The first gapless, reference-quality, fully annotated
genome from a Southern Han Chinese individual

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Abstract

We used long-read DNA sequencing to assemble the genome of a Southern Han Chinese male. We organized the sequence into chromosomes and filled in gaps using the recently completed T2T-CHM13 genome as a guide, yielding a gap-free genome, Han1, containing 3,099,707,698 bases. Using the T2T-CHM13 annotation as a reference, we mapped all genes onto the Han1 genome and identified additional gene copies, generating a total of 60,708 putative genes, of which 20,003 are protein-coding. A comprehensive comparison between the genes revealed that 235 protein-coding genes were substantially different between the individuals, with frameshifts or truncations affecting the protein-coding sequence. Most of these were heterozygous variants in which one gene copy was unaffected. This represents the first gene-level comparison between two finished, annotated individual human genomes.

Keywords: genome assembly, annotation, DNA sequencing, reference genome, variant calling

Introduction

Over the past 20 years, the biomedical research community has relied on a single human reference genome, which was first published in 2001 (International Human Genome Sequencing Consortium 2001; Venter et al. 2001) when it was less than 90% complete, and which has steadily improved since then. That reference genome, currently version GRCh38, is a mosaic of many individuals, which means that it does not capture the actual genome of any single human. In addition, the GRCh38 genome has hundreds of gaps, including very large gaps for all of the centromeres and the short arms of acrocentric chromosomes. In 2022, a breakthrough publication from the Telomere-to-Telomere (T2T) consortium described the first-ever complete human genome, T2T-CHM13, in which all gaps had been filled and which represented a single individual of northern European descent (Nurk et al. 2022). Although CHM13 describes a female and is missing the Y chromosome, the T2T consortium finished a gap-free Y chromosome from another individual, HG0002, and released that as part of the T2T-CHM13 assembly.

The existence of a finished human genome, together with highly accurate long-read sequencing technology, now allows the assembly of additional human genomes of similarly high quality and completeness. As demonstrated first by the assembly of an Ashkenazi individual, Ash1 (Shumate et al. 2020), and more recently by the assembly of a Puerto Rican individual, PR1 (Zimin et al. 2021), long-read data (either on its own or in combination with short reads) can be used to generate highly accurate contigs covering most of the human genome, and these can then be scaffolded using either GRCh38 or T2T-CHM13 as a guide. Following scaffolding, the CHM13 sequence can then be used to fill in all gaps, including the centromeres, to produce a gap-free assembly.

An individual genome is far more useful as a research resource if it is annotated. Several computational pipelines can produce annotation de novo for a newly assembled genome, but when the genome represents another individual human, as opposed to a new species, comprehensive annotation is not usually necessary. Instead, one can simply map (or “lift over”) the annotation from a reference human genome onto the new assembly, which is usually a superior approach when the reference is of high quality and has reliable annotations. This is the approach we followed.

The first genome sequenced from a Han Chinese individual (designated YH), which was also the first Asian individual genome assembled, was published over a decade ago (Wang et al. 2008). Subsequently, another Southern Han (SH) Chinese individual genome, HX1, was de novo assembled using single-molecule real-time long reads (Shi et al. 2016). Research then shifted toward northern individuals: the first Northern Han (NH) Chinese individual genome, NH1.0, appeared in 2019 (Du et al. 2019), and the most recent Han Chinese haplotype-resolved genome, HJ-H1 and HJ-H2, was released in early 2022 (Yang et al. 2022). In addition to these four Han Chinese genomes, two other individual genomes, each representing an ethnic group closely related to Han, have been assembled: ZF1 (a Tibetan individual) (Yang et al. 2022) and TJ1.p0/TJ1.p1 (a Tujia individual) (Lou et al. 2022). However, all these
Table 1. A comparison among Han1, GRCh38, and the assemblies of previously released Chinese genomes.

| Genomic Assembly | Ethnicity | Contig N50 (Mb) | Number of contigs | Number of gaps | Assembly size (Gb) |
|------------------|-----------|----------------|-------------------|----------------|-------------------|
| Han1             | SH Chinese| 148.02         | 25                | 0              | 3.10              |
| HG00621 (hifiasm) | SH Chinese| 95.77          | 182               | 157            | 3.11              |
| T2T-CHM13v2.0    | Northern European| 150.62 | 25                | 0              | 3.12              |
| HJ-H1            | NH Chinese| 28.15          | 1,330             | 427            | 3.07              |
| HJ-H2            | NH Chinese| 25.90          | 896               | 390            | 2.91              |
| NT1              | NH Chinese| 3.60           | 11,019            | 8,484          | 2.89              |
| HX1              | SH Chinese| 8.33           | 5,843             | 4,025          | 2.93              |
| YH2.o            | SH Chinese| 0.02           | 361,157           | 235,514        | 2.91              |
| TJ1.p0           | Tuja      | 13.67          | 1,430             | 907            | 2.87              |
| TJ1.p1           | Tuja      | 13.70          | 1,426             | 873            | 2.87              |
| ZF1              | Tibetan   | 23.62          | 1,384             | 1360           | 2.85              |
| Mixed            |           | 57.88          | 994               | 804            | 3.10              |

Assembly sizes include estimated gaps.
1: Statistics based on chromosomes 1–22, X, Y, and mitochondrial DNA of Han1 (CHM13 reference-guided assembly).
2: Statistics based on primary contigs of HG00621 hifiasm de novo assembly.
3: Statistics calculated directly from the T2T-CHM13 assembly, v2.0.
4: Statistics taken from Table S2 of Yang et al. (2022).
5: Statistics calculated directly from the YH2.o assembly, downloaded from NCBI at https://www.ncbi.nlm.nih.gov/assembly/GCA_000004845.2/.
6: Statistics calculated directly from the ZF1 assembly, downloaded from the ZF1 Genome Sequence Archive page at https://ngdc.cncb.ac.cn/bioproject/browse/PRCA000936.
7: Statistics calculated using the primary assembly only, excluding alternative scaffolds. Scaffolds were split into contigs at position where the number of Ns was greater than 10.

