

Europe. Even the previously reported distributions of autism risk score of AGRE individuals with and without the disorder¹ are consistent with this explanation (Supplementary Data).

As we found that autism risk scores based on the publicly available SNPs did not distinguish independent cases from controls, we asked if these score distributions differed between European populations. CEU (the control group used to train the classifier) had the lowest median and mean autism risk scores of these European populations (1.3 and 1.4, respectively) whereas Finns, a representative Northeastern European population, had the highest median and mean autism risk scores (2.8 and 2.7, respectively), as would be expected if the classifier were confounded by population structure. Their overall distributions also differed (two-sample K-S test, $P=0.0005$).

In the publication describing the classifier, an autism risk score cutoff of 3.93 was used to predict affectation status. We examined the properties of our populations using this cutoff, although we note that as we had data only on 19 of the 30 SNPs, it is an approximation of the results based on the 30 SNP classifier.¹ Importantly, the proportion of Finns above this autism risk score cutoff (29%) differed neither from AGRE cases (28%) nor AGRE controls (31%) (two-tailed Fisher's exact tests $P=0.89$ and $P=0.81$, respectively). In contrast, more Finns were classified as autistic than the training HapMap3 population CEU (12%; two-tailed Fisher's exact test $P=0.0054$), the independent 1000 Genomes British population GBR (17%; two-tailed Fisher's exact test $P=0.055$) and the HapMap3 Italian population TSI (16%; two-tailed Fisher's exact test $P=0.039$). These analyses lead to the conclusion that the autism risk scores based on the publicly available SNPs effectively separate European populations from one another, but do not separate cases from controls. Moreover, as Northeastern Europeans generally had higher scores than Western or Southern Europeans, this would result in inflated measures of accuracy in the previously reported independent validation that used diverse European Americans as cases and Northwestern Europeans as controls.¹

Whereas these strongest contributors to the classifier are more consistent with artifacts of population structure than with true autism spectrum disorder signal, it remains possible that there are some true signals differentiating cases and controls, particularly among the 207 weaker SNPs that are not currently publicly available. However, until more evidence can be provided, we favor the more conservative interpretation that these associations are due to previously unobserved population stratification in the cases and controls, and do not contribute meaningfully to a diagnostic classifier.

CONFLICT OF INTEREST

DHG is on the scientific advisory board of Synapdx. All other authors declare no conflict of interest.

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REFERENCES

- 1 Skafidas E *et al.* *Mol Psychiatry* 2012, e-pub ahead of print 11 September 2012; doi:10.1038/mp.2012.126.
- 2 Geschwind DH, Sowiński J, Lord C, Iversen P, Shestack J, Jones P *et al.* *Am J Hum Genet* 2001; **69**: 463–466.

3 Lajonchere CM, AGRE Consortium. *Neuron* 2010; **68**: 187–191.

4 Yang W-Y, Novembre J, Eskin E, Halperin E. *Nature* 2012; **44**: 725–731.

5 Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM *et al.* *Nucleic Acids Res* 2001; **29**: 308–311.

6 Kidd KK *et al.* *Am J Phys Anthropol* 2003, **120** (Suppl S36): 128.

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Response to Belgard *et al.*

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We thank the Editor for the opportunity to respond to the letter from Belgard *et al.*¹ In their letter, these authors consider that the issue of ethnic population stratification may have negatively impacted the findings in our original manuscript.² We agree that population stratification is an important issue that needs to be accounted for in such analyses.

We wrote to Dr Belgard who kindly provided the 19 single-nucleotide polymorphisms (SNPs) used in their analysis.¹ These 19 SNPs were derived from the 30 SNPs provided in our original article. Of these 19 SNPs, the number of SNPs with positive weights exceeded the number of SNPs with negative weights, including the second most negative weighted SNP, rs12317962, on KCNMB4, which would bias the classifier score. Our original analyses included a total of 237 SNPs. In order to address the issue of ethnic population stratification, we downloaded data from the 1000 genome cohort,³ including Central European (CEU), Finnish (FIN), Great British (GBR) and Iberian Spanish (IBS) populations.

