Supplementary Note

Unravelling the genetic architecture of musical rhythm

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Glossary

Music cognition terminology

beat perception: perceptual inference of a pulse given a rhythmic pattern (source: Kotz et al, 2018 https://www.cell.com/trends/cognitive-sciences/fulltext/S1364-6613(18)30191-8)

beat synchronization, also known as auditory-motor entrainment: production of periodic motor actions synchronized to a perceptual beat, inferred from quasi-periodic auditory stimulus (source: Kotz et al, 2018).

meter: "hierarchical structuring of a series of events (which may or may not be strictly isochronous) into higher-order groupings." (source: Kotz et al, 2018)

motor periodicity: repetitive action with an identifiable frequency and phase; motor periodicity is "ubiquitous in biology, including heartbeat, breathing, running, swimming, chewing, wake/sleep cycle". (source: Kotz et al, 2018)

musicality: set of traits allowing for the perception and production of music, constrained by our cognitive and biological systems (Honing, 2018; https://nyaspubs.onlinelibrary.wiley.com/doi/full/10.1111/nyas.13638)

rhythm discrimination task: experimental paradigm to test perception/differentiation of musical rhythms; usually administered as a same-different task.

rhythm: "systematic pattern of events in terms of timing, accent, and grouping" (Patel 2008, Chap. 3 https://psycnet.apa.org/record/2008-04843-000

Computational genetics terminology

chromosomal inversion: change in orientation of a segment of DNA within a chromosome. source: Puig et al, 2015 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4576756/

chronotype. Individual preference for sleep patterns, i.e. behavioral manifestation of circadian rhythms resulting in morning types and evening types. (source: https://en.wikipedia.org/wiki/Chronotype)

complex trait "A trait that does not follow Mendelian Inheritance patterns, is likely derived from multiple genes, and exhibits a large variety of phenotypes. (source: https://www.nature.com/scitable/definition/complex-trait-82/ Nature Education)"

electronic health record (EHR): electronically stored and managed medical chart.

gene regulation: mechanisms that act to induce or repress the expression of a gene **(source:** https://www.nature.com/subjects/gene-regulation)

Generalized Summary-data-based Mendelian Randomization (GSMR): statistical method for testing for causal influence between phenotypes, using GWAS data. (Source: Zhu et al, 2018, Nature communications).

Genetic correlations. Genetic relationship between two traits, related to the concept of **pleiotropy** (a genetic locus that affects more than one trait). With LDscore regression software, it is possible to test genetic correlations between complex traits measured in separate samples. (sources: https://www.nature.com/articles/ng.3406 and https://www.nature.com/articles/s41576-019-0137-z)

genome-wide association study (GWAS): an approach that scans markers across genomes of many people to find common genetic variants associated with diseases or complex traits (source: https://www.genome.gov/about-genomics/fact-sheets/Genome-Wide-Association-Studies-Fact-Sheet)

genomic locus: location of a gene or DNA sequence. source: https://www.cancer.gov/publications/dictionaries/genetics-dictionary/def/locus and https://doi.org/10.1016/j.mehy.2011.01.019. In the present study, each locus is defined using FUMA's mapping of independent SNPs.

handgrip strength. A proxy for muscular fitness; handgrip strength is also predictive of other health and fitness traits (sources: https://www.nature.com/articles/ncomms16015 and https://www.nature.com/articles/s41598-018-24735-y)

heritability on the liability scale: method of adjusting heritability estimates in the research study sample to account for population prevalence of a given trait (source: https://www.cell.com/ajhg/fulltext/S0002-9297(11)00020-6)

heritability: the estimate of how much of the variation in a given trait can be attributed to genetic variation. source: https://ghr.nlm.nih.gov/primer/inheritance/heritability)

Human Accelerated Regions (HARS): "DNA sequences that changed very little throughout mammalian evolution, but then experienced a burst of changes in humans since divergence from chimpanzees"
source: https://www.ncbi.nlm.nih.gov/pubmed/25156517

linkage disequilibrium (LD): "correlation between nearby variants such that the alleles at neighboring polymorphisms (observed on the same chromosome) are associated within a population more often than if they were unlinked."
source: https://www.sciencedirect.com/topics/neuroscience/linkage-disequilibrium

Partitioned heritability: "the proportion of genome-wide SNP heritability attributable to various functional categories" source: Finucane et al 2015 https://www.nature.com/articles/ng.3404

polygenic risk scores (also called polygenic scores or genetic risk scores or genetic risk profile scores): "The cumulative risk derived from aggregating contributions of the many DNA variants associated with a complex trait or disease" (source: (https://jamanetwork.com/journals/jama/fullarticle/2730627)

single nucleotide polymorphism (SNP): type of common genetic variation representing differences in building blocks of DNA (nucleotides). Source: https://ghr.nlm.nih.gov/primer/genomicresearch/snp

SNP-based heritability: total phenotypic variance explained by the aggregate of SNPs in a GWAS. source: https://www.nature.com/articles/ng.3941

UK Biobank: a large-scale community-based data repository, which houses many genetic, phenotypic, and other variables. https://www.ukbiobank.ac.uk/

Estimation of population prevalence of rhythm deficits.

In order to adjust heritability on the liability scale, we estimated the population prevalence of poor rhythm as between 3.5%-6.5% from existing data reported in two related large-scale studies [1,2]. Data from two rhythm tasks reported in Mosing et al. (2016) in nearly 7,000 Swedish twins was accessed and analyzed to determine the prevalence of individuals scoring more than 2 SD's below the mean. The rhythm perception task was the Rhythm scale of the Swedish Music Discrimination Test. In each of 18 items, participants are instructed to indicate whether two consecutively presented rhythmic sequences are the same or different. For a detailed description and psychometric validation of this test, see [3]. The Rhythm score was calculated as the number of correct responses. 3.95% of the participants scored less than two SDs below the mean. Rhythm production (motor timing) in the Swedish Twin cohort was measured using the ISIP task [4]. As described previously (see e.g. [4]), each ISIP trial consisted of a synchronization phase where the participant taps in synchrony with a regular, auditory metronome, followed by a continuation phase where the participant continues to tap self-paced with no metronome. Only data from the continuation phases of the experimental trials were used in the analyses. Because the SD is approximately linearly related to the IOI, the coefficient of variation (SD/mean) was used as a variability measure. The variable ISIP was calculated as the mean coefficient of variation across the six trials. 3.66% of the participants scored more than 2 SD's below the mean.

In addition, Peretz & Vuvan (2017) identified N=457 time-based amusics and N=569 individuals with an "uncategorized" musical deficit (including failing the Off-beat Test) out of a total N=16,625; the combined N=1026 with poor rhythm yields a

prevalence of 6.17% [2].Thus considering evidence on rhythm perception production deficits from both studies together, we chose to use a range of values (3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%) in our study for the heritability adjustment.

Test of association with chr17q21 inversion

Given that the genome-wide significant locus on chromosome 17q21 is located within a well-established inversion region that may also be associated with local population substructure, we conducted additional analyses focused on the region. First, similar to procedures described in [5], we conducted a principal component analysis (PCA) of subset of 10,000 samples from the GWAS samples and samples from the 1000 genomes project (Supplementary Figure 6). The SNPs that were genome-wide significant and tagged the inversion were not correlated with PC1, suggesting that the inversion polymorphism is not related to local subpopulation ancestry (Supplementary Table 16).

Sensitivity analyses of 'clap to beat' phenotype and Parkinson's Disease

One of the associated loci in the GWAS study is the *MAPT* locus (17q21 locus), known to be associated with Parkinson's disease (PD) [6]. The independent SNP in the locus, rs4792891, is in mild LD with the independent SNP associated with PD (rs365825, [7], r2=0.55). In light of prior research in PD patients showing lower rhythm perception task performance [8], they may have also have difficulty clapping in time with a musical beat. Here we found that PD status is significantly associated with difficulty in clapping to the beat (OR=0.5996, p=7.93e-35, adjusting for age, sex, 5 PCs). Due to the 23andMe-Michael J Fox Foundation collaboration, PD patients are over-represented in the 23andMe database. We tested for the possibility that the presence of PD patients in our study sample (less than 1% of the total) could account for the *MAPT* associations by removing PD cases (N=5,644) and fitting the same association model adjusting for age,

sex, five principal components, and genotyping platforms. The result shows the variant rs4792891 is still associated with the clap-to-beat phenotype ($p=1.61x10^{-13}$), thus showing that the *MAPT* association with the clap-to-beat phenotype is not driven by the presence of PD cases in our sample (Supplementary Table 17).