Genomes still contain gaps and none were fully annotated. Table 1 provides a comparison among these genomes and the new Han1 assembly.

Here we describe the assembly of deep-coverage long-read data to produce the genome of a Southern Han Chinese male individual, which we designate Han1. We filled in all gaps using CHM13 sequence where necessary, and then annotated the genome by mapping over the latest RefSeq annotation from T2T-CHM13 onto Han1. We then compare the annotation between Han1 and T2T-CHM13 and report on some of the key differences.

Materials and methods

Sample background

The data for the HG00621 genome were generated and made publicly available by the Human Pangenome Reference Consortium (https://github.com/human-pangenomics/hpgp-data). HG00621 is a Southern Han Chinese male from Fu Jian Province located on the southeastern coast of China. We chose HG00621 for this study because it represents a population for which no annotated genome previously existed, and because Han is the largest ethnic group in the world. The estimated population size of Han people is approximately 1.4 billion, with around 1.29 billion living in China (National Bureau of Statistics of China 2021) and 21.8 million living in Taiwan (National Bureau of Statistics of China 2021) and 21.8 million living in Taiwan (National Bureau of Statistics of China 2021) and 21.8 million living in Taiwan (National Bureau of Statistics of China 2021). Moreover, as most Han immigrants to the United States of America, Taiwan, Singapore, Malaysia, Philippines, and other territories originated from southern China (Cavalli-Sforza 1998). A complete and accurate Han Chinese reference genome should be a valuable resource for genetic studies in this population. Another benefit of selecting HG00621 is that sequencing data are available from both of his parents, HG00619 and HG00620, which provides opportunities to study allelic variations in the future. Also it is worth noting that at least three of HG00621’s grandparents are confirmed SH Chinese individuals.

Genome assembly

Our main goal in this study was to create a gapless, reference-quality, haplotype-merged assembly of the HG00621 genome with 25 sequences representing chromosomes 1 to 22, X, Y, and the mitochondrial DNA. We assembled contig sequences de novo, and we used the T2T-CHM13 genome recently published by the T2T consortium to guide the scaffolding process. The T2T-CHM13 genome is far more complete and accurate than the previous standard, GRCh38 (Nurk et al. 2022). CHM13 represents a northern European individual, and because it is a female sample, it lacks a Y chromosome. We used version 1.1 of the T2T-CHM13 assembly (https://github.com/marbl/CHM13), a gap-free version augmented with the complete Y chromosome from HG002, an individual of Ashkenazi Jewish descent.

The initial Han1 assembly was created using PacBio high-fidelity (HiFi) reads and Oxford Nanopore Technology (ONT) reads, with approximately 39x coverage in HiFi reads and 35x coverage in ONT reads (Table 2). The basecallers were ccs v4.0.0 (HiFi) and Guppy v4.0.11 (ONT). We assembled all HiFi reads with Hifiasm (Cheng et al. 2021) version 0.16.1-r375 and all ONT reads with Flye (Kolmogorov et al. 2019) version 2.5. The statistics of these two preliminary assemblies are shown in Table 3. Comparing both assemblies, the HiFi draft assembly had substantially higher contiguity with an N50 contig size of 95.8 Mb and only 182 contigs, while the Flye assembly had an N50 of 40.9 Mb and 1,658 contigs. We, therefore, chose the Hifiasm output as the main assembly for the subsequent steps.

For the scaffolding step, we first used the MaSuRCA chromosome scaffolder module (Zimin et al. 2017) (chromosome_scaffolder.sh) with the T2T-CHM13 reference to order and orient contigs generated by Hifiasm. This process is similar to the scaffolding procedures we used in building the Ashkenazi (Ash1) (Shumate et al. 2020) and Puerto Rican (PR1) (Zimin et al. 2021) reference genomes. The chromosome scaffolder identified 12 misassembled contigs that we had to split before scaffolding. A list of alignments
of these 12 contigs to T2T-CHM13 appears in Supplementary Table S1.

After scaffolding, we had 25 scaffolds (with gaps) representing nuclear chromosomes 1–22, X and Y, plus 77 unplaced contigs containing an additional 38,815,834 bp. The chromosome sequences contained only 101 gaps.