In their analysis using 19 SNPs, Belgard *et al.* indicated that in Finns (non-autism spectrum disorder (ASD)), our classifier had a higher chance of classifying individuals as ASD compared with CEU (non-ASD) individuals. They concluded that our classifier might be better at separating between European subpopulations than cases from controls. In order to examine this in detail, we tested our classifier performance in correctly identifying control individuals from the CEU, FIN, GBR and IBS control populations. As not all SNPs were available across all data sets, we retrained the classifier using the common SNPs on our training set and then applied the classifier on unseen validation data from the FIN, GBR and IBS control cohorts. Comparing these ethnic European subpopulations, we found that greater differences in classifier score between these populations occurred when only part of the classifier was used (a difference as high as 25% was observed between the FIN and GBR groups). However, using the full classifier, the effects of ethnic population contributed to < 6% of the total difference in classifier score. We also provide the full 237 SNPs relevant to our classifier (Table 1). The full code used in the generation of the classifier has been made available on the Autism Genetic Resource Exchange (AGRE) website (<http://agre.org>), together with testing of the classifier on other ASD data sets.

Using our SNPs, we then examined their predictive accuracy in classifying control individuals from the FIN and GBR (non-ASD) populations, as well as SFARI (Simons Foundation Autism Research Initiative) ASD probands (the independent validation sample in our paper). We plotted the percentage of individuals classified as

Table 1. List of all 237 SNPs for ASD classifier in the CEU Cohort,² organised from highest to lowest median weightings

SNP	Weight lower	Weight median	Weight upper	Gene no.	Gene symbol
rs968122	1.5465	1.5555	1.5645	27345	KCNMB4
rs876619	0.9476	1.2092	1.4708	2775	GNAO1
rs11020772	0.8553	0.8641	0.8729	2915	GRM5
rs9288685	0.5856	0.5998	0.614	3635	INPP5D
rs10193128	0.5836	0.5946	0.6056	3635	INPP5D
rs7842798	0.5298	0.5386	0.5474	114	ADCY8
rs3773540	0.5125	0.5208	0.5291	55799	CACNA2D3
rs1818106	0.5002	0.5161	0.532	80310	PDGFD
rs2384061	0.4195	0.4306	0.4417	109	ADCY3
rs12582971	0.3983	0.4295	0.4607	5288	PIK3C2G
rs10409541	0.4067	0.4189	0.4311	773	CACNA1A