Proof-of-concept of the genetics of musicality in a health care context

We examined whether common alleles associated with rhythm en masse (also known as genetic profile risk scores or polygenic risk scores (PGS)) predict the presence of "musician" keyword ("musician status") recorded in a health care context. Our hypothesis was that higher PGS for rhythm will be associated with higher likelihood of having the keyword "musician" recorded in an individual's electronic health record in Vanderbilt University Medical Center's Biobank (VUMC BioVU). The PGS was standardized to have a mean of 0 and SD of 1.

Genotyping

The VUMC BioVU MEGA^{EX} project genotyped ~100,000 samples over a period of 2.5 years. DNA was obtained from blood samples from the Biobank participants and were assayed using Illumina bead arrays (MEGA^{EX}) containing more than 2 million markers. We only selected individuals of European ancestry with genetic data that met standard quality control thresholds.

The pre-imputation procedures followed standard quality control procedures including SNP pre-cleaning, filtering at an individual call rate <0.98, clarifying sex discrepancies, |Fhet| >0.2. Data was imputed to the Haplotype Reference Consortium panel using the Michigan Imputation Server and converted from dosage to hard calls using PLINK's default settings. It was then filtered to include only biallelic SNPs, Minor Allele Frequency (MAF)<=0.005, R^2>=0.3 and call rates <0.98. SNPs were filtered for batch

effects using logistic regression of paired imputation batches. SNPs were also filtered when MAFs within ancestry >0.1 difference from MAFs of corresponding 1000genomes MAFs. Finally, SNPs were also filtered when Hardy Weinberg Equilibrium p-value <10-[10] within ancestry.

Genome Profile Risk Scores

We generated PGS using the rhythm GWAS summary statistics. We used PRS_CS [9] to calculate the PGS. Briefly, this method uses a Bayesian regression framework and places continuous shrinkage (CS) prior on SNP effect sizes. This method outperforms previous methods in terms of prediction accuracy especially when the training sample size is large [9], as is the case with the rhythm GWAS.

Musician status in BioVU

Musician status in patient records was determined through keyword searches that represent a variety of musical professions (musician OR guitarist OR drummer OR pianist OR saxophonist OR vocalist) in patients' de-identified EHRs from the Synthetic Derivative database associated with genotyped data in the BioVU resource. We identified 864 individuals (541 (63%) males, mean age (SD)=49.2(19.3)) as musician "cases" that we compared with 66,577 controls (29,252(44%) males, mean age (SD)=48.2(22.7)), defined as absence of the above-listed musician keywords in their EHR. Although it is certainly possible that there are professional musicians within the Control group that do not have any indication of their musician profession associated with our particular keyword search in their EHR, the presence of such individuals within

the control sample will reduce power and increase the false negative rate of the analyses [10,11].

Control traits in BioVU

As negative control phenotypes we selected the following traits: Iron deficiency anemias (phecode:280, cases=4958, controls=62483), carcinoma in situ of skin (phecode:172.3, cases=580, controls=66861), and cancer of the brain (phecode:191.11, cases=1016, controls=66425).

Data analysis

We conducted a logistic regression where the outcome variable was keyword "musician" (yes vs no) and the predictor variable was PGS for rhythm, while also adjusting for median age, sex, 10 Principal Components and genotyping batch. The same process was followed when the outcome variables were iron deficiency anemias, carcinoma in situ of skin and cancer of the brain (cases vs. controls).

Results

We found evidence that the PGS for rhythm predicted musician status in BioVU (OR per SD increase in PGS, 1.30, 95%CI:1.20-1.38, $p<2.5 \times 10^{-13}$, Nagelkerke's R^2=1%), supporting our hypothesis. As expected, we did not find evidence for associations of the rhythm PGS with the negative control phenotypes (i.e., iron deficiency anemias, carcinoma in situ of skin, and cancer of the brain (Supplementary Table 9, Figure 3).

Mendelian Randomisation (MR) analyses

Given that musicality has been linked to speech/language skills [12], and executive function [13], that musicians have been found to have a higher prevalence of insomnia and sleep difficulties [14] and that listening to music can enhance hand grip strength [15], out of the traits that were significantly associated with rhythm in the genetic correlation analyses, we selected processing speed, chronotype (a sleep related trait) and hand grip strength to examine in an MR framework.

We conducted MR analyses using the GSMR [16] method, with filtering to remove SNPs with pleiotropic outliers using the Heidi test (Supplementary Table 15). We also present the estimates in the liability scale as per [17].

Bi-directional significant associations were observed between rhythm and all three traits tested. Specifically, when rhythm was an outcome, there was an increase of 0.08 in liability to rhythm per 1 SD increase in liability to chronotype ($b_{rhythm|chronotype}$=0.08, s.e.=0.04, p=1 x 10^{-2}, number of independent SNP instruments (nSNP=97). When rhythm was an exposure, there was an increase of 0.01 in liability to chronotype per 1 SD increase in liability to rhythm ($b_{chronotype|rhythm}$=0.01, s.e.=0.01, p=0.04, nSNP=65).

In terms of processing speed, when rhythm was an outcome, for 1 standard deviation change in processing speed, the odds of liability to rhythm increased per 1.79 ($b_{rhythm|processingspeed}$=1.79, s.e.=0.1, p=7.8 x 10^{-9}, nSNP=19). When rhythm was an exposure, the log of the odds ratio of processing speed was 0.07 per 1 SD increase in liability in rhythm ($b_{processingspeed|rhythm}$=0.07, s.e.=0.01, p=1.44 x 10^{-5}, nSNP=68).

Finally, when rhythm was an outcome, for 1 standard deviation change in hand grip strength, the odds of liability to rhythm increased per 1.34 ($b_{rhythm|handgrip}$=1.34, s.e.=0.1, p=2.4 x 10^{-5}, nSNP=111). When rhythm was an exposure, the increase in liability to hand grip strength per 1 SD increase in liability to rhythm was 0.09 ($b_{handgripstrength|rhythm}$=0.09, s.e.=0.1, p=3.3 x 10^{-14}, nSNP=64).

Genes previously implicated in musicality studies

Taking a list of genes previously implicated in musicality studies [18] as well as *GATA2*, *PCDH7* [19] and *UGT8* [20], we examined our current data for replications of these associations by looking at the p-values of each gene from the MAGMA, gene-based analysis (Supplementary Table 3). Although none of the genes reached the statistical significance threshold used for gene-based analysis ($p<3x10^{-6}$), several are located nearby our top gene in the MAGMA analysis, *CCSER1* in the 4q22-24 region. The list of genes and their p-values in our analysis are reported in Supplementary Table 4**.**

