

Benchmarking Twist Genotyping-by-Sequencing Against Whole-Genome Sequencing in Nuclear Families

Authors

Jacob Klugerman, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, ijklugerman@uchicago.edu

Ivan Iossifov, Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY 11724, iossifov@cshl.edu

Kenny Ye, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, kenny.ye@einsteinmed.edu*

* *Corresponding author*

Abstract

Genome-wide genotyping is widely used in human genetics research, and targeted sequencing-based approaches such as the Twist Bioscience genome-wide SNP capture platform (GxS) have emerged as alternatives to conventional SNP arrays. Here, we evaluated GxS genotype calls from 555 individuals in 184 nuclear families against matched whole-genome sequencing (WGS) calls and compared platform performance with that of the Illumina Infinium Global Screening Array-24 (GSA), which was evaluated in 987 individuals from 279 nuclear families. Genotype data were harmonized across platforms, and analyses were restricted to overlapping SNP loci. Across all callable positions, mean per-SNP call rates were 98.26% for GxS and 98.67% for GSA. Overall SNP concordance with WGS was 99.79% for GxS and 99.87% for GSA, and mean per-individual concordance was also 99.79% and 99.87%, respectively. Per-trio Mendelian violation rates of GxS are about 10 times those of WGS, while those of GSA are about 4 times those of WGS on average. These results indicate that GxS performs slightly worse than GSA by key concordance and inheritance metrics, while still showing strong overall agreement with WGS.

Ethics approval and consent to participate

All data were obtained from the SPARK cohort under an institutional data access agreement. The original SPARK study received IRB approval from SFARI. No additional ethics approval was required for this secondary analysis.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed in this study are available from the Simons Foundation Autism Research Initiative (SFARI) upon approved application at <https://base.sfari.org>. The individual ID and family ID of the subjects included in the study are provided in the Supplementary materials in the PLINK .fam format. Computer programming code is available from the first author upon reasonable request.

Competing interests

JK, II, and KY declare that they have no competing interests.

Funding

The work of Iossifov and Ye is supported in part by Simons Foundation (SFARI SF497800)

Authors' contributions

JK performed data analysis and drafted the manuscript. II contributed to the conception and design of the study. KY conceived and designed the study and supervised the data analyses. All authors read and approved the final manuscript.

Acknowledgements

The authors acknowledge using OpenAI's ChatGPT (GPT-5.5) to generate an initial draft of portions of the introduction. The authors reviewed, edited, and revised all generated text and take full responsibility for the accuracy and integrity of the final manuscript. We are grateful to all the families at the participating SPARK sites as well as the SPARK Consortium for contributing the genetic data that made this research possible. We appreciate the opportunity to access phenotypic and genomic sequencing datasets (including SPARK iWGS release v1.1 and iWES release 3) on SFARI Base. We thank the Simons Foundation Autism Research Initiative (SFARI) for establishing and funding the SPARK cohort [5].

Background

Genome-wide genotyping is widely used in human genetics research, including genome-wide association studies (GWAS) and polygenic risk prediction. SNP array platforms such as the Illumina Infinium Global Screening Array-24 have been widely adopted for their low cost, high reproducibility, and established analytical workflows. Combined with genotype imputation, SNP arrays can capture a large proportion of common human genetic variation [1,2].

More recently, targeted sequencing-based genotyping approaches have emerged as alternatives to conventional SNP arrays. The Twist Bioscience genome-wide SNP capture (GxS) platform uses hybridization-based enrichment to interrogate genome-wide SNP loci and may offer advantages in assay flexibility and compatibility with sequencing-based workflows. A recent study demonstrated the utility of the Twist GxS platform for genotyping challenging and degraded DNA samples [4].

However, we were unable to find a third-party evaluation of the Twist Bioscience SNP capture platform or any similar platforms in the existing literature. In this study, we evaluate genotypes called by the Twist Bioscience genome-wide SNP capture platform (GxS) on 555 individuals comprised of 184 nuclear families, with genotype calls by whole-genome sequencing (WGS) [3] on the same individuals serving as a benchmark. Moreover, we compare performance metrics of GxS, such as genotype concordance with WGS and Mendelian violation frequency, to those of the Illumina GSA-24 platform (GSA), which will

be evaluated similarly on 987 individuals of 279 nuclear families, with no overlap with the 555 individuals genotyped by GxS.