We then proceeded with three gap closing steps. For these steps, we used MaSuRCA’s intra-scaffold gap-closer script (close_scaffold_gaps.sh), which is a wrapper for the SAMBA scaffold folder (Zimin and Salzberg 2022). In the first step, we used HiFi reads and default parameters, which closed 24 gaps. The second step used contigs generated by Flye from the ONT reads with modified parameters “--m 2500 --o 10000,” which set the thresholds for the minimum match length on both sides of a gap (2,500bp) and the maximum overhang (10,000bp). This step closed an additional 19 gaps. The third and final step of automated gap closing used the CHM13 sequence to attempt closing the remaining 58 gaps. This step closed another 45 gaps with only 7 gaps remaining in chromosomes 13, 14, 15, and 22. Manual inspection of these gaps identified repeat-induced misassemblies in pericentromeric regions in chromosomes 13 and 15, which we corrected manually, and redundant haplotype-variant contigs in chromosomes 14 and 22 that we deleted. A more detailed strategy for solving misassembled contigs is shown in Supplementary Figure S1 and Supplementary Materials, and we listed their positions on Han1 draft assembly in Supplementary Table S1 and Supplementary Materials, and we listed their positions on Han1 draft assembly in Supplementary Table S2.

After fixing the misassemblies and eliminating redundant haplotype contigs, we re-ran gap closing and ended up with a single contig for every chromosome. We observed that one of the unplaced contigs contained several copies of the mitochondrial sequence, and we extracted a single circular one of the unplaced contigs contained several copies of the mitochondrial sequence, and we extracted a single circular

Table 2. Sequencing data from HG00621 used for assembly of the Han1 genome.

| Sequence type | Basecaller | Number of reads | N50 read length (bp) | Mean read length (bp) | Maximum length | Total length (Gbp) | Genome coverage |
|---------------|------------|-----------------|----------------------|-----------------------|-----------------|--------------------|-----------------|
| PacBio HiFi   | ccs v4.0.0 | 5,570,675       | 21,499               | 21,989                | 50,388          | 122.50             | 39.45x          |
| ONT           | Guppy      | 3,110,293       | 83,822               | 34,136                | 2,495,296       | 106.17             | 34.75x          |
| Ultralong     | v4.0.11    | (9.11% > 100 Kb) |                      |                       |                 |                    |                 |

We used three programs in the MUMmer4 package (Marçais et al. 2018) to compare the Han1 and T2T-CHM13 genomes: we aligned Han1 to T2T-CHM13 using nucmer, selected the best 1-to-1 alignment using delta-filter, and created dotplots using mummerplot.

Gene annotation

We used Liftoff (Shumate and Salzberg 2021) version 1.6.2 to map genes from T2T-CHM13 onto Han1. The T2T-CHM13 annotation was generated by mapping RefSeq (O’Leary et al. 2016) release 110 of GRCh38.p14 onto T2T-CHM13 version 2.0. The T2T-CHM13 annotation contains a total of 61,140 genes, including 20,022 protein-coding genes and 18,389 lncRNA genes. It includes 181,713 transcripts, of which 129,878 are protein-coding.

The T2T-CHM13 annotation used here is available at ftp://ftp.ccb.jhu.edu/pub/data/T2T-CHM13/.

Although Liftoff maps most genes automatically, the ribosomal DNA (rDNA) regions present a problem because they occur in very long tandem arrays of near-identical copies. Each rDNA unit is composed of three ribosomal RNA (rRNA) genes, 18S, 5.8S, and 28S, separated by two flanking spacers (5′- and 3′-ETS) and two internal transcribed spacers (ITS-1 and 2) (Agrawal and Ganley 2018). The arrays are located on the acrocentric chromosomes 13, 14, 15, 21, and 22. To accurately map the rDNA arrays onto Han1, we adopted a two-pass Liftoff process.

First, we ran Liftoff to map all genes except the rDNA arrays onto Han1, as follows. We aligned a 44,838 bp reference rDNA sequence from the KY962518 locus (Kim et al. 2018) to Han1 using Nucmer (Marçais et al. 2018) with the parameters “-maxmatch -l 31 -c 100,” which identified 1,196 preliminary rDNA locations. We then filtered these matches to retain only those that were >1,000 bp and >98.5% identical to the reference rDNA sequence, which yielded 257 approximate rDNA array locations. We used bedtools (Quinlan and Hall 2010) maskfasta to mask the locations of the 257 potential rDNA arrays, replacing them with Ns, and then ran Liftoff with parameters: “-chroms chromosomes.csv -copies -polish” to map all CHM13 genes except the rDNA arrays. This Liftoff first-pass procedure mapped 60,099 genes, including 20,003 protein-coding genes, 18,321 lncRNAs, and 51 non-rDNA array rRNAs, as well as many other gene types including microRNAs and pseudogenes.

In our second-pass process, we only lifted over the rDNA annotations from T2T-CHM13 onto Han1. We first extracted all rDNA annotations on T2T-CHM13 into an annotation (GFF) file, which included 219 rDNA units with three subunits each, and thus 657 subunits in total. Then we ran Liftoff with parameters: “-f features.txt -mm2_options “-N 250” -copies -sc 0.95.” The features.txt restricted the mapping to locations where all three subunits mapped onto Han1 at the same locus in the correct order, 18S, 5.8S, and 28S, which we called a valid rDNA unit lift-over condition. One additional note is that the rDNA morphotypes on each chromosome are structurally distinct (Nurk et al. 2022) which can create a mapping bias. We checked all 219 rDNA arrays during the mapping process to ensure that each copy was mapped to the...
best-matching chromosome. The Liftoff second-pass procedure mapped a total of 203 rDNA units, which is 609 subunits in total.