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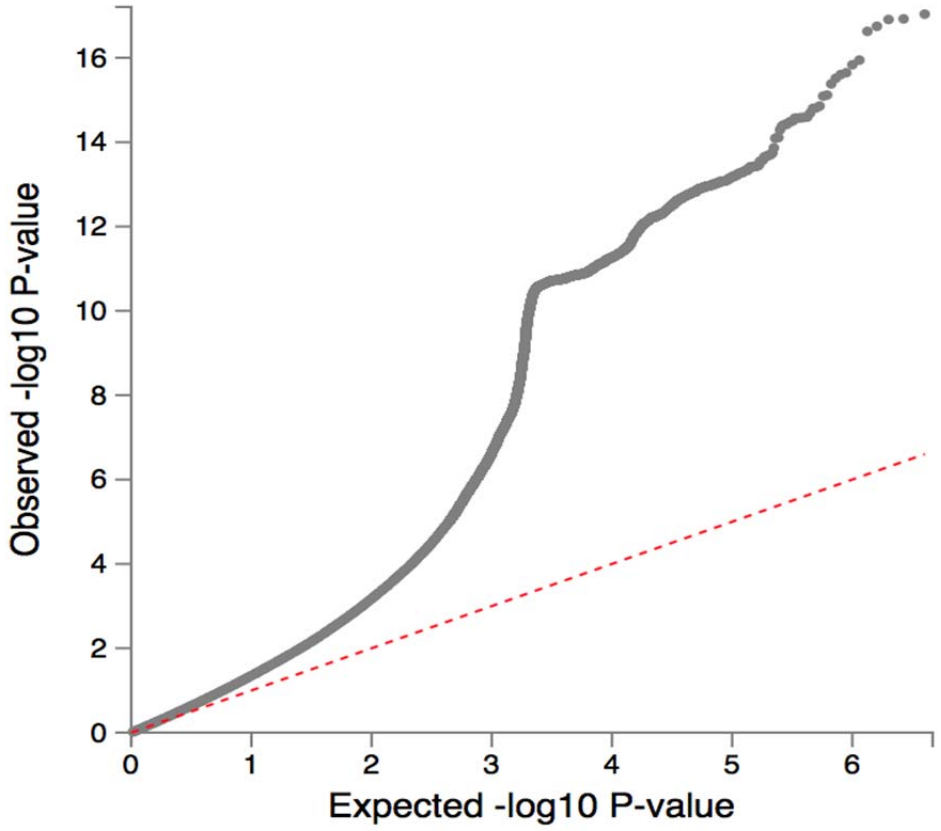
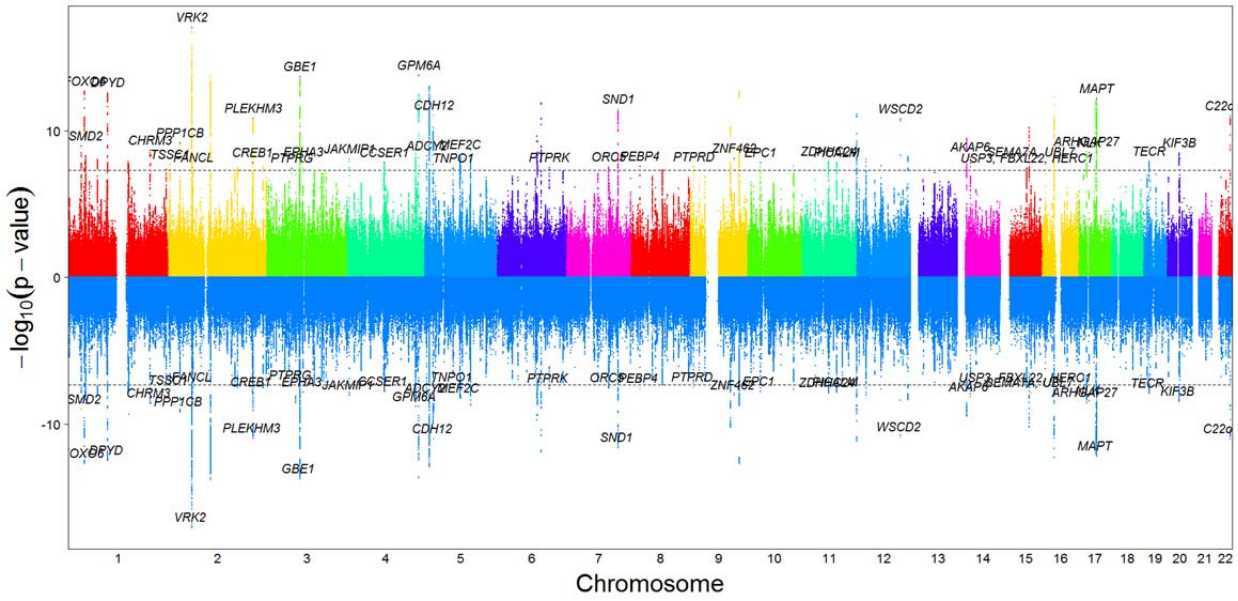
Supplementary Figures

Supplementary Figure 1. Q-Q plot of rhythm GWAS results. The Quantile-Quantile (QQ) plot shows observed vs. expected -$\log_{10}$p-values. The inflation observed in the QQ plot is likely due to polygenicity of the trait rather than to population stratification, given that the lambda and intercept indexes are within the expected range, and that when adjusting for population substructure (see **Supplementary Note and Supplementary Table 14**), the betas of the GWAS remained virtually identical.

