multitude of small, possibly interacting genetic and non-genetic influences. However, these studies typically are not capable of detecting influences of rare mutations on handedness. Here, we used whole exome sequencing in a Turkish family with history of consanguinity and overrepresentation of left-handedness and performed quantitative trait analysis with handedness lateralization quotient as a phenotype. While rare variants on different loci showed significant association with the phenotype, none was functionally relevant for handedness. This finding was further confirmed by gene ontology group analysis. Taken together, our results add further evidence to the suggestion that there is no major gene or mutation that causes left-handedness.

Keywords: handedness; hemispheric asymmetries; genetics; ontogenesis; consanguineous marriage

1. Introduction

Handedness is a heritable trait [1] and, historically, it was thought that left-handedness was determined by a major gene effect [2]. This idea was based on the statistical distribution of the phenotype, but has since been refuted by molecular studies. In particular, the fact that genome-wide associations studies (GWAS) consistently failed to identify a gene that explains enough phenotypic variance to qualify as a single-gene explanation has disproven single gene theories [3,4]. Thus, most authors today agree that handedness is likely to be a multifactorial trait that is determined by several different genetic and non-genetic factors (e.g., [5–8]). A number of contributing loci have been identified by GWAS and candidate gene studies using handedness questionnaires or hand skill tests like the pegboard test as phenotypes, e.g., *LRRTM1*, *PCSK6* and *AR* [9–15]. However, the general understanding is that there is likely a large number of yet unidentified genetic contributions to handedness [5]. Besides replication of published loci, identification of new candidate genes therefore is one of the major aims of current research on handedness genetics. Since GWAS in healthy cohorts

are unlikely to identify rare genetic variants relevant for handedness, other methods to identify candidate genes should also be considered.

One possible way to increase statistical power to detect relevant candidate genes for handedness without the need for overly large cohorts is testing population isolates with reduced genetic heterogeneity and overrepresentation of left-handedness. For example, Somers et al. [16] performed a genome-wide genetic linkage study of left-handedness and language lateralization in a sample of 368 subjects from a population isolate in the Netherlands. Due to the geographical isolation of the town that the subjects were recruited from, as well as a genetic bottleneck event in the early 17th century, founders in the sample of Somers et al. [16] showed lower genetic heterogeneity than random samples from the Dutch population. The sample was deliberately enriched for left-handedness, as the authors only selected families that had left-handed subjects in at least two generations, with at least two left-handed family members per generation. This resulted in a sample in which 24% of participants were left-handed, roughly 2.5 as many as in the general population. While Somers et al. [16] did not observe any genome-wide evidence for linkage in handedness, there was at least suggestive evidence for linkage for left-handedness in the 22q13 region. Somers et al. [16] argued that the absence of any significant linkage indicates that there is no major gene coding for handedness and it is likely to be a polygenic complex trait.

In addition to testing populations that show lower genetic heterogeneity than the general population due to a genetic bottleneck in the past and a more or less isolated way of living, another methodological option to detect genetic variants that influence handedness is to test families with a history of consanguineous marriage and an overrepresentation of left-handedness. This method has for example been used by Kavaklioglu et al. [17]. These authors used whole exome sequencing in 17 members of an extended family from Pakistan that practiced consanguineous marriage and had an overrepresentation of non-right-handed members (about 40%). Neither multipoint linkage analysis across all autosomes nor single-point analysis of exomic variation resulted in any clear candidate genes or mutations, leading Kavaklioglu et al. [17] to conclude, similar to Somers et al. [16], that handedness is a polygenic complex trait and not driven by a major gene or single mutation.

Although neither of these studies observed any significant effects, this does not necessarily imply that rare mutations could not affect handedness in other samples. Thus, more research in similar samples in other regions is needed. Also, previous studies in bottleneck populations analyzed handedness as a dichotomous variable (e.g., right-handedness/non-right-handedness). However, it is commonly measured as a continuous variable using a lateralization quotient (LQ) [18], ranging from −100 (consistent left-handedness) to +100 (consistent right-handedness). Interestingly, findings from a recent PCSK6 candidate gene study on handedness showed that the direction and degree of handedness might underlie differential genetic influences [9]. Thus, using the LQ as a phenotype instead of differentiating between left- and right-handers could potentially yield interesting insights into the genetics of handedness. To this end, we performed whole exome sequencing in nine members of an extended Eastern Turkish family that practices consanguineous marriage and has an overrepresentation of left-handedness. We then conducted a quantitative trait analysis with handedness LQ as a trait. Our hypothesis was that if there was indeed a major gene effect of a rare variant in this cohort, this variant should be significantly related to handedness LQ. If no such association was found, this would further confirm the idea that handedness is not driven by a major gene effect.

2. Materials and Methods

2.1. Participants

All participants were from Turkey, specifically from the vicinity of Şanlı Urfa, a city in the east of Turkey. This area was chosen as it has a higher prevalence of kin marriage compared to other regions of Turkey. The study was approved by the ethics committee of Dokuz Eylül University, Faculty of Medicine, İzmir, Turkey. All participants were treated in accordance with the declaration of Helsinki. All participants gave written informed consent, and in case of participants younger than

18 years, the parents also gave written informed consent. Subjects were compensated for participating in the experiment with a gift of high quality Turkish sweets, as they refused to take money as reimbursement. Nine members of the family, two female and seven male, with a mean age of 29.33 (SD = 13.07; range: 11–46 years) agreed to participate in the study (Figure 1). Verbal interviews confirmed at least four consanguineous marriages between living family members and a family history of previous consanguineous marriages. None of the participants had a history of any psychiatric diseases or neurological diseases.

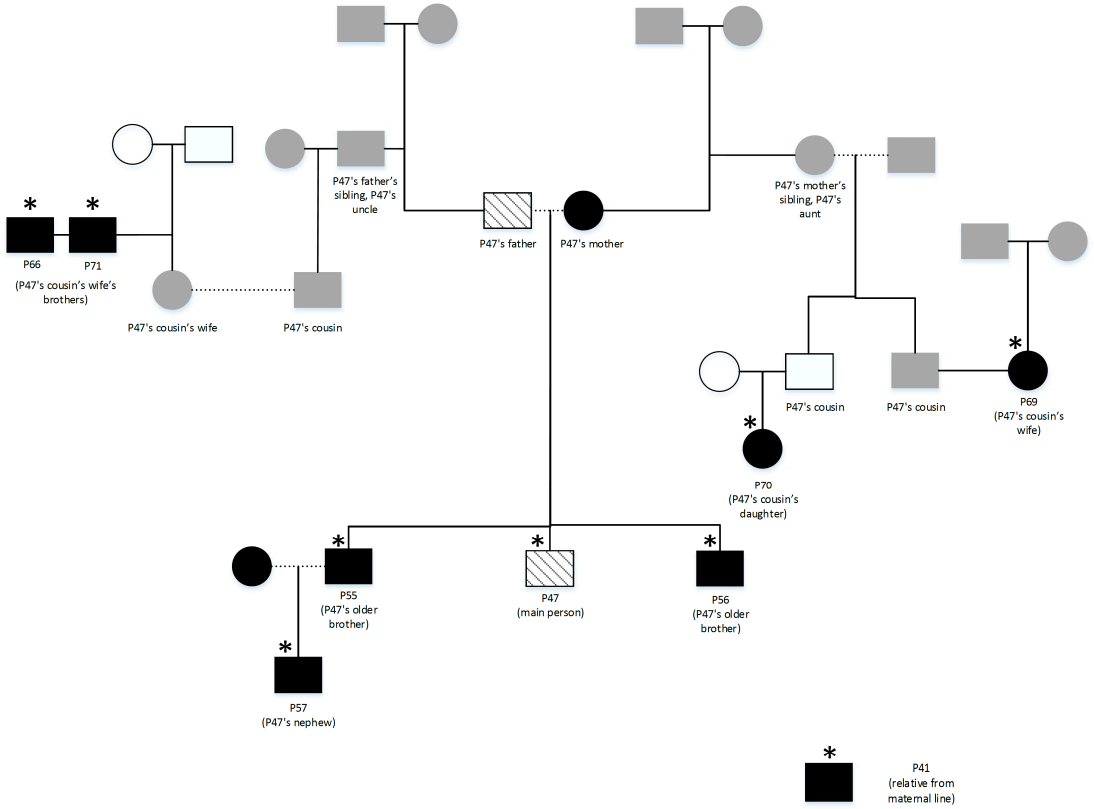

Figure 1. Family tree for the investigated cohort. Squares indicate male family members, circles indicate female family members. Asterisks indicate family members that participated in the present study. For these family members, handedness was determined using the Edinburgh Handedness Inventory (EHI). For other family members shown in the figure, handedness was assessed by verbal report. Black indicates left-handedness, white right-handedness and white with black shading ambidexterity. For family members with grey symbols, no information about handedness could be obtained. Consanguineous marriages are indicated by dotted lines. Consanguineous marriages were also performed by several family members of earlier generations not shown in this figure, as confirmed by verbal report.

2.2. Phenotyping

2.2.1. Edinburgh Handedness Inventory

Handedness was assessed with a Turkish translation of the EHI [18]. In this questionnaire, participants have to indicate whether they prefer to use left or right hand for ten different activities which are hand preference in writing, drawing, throwing a ball, using scissors, a toothbrush, a knife (without fork), a spoon, and a broom (upper hand), striking a match, and opening a box. An individual LQ can be calculated using the Formula LQ = [(R − L)/(R + L)] × 100 (R = the number of right-hand preferences; L = the number of left-hand preferences) as based on participants' answers. The LQ has a range between +100 and −100. Positive values indicate right-handedness and negative

values indicate left-handedness. At the same time, higher absolute values indicate more consistent handedness and lower absolute values indicate more inconsistent handedness or ambidexterity.