To verify the rDNA mapping result, we intersected the mapped annotations from the first and second passes using bedtools (Quinlan and Hall 2010) intersect version 2.30.0 with parameters: "-wa -wb." None of the 203 rDNA units overlap with any other genes mapped to Han1, which shows no sign of mismapped rDNA units. In the final step, we merged the two GFF files and ran gffread (Pertea and Pertea 2020) version 0.12.7 with parameters: "-O -F --keep-exon-attrs" to sort the final annotation and set the phases of CDS features.

**T2T-CHM13 and Han1 annotation comparison**

We compared the gene annotations between T2T-CHM13 and Han1 by running LiftoffTools (Shumate and Salzberg 2022) version v0.2.0, which performed a transcript-level comparison between all transcripts on the two genomes. For protein-coding transcripts, LiftoffTools determines the effects of any DNA differences on the protein sequence, which it categorizes into 10 groups: synonymous, nonsynonymous, in-frame deletion, in-frame insertion, start codon loss, 5′ truncation, 3′ truncation, frameshift, and stop codon gain.

LiftoffTools reported comparisons between 181,029 transcripts between T2T-CHM13 and Han1, with only 702 transcripts unmapped. For 130,909 transcripts with CDS features (which included protein-coding genes, VDJ segments, and some pseudogenes), this step computed DNA sequence identity, amino acid sequence identity, and any of the 10 variant effects aforementioned. For the 50,120 transcripts lacking CDS features, only the DNA sequence identity was computed.

To analyze mutations at the gene level, we created a transcript-gene look-up table by their IDs, mapped 130,909 transcripts back to their gene names and obtained a list of 19,706 protein-coding genes, 25 C regions, 75 J segments, and 403 V segments, for a total of 20,209 genes. For each of these, we selected the transcript with the highest amino acid sequence identity score as representative and categorized the gene into the variant effect group of that transcript. The number of genes in each group is summarized in Table 4.

We were particularly interested in genes containing deleterious mutations. We assumed that nonsynonymous mutations, in-frame insertions, and in-frame deletions are relatively harmless (Dunkle and Dunham 2015), and we focused on mutations that created major differences between the T2T-CHM13 and Han1 protein sequences, which included frameshifts (an insertion or deletion whose length is not a multiple of 3), truncations that removed either the 5′ or 3′ end of the transcript, including part of the CDS, mutations that changed the start codon to another codon, and mutations that created a premature stop codon. This gave us a target list of 235 genes for further investigation.

Because many of the 235 genes with deleterious mutations might represent cases where the Han1 genome is heterozygous, and where one haplotype contains a normal copy of the gene, we went back to the raw HiFi data and used it to identify heterozygous and homozygous variants, as follows. We first aligned all HiFi reads to the T2T-CHM13 genome using Minimap2 (Li 2018) version 2.24-r1122, and then used SAMtools (Li et al. 2009) version 1.13 to sort and index the aligned BAM file. We called variants using the "mpileup" and "call" commands from BCFtools (Li 2011) version 1.15.1 and selected heterozygous and homozygous variants by running "bcftools filter" with parameters "\"GT=het\"" and "\"GT=hom\"", respectively. This step identified 4,331,520 heterozygous sites and 2,285,451 homozygous sites.

We manually inspected a sample of altered genes with heterozygous mutations and found in each case that the alternative haplotype lacked the deleterious mutation. Therefore, we focused the remainder of our analysis on genes with homozygous mutations. We filtered out single nucleotide polymorphisms (SNPs) and kept mutations that might contribute to frameshifts, leaving us with 204,706 homozygous mutation sites.

Next, we extracted all CDS regions from all transcripts of the 235 genes with the most deleterious mutations, which yielded a total of 8,578 coding exons. We intersected these with the 204,706 homozygous, non-SNP mutation sites using bedtools intersect. After removing repeats caused by exon sharing between isoforms, 54 distinct mutation sites in 46 distinct genes were identified, where each gene contained at least 1 homozygous, non-SNP mutation as compared to T2T-CHM13.

**Protein-level analysis of altered genes**

We further investigated our list of 46 altered genes. We first checked the reported homozygous mutation sites in IGV (Robinson et al. 2011), extracted the GFF entries with target gene names, and translated their CDS into amino acid sequences using gffread (Pertea and Pertea 2020). We then compared the protein sequence of Han1 and T2T-CHM13 to RefSeq using BLASTP

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**Table 3. Statistics for the preliminary Han1 assemblies.**

| Assembler   | Sequencing data | assembled sequence (bp) | Contig N50 | Number of contigs | Quality value |
|-------------|-----------------|--------------------------|------------|-------------------|---------------|
| Hifiasm v0.16.1-r375 | PacBio HiFi | 3,110,501,483 | 95,769,069 | 182 | 57.8* |
| Flye v2.5  | ONT Ultralong   | 2,974,205,132 | 40,850,737 | 1,658 | 25.6* |

*Quality values were calculated using JASPER v1.0.0.