Methods

Study Design and Data Sources

This study used genetic data from the SPARK cohort [5], including whole-genome sequencing (WGS) data from SPARK iWGS release v1.1 [6] and whole-exome sequencing (WES) data from SPARK iWES release 3 [7]. Within the iWES release, samples from Batches 1–4 were additionally genotyped using Illumina Global Screening Array version 1 (GSAv1) or version 2 (GSAv2), whereas samples from Batches 5–9 were genotyped using the Twist Bioscience Genomic Screening (GxS) platform. Among subjects genotyped using the GxS platform, 184 nuclear families with a total of 555 individuals were also included in the iWGS release. Among subjects genotyped using the GSAv1 or GSAv2 platforms, 279 nuclear families with a total of 987 individuals were also included in the iWGS release. The performance of GxS was evaluated using WGS calls as a benchmark on these nuclear families and compared to the performance of GSA, which was evaluated in the same way.

Genotype harmonization

Genotype data generated from the Twist Bioscience genome-wide SNP capture platform (GxS) and the Illumina Infinium Global Screening Array-24 (GSA) were compared against whole-genome sequencing (WGS) genotype calls. Note that both platforms only target SNP

markers and no indels were target. For GSA, only those SNP markers present in both GSAv1 and GSAv2 platforms were retained in our analysis.

Using PLINK v2.0 [8], GxS and GSA genotype data in binary PLINK format included in the iWES release v.1.1 were converted into PED/MAP text formats. WGS genotype calls of iWGS provided in the variant call format (.vcf) were similarly converted into PED/MAP formats to facilitate comparison across platforms. Genotype harmonization was performed by matching SNP loci of GxS (or GSA) to WGS variants using chromosome and base-pair position information. Among SNPs on the GxS (or GSA) platform, only those variants whose position had an exact match in WGS genotyping calls were retained for downstream analyses.

Concordance and Call Rates

For each SNP in GxS (or GSA) that had a WGS variant matched by exact position and matched alternative alleles, we compared the genotype calls on each individual directly where both platforms made eligible SNP calls. Insertions, deletions, and other non-single-base variants call by WGS were treated as missing, as well as no-calls made by either WGS or the platform being evaluated. Matching genotypes were classified as concordant, and all others as discordant. Concordance and call rates were aggregated across all SNP positions and individuals. We further classified concordance rates by six SNP variant types (A/C, A/G, A/T, C/G, C/T, G/T). To assess systematic genotype-specific error patterns between the tested platforms and WGS, genotype confusion matrices were generated for each of six variant types.

Mendelian violations

Mendelian inheritance consistency was evaluated using parent–child trios available within each dataset. Mendelian violations were checked only on SNP loci for which genotype calls were available for the child and both parents in both the tested platform and WGS.

Mendelian violation rates were aggregated for each trio and normalized by the number of eligible loci.

Filtering by concordance and call rate

The performances of GxS and GSA were re-evaluated on a subset of SNPs that had greater than 99% concordance with WGS and call rates greater than 99%.

Results

Call rates

Across all positions in both datasets, the mean per-SNP call rate, aggregated over all individuals, was 98.26% for GxS, with a median of 100% and a 90th percentile of 97.84%. In comparison, the mean, median, and 90th percentile rates for GSA were 98.67%, 99.80%, and 97.16%, respectively. In GxS, 54.77% of positions had a call rate of 100%, and 84.57% had a call rate of at least 99%. In GSA, 28.21% of positions had a call rate of 100%, and 78.03% had a call rate of at least 99%.

The mean and median per-individual call rates were 98.26% and 98.53% for GxS, and 98.67% and 98.99% for GSA. Among 555 individuals genotyped on GxS, 121 (21.8%) exhibited call rates exceeding 99% across all positions; out of 987 individuals genotyped on GSA, 483 (48.94%) had call rates greater than 99%. On the other hand, only 1 out of 555 (0.18%) GxS individuals had a call rate below 95%, while 18 out of 987 (1.8%) GSA individuals did.