2.2.2. Pegboard Test

In addition to questionnaires like the EHI that assess hand preference, hand skill can be assessed with motor tasks such as placing dots in squares or circles on a sheet of paper as quickly as possible [19,20], or picking up matches placed on a table as quickly as possible [19]. The most commonly used measure is the so-called "pegboard task" (e.g., [15,21,22]) that was also utilized to determine participants manual hand skills in the present study. The test consists of measuring the time taken by the subjects to move, with each hand separately, a row of 10 pegs on a board from one location to another. The test is repeated three times for each hand. The measure of relative hand skill (PegQ) is calculated as the difference between the average times for the left hand (L) and the right hand (R), (L − R), divided by the average time for both hands combined, (L + R)/2 [15]. A positive PegQ demonstrates superior relative right-hand skill, and a negative PegQ demonstrates superior relative left-hand skill.

2.2.3. Dichotic Listening Task

The Dichotic Listening Task is a noninvasive behavioral test to determine language lateralization. During a dichotic listening test, two different consonant-vowel (CV) syllables are presented to participants simultaneously using headphones, one to the right ear and one to the left ear. The syllables used in the present study were "BA, DA, GA, KA, PA, TA" [23]. Participants are instructed to indicate the syllable which they heard best by pressing a button [23]. Overall, 72 stimulus pairs were presented with Sony stereo headphones type MDR-ZX100 using Presentation software (https://www.neurobs.com/). The stimuli consisted of two times presenting all possible 36 combinations of the six syllables, including homonyms (e.g., BA-BA). Syllables were spoken by a native Turkish speaker and were provided by Dokuz Eylül University, Faculty of Medicine, Biophysics Department. Voice-onset times were controlled for.

2.3. Collection of DNA Samples

For the non-invasive collection of high quality DNA, saliva samples were collected using Oragene-DNA OG-500 saliva self-collection kits. These kits were used since they ensure DNA sample stability at room temperature for a prolonged time, which was essential since data collection took place in a field study without permanent access to refrigeration. From each participant, 2 mL of saliva were collected.

2.4. Whole Exome Sequencing

DNA was extracted from saliva samples and purified according to the kit protocol. All samples passed initial quality control with OD260/OD280 ratios between 1.6 and 2.0, and were then shipped to GATC Biotech AG (Konstanz, Germany), a service provider for DNA sequencing and bioinformatics (www.gatc-biotech.com). In addition to the nine samples from the family, we also included one sample of an unrelated right-hander from Turkey, to differentiate possible regional exome variation from true rare variants specific for the family, in addition to comparison against other reference genomes (see below). All samples passed a second DNA quality control performed by GATC. "INVIEW HUMAN EXOME" (http://www.gatc-biotech.com/de/produkte/inview-applikationen/inview-human-exome.html) was chosen as the whole exome sequencing platform. The array used was an Agilent Genomics SureSelectXT All Exon V5 (Agilent Technologies, Santa Clara, CA, USA). Mapping to the UCSC Genome Browser *Homo Sapiens* reference genome (hg19) was performed using BWA (Burrows-Wheeler Aligner; http://bio-bwa.sourceforge.net/[24], with default parameters. On average, 99.13% of high quality reads were mapped to the reference genome (see Table S1 for mapped read metrics for all samples). Removal of polymerase chain reaction (PCR) duplicates was conducted using Picard (http://broadinstitute.github.io/picard/) and local

realignment using GATK (Genome Analysis Toolkit; https://software.broadinstitute.org/gatk/) [25]. On average, 93.99% of the exome was covered with a sequence depth read of at least 10× (see Table S2 for the depth of coverage summary). single-nucleotide polymorphism (SNP) and InDel calling was performed using GATK's UnifiedGenotyper (https://software.broadinstitute.org/gatk/documentation/tooldocs/current/org_broadinstitute_gatk_tools_walkers_genotyper_UnifiedGenotyper.php) [25], with a Bayesian genotype likelihood model. Subsequently, variant annotations were performed using snpEff (http://snpeff.sourceforge.net/) [26]. Further analysis of exome data and quantitative trait analysis was performed using "QIAGEN Ingenuity Variant Analysis" (http://www.ingenuity.com/products/variant-analysis) (see results for analysis pathway). The quantitative trait test that was used represents a continuous version of the Sequence Kernel Association Test (SKAT) where each sample is associated with a continuous quantity (in our case handedness LQ) instead of a case and control label. The underlying test is a variance component score test, based on a linear mixed effects model where the impact of rare variants is taken into account as random effects and co-variants are included as fixed affects. The quantitative trait test determines asymptotic p-values that are calculated approximately using Kuonens saddlepoint method. Furthermore, Gene ontology (GO) analysis was performed using the webtool WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt/). This was done in order to identify whether associated gene variants were involved in GO groups with functional significance for handedness development (e.g., left-right axis differentiation or nervous system development). The minimum number of genes included in each GO group was set to five, and analyses were corrected for hypergeometric testing ($p < 0.001$) using false discovery rate (FDR) correction [27].