rs2300497	0.3782	0.3889	0.3996	801	CALM1
rs7562445	0.3741	0.3843	0.3945	2066	ERBB4
rs7313997	0.3382	0.3567	0.3752	5801	PTPRR
rs2239118	0.3348	0.3552	0.3756	775	CACNA1C
rs4688054	0.1801	0.3476	0.515	2932	GSK3B
rs10823195	0.2597	0.3445	0.4294	1763	DNA2
rs9798267	0.2759	0.3388	0.4017	84083	ZRANB3
rs1075354	0.4236	0.3177	0.6402	55799	CACNA2D3
rs1942052	0.2641	0.3088	0.3535	130013	ACMSD
rs4696443	0.2525	0.3047	0.3569	23321	TRIM2
rs243196	0.2402	0.2976	0.3549	1112	FOXN3
rs16929470	0.1854	0.2712	0.3571	775	CACNA1C
rs7580690	0.1647	0.2248	0.285	83439	TCF7L1
rs7145618	0.1515	0.2238	0.296	5528	PPP2R5C
rs3770132	0.1514	0.2093	0.2673	3676	ITGA4
rs3790095	0.1215	0.2017	0.2819	2775	GNAO1
rs1013459	0.1417	0.1969	0.2522	2774	GNAL
rs11001056	0.1519	0.1891	0.2263	5592	PRKG1
rs10952662	0.148	0.1868	0.2257	26047	CNTNAP2
rs7756516	0.152	0.1853	0.2186	3120	HLA-DQB2
rs8054767	0.1322	0.1803	0.2284	5579	PRKCB
rs2239028	0.1121	0.1763	0.2405	775	CACNA1C
rs3935743	0.0969	0.1737	0.2505	5336	PLCG2
rs1928168	0.0657	0.099	0.1322	401237	LINC00340
rs7100765	0.0434	0.0935	0.1436	5593	PRKG2
rs1369450	0.0563	0.0924	0.1285	114	ADCY8
rs1040336	-0.0615	0.091	0.2435	2272	FHIT
rs10407144	0.0434	0.0872	0.131	773	CACNA1A
rs10794197	0.045	0.0869	0.1287	1488	CTBP2
rs3734464	0.0247	0.0868	0.149	5071	PARK2
rs7864216	-0.0072	0.0863	0.1798	9630	GNA14
rs4254056	0.0432	0.0846	0.126	338751	OR52L1
rs988920	0.0453	0.0842	0.1232	9229	DLGAP1
rs12393998	0.0536	0.0839	0.1142	8450	CUL4B
rs872794	0.0413	0.0813	0.1213	3778	KCNMA1
rs2503220	-0.0527	0.0806	0.214	5142	PDE4B
rs10468681	0.0356	0.08	0.1243	2774	GNAL
rs7258489	0.0428	0.079	0.1152	808	CALM3
rs153968	0.0379	0.0765	0.115	5144	PDE4D
rs944761	0.0361	0.076	0.1159	9568	GABBR2
rs2161630	0.0232	0.0754	0.1276	10725	NFAT5
rs7097311	0.0294	0.0703	0.1111	5593	PRKG2
rs2088747	-0.0137	0.0693	0.1522	11060	WWP2
rs9832697	-0.0766	0.0689	0.2144		KCNMB2
rs7731023	0.0343	0.0683	0.1023	6502	SKP2
rs7120612	0.0224	0.0659	0.1094	390055	OR52A6
rs2033655	0.0277	0.0647	0.1017	109	ADCY3
rs1453541	-0.1057	0.0354	0.1766	219983	OR4D6
rs3746821	-0.0262	0.0335	0.0932	958	CD40
rs220740	-0.0085	0.0332	0.0749	10846	PDE10A
rs2299679	-0.014	0.0331	0.0801	5332	PLCB4
rs887387	-0.0028	0.0317	0.0662	489	ATP2A3
rs7174459	-0.0092	0.0288	0.0669	4735	NEDD5
rs884399	-0.0073	0.0281	0.0634	5581	PRKCE
rs5021051	-0.0146	0.027	0.0686	2895	GRID2
rs2903813	-0.0208	0.0252	0.0711	3315	HSPB1
rs1062935	-0.0207	0.0245	0.0697	57521	RPTOR

Table 1. (Continued)

SNP	Weight lower	Weight median	Weight upper	Gene no.	Gene symbol
rs9347553	-0.0154	0.0228	0.0609	5071	PARK2
rs11072416	-0.0259	0.0222	0.0703	6263	RYR3
rs4553343	-0.0304	0.0204	0.0712	2977	GUCY1A2
rs7146234	-0.0132	0.0202	0.0535	5495	PPM1A
rs848282	-0.0191	0.0172	0.0536	55120	FANCL
rs7962764	-0.0495	0.0126	0.0748	5801	PTPRR
rs12726519	-0.0377	0.0098	0.0572	5321	PLA2G4A
rs718949	-0.0303	0.0093	0.0489	1488	CTBP2
rs1954787	-0.0264	0.0089	0.0441	2900	GRIK4
rs2238079	-0.0283	0.0084	0.045	775	CACNA1C
rs1337420	-0.0398	0.008	0.0558	2898	GRIK2
rs917948	-0.0553	0.0075	0.0704	5536	PPP5C
rs3817222	-0.1848	0.0055	0.1957	4660	PPP1R12B
rs17531147	-0.0612	0.003	0.0672	55970	GNNG12
rs11048476	-0.0801	-0.0384	0.0033	3709	ITPR2
rs4145903	-0.0762	-0.0395	-0.0028	783	CACNB2
rs10505029	-0.1011	-0.0404	0.0203	51366	UBR5
rs1122838	-0.1213	-0.0408	0.0396	9630	GNA14
rs1993477	-0.0818	-0.0434	-0.0049	51366	UBR5
rs2179871	-0.0912	-0.0454	0.0005	10369	CACNG2
rs10740244	-0.0892	-0.0467	-0.0041	5592	PRKG1
rs2503220	-0.1151	-0.0472	0.0207	5142	PDE4B
rs1065657	-0.0838	-0.0488	-0.0139	51465	UBE2J1
rs12714137	-0.1234	-0.0528	0.0179	83439	TCF7L1
rs7176475	-0.1275	-0.0537	0.0201	123746	PLA2G4E
rs1937671	-0.0953	-0.0545	-0.0138	5592	PRKG1
rs7079293	-0.0902	-0.0549	-0.0196	10581	SORBS2
rs1003854	-0.1288	-0.0551	0.0187	326	AIRE
rs919741	-0.0962	-0.0565	-0.0169	815	CAMK2A
rs750438	-0.1075	-0.0574	-0.0074	11184	MAP4K1
rs6139034	-0.0997	-0.0576	-0.0154	3704	ITPA
rs1554606	-0.1087	-0.0599	-0.0111	6018	IL6
rs7108524	-0.0938	-0.0603	-0.0267	81286	OR51E3
rs1002424	-0.1023	-0.0626	-0.0229	5562	PRKAA1
rs2239316	-0.1033	-0.0631	-0.0228	1387	CREBBP
rs5030949	-0.157	-0.0653	0.0264	3098	HK1
rs17682073	-0.1006	-0.066	-0.0315	6262	RYR2
rs1872902	-0.1108	-0.0665	-0.0221	80310	PDGFD
rs11602535	-0.166	-0.1236	-0.0812	219981	OR5A2
rs11644436	-0.1733	-0.1253	-0.0774	5336	PLCG2
rs10762342	-0.1909	-0.1283	-0.0658	5592	PRKG1
rs11583646	-0.2023	-0.1311	-0.0599	6262	RYR2
rs6118611	-0.1819	-0.1321	-0.0822	5332	PLCB4
rs2587891	-0.1722	-0.1322	-0.0922	2775	GNAO1
rs4651343	-0.1739	-0.1333	-0.0926	5321	PLA2G4A
rs1659506	-0.1761	-0.1363	-0.0966	23295	MGRN1
rs2271986	-0.1968	-0.1367	-0.0767	4842	NOS1
rs2302898	-0.1775	-0.1375	-0.0975	10381	TUBB3
rs6971999	-0.2088	-0.1425	-0.0763	26212	OR2F2
rs2272197	-0.1896	-0.1485	-0.1073	4216	MAP3K4
rs4947963	-0.1867	-0.1493	-0.1119	1956	EGFR
rs7536307	-0.1876	-0.1507	-0.1138	26289	AK5
rs12462609	-0.2085	-0.151	-0.0936	773	CACNA1A
rs1517521	-0.2925	-0.152	-0.0114	23180	RFTN1
rs8063461	-0.1865	-0.1534	-0.1203	7249	TSC2
rs888817	-0.1937	-0.1604	-0.1272	5924	RASGRF2
rs922445	-0.2435	-0.1659	-0.0883	2775	GNAO1
rs339408	-0.203	-0.167	-0.131	9322	TRIP10
rs7512378	-0.2068	-0.1691	-0.1314	55811	ADCY10
rs7870040	-0.2408	-0.1892	-0.1376	774	CACNA1B
rs3904668	-0.2423	-0.2069	-0.1715	29993	PACSIN1
rs12716928	-0.2784	-0.2073	-0.1362	5336	PLCG2

Abbreviations: ASD, autism spectrum disorder; CEU, Central European; SNP, single-nucleotide polymorphism. Weight indicates the contribution of each SNP to ASD clinical status. The lower and upper weights represent the 95% confidence intervals (CIs) of the distribution of weights for each SNP.

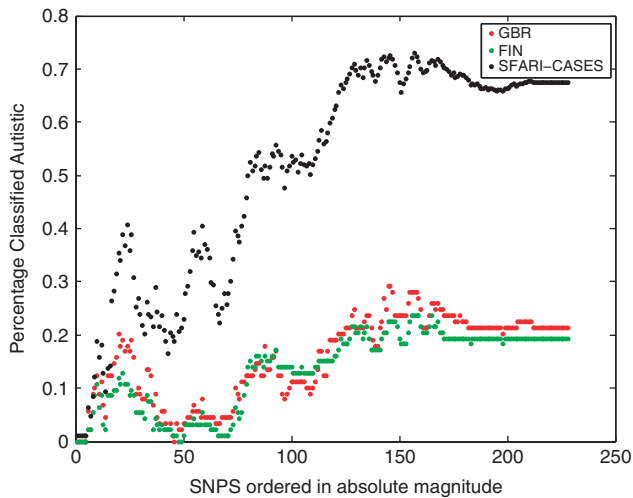


Figure 1. Percentage of individuals classified as ASD as a function of the number of single-nucleotide polymorphisms (SNPs) ordered in decreasing absolute magnitude. Significant variance was observed at smaller number of SNPs (not plotted). Note the gradient differential between SFARI cases versus FIN and GBR between SNPs 80 and 150. ASD, autism spectrum disorder; SNPs, single-nucleotide polymorphisms; SFARI-CASES, Simons Foundation Autism Research Initiative ASD probands; population samples from the 1000 genome cohort³: GBR, Great British; FIN, Finnish.

ASD against the number of SNPs used in the classifier, with SNPs ordered by absolute magnitude of their weightings. As can be seen in Figure 1, while population stratification may have an influence at lower SNP numbers with regard to differences in classifier accuracy between populations, such an effect is diminished as a greater number of SNPs are included. The separation in percentage classified as ASD between the SFARI/ASD and the FIN/GBR groups occurred with increasing gradient between 50 and 100 SNPs, whereas at >150 SNPs the separation between these groups plateaus. This is to be expected, as these SNPs have the smallest weightings within the classifier. Therefore, in keeping with Belgard *et al's* analysis, we show that at low SNP numbers, population effects may influence classification accuracy, but these effects are of second order to the ASD signal as the number of SNPs increases.

Using the classifier, as described above, we tested its accuracy in correctly classifying controls (non-ASD) within individual European cohorts. We achieved accuracies (that is, correct classification as non-ASD) of 82% for the FIN, 78% for GBR and 67% for the Spanish cohorts. In addition, to determine classifier performance confidence intervals, we performed a bootstrap analysis (1000 permutations were undertaken; 80% of the data was used to train a classifier to predict the remaining 20%) on all white non-hispanic populations, including all available populations (that is, SFARI and Autism Genetic Resource Exchange probands, and WTBC, CEU, FIN, GBR and IBS Controls). Diagnostic accuracy for ASD was 66.0% (90% CI: 61.5–71.9), with a sensitivity of 63.4% (90% CI: 54.3–75.9) and specificity of 67.2% (90% CI: 59.5–74.3). This equates to a positive likelihood ratio of 1.9 (90% CI: 1.3–3.0).

In our paper, we reported positive and negative predictive accuracies that were 70.8% and 71.8%, respectively.² Based on a

population prevalence of 1:88 cases of ASD in the US population,⁴ this equates to a positive predictive value (that is, precision) of 2.8% and a negative predictive value of 99.5%. This suggests that the classifier is not suitable as a general screening method, rather it should only be considered in high-risk populations where the base rate of ASD is high and produces acceptable positive and negative predictive values.

In conclusion, we demonstrate that the SNPs in our classifier show some ability to non-randomly distinguish between ASD and controls and that our results are not merely explained by population stratification as demonstrated in our analyses in independent cohorts of individuals of European ancestry. Further work on such approaches is needed in order to validate these findings, for example, prospective studies that examine children at risk for ASD (such as families with an affected member).

CONFLICT OF INTEREST

A patent application has been filed by The University of Melbourne.

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REFERENCES

- Belgard TG, Jankovic I, Lowe JK, Geschwind DH. *Mol Psychiatry* advance online publication, 2 April 2013; doi:10.1038/mp.2013.34 (e-pub ahead of print).
- Skafidas E, Testa R, Zantomio D, Chana G, Everall IP, Pantelis C. *Mol Psychiatry* advance online publication, 11 September 2012; doi:10.1038/mp.2012.126 (e-pub ahead of print).
- Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE *et al.* *Nature* 2012; **491**: 56–65.
- Centers for Disease Control and Prevention. *Surveillance Summaries* 2012; **61**: 1–19.



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