Concordance with WGS genotyping Calls

Out of 1,414,695 GxS SNP markers, 1,355,694 were matched to WGS variants by their positions. Of 604,929 GSA SNP markers present in both GSAv1 and GSAv2 platforms, 560,450 were matched to WGS variants. The overall concordance rates across all individuals and all positions for GxS and GSA were 99.79% and 99.87%, respectively. Concordance rates varied slightly by variant type (Fig. 1a), with variant A/T having the lowest concordance rate in both GxS and GSA at 99.74% and 99.81% respectively. Overall, GSA is slightly better than GxS across all six SNP variant types.

The cumulative density plots of non-concordance rates over all positions, shown in Fig. 1b, display that GxS had a higher proportion of lower concordance positions than GSA. 0.59% of GxS positions had a concordance rate below 95%, compared to GSA's 0.29%, and 4.41% of GxS positions had a concordance rate below 99% compared to 2.45% of GSA positions.

The mean per-individual concordance rates with WGS were 99.79% in GxS and 99.87% in GSA, with medians of 99.80% and 99.92%, respectively. All individuals in GxS and 984 out of 987 individuals in GSA had genotype concordance rates greater than 99%. As shown in

Figure 1d, per-individual non-concordance rates were positively associated with per-individual no-call rates among both GxS and GSA. The relationship is non-linear but tight, with Spearman correlations of 0.82 for GxS and 0.92 for GSA. Moreover, it is also clear from the figure that GxS per-individual call-rates and concordance rates show less variation than those of GSA.

Mendelian violations

There are 187 trios from 181 nuclear families for GxS, and 429 trios from 279 nuclear families for GSA. Among the 187 mother-father-child trios present in GxS data, the average Mendelian violation rate across all valid positions was 0.108%. In contrast, the Mendelian violation rate was 0.005% in matched WGS calls, more than 20 times lower. The average Mendelian violation rate in GSA was 0.035% across 429 family trios and 0.008% in matched WGS calls. These observations confirm that WGS genotyping calls are superior to those from either GxS or GSA.

As shown in Figure 1c, GxS mendelian violation rates for each parent-child trio were consistently above the corresponding rates of WGS, whereas the rates of GSA showed greater variability across trios, and in many cases were on par or even lower than those of WGS.

Filtering

GxS and GSA call data were filtered to exclude SNP positions with a concordance rate less than 99% and calls for fewer than 99% of individuals by either the platform being evaluated or WGS. After filtering, 1,128,522 positions were retained for GxS and 439,590 for GSA. The

concordance rates with WGS for the retained SNPs increased to 99.22% for GxS and 99.99% for GSA. The concordance rates also become more consistent across the six variant types (Fig. 1a).

Per-family Mendelian violation rates for GxS, GSA, and WGS were recalculated after the filtering. Among the 187 trios in GxS, the Mendelian violation rate for retained positions decreased by a factor of 2 to 0.0487%, and in GSA, the per-family Mendelian violation rate decreased by a factor of 5 to 0.0064%. However, the per-family rates of GxS remained consistently higher than the corresponding WGS rates, whereas those of GSA were similar to WGS (Fig. 1c).

Discussion

The SPARK WES release includes whole-genome SNP genotyping data but switches genotyping platforms from GSA to GxS in Batches 5-9. This study is motivated by the lack of published third-party studies on Twist GxS platforms.

We only used variants that had an exact match within the WGS variant calls provided by the iWGS release. A small but non-trivial proportion of SNPs on either GxS (4.2%) and GSA (7.3%) platforms did not match WGS variant calls because these positions contained indels and were annotated to different positions by the WGS variant calling pipeline. Realigning these variants would require additional work, and we believe that excluding them is unlikely to alter our conclusion. We computed the average call rates for these unmatched variants, and they are slightly lower than those for the matched variants

(96.32% compared to 98.34% in GxS and 96.91% compared to 98.79% in GSA, respectively). The slight decrease in call rates is not surprising given the complications of having indels at those positions.

For the matched positions, we removed WGS indels from the comparison, since by design, GxS and GSA only call SNPs. Removed indel calls represent less than 0.3% of total calls at these positions and are less than 20% of the number of no-call WGS results.

Our analysis showed that GSA has a slightly better average concordance rate than GxS. However, these rates were aggregated over largely different targeting. Therefore, we compared average concordance rates for the 560,450 variants that were present in both platforms, stratifying by six variant types. As shown in Supplementary Figure 1, GSA still performs slightly better than GxS at these overlapped positions.