3. Results

3.1. Phenotyping

All nine family members investigated were left-handed according to EHI results (mean LQ: −84.44, standard deviation: 26.51; range: −100 to −20). The person from whom the control sample was obtained was right-handed (LQ: 100). Analysis of pegboard data showed that seven family members showed superior left hand skill and two family members slightly superior right hand skills (mean PegQ: −0.17, standard deviation: 0.15; range: −0.45 to 0.04). The control person showed superior right hand skills (PegQ: 0.19). For the dichotic listening data, three family members showed a left ear advantage (33.33%) and six showed the typical right ear advantage (66.66%). Dichotic listening data were analyzed non-parametrically due to the small sample size. In absolute number, family members on average reported more syllables presented to the right ear (35.67, standard deviation: 9.72) than to the left ear (29.56, standard deviation: 6.50), but this difference failed to reach significance (Z: −1.31, $p = 0.19$). To determine whether this nonsignificant result was indicating a real absence of an effect or rather was an artefact due to the small sample size, we also analyzed the data with a bootstrapped t-test for dependent comparisons with 5000 iterations. As this comparison also failed to reach significance ($p = 0.26$) it is likely that family members indeed did not show the typical right ear advantage found in the population.

3.2. Sequencing Results

Overall, the analysis detected 299,431 variants on 19,576 genes in family members that were non-identical to the reference genome. As a first step, variants with a call quality less than 20 and all variants in highly variable exonic regions were excluded, narrowing down the number of variants to 235,339 on 19,075 genes. We then excluded all variants that were present in less than at least seven of the nine family members (77.78%), resulting in 9714 variants on 4376 genes. This was done in order to include only variants that were consistently typical for the sample. Furthermore, all variants with a frequency higher than 3% in the 1000Genomes project (http://www.1000genomes.org/) were excluded, as we focused on detecting rare variants. This step resulted in 810 variants on 411 genes left in the analysis. Afterwards, only variants likely to cause loss of function of a gene were included using the "Predicted deleterious" filter, resulting in 116 variants on 69 genes. This was done to only

include causal genetic variants that affect protein function. As a last step, quantitative trait analysis was performed to include only variants that showed significant relations with handedness LQ with p-values of at least $p < 0.01$. This analysis revealed 49 variants on 26 genes that were significantly associated with the phenotype (see Table 1). Most of these genes were involved in general cellular processes and only very few were associated with the brain or neuronal processes specifically.

Table 1. Rare gene variants statistically associated with the phenotype. IDs from the Single Nucleotide Polymorphism Database (dbSNP) are given when available. Likely gene functions were determined using PubMed (http://www.ncbi.nlm.nih.gov/gene). (Chr. = chromosome).

Chr.	Gene	dbSNP ID	Likely Function
2	*ANKRD36C*	202102082	Ion channel inhibitor activity
3	*MUC20*	2688539 3828408	Cellular protein metabolism
4	*ZNF595*	-	Regulation of DNA transcription
4	*FRG1*	199978807 201142987	Associated with facioscapulohumeral muscular dystrophy
7	*MUC3A*	71540917 775174499 747768677 759956700 796070497 796719496 796627084 796799995 796422604 796558082 796345426 796976589 62483696	Cellular protein metabolism
10	*FRG2*	200347477	Protein coding in the nucleus
11	*MUC6*	770290437 34490696 200644196 796934918 111641154 112301388 78265558	Cellular protein metabolism/ production of gastric mucin
11	*MUC5AC*	74390930 749291344	Cellular protein metabolism
11	*TRIM49*	74584169	Protein-protein interactions, preferentially expressed in testis
14	*HOMEZ*	148005528	Regulation of DNA transcription
15	*GOLGA6L2*	76062343	Protein binding
16	*CBFA2T3*	71395351 71395352	Transcription corepressor activity
17	*CCDC144NL*	73298040	Affects blood copper, selenium and zinc
17	*KCNJ12*	77987694 80335301	Encodes an inwardly rectifying K+ channel in neurons, heart and muscle cells.
17	*RECQL5*	142406301	DNA helicase activity
18	*CNDP1*	10663835	Encodes a member of the M20 metalloprotease family that is specifically expressed in the brain
19	*MUC16*	4992693	Cellular protein metabolism
19	*ZNF443*	62114866	Regulation of DNA transcription
19	*SIGLEC11*	9676436 78673790	Anti-inflammatory and immunosuppressive signaling
21	*BAGE2*	9808647	Melanoma antigen
21	*BAGE5*	113315187	Melanoma antigen
X	*RBMX*	76876438 74463481 74667874 35899675 77794331	RNA binding

Gene ontology (GO) analysis showed that the identified genes were significantly enriched within nine GO groups. The majority of these GO groups were related to protein glycosylation (see Table 2). The remaining GO group was "Golgi lumen".