**Table 4. Comparison of genes with CDS features mapped from T2T-CHM13 to Han1.**

|                     | Mapped genes with CDS features |
|---------------------|-------------------------------|
|                     | Conserved genes (82%) | Altered genes (18%) |
|                     | Identical | Synonymous | Nonsynonymous | Start lost | Stop gained | Truncated | Frameshift | In-frame insertion | In-frame deletion |
| 14,130 | 2,442 | 3,200 | 21 | 31 | 1 + 39 (5′ + 3′) | 143 | 110 | 92 |
We compared copy number variation between the Han1 and T2T-CHM13 by grouping genes into paralogous groups using Liftofftools, which uses the “linclust” function in MMSeq2 (Steinegger and Söding 2017) version 13.45111. Protein-coding genes were clustered based on their amino acid sequences, and non-coding genes were clustered based on their nucleotide sequences, where genes in a cluster needed to be at least 90% identical over 90% of their lengths to at least one other cluster member.

### Results and discussion

#### Genome assembly

The Han1 genome (version 1.0) contains 3,099,707,698 bp, and the autosomes (1–22), the sex chromosomes (X and Y), and the mitochondrial genome were assembled end-to-end with no gaps. Approximately 120 Mb in gaps was filled using sequence from the T2T-CHM13 genome, illustrated in Fig. 1. The sizes of each chromosome and the amount of non-HG00621 sequence per chromosome are summarized in Table 5. Han1 is slightly smaller than T2T-CHM13, which has a total size of 3.117 Gbp. Chromosomes 9 and Y have the lowest proportion of HG00621 sequence compared to other chromosomes. Chromosome 9 has the largest centromeric region among all autosomes, as has been previously reported (Humphray et al. 2004). The centromeric region spans approximately 35 Mb, which was inserted entirely from T2T-CHM13. Chromosome Y contains a number of particularly challenging regions for assembly, including the male-specific region, MSY, which is composed of eight very long palindromes ranging from 9 Kb (P7) to 1.45 Mb (P1) with arm-to-arm sequence identities of 99.94–99.997% (Rozen et al. 2003; Skaletsky et al. 2003), as well as a lengthy X-transposed region, XTR, sharing 99% sequence identity to chromosome X (Skaletsky et al. 2003; Ross et al. 2005), which led us to rely on the T2T-CHM13 assembly for 27% of chromosome Y. Note that the T2T-CHM13 Y chromosome was derived from a separate individual, HG002, of Ashkenazi Jewish descent.

We identified two nuclear mitochondrial sequences (NUMTs) (Lopez et al. 1994) in Han1. The first one, an 866 bp insertion on chromosome 13 from 12,339,933 to 12,340,799 is present in T2T-CHM13, but absent from GRCh38. The second NUMT is a longer 13,781 bp insertion on chromosome 20 from 21,541,750 to 21,555,531, and it is unique to the Han1 genome, i.e. it is absent from both T2T-CHM13 and GRCh38. We validated these two NUMTs by aligning the ONT reads back to the Han1 genome using minimap2 with the parameter “--secondary=no,” requiring that the reads contained the entire NUMT and that the alignments extended at least 500 bp beyond it on both ends. We found 15 ONT reads supporting the NUMT on chromosome 13, suggesting that the reads contained the entire NUMT and that the alignments extended at least 500 bp beyond it on both ends. We found 26 ONT reads supporting the chromosome 20 NUMT. The chromosome 20 NUMT includes 26 genes, of which 8 are protein-coding genes, 16 are tRNAs, and 2 are rRNAs.

A dot plot comparing the entire sequence of Han1 and T2T-CHM13 genomes is shown in Fig. 2, which illustrates the

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### Table 5. Han1 chromosome sizes and the amount of non-HG00621 sequence per chromosome in the final Han1 assembly.

| Chromosome | Han1 total (bp) | Non-HG00621 sequence (inserted from CHM13) (bp) | Ratio of source sequences (HG00621:CHM13) |
|------------|----------------|-----------------------------------------------|----------------------------------------|
| 1          | 249,525,787    | 119,184                                       | 0.9995                                 |
| 2          | 242,739,747    | 2,482,037                                     | 0.9898                                 |
| 3          | 200,211,729    | 377,991                                       | 0.9981                                 |
| 4          | 192,045,028    | 518,393                                       | 0.9973                                 |
| 5          | 181,667,637    | 494,129                                       | 0.9973                                 |
| 6          | 170,861,069    | 314,798                                       | 0.9973                                 |
| 7          | 160,865,769    | 107,243                                       | 0.9993                                 |
| 8          | 145,880,131    | 791,768                                       | 0.9946                                 |
| 9          | 148,018,047    | 35,504,706                                    | 0.7601                                 |
| 10         | 135,316,043    | 585,347                                       | 0.9957                                 |
| 11         | 135,129,219    | 874,841                                       | 0.9955                                 |
| 12         | 134,132,185    | 102,971                                       | 0.9992                                 |
| 13         | 111,903,191    | 10,782,722                                    | 0.9036                                 |
| 14         | 101,435,482    | 5,090,291                                     | 0.9498                                 |
| 15         | 101,210,777    | 12,429,469                                    | 0.8772                                 |
| 16         | 95,412,483     | 13,280,238                                    | 0.8608                                 |
| 17         | 83,450,189     | 1,080,955                                     | 0.9870                                 |
| 18         | 78,966,361     | 210,798                                       | 0.9973                                 |
| 19         | 61,978,944     | 1,089,081                                     | 0.9824                                 |
| 20         | 65,189,243     | 963,587                                       | 0.9852                                 |
| 21         | 45,827,290     | 5,613,897                                     | 0.8775                                 |
| 22         | 50,610,422     | 3,397,082                                     | 0.8934                                 |
| X          | 154,227,164    | 7,056,525                                     | 0.9542                                 |
| Y          | 53,057,190     | 14,607,629                                    | 0.7247                                 |
| M          | 16,571         | 0                                             | 1.0000                                 |
| Total      | 3,099,707,698  | 119,875,682                                   | 0.9614                                 |

Every chromosome is in a single contig.
overall high collinearity of all chromosomes. One interesting finding is that Han1 contains an inversion at chr8: 7,306,407–11,410,283 (4,103,876 bp) which corresponds to T2T-CHM13 chr8: 11,717,452–7,617,994 (4,099,458 bp). This inversion is one of the largest common inversion polymorphisms in humans (Logsdon et al. 2021), and the arrangement in Han1, shown in more detail in Fig. 3, matches GRCh38 but disagrees with T2T-CHM13. A more detailed dotplot of the inversion is shown in Supplementary Figure S2. This region includes the β-defensin gene cluster locus and has been described as one of the most structurally dynamic regions in the human genome (Ganz 2003; Hollox et al. 2003, 2008; Mohajeri et al. 2016).

Fig. 2. a) A dot plot between the DNA sequences of Han1 and T2T-CHM13 shows the high collinearity of every chromosome between the two genomes. The segments in purple color mean sequences in T2T-CHM13 and Han1 are in the same direction, whereas the blue color means they are in the reverse direction. b) A gene order plot, where genes were numbered from 1 to N in order along all chromosomes of both T2T-CHM13 and Han1, and color-coded according to sequence identity. For both a) and b), the X axis is the T2T-CHM13 reference, and the Y axis is Han1. Chromosomes are in the order of 1 to 22, X, Y, and M. The alignments and dot plot were produced using the MUMmer4 software, and the gene order plot was generated by Liftofftools v0.2.0.

Fig. 3. A detailed view of an inversion between Han1 (top, in blue color) and T2T-CHM13 (bottom, in orange color), spanning the region from approximately 7.5 to 11.5 Mbp on chromosome 8. Figure created with Plotsr (Goel and Schneeberger 2022) version 0.5.4.
Gene content differences between Han1 and T2T-CHM13

The recent completion of the genome of a single individual, T2T-CHM13, along with the results here for Han1, provided us with the opportunity to do the first-ever direct comparison between the gene content of two complete individual human genomes. Because Han1 was assembled using T2T-CHM13 as a guide, it may have a gene content bias toward T2T-CHM13. In total, the Han1 annotation contains 60,708 genes, including 20,003 protein-coding genes, 18,321 IncRNAs, and 658 rRNAs. The number of genes on Han1 is slightly lower than the 61,140 genes and 20,022 protein-coding genes on T2T-CHM13, although the small differences could be due to mapping artifacts. An overview of the comparison between Han1 and T2T-CHM13 by gene categories, along with a comparison to the gene content of GRCh38, is shown in Table 6.

As described in Materials and methods section, we identified 46 distinct protein-coding genes that appear to have deleterious mutations in both copies of the gene. Among these, 27 are frame-shifts, 14 are 3' truncations, 3 have lost their start codons, and 2 have gained an early stop codon. A list of the genes in each category appears in Supplementary Table S3. Out of these 46 genes, 24 are hypothetical proteins with no known function, raising the question of whether they are actually functional proteins. Six of these are olfactory receptors, which might indicate a genuine difference in the number of olfactory receptors, or might indicate that those copies are pseudogenes. Three are not actual genes but VDJ segments, which are highly diverse among individuals.

Table 6. A comparison of major gene categories in the annotations of GRCh38.

| Gene biotype          | Count in GRCh38 | Count in T2T-CHM13 | Count in Han1 |
|-----------------------|-----------------|-------------------|--------------|
| Protein coding        | 19,871          | 20,022            | 20,003       |
| IncRNA                | 17,793          | 18,389            | 18,321       |
| Pseudogene            | 15,357          | 16,027            | 15,881       |
| miRNA                 | 1,914           | 2,046             | 2,058        |
| Transcribed pseudogene| 1,221           | 1,262             | 1,232        |
| rRNA                  | 38              | 765               | 658          |
| Other                 | 2,506           | 2,629             | 2,556        |
| Total                 | 58,700          | 61,140            | 60,708       |

Table 7. Protein level comparison between the Han1 and T2T-CHM13 genomes, showing genes with homozygous mutations in Han1 that are either frameshifted or truncated with respect to T2T-CHM13.

The remaining 13 genes are listed in Table 7. Two of these genes, MUC19 and AQP12A, contain premature stop codons and appeared to be severely truncated in Han1, with protein lengths far shorter than the corresponding proteins in both T2T-CHM13 and GRCh38.

Five genes, RETNLB, TCP11X1, DEFB126, TPSB2, and PBOV1, have protein sequences that are well-conserved compared to GRCh38, with 93–100% identity. These genes appeared to be frameshifted in T2T-CHM13, creating protein products in T2T-CHM13 that are either much shorter or frameshifted, with amino acid identity ranging from 30% to 88%. These results indicate that the deleterious mutations occur in the T2T-CHM13 genome (which we note was derived from a complete hydatidiform mole, not from a healthy adult) rather than in Han1.

For the other six genes, GOLGA6L10, KLHDC7B, NBFF19, RPL11, TMEM82, and KIR2DL3, the translations of their CDS regions are well-conserved despite the mutations, with all of their amino acid identity scores higher than 92% and protein length ratios close to one.

Conserved synteny

Figure 2b shows the overall conserved gene order between the Han1 and T2T-CHM13 genomes. The figure shows 60,746 points, each representing a distinct gene, plotted in their order along the chromosomes. The color coding indicates the sequence identity score between T2T-CHM13 genes and Han1 genes, with green and red lower. Among the 60,746 genes, around 98.2% of genes (59,654) have sequence identity scores greater than 95%. As the figure shows, nearly all genes occur in exactly the same order between the two genomes.

Gene family expansions

Liftofftools clustered all protein sequences using relatively strict criteria, which identified 19,653 clusters of near-identical proteins, of which 122 clusters had at least three genes in T2T-CHM13. In total, 94 of the 19,653 clusters have copy number changes, with all changes being copy number losses in Han1.

We looked more closely at the TSPY gene family, which occurs as a large tandem array with interspersed repeats on chromosome Y in both T2T-CHM13 and Han1. Both genomes have 46 copies of TSPY, and CHM1 has 20 pseudogenes, while Han1 has 24. It is worth noting that one copy of TSPY3 in T2T-CHM13, although

The RefSeq protein length is based on the RefSeq v110 annotation of GRCh38.
annotated as a protein-coding gene, has a premature stop codon that creates a truncated 237 amino acids (aa) protein, whereas the same gene on Han1 is full length at 308 aa. Thus, T2T-CHM13 appears to have lost one copy of the TSPY gene family.

In the 94 clusters with copy number loss, we found 46 clusters in T2T-CHM13 that had no corresponding genes in Han1, 43 clusters that lost a single gene copy, and five clusters that had lost more than one copy. The last groups (clusters that lost >1 gene copy) are shown in Supplementary Table S4.

**Conclusion**

In this study, we assembled and annotated the first gap-free genome, Han1, from a SH Chinese individual. The genome has a total size of 3,099,707,698 bp, and the autosomes (1–22), the sex chromosomes (X and Y), and the mitochondrial genome are assembled end-to-end with no gaps. The vast majority of the sequence is colinear with the T2T-CHM13 and GRCh38 genomes, although we did observe at least one novel mitochondrial insertion and a number of small-scale sequence duplications. Annotation of Han1 generated 60,708 putative genes, of which 20,003 are protein-coding genes. Finally, we conducted the first-ever direct comparison between the gene content of two complete individual human genomes, Han1 and T2T-CHM13 to determine how many genes are truly different between individuals. This analysis revealed two protein-coding genes in Han1 and five genes in T2T-CHM13 that appear to be severely truncated in comparison to the full-length version of the protein.

**Data availability**

All raw reads for HG00621 were generated and released by the Human Pangenome Reference Center, and are available as an AWS Open Data set from https://github.com/human-pangenomics/hpgg-data. The Han1 assembly (v1.0) is available from GenBank under accession JANJEX000000000 and on Github at https://github.com/JHUCCB/ChineseHanSouthGenome. Supplementary material are available at G3 online.

**Funding**

This research was supported in part by the U.S. National Institutes of Health [grant numbers R01-HG006677 and R35-GM130151] and by the U.S. National Science Foundation [grant numbers IOS-1744309 and DBI-1759518].

**Conflicts of interest**

The authors declare no conflict of interest.

**Author contributions**

K.C., M.P., and S.I.S. designed the research. K.C. and A.V.Z. performed assembly, scaffolding, and error correction. K.C. did the annotation. K.C., A.V.Z., M.P., and S.I.S. analyzed the results. K.C., A.V.Z., M.P., and S.I.S. wrote the manuscript. The authors thank Alaina Shumate for help with the RNAseq.

**Literature cited**

Agrawal S, Ganley AR. The conservation landscape of the human ribosomal rna gene repeats. PLoS ONE. 2018;13:e0207531.
Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetic parameter estimation from sequencing data. Bioinformatics. 2011;27:2987–2993. doi:10.1093/bioinformatics/btr509

Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34:3094–3100. doi:10.1093/bioinformatics/bty191

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The sequence alignment/map format and samtools. Bioinformatics. 2009;25:2078–2079. doi:10.1093/bioinformatics/btp352

Logsdon GA, Vollger MR, Hsieh P, Mao Y, Liskovyykh MA, Koren S, Nurk S, Mercuri L, Dishuck PC, Rhie A, et al. The structure, function and evolution of a complete human chromosome 8. Nature. 2021;593:101–107. doi:10.1038/s41586-021-03420-7

Lopez JV, Yuhki N, Masuda R, Modi W, O’Brien SJ. Numt, a recent taxonomic expansion, and functional annotation. Nucleic Acids Research. 2016;44:D733–D745.

Luo H, Gao Y, Xie B, Wang Y, Zhang H, Shi M, Ma S, Zhang X, Liu C, Xu S. Haplotype-resolved de novo assembly of a Tuja genome suggests the necessity for high-quality population-specific genome references. Cell Syst. 2022;13:321–333. doi:10.1016/j.cels.2022.01.006

Marçais G, Delcher AL, Phillippy AM, Coston R, Salzberg SL, Zimin A. Mummer4: a fast and versatile genome alignment system. PLoS Comput Biol. 2018;14:e1005944.

Mohajer K, Cantsilieris S, Huddleston J, Nelson BJ, Coe BP, Campbell CD, Baker C, Harshman L, Munson KM, Kronenberg ZN, et al. Interc chromosomal core duplicons drive both evolutionary instability and disease susceptibility of the Chromosome 8p23.1 region. Genome Res. 2016;26:1453–1467. doi:10.1101/gr.211284.116

National Bureau of Statistics of China. Communiqué of the Seventh National Population Census (No. 2); 2021 [online; accessed 2022 Aug 4]. http://www.stats.gov.cn/english/PressRelease/202105/t20210510_1817187.html

Nurk S, Koren S, Rhie A, Rautiainen M, Bzikadze AV, Mikheenko A, Vollger MR, Allernose N, Uralsky L, Gershman A, et al. The complete sequence of a human genome. Science. 2022;376:44–53. doi:10.1126/science.abf6878

O’Leary NA, Wright MW, Brister JR, Ciufro S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016;44:D733–D745.

Pertea G, Pertea M. GFF utilities: GffRead and GffCompare. F1000Research. 2020;9:304. doi:10.12688/f1000research.23297.2

Quinlan AR, Hall IM. Bedtools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 2010;26:841–842. doi:10.1093/bioinformatics/btq303

Robinson JT, Thorvaldsdóttir H, Winckler W, Gutmann M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. Nat Biotechnol. 2011;29:24–26. doi:10.1038/nbt.1754

Ross MT, Graffham DV, Coffey AJ, Scherer S, McLaren K, Muzny D, Flatzer M, Howell CR, Burrows C, Bird CP, et al. The DNA sequence of the human X chromosome. Nature. 2005;434:325–337. doi:10.1038/nature03440

Rozen S, Skal elska H, Marszalek JD, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Page DC. Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. Nature. 2003;423:873–876. doi:10.1038/nature01723

Shi L, Guo Y, Dong C, Huddleston J, Yang H, Han X, Fu A, Li Q, Li N, Gong S, et al. Long-read sequencing and de novo assembly of a Chinese genome. Nat Commun. 2016;7:1–10.

Shumate A, Salzberg SL. Liftofftools: a toolkit for comparing gene annotations mapped between genome assemblies. F1000Research. 2022;11:1230. doi:10.12688/f1000research.124059.1

Shumate A, Salzberg SL. Liftoff: accurate mapping of gene annotations. Bioinformatics. 2021;37:1639–1643. doi:10.1093/bioinformatics/btaa1016

Shumate A, Zimin AV, Sherman RM, Puiu D, Wagner JM, Olson ND, Pertea M, Salit ML, Zook JM, Salzberg SL. Assembly and annotation of an Ashkenazi human reference genome. Genome Biol. 2020;21:1–18. doi:10.1186/s13059-020-02047-7

Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Nykti kova T, Ali J, Bieri T, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature. 2003;423:825–837. doi:10.1038/nature01722

Steinegger M, Söding J. MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. Nat Biotechnol. 2017;35:1026–1028. doi:10.1038/nbt.3988

Tongmiao Z, Gongliang Z, Yongming Z, Suqin Z, Dingyuan L, Qi C, Xia Z. The distribution of immunoglobulin Gm allotypes in forty Chinese populations. Acta Anthropol Sinica. 1987;6:1.

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al. The sequence of the human genome. Science. 2001;291:1304–1351. doi:10.1126/science.1058040

Wang J, Wang W, Li R, Li Y, Tian G, Goodman L, Fan W, Zhang J, Li J, Zhang J, et al. The diploid genome sequence of an Asian individual. Nature. 2008;456:60–65. doi:10.1038/nature07484

Yang X, Zhao X, Qu S, Jia P, Wang B, Gao S, Xu T, Zhang W, Huang J, Ye K. Haplotype-resolved Chinese male genome assembly based on high-fidelity sequencing. Fundam Res. 2022;2:946–953. doi:10.1016/j.fmre.2022.02.005

Zhang F, Su B, Zhang Yp, Jin L. Genetic studies of human diversity in east Asia. Philos Trans R Soc B Biol Sci. 2007;362:987–996. doi:10.1098/rstb.2007.2028

Zhang J, Tao D, Lee T. Gm and Km allotypes in 74 Chinese populations: a hypothesis of the origin of the Chinese nation. Hum Genet. 1989;83:101–110. doi:10.1007/BF00286699

Zimin AV, Puiu D, Luo MC, Zhu T, Koren S, Marçais G, Yorde JA, Dvofák J, Salzberg SL. Hybrid assembly of the large and highly repetitive genome of Aleiopsis tauschii, a progenitor of bread wheat, with the MaSuRCa mega-reads algorithm. Genome Res. 2017;27:787–792. doi:10.1101/gr.213405.116

Zimin AV, Salzberg SL. The samba tool uses long reads to improve the contiguity of genome assemblies. PLoS Comput Biol. 2022;18:e1009860. doi:10.1371/journal.pcbi.1009860

Zimin AV, Shumate A, Shinder I, Heinz J, Puiu D, Pertea M, Salzberg SL. A reference-quality, fully annotated genome from a Puerto Rican individual. Genetics. 2021;220:iyab227. doi:10.1093/genetics/iyab227

Communicating editor: J. J. Emerson