We further investigated discordances between GxS and WGS and compared them to discordances between GSA and WGS. Table 1 shows confusion matrices for each variant type. As can be seen, GxS is much more likely to incorrectly call heterozygous genotypes homozygous, but also much less likely to call homozygous genotypes heterozygous, compared to GSA. This difference can be explained by the different technology used by these two platforms. GxS is sequence-based, so if one of two alleles at a heterozygous site is not observed, a homozygous call is made. The chance of this happening is $1/2^{n-1}$, where n is the number of reads observed at this site. At $n=10$, this is approximately .2%. This vulnerability also explains the high Mendelian violation rate observed in GxS calls, as it can result when a heterozygous parent genotype is called homozygous.

Conclusion

Overall, we found that the Twist GxS genotyping platform is highly accurate, although slightly less accurate than the more traditional Illumina array platform. In addition, compared to GSA, GxS has lower assay-to-assay variation, as per-individual call rates and concordance rates are more consistent. Nonetheless, we also observed a higher proportion of unreliable SNP variants than GSA and would recommend that those positions be filtered out for genetic analysis.

References

1. Geibel J, Reimer C, Weigend S, Weigend A, Pook T, Simianer H. How array design creates SNP ascertainment bias. *PLoS One*. 2021, 16(3):e0245178. <https://doi.org/10.1371/journal.pone.0245178>
2. Verlouw JAM, Clemens E, de Vries JH, Zolk O, Verkerk AJMH, am Zehnhoff-Dinnesen A, et al. A comparison of genotyping arrays. *Eur J Hum Genet*. 2021, 29(11):1611-1624. <https://doi.org/10.1038/s41431-021-00917-7>
3. Telenti A, Pierce LCT, Biggs WH, di Iulio J, Wong EHM, Fabani MM, et al. Deep sequencing of 10,000 human genomes. *Proc Natl Acad Sci U S A*. 2016, 113(46):11901-11908. <https://doi.org/10.1073/pnas.1613365113>
4. Staadig A, Ballard D, Tillmar A, Hedman J, Krzewinska M, Sidstedt M, et al. Comparative assessment of SNP genotyping assays for challenging forensic samples utilizing ancient DNA methods. *Genome Biol*. 2025, 26(1):433. <https://doi.org/10.1186/s13059-025-03912-z>
5. SPARK Consortium. SPARK: A US cohort of 50,000 families to accelerate autism research. *Neuron*. 2018, 97(3):488-493. <https://doi.org/10.1016/j.neuron.2018.01.015>
6. Simons Foundation Autism Research Initiative (SFARI). SPARK iWGS release v1.1 release notes/documentation. 2026. <https://www.sfari.org/resource/spark-whole-genome-sequencing-data/>. Accessed 11 June 2026.

7. Simons Foundation Autism Research Initiative (SFARI). SPARK iWES release 3 release notes/documentation. 2026. <https://www.sfari.org/resource/spark-whole-exome-sequencing-data/>. Accessed 11 June 2026.
8. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*. 2015, 4(1):7. <https://doi.org/10.1186/s13742-015-0047-8>