Table 2. Results of the GO group analysis. *p*-values are Benjamini-Hochberg corrected.

GO Group	Genes	Adjusted *p*-Value
O-glycan processing	5	0.0000002
Protein O-linked glycosylation	5	0.0000005
Post-translational protein modification	5	0.00005
Protein glycosylation	5	0.0001
Macromolecule glycosylation	5	0.0001
Glycosylation	5	0.0001
Glycoprotein biosynthetic process	5	0.0002
Glycoprotein metabolic process	5	0.0005
Golgi lumen	5	0.0000007

4. Discussion

Handedness is a trait that has been related to both cognitive ability [28] and psychopathology [29], making the identification of genetic factors underlying its ontogenesis highly interesting for cognitive neuroscientists and clinical psychologists alike. Here, we performed whole exome sequencing in nine members of an extended Eastern Turkish family with a long history of consanguineous marriage and an overrepresentation of left-handedness. For the first time, we used quantitative trait analysis in such a cohort in order to identify rare genetic variants that were associated with handedness.

The results from the EHI clearly revealed that all nine tested family members were left-handers and, for most family members, these findings were also supported by the results of the pegboard test. Family members showed reduced language lateralization. While in the general population about 95% of individuals show left hemispheric language dominance, in our sample only 66.66% of individuals showed a right-ear advantage during dichotic listening and there was no significant right-ear advantage. This number is however only slightly lower than the 70–80% observed in left-handed samples [30]. Given the small sample size of the present study, we would assume that our data are within the normal range for left-handed populations.

The quantitative trait analysis revealed rare variants on 49 loci on 26 genes that were significantly associated with the EHI LQ. However, the biological significance of these genes for handedness remains unclear. As handedness represents a functional asymmetry between the left and right motor cortices in controlling for fine motor skills [6], one would expect genes involved in shaping this phenotype to be specifically expressed in the brain or spinal cord. Moreover, they should have functional relevance for left-right axis development or nervous system development or function in the broadest sense. Almost all of the genes that were associated with handedness LQ in the present study did not meet these criteria, as they were involved in general cellular or regulatory processes not specific for nervous tissue. Furthermore, some genes clearly were relevant for function in body parts other than the brain, making an involvement in handedness development highly unlikely. Only two out of 26 genes showed a functional relevance for neuronal functioning in the broadest sense. The first of these genes, *KCNJ12* (potassium voltage-gated channel subfamily J member 12), encodes a functional inward rectifier potassium channel [31]. Functionally, most studies have linked it to the heart (e.g., [32]) or muscle [33] function, but also tumerogenesis [34]. While a recent study suggested that protein-protein interactions between a G protein-gated inwardly rectifying potassium channel (Kir3), G proteins and G protein-coupled neurotransmitter receptors might be functionally relevant for GABA-B receptors [35], direct evidence linking *KCNJ12* to a specific function in the central nervous system is sparse. While Stonehouse et al. [36] could show that the inwardly rectifying potassium ion channel encoded by *KCNJ12* in humans can be localized in sections of rat hindbrain and dorsal root ganglia tissue, there is no evidence for a functional link to handedness development so far. The second gene, *CNDP1* (carnosine dipeptidase 1), encodes a member of the M20

metalloprotease family which acts as carnosinase. While it is expressed in the brain, most studies have linked it to susceptibility for diabetic nephropathy in human diabetic patients (e.g., [37]), with no evidence for a direct functional link to handedness. Thus, the analysis of functionally relevant rare variants did not result in any evidence for a major gene or mutation determining handedness in our cohort.

This interpretation was further supported by the result of the GO analysis. Out of nine GO groups that reached significance, seven were linked to glycosylation, an enzymatic process that attaches glycans to other molecules. Glycosylation represents an important post-translational modification of proteins in a vast number of different tissues. While congenital disorders of glycosylation have been shown to affect central nervous function [38], glycosylation has also been related to the development and progression of several different types of cancer and other diseases unrelated to the brain [39]. Interestingly, it has been shown that inbreeding in human populations strongly affects the glycosylation of human plasma proteins, potentially leading to the increased prevalence of tumors that has been reported in certain isolated populations as well as other phenotypic changes [40]. Thus, it is likely that the significant effects for glycosylation-related GO groups were an effect of inbreeding and only by happenstance were associated with the handedness phenotype. The other three significant GO groups also were unlikely to affect handedness, as they either represented processes unrelated to the brain or were too general ("Golgi lumen" "post-translational protein modification") to specifically be involved in the formation of the functional motor cortex asymmetry underlying handedness.

The present study contains several methodological aspects that have the potential to be optimized in future studies. Clearly, testing a larger group of family members with a consanguineous background would be ideal. Unfortunately, we were only able to recruit left-handed family members in the present study, but for future studies including both left-and right-handers from the same family would by optimal. Also, for quantitative trait analyses, larger cohorts would be favorable, if recruitment is possible. This would be particularly important as the GATK protocol used for variant calling in the present study gives optimal results with sample sizes of 30 or larger. Moreover, in our cohort there was the possibility that some of the individuals (e.g., P69, see Figure 1) married in with potentially their own forms of left-handedness, and do not necessarily share a genetic basis with the other members of the family. This could have confounded the analysis and should be controlled for when recruiting cohorts for future studies. Moreover, the test used to determine quantitative trait association did not account for different degrees of relatedness, but for a weakly heritable trait this is unlikely to bias the results. As rare variants might be highly cohort-specific, more studies in cohorts with diverse ethnic backgrounds are needed to completely exclude a possible influence of major rare variants on handedness. Another possible criticism of our data could be that it is unclear to what extent an overrepresentation of left-handedness is a specific characteristic of the sample that was investigated in our study or the general population it comes from. While there is no specific published data on handedness in the vicinity of Şanlı Urfa, studies in Turkish samples indicate that the frequency of left-handedness in Turkey is between 6% and 11% [41–43], which is in line with what has been found in other populations worldwide (around 10%). Tan reports the incidence of familial left-handedness in Turkey to be around 28.4% [43], which is lower than the 39.3% that has been reported in a large American sample [44]. Thus, the over-representation of left-handedness observed in our sample is typical for this family, not the general population in Turkey.

5. Conclusions

Taken together, both the analysis of single rare variants and the analysis of GO groups revealed no indication for a rare variant that could realistically determine handedness. Thus, our analysis in a Turkish cohort with lower genetic heterogeneity than the general population independently replicates previous findings from similar studies in Dutch [16] and Pakistani [17] cohorts. Thus, our study supports the conclusions of these studies that handedness is likely to be determined by complex polygenic and/or epigenetic factors [45].

Supplementary Materials: The following are available online at www.mdpi.com/link, Table S1: Mapped read metrics for all samples, Table S2: Depth of coverage summary with total and average bases and the percentage of the exome covered with at least 2×, 5×, 10, 20× and 30× sequence depth read.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft through Gu 227/16-1 to O.G. The authors also acknowledge support by the DFG Open Access Publication Funds of the Ruhr-Universität Bochum. We would like to thank Ahmet Çini, Macide Barutçuoğlu and Monika Güntürkün for their help with data collection.

Author Contributions: Onur Güntürkün and Sebastian Ocklenburg conceived and designed the experiments; Ceren Barutçuoğlu and Onur Güntürkün performed the experiments; Ceren Barutçuoğlu, Judith Schmitz and Sebastian Ocklenburg analyzed the data; Adile Öniz Özgören, Murat Özgören, Esra Erdal, Robert Kumsta and Dirk Moser contributed reagents/materials/analysis tools; Sebastian Ocklenburg wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Lien, Y.J.; Chen, W.J.; Hsiao, P.C.; Tsuang, H.C. Estimation of heritability for varied indexes of handedness. *Laterality* **2015**, *20*, 469–482.
2. Annett, M. Tests of the right shift genetic model for two new samples of family handedness and for the data of McKeever (2000). *Laterality* **2008**, *13*, 105–123.
3. Armour, J.A.; Davison, A.; McManus, I.C. Genome-wide association study of handedness excludes simple genetic models. *Heredity* **2014**, *112*, 221–225.
4. Eriksson, N.; Macpherson, J.M.; Tung, J.Y.; Hon, L.S.; Naughton, B.; Saxonov, S.; Avey, L.; Wojcicki, A.; Pe'er, I.; Mountain, J. Web-based, participant-driven studies yield novel genetic associations for common traits. *PLoS Genet.* **2010**, *6*, e1000993
5. McManus, I.C.; Davison, A.; Armour, J.A. Multilocus genetic models of handedness closely resemble single-locus models in explaining family data and are compatible with genome-wide association studies. *Ann. NY Acad. Sci.* **2013**, *1288*, 48–58.
6. Ocklenburg, S.; Beste, C.; Güntürkün, O. Handedness: A neurogenetic shift of perspective. *Neurosci. Biobehav. Rev.* **2013**, *37*, 2788–2793.
7. Ocklenburg, S.; Beste, C.; Arning, L.; Peterburs, J.; Güntürkün, O. The ontogenesis of language lateralization and its relation to handedness. *Neurosci. Biobehav. Rev.* **2014**, *43*, 191–198.
8. Rentería, M.E. Cerebral asymmetry: A quantitative, multifactorial, and plastic brain phenotype. *Twin Res. Hum. Genet.* **2012**, *15*, 401–413.
9. Arning, L.; Ocklenburg, S.; Schulz, S.; Ness, V.; Gerding, W.M.; Hengstler, J.G.; Falkenstein, M.; Epplen, J.T.; Güntürkün, O.; Beste, C. PCSK6 VNTR Polymorphism Is Associated with Degree of Handedness but Not Direction of Handedness. *PLoS ONE* **2013**, *8*, e67251.
10. Arning, L.; Ocklenburg, S.; Schulz, S.; Ness, V.; Gerding, W.M.; Hengstler, J.G.; Falkenstein, M.; Epplen, J.T.; Güntürkün, O.; Beste, C. Handedness and the X chromosome: The role of androgen receptor CAG-repeat length. *Sci. Rep.* **2015**, *5*, doi:10.1038/srep08325.
11. Brandler, W.M.; Morris, A.P.; Evans, D.M.; Scerri, T.S.; Kemp, J.P.; Timpson, N.J.; St. Pourcain, B.; Smith, G.D.; Ring, S.M.; Stein, J.; Monaco, A.P.; et al. Common variants in left/right asymmetry genes and pathways are associated with relative hand skill. *PLoS Genet.* **2013**, *9*, e1003751.
12. Francks, C.; Maegawa, S.; Laurén, J.; Abrahams, B.S.; Velayos-Baeza, A.; Medland, S.E.; Colella, S.; Groszer, M.; McAuley, E.Z.; Caffrey, T.M.; et al. LRRTM1 on chromosome 2p12 is a maternally suppressed gene that is associated paternally with handedness and schizophrenia. *Mol. Psychiatr.* **2007**, *12*, 1129–1139.
13. Leach, E.L.; Prefontaine, G.; Hurd, P.L.; Crespi, B.J. The imprinted gene LRRTM1 mediates schizotypy and handedness in a nonclinical population. *J. Hum. Genet.* **2014**, *59*, 332–336.
14. Robinson, K.J.; Hurd, P.L.; Read, S.; Crespi, B.J. The PCSK6 gene is associated with handedness, the autism spectrum, and magical ideation in a non-clinical population. *Neuropsychologia* **2016**, *84*, 205–212.
15. Scerri, T.S.; Brandler, W.M.; Paracchini, S.; Morris, A.P.; Ring, S.M.; Richardson, A.J.; Talcott, J.B.; Stein, J.; Monaco, A.P. PCSK6 is associated with handedness in individuals with dyslexia. *Hum. Mol. Genet.* **2011**, *20*, 608–614.