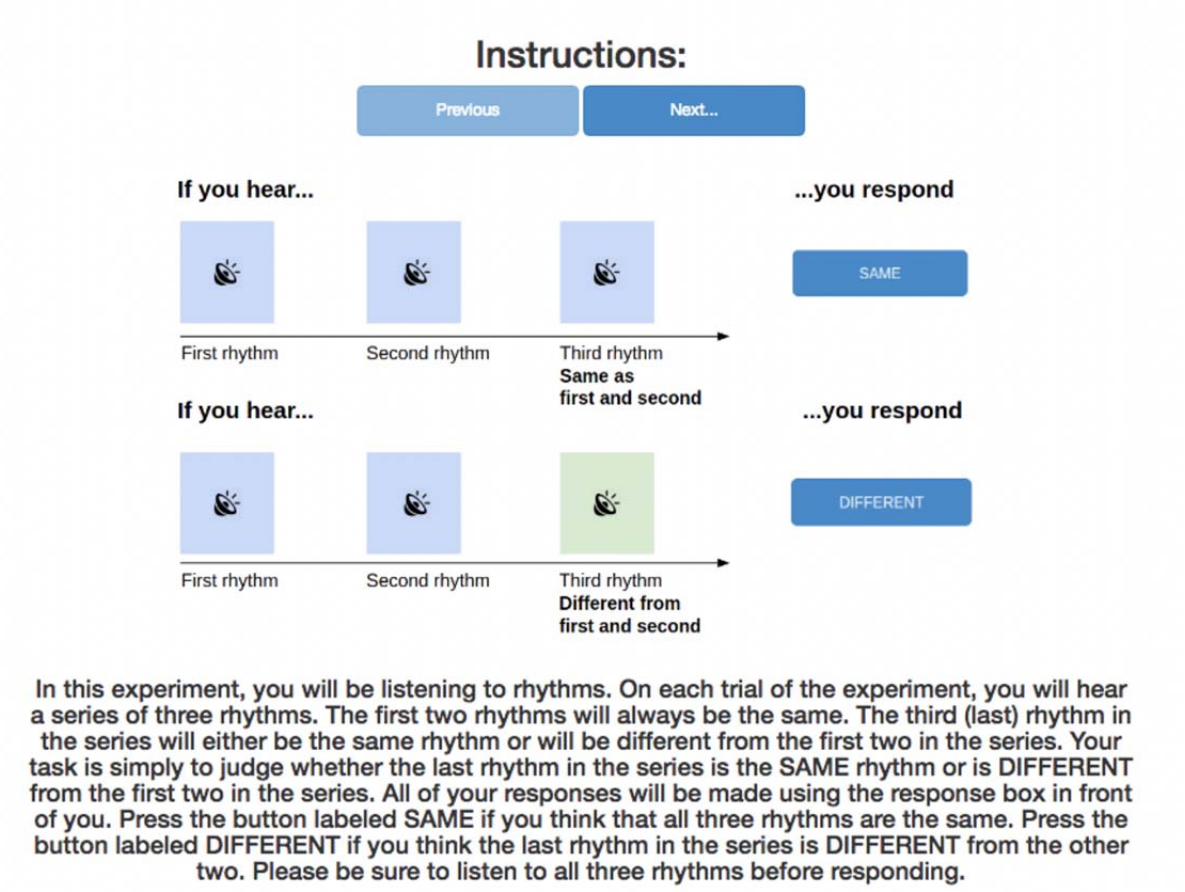


Lambda GC: 1.47
Intercept: 1.02(0.009)

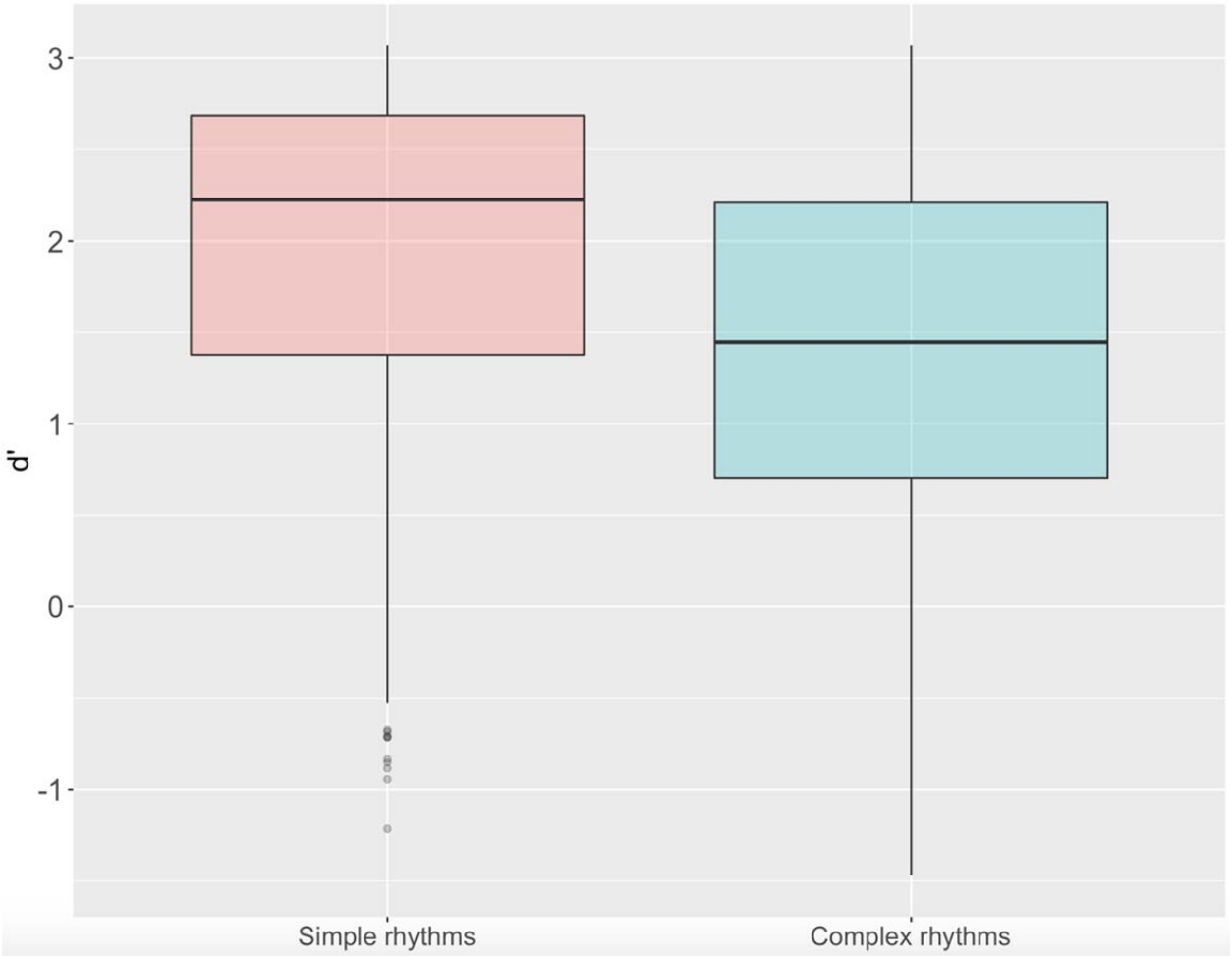
Supplementary Figure 2. Miami plot showing the rhythm GWAS results unadjusted (top) and adjusted (bottom) for IQ. We used mtCOJO to condition the rhythm GWAS summary statistics on GWAS of intelligence[21]. The results remain largely unchanged; 66 of the original 68 genomic loci still surpass the criteria for genome-wide significance ($p<5x10^{-8}$) after adjusting for IQ (**see Supplementary Table 10**). For illustration purposes we only present 500,000 SNPs with p<0.1



Supplementary Figure 3. Task instructions for internet-based rhythm perception task in phenotype validation study. Participants were instructed that in each trial, they would listen to the series of three rhythms (the first two were always identical, and the third could be the same or different) and have to indicate if the third rhythm was the same or different.

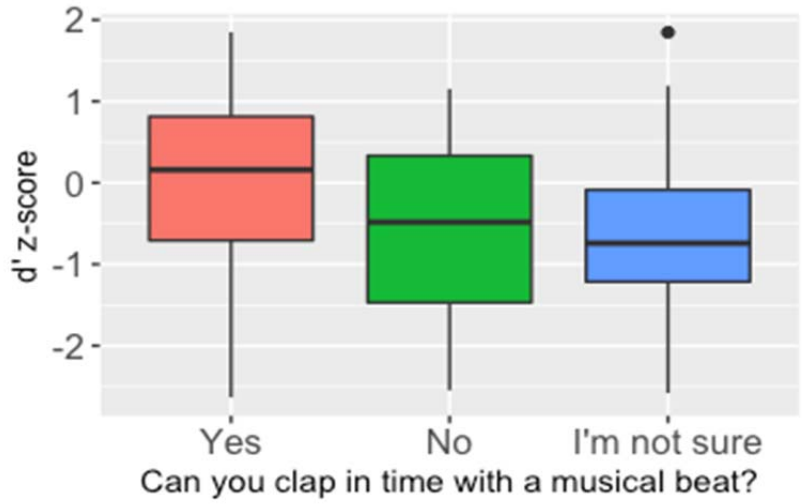


Supplementary Figure 4: Discrimination performance for simple vs. complex rhythm trials in the phenotype validation study. In line with prior work (Grahn & Brett, 2007), participants (N=724 individuals who participated via Amazon's Mechanical Turk) performed better ($t(724)=11.11$, $p<2.2 \times 10^{-16}$, Cohen's $d=0.58$) on simple, beat-based rhythms vs. complex rhythms (which may be syncopated and have a less prominent beat structure). Discrimination performance (*d'*) is indicated on the y-axis.



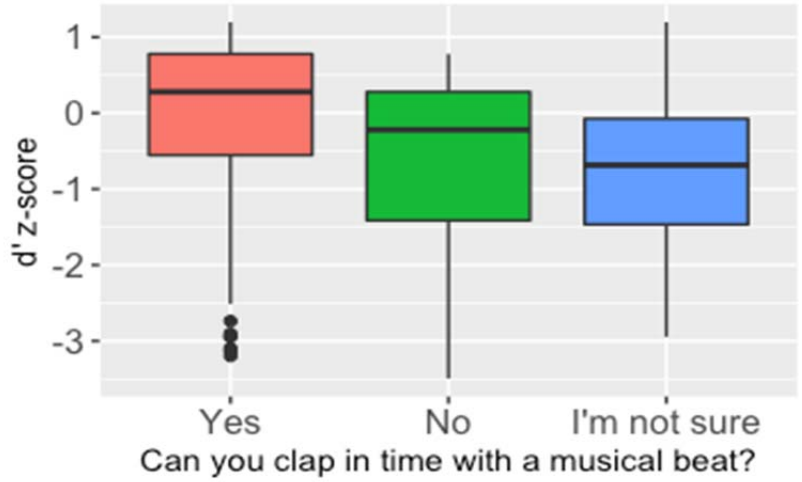
Supplementary Figure 5. Phenotype validation study results, showing a) Performance on the rhythm perception task (z-scored total *d'*) in relation to self-reported rhythm (beat synchronization) ability. b) Performance on simple rhythm trials in the rhythm perception task in relation to self-reported rhythm ability. Box plots represent the median values and interquartile range of values.

a.



OR(95%CI)=1.94(1.28 to 3.01), p=0.002, McFadden's R^2=0.39

b.



OR(95%CI=1.99(1.36-2.90), p<0.001, McFadden's R^2=0.40

Supplementary Figure 6. PCA plot of the study population.