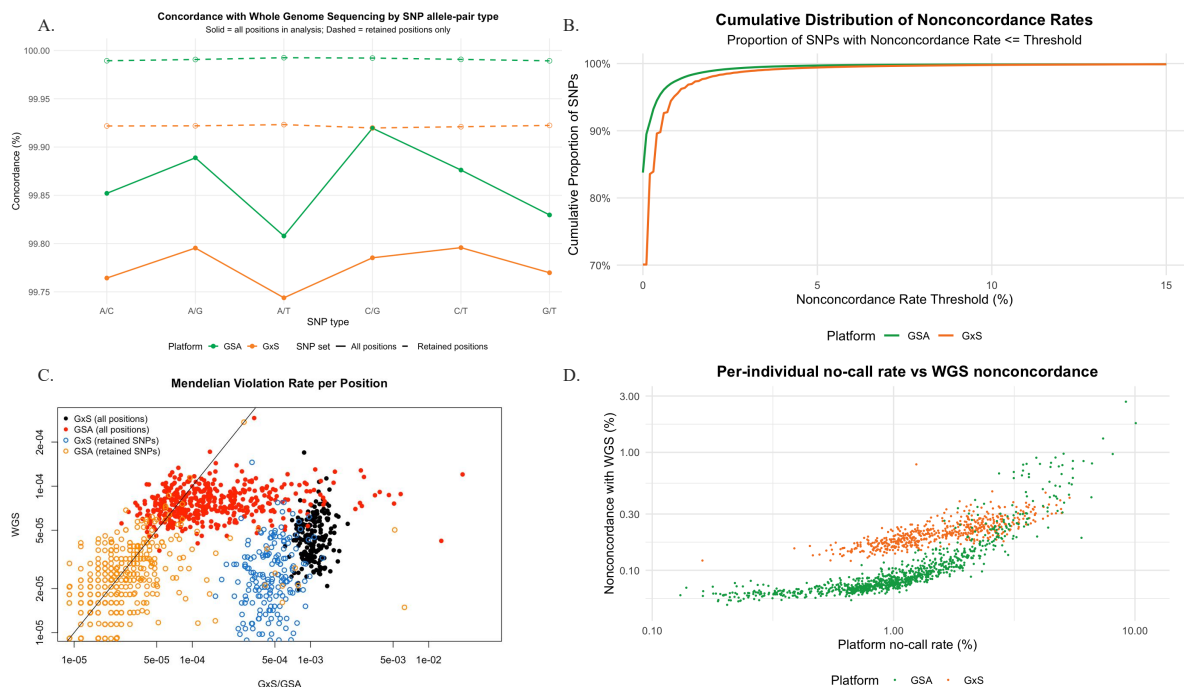
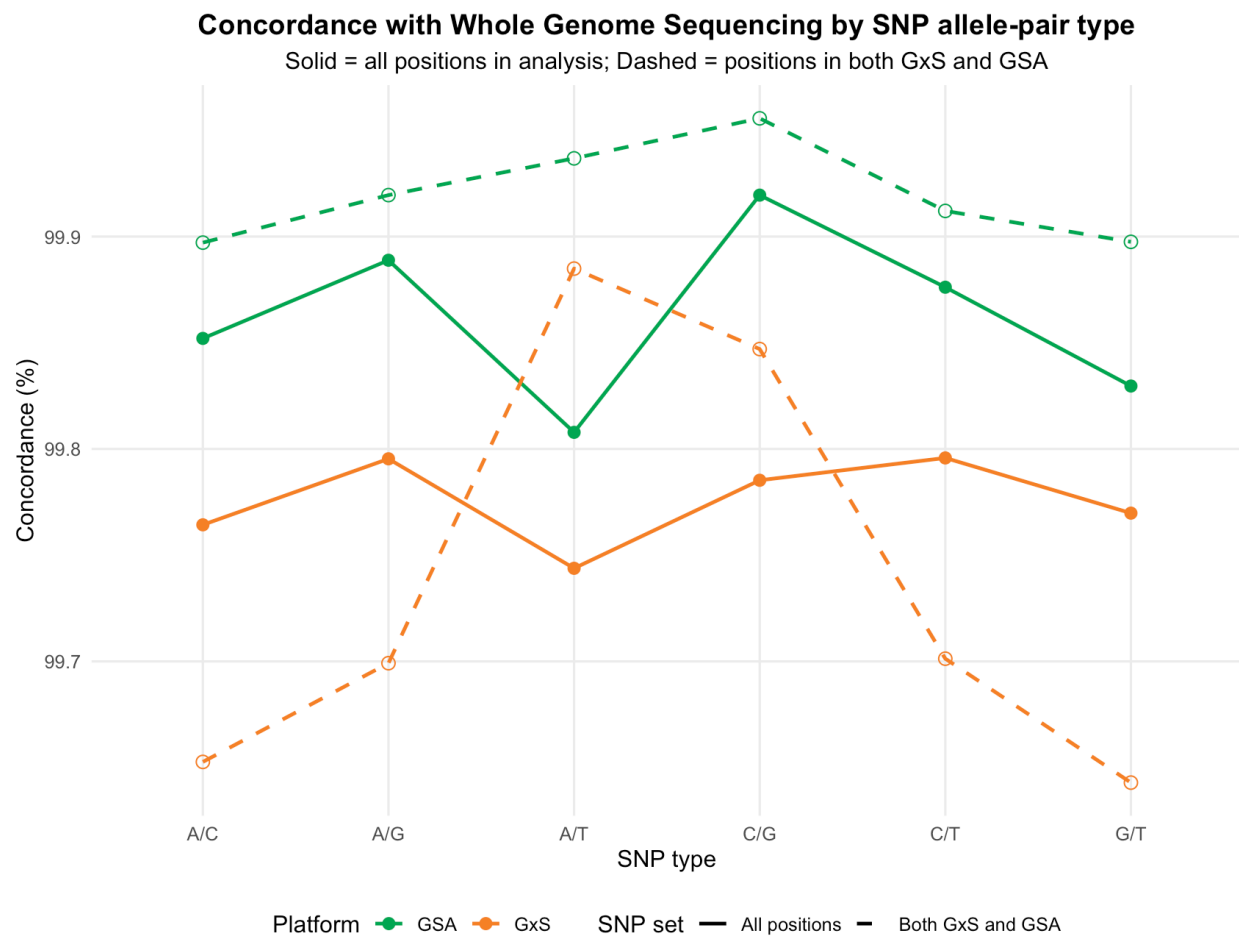


Figure 1. Comparison of genotype performance metrics between Twist Genotyping-By-Sequencing (GxS) and the Global Screening Array (GSA) using whole-genome sequencing (WGS) as the reference standard. **(A)** Genotype call concordance rates for GxS and GSA relative to WGS, stratified by SNP position type based on unordered reference/alternate (REF/ALT) allele pairs. **(B)** Cumulative distribution of per-SNP nonconcordance rates for GxS and GSA compared with WGS. **(C)** Per-family Mendelian violation rates for GxS and GSA relative to WGS across all analyzed variant positions and across the subset of positions with genotype call rates $\geq 99\%$. **(D)** Per-individual no-call rates and per-individual non-concordance rates with WGS for GxS and GSA.



Supplementary Figure 1. Per-position concordance rates with Whole Genome Sequencing by SNP allele-pair type including overlapping SNP positions between Twist Genotyping-By-Sequencing (GxS) and Illumina Global Screening Array (GSA). The dashed line represents concordance rates for positions that were present in both GxS and GSA comparisons with WGS.

GxS/GSA vs WGS calls by variant type

shaded diagonal = concordant

GxS – A/C				GxS – A/G				GxS – A/T			
	AA	AC	CC		AA	AG	GG		AA	AT	TT
AA	22,991,438	15,121	11	AA	87,824,056	58,529	51	AA	20,804,649	15,075	10
AC	51,609	10,501,432	56,599	AG	167,323	42,968,015	203,451	AT	48,411	8,471,084	48,461
CC	17	20,149	28,051,990	GG	48	81,053	119,411,629	TT	5	15,818	20,678,306

GxS – C/G				GxS – C/T				GxS – G/T			
	CC	CG	GG		CC	CT	TT		GG	GT	TT
CC	27,505,748	18,635	17	CC	119,315,368	79,022	54	GG	27,923,697	19,834	12
CG	52,798	11,347,842	49,255	CT	202,966	42,945,626	167,886	GT	56,655	10,421,795	47,833
GG	17	18,522	27,253,756	TT	62	58,183	87,362,532	TT	184	14,373	22,492,981

GSA – A/C				GSA – A/G				GSA – A/T			
	AA	AC	CC		AA	AG	GG		AA	AT	TT
AA	18,700,557	18,116	3,903	AA	76,083,000	56,096	8,522	AA	552,122	308	8
AC	6,306	9,112,407	10,028	AG	21,132	38,893,576	25,694	AT	81	179,687	100
CC	4,765	29,811	22,403,551	GG	13,195	120,726	105,800,155	TT	1,566	421	562,501

GSA – C/G				GSA – C/T				GSA – G/T			
	CC	CG	GG		CC	CT	TT		GG	GT	TT
CC	1,156,737	648	20	CC	105,732,256	120,464	10,398	GG	22,037,106	30,722	3,287
CG	183	301,766	115	CT	29,035	39,136,090	24,683	GT	13,349	9,039,106	7,460
GG	603	543	1,206,187	TT	14,460	74,060	76,026,669	TT	6,181	22,872	18,535,200

Table 1. Confusion matrices of genotype calls for GxS and GSA relative to WGS, stratified by SNP variant type.