16. Somers, M.; Ophoff, R.A.; Aukes, M.F.; Cantor, R.M.; Boks, M.P.; Dauwan, M.; de Visser, K.L.; Kahn, R.S.; Sommer, I.E. Linkage analysis in a Dutch population isolate shows no major gene for left-handedness or atypical language lateralization. *J. Neurosci.* **2015**, *35*, 8730–8736.
17. Kavaklioglu, T.; Ajmal, M.; Hameed, A.; Francks, C. Whole exome sequencing for handedness in a large and highly consanguineous family. *Neuropsychologia* **2015**, *93*, 342–349.
18. Oldfield, R.C. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* **1971**, *9*, 97–113.
19. McManus, I.C. Right- and left- hand skill: Failure of the right shift model. *Br. J. Psychol.* **1985**, *76*, 1–34.
20. Bryden, M.P.; Tapley, S.M. A group test for the assessment of performance between the hands. *Neuropsychologia* **1985**, *23*, 215–221.
21. Annett, M. *Left, Right, Hand and Brain: The Right Shift Theory*; Lawrence Erlbaum Associates: Mahwah, NJ, USA, 1985.
22. Annett, M. *Handedness and Brain Asymmetry: The Right Shift Theory*; Psychology Press: Abingdon, UK, 2002.
23. Hugdahl, K.; Eichele, T.; Rimol, L.M. The effect of voice-onset-time on dichotic listening with consonant–vowel syllables. *Neuropsychologia* **2006**, *44*, 191–196.
24. Li, H.; Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **2009**, *25*, 1754–1760.
25. DePristo, M.A.; Banks, E.; Poplin, R.; Garimella, K.V.; Maguire, J.R.; Hartl, C.; Philippakis, A.A.; del Angel, G.; Rivas, M.A.; Hanna, M.; et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.* **2011**, *43*, 491–498.
26. Cingolani, P.; Platts, A.; Wang, L.L.; Coon, M.; Nguyen, T.; Wang, L.; Land, S.J.; Lu, X.; Ruden, D.M. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly* **2012**, *6*, 80–92.
27. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Statist. Soc. B* **1995**, *57*, 289–300.
28. Somers, M.; Shields, L.S.; Boks, M.P.; Kahn, R.S.; Sommer, I.E. Cognitive benefits of right-handedness: A meta-analysis. *Neurosci. Biobehav. Rev.* **2015**, *51*, 48–63.
29. Hirnstein, M.; Hugdahl, K. Excess of non-right-handedness in schizophrenia: Meta-analysis of gender effects and potential biases in handedness assessment. *Br. J. Psychiatr.* **2014**, *205*, 260–267.
30. Foundas, A.L.; Corey, D.M.; Hurley, M.M.; Heilman, K.M. Verbal dichotic listening in right and left-handed adults: laterality effects of directed attention. *Cortex* **2006**, *42*, 79–86.
31. Kaibara, M.; Ishihara, K.; Doi, Y.; Hayashi, H.; Ehara, T.; Taniyama, K. Identification of human Kir2.2 (KCNJ12) gene encoding functional inward rectifier potassium channel in both mammalian cells and Xenopus oocytes. *FEBS Lett.* **2002**, *531*, 250–254.
32. Kiesecker, C.; Zitron, E.; Scherer, D.; Lueck, S.; Bloehs, R.; Scholz, E.P.; Pirot, M.; Kathöfer, S.; Thomas, D.; Kreye, V.A.; et al. Regulation of cardiac inwardly rectifying potassium current IK1 and Kir2.x channels by endothelin-1. *J. Mol. Med.* **2006**, *84*, 46–56.
33. Karkanis, T.; Li, S.; Pickering, J.G.; Sims, S.M. Plasticity of KIR channels in human smooth muscle cells from internal thoracic artery. *Am. J. Physiol. Heart. Circ. Physiol.* **2003**, *284*, H2325–H2334.
34. Lee, I.; Park, C.; Kang, W.K. Knockdown of inwardly rectifying potassium channel Kir2.2 suppresses tumorigenesis by inducing reactive oxygen species-mediated cellular senescence. *Mol. Cancer Ther.* **2010**, *9*, 2951–2959.
35. Fowler, C.E.; Aryal, P.; Suen, K.F.; Slesinger, P.A. Evidence for association of GABA(B) receptors with Kir3 channels and regulators of G protein signalling (RGS4) proteins. *J. Physiol.* **2007**, *580*, 51–65.
36. Stonehouse, A.H.; Grubb, B.D.; Pringle, J.H.; Norman, R.I.; Stanfield, P.R.; Brammar, W.J. Nuclear immunostaining in rat neuronal cells using two anti-Kir2.2 ion channel polyclonal antibodies. *J. Mol. Neurosci.* **2003**, *20*, 189–194.
37. Sauerhöfer, S.; Yuan, G.; Braun, G. S.; Deinzer, M.; Neumaier, M.; Gretz, N.; Floege, J.; Kriz, W.; van der Woude, F.; Moeller, M.J. L-carnosine, a substrate of carnosinase-1, influences glucose metabolism. *Diabetes* **2007**, *56*, 2425–2432.
38. Barone, R.; Fiumara, A.; Jaeken, J. Congenital disorders of glycosylation with emphasis on cerebellar involvement. *Semin. Neurol.* **2014**, *34*, 357–366.
39. Drake, R.R. Glycosylation and cancer: moving glycomics to the forefront. *Adv. Cancer Res.* **2015**, *126*, 1–10.

40. Polašek, O.; Leutenegger, A.L.; Gornik, O.; Zgaga, L.; Kolcic, I.; McQuillan, R.; Wilson. J.F.; Hayward, C.; Wright, A.F.; Lauc, G.; et al. Does inbreeding affect N-glycosylation of human plasma proteins? *Mol. Genet. Genom.* **2011**, *285*, 427–432.
41. Ertas, U.; Canakçi, E. Prevalence and handedness correlates of recurrent aphthous stomatitis in the Turkish population. *J. Publ. Health Dent.* **2004**, *64*, 151–156.
42. Canakci, V.; Akgül, H.M.; Akgül, N.; Canakci, C.F. Prevalence and handedness correlates of traumatic injuries to the permanent incisors in 13–17-year-old adolescents in Erzurum, Turkey. *Dent. Traumatol.* **2003**, *19*, 248–254.
43. Tan, U. The distribution of the Geschwind scores to familial left-handedness. *Int. J. Neurosci.* **1988** *42*, 85–105.
44. Spiegler, B.J.; Yeni-Komshian, G.H. Incidence of left-handed writing in a college population with reference to family patterns of hand preference. *Neuropsychologia* **1983**, *21*, 651–659.
45. Ocklenburg, S.; Schmitz, J.; Moinfar, Z.; Moser, D.; Klose, R.; Lor, S.; Kunz, G.; Tegenthoff, M.; Faustmann, P.; Francks, C.; et al. Epigenetic regulation of lateralized fetal spinal gene expression underlies hemispheric asymmetries. *eLife* **2017**, *1*, e22784.