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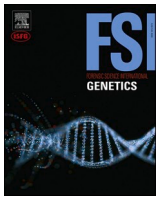

Uncovering genetic signatures of the Walser migration in the Alps: Patterns of diversity and differentiation

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ABSTRACT

Since leaving Africa, human populations have gone through a series of range expansions. While the genomic signatures of these expansions are well detectable on a continental scale, the genomic consequences of small-scale expansions over shorter time spans are more challenging to disentangle. The medieval migration of the Walser people from their homeland in ssouthern Switzerland (Upper Valais) into other regions of the Alps is a good example of such a comparatively recent geographic and demographic expansion in humans. While several studies from the 1980s, based on allozyme markers, assessed levels of isolation and inbreeding in individual Walser communities, they mostly did so by focusing on a single community at a time. Here, we provide a comprehensive overview of genetic diversity and differentiation based on samples from multiple Walser, Walser-homeland, and non-Walser Alpine communities, along with an idealized (simulated) Swiss reference population (Ref-Pop). To explore genetic signals of the Walser migration in the genomes of their descendants, we use a set of forensic autosomal STRs as well as uniparental markers. Estimates of pairwise F_{ST} based on autosomal STRs reveal that the Walser-homeland and Walser communities show low to moderate genetic differentiation from the non-Walser Alpine communities and the idealized Ref-Pop. The geographically more remote and likely more isolated Walser-homeland community of Lötschental and the Walser communities of Vals and Gressoney appear genetically more strongly differentiated than other communities. Analyses of mitochondrial DNA revealed the presence of haplogroup W6 among the Walser communities, a haplogroup that is otherwise rare in central Europe. Our study contributes to the understanding of genetic diversity in the Walser-homeland and Walser people, but also highlights the need for a more comprehensive study of the population genetic structure and evolutionary history of European Alpine populations using genome-wide data.

1. Introduction

The history of most human populations is characterized by both range expansion and gene flow, with range expansion typically leading to increased genetic drift [1–3]. Both gene flow and genetic drift, alongside mutation and natural selection, are fundamental evolutionary processes that shape population genetic diversity over time [4]. While range expansion is associated with multiple founder events, which may lead to decreasing genetic diversity along the expansion axis [2], gene flow may attenuate this effect and thus homogenize populations. Conversely, restricted gene flow between populations resulting from isolation promotes population divergence through mutation, natural selection and genetic drift. Understanding the extent to which these processes shape genetic diversity and differentiation provides insights

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into our own past and the intricate historical dynamics that have influenced human populations. Furthermore, genetic data can be used to complement archaeological and linguistic evidence and build a more complete picture of our past [5,6]. Beyond the study of human evolutionary history, an investigation of population structure and evolutionary processes may provide valuable insights for applications in medical genetics [7] and forensic genetics [8] as well as fundamental research on the spread and diversification of human languages [9].

In Europe, an example of recent human population range expansion that has repeatedly attracted the interest of anthropologists [10–12], historians [13] and linguists [14,15] is the migration of the Walser people in the Alps. The Walser are descendants of the Alemanni, a confederation of Germanic tribes who established their presence in the region of Valais (now a Swiss canton) in the 8th and 9th century [11]. Given the presence of the Burgundians in the western part of the Valais region, the Alemannic tribes occupied its eastern part (Upper Valais). In the 12th and 13th century, inhabitants of these Alemannic settlements migrated from their homeland in Upper Valais to various regions across the Alps. This migration resulted in the establishment of multiple settlements in the Swiss cantons of Ticino, Grisons, and St. Gallen as well as in northern Italy, Liechtenstein, and Austria (Fig. 1) [11]. These settlements were predominantly in high mountain valleys and remote Alpine areas, often in regions that were sparsely inhabited or less accessible at the time. The migrants were referred to as the 'Walser', a term derived from their homeland in the canton of Valais ('Wallis' in German). Both the native inhabitants of Upper Valais (Walser-homeland) and the Walser people share a distinct culture and speak related dialects, namely Walliser-German and Walser-German, respectively [16]. These dialects have developed distinct regional varieties influenced by the local languages and cultures of the areas where they settled, while retaining their shared heritage.

The reasons behind the 12th and 13th-century migration of the Walser out of Upper Valais remain a subject of debate among historians [14]. A possible driver could have been the search for new agricultural lands in response to growing population density, climate change, or natural disasters in the Upper Valais [17]. Another possibility is that the Walser were compelled to relocate to new areas by feudal landlords. Their primary role in these new domains was to defend strategic points, such as vital trade routes and Alpine passes, and to oversee the management of pasturelands in exchange for freedom [14]. Although most of the regions occupied were sparsely inhabited, in some regions there were already local inhabitants, for instance in areas of Grisons occupied by the Romansh, a Gallo-Romance-speaking group in Switzerland [11].

It has been reported that genetic exchange was a recurring phenomenon, occurring not only among Walser-homeland and Walser communities but also involving non-Walser populations in the Alpine region such as the aforementioned Romansh in the Swiss canton of Grisons [11]. To what extent such genetic exchange occurred is unclear. Nonetheless, the Walser have maintained a strong sense of cultural and linguistic identity throughout their history and have developed distinct local material cultures and customs, contributing to the cultural diversity of the Alpine regions [18]. Overall, it is unclear to what extent

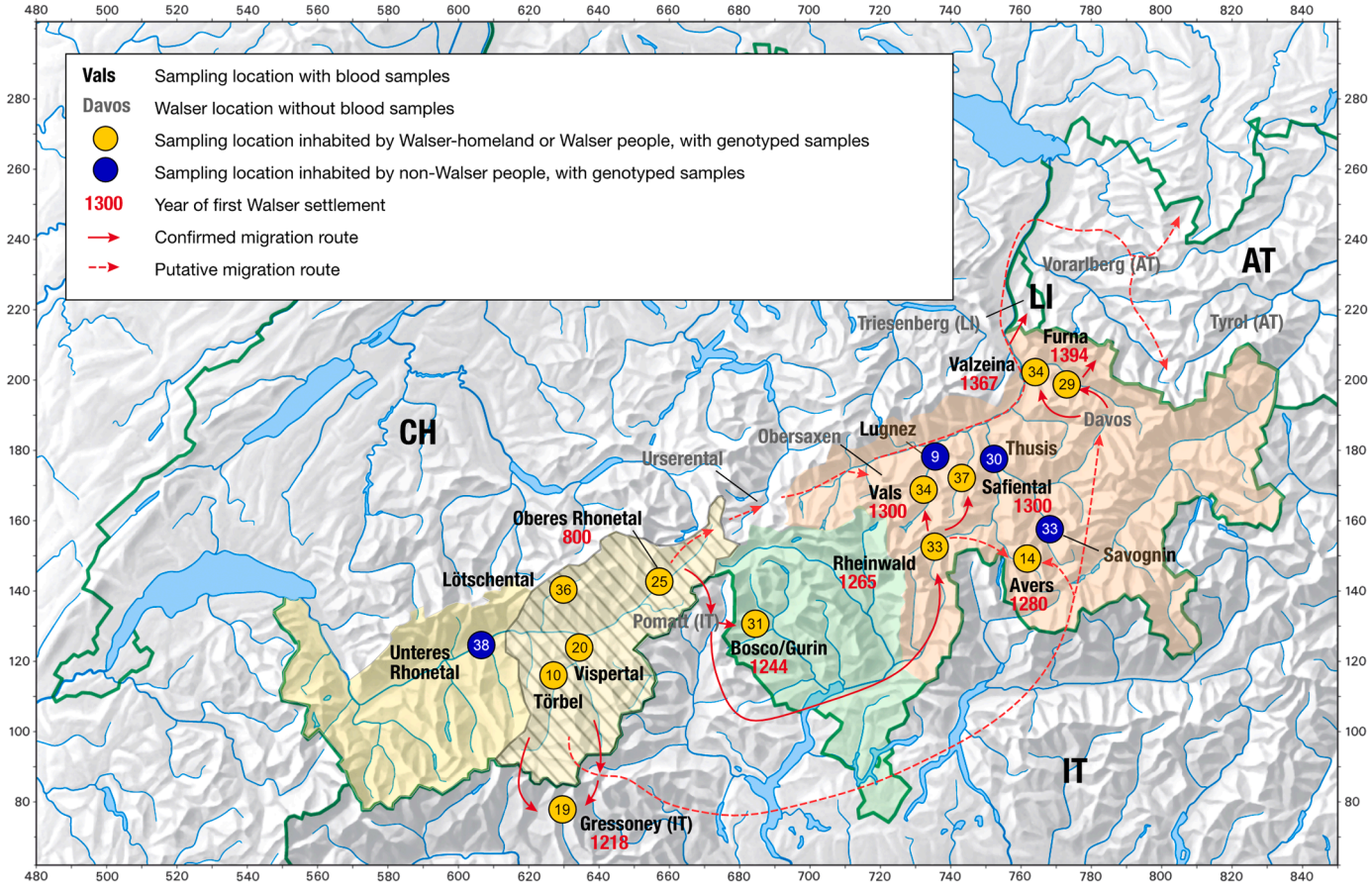

Fig. 1. : Study area with sampling locations, sample sizes and historical routes of migration of the Walser people in the Alps. The map shows Switzerland (CH) and relevant parts of Italy (IT), Liechtenstein (LI), and Austria (AT) bordering the Swiss Alps. Areas coloured in yellow, green, and red represent the Swiss cantons of Valais, Ticino, and Grisons, respectively. The hatched area in Valais indicates the homeland of the Walser (Walser-homeland; WHL) in Upper Valais. Sampling locations are indicated as yellow circles for the Walser-homeland and the Walser people, and as blue circles for non-Walser. Numbers in the circles indicate the number of samples typed for autosomal STRs in this study. Migration routes, both confirmed and putative, are based on Fibicher [19]. The x- and y-axes display the Swiss national coordinates (CH1903) in kilometers, with the origin point near Bordeaux (France).

genetic signals of the Walser migration can still be detected. Geographic barriers, particularly due to settlement at higher altitudes, and strong cultural identity may have resulted in overall genetic isolation and differentiation of the individual Walser communities since their expansion out of their homeland in Upper Valais. Yet, migrations to lower altitudes from the 15th century onwards, spurred by the Little Ice Age, as well as the improved connectedness of communities induced by Industrial Revolution may have attenuated and eradicated the signals of initial migration and subsequent isolation. How much these aspects shaped the genetic diversity of the Walser is unclear and deserves exploration.

In the past, several studies have genetically characterized individual Walser settlements and their homeland communities in Upper Valais, including the Lötschental valley [20], the village of Törbel [21], the valleys of the Visp (Vispertäler, i.e. Vispertal, Muttertal, and Saasertal) [22], or the Rheinwald valley [23] among others. These studies examined blood and enzyme markers and found varying degrees of differentiation among these communities, from elevated differentiation in Lötschental to limited differentiation in the Vispertäler. Currat [24] was the first to study the genetic makeup of multiple communities from the canton of Valais and Grisons, typing 13 protein markers. His analyses of various alpine communities indicated that the Walser do not form a genetically uniform group; instead, differentiation is more strongly associated with geographical isolation in mountains than with linguistic or cultural affiliation. Overall, Currat did not detect signatures of the Walser migration from the Walser-homeland in Upper Valais into Grisons. The lack of such signatures might be partially explained by the fact that the protein markers examined are expected to evolve under evolutionary pressures (natural selection), which might have biased the results [24]. In a demographic study, Boattini et al. [25] explored surname structures and marriage patterns in northern Italy, focusing on three Walser-German-speaking and one Romance-speaking community (Gaby). Their findings showed that despite maintaining linguistic traditions, the Walser communities did not exhibit elevated levels of consanguinity. Specifically, in the German-speaking Walser communities of Issime, Gressoney-Saint-Jean, and Gressoney-La-Trinité, rates of endogamy were found to be lower than those detected in the Gaby community [25]. Overall, these studies suggest that the Walser-homeland and Walser communities maintained a certain level of genetic exchange amongst them as well as other communities, and that such gene flow may have eroded the footprint of the Walser migration in allozyme variation.

The goal of this study is to gain a more comprehensive understanding of the genetic structure of the Walser-homeland and Walser people in the Alps. We explore whether the medieval Walser range expansion is still detectable in the genetic structure of contemporary Walser communities. To this end, we examine four Walser-homeland communities in Upper Valais, eight Walser communities in neighboring cantons, and four alpine communities that are considered to be non-Walser. We ask the following questions: i) What is the population structure and relatedness of the twelve focal Walser-homeland and Walser communities; ii) how do the Walser-homeland and Walser communities compare to other, to non-Walser Alpine communities and to an idealized Swiss reference population (Ref-Pop) in terms of genetic diversity; and iii) how do patterns of population structure at uniparental genetic markers compare to those at autosomal markers? To answer these questions, we type 23 neutral autosomal forensic STRs in over 432 individuals, selecting a subset of unrelated individuals for further analyses of 23 Y-STRs, 5 G-haplogroup specific Y-SNPs, and the whole mtDNA. By integrating data from both autosomal and uniparental markers, our study broadens the scope of population genetic analysis beyond the limitations of classical markers and historical records. Our study represents an important investigation of potential genetic signals persisting from the Walser medieval migration.

2. Materials and methods

2.1. Samples and ethical compliance

We obtained blood samples from the Walser project collection available at the Department of Evolutionary Anthropology at the University of Zurich (UZH). These samples were collected during blood donation campaigns organized by the Swiss Red Cross in the late 1980s, as part of a previous research project on the history and diversity of the Walser communities. The populations sampled include four Walser-homeland communities in Upper Valais, eight Walser communities located in Ticino, Grisons, and Valle d′Aosta in Italy, as well as four non-Walser communities, i.e. one community in Lower Valais (Unteres Rhonetal) and three communities in Grisons (Fig. 1). Further information on the census sizes and elevation of these communities is provided in Table 1. Different numbers of samples were available for each location (ranging from 18 to 437), and at the time of this study, only those samples with sufficient volume of blood were considered for downstream analyses of autosomal, Y-chromosomal and mtDNA markers. For the autosomal genetic analyses of this study, we targeted a minimum of 30 individuals per community, distributed roughly equally between sexes. An exception was the community of Avers for which only 18 samples were available. Given that many samples had insufficient DNA quantity or quality, the target was not reached for all locations (see numbers of samples typed in Fig. 1 and Table 1). For the Y chromosomal markers, we focused on all unrelated males from our dataset. For the mtDNA analyses, which were part of a separate but related project, we followed a different approach, targeting a subset of 48 individuals. Ethical compliance was evaluated and approved by the CEBES review board at the University of Zurich (case number 2022–08, https://www.med.uzh.ch/en/Ethikkommission.html). The evaluation took into account the sampling performed in the 1980s, which was informed and with anonymous participation, but without signed informed consent forms. In addition, a declaration of non-objection was obtained from the cantonal ethics committee (case number 2016–00993, https://www.zh.ch/de/gesundheitsdirektion/ethikkommission.html)

2.2. Laboratory analyses

2.2.1. DNA extraction and quantification

We extracted DNA from 300 µl of blood using the Puregene Blood Kit (Qiagen, Hilden, Germany) following the DNA purification protocol for whole blood. DNA quantity was assessed using the Quantifiler Trio Quantification Kit (Thermo Fisher Scientific, Massachusetts, USA; abbreviated as TFS in the following).

2.2.2. Autosomal STR typing

For autosomal STR typing, we employed the PowerPlex® Fusion 6 C Kit (Promega, Wisconsin, US) following the manufacturer's instructions [27]. This kit allows for the co-amplification of 27 markers, encompassing the expanded Combined DNA Index System (CODIS) core loci and additional markers such as Amelogenin and DYS391 for sex determination, as well as Penta D, Penta E, SE33, and two rapidly mutating Y-STR loci (DYS570 and DYS576).

2.2.3. Y-STR typing

For those samples for which autosomal typing was successful and indicated that the individual was a male, amplification of Y-STR markers was conducted with the PowerPlex® Y23 System (Promega, Wisconsin, US), following the manufacturer's instructions [28]. The kit includes a set of essential haplotype STRs, DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, along with highly variable marker DYS385 a/b. Additional markers included in the kit are DYS448, DYS438, DYS437, DYS635, DYS439, DYS458, DYS456 and Y-GATA-H4 as well as six highly discriminating STRs DYS481, DYS533, DYS549, DYS570, DYS576, and DYS643.

Table 1
Elevation (in meters above sea level [masl]) and census size in 1980s (at the time of sampling) and in 2023 for the communities analyzed in this study [26].

Group	Location/Community	Sample size	Census Size in 1980 vs. 2023	Description	Elevation [masl]
Non-Walser in Lower Valais	Unteres Rhonetal	38	23.775/36.612 (Sion)	From Saint-Maurice to Sierre	414–533
Walser-Homeland	Lötschental	36	~1500*	Region	634–1375
	Oberes Rhonetal	25	> 18,000*	Section of valley from Sierre to Obergoms (Visp, Brig, Goms, Obergoms)	533–1377
	Vispertal	20	~3200*	Stalden(ried), Visperterminе, Zeneggen	795–1378
	Törbel	10	495/494	Village	1506
Walser	Gressoney	19	908/9310	Gressoney Saint-Jean, Gressoney La-Trinité	1385–1635
	Bosco Gurin	31	65/53	Village	1506
	Rheinwald	33	626/576	Region	1516
	Vals	34	929/949	Village	1252
	Safiental	37	994/963	Region	1315
	Avers	14	140/168	Region	1960
	Furna	29	181/203	Village	1351
	Valzeina	34	140*	Village	1114
Non-Walser in Grisons	Lugnez	9	2262/2072	Region	1244
	Thusis	30	2608/3458	Village	720
	Savognin	33	2172/2424	Village	1207

* only census sizes from 2023 were available

2.2.4. Y-SNP typing

We also examined the G haplogroup in more detail. We targeted five SNPs (P15, M286, L32, L497, and L91) on the Y-chromosome specific to the G2a clade as previously described in Berger et al. [29]. Polymerase chain reaction (PCR), single-base extension (SBE) and clean-up reactions were performed according to instructions specified in Supplementary Table S2 [30,31].

2.2.5. Fragment analysis

Capillary electrophoresis for both autosomal and Y-chromosomal markers (Y-STRs and Y-SNPs) was conducted on the 3130xl, 3500, or SeqStudio Genetic Analyzer (TFS) and data analysis carried out with Genemapper ID-X Software Version 1.4 (TFS). We used a peak detection threshold of 50 relative fluorescent units (RFU) for allele calling.

2.2.6. Y-STR haplogroup prediction

Y-STRs were used to infer haplogroups with NEVGEN [32] and Whit Athey's [33]. We used two haplogroup predictors to maximize accuracy of the results. In those cases where the haplogroup predictions between the two tools did not agree, we designated the haplogroup as "Undetermined". If the predictions did not match at the sub-haplogroup level, the haplogroup of the most recent common ancestor (MRCA) was selected as the consensus. For instance, if NEVGEN predicted I2a2a and Whit Athey's predicted I2b1, we assigned the haplotype to I2 and considered the sub-haplogroup undetermined.

2.2.7. mtDNA sequencing

The whole mitochondrial DNA (mtDNA) was sequenced using the Precision ID mtDNA Whole Genome Panel (TFS) and massively parallel sequencing technology on an IonTorrent S5 instrument (TFS) following the steps described in Alterauge et al. [34] except for the following modifications. We used 6000, 12,000 or 24,000 mtDNA copies amplified in 19, 18 or 17 cycles, respectively, instead of 1500 copies in 21 cycles. In addition, after pooling of the two separate 10 µl amplification reactions with primer pools 1 and 2, only 10 µl were used for subsequent library preparation and, accordingly, half of the recommended volumes for all library preparation steps. Sequenced reads were mapped against the revised Cambridge reference sequence (rCRS) [35] and, concurrently, to the human genome build 19 (hg19) with Torrent Suite v5.10 (TFS) in order to remove nuclear mitochondrial elements (NUMTs). Variant calling was performed as described in [34]. All variants were checked manually with the Integrative Genomics Viewer IGV v2.8.9 [36]. Quality control of the mitotypes, their phylogenetic alignment, and haplogroup assignment were conducted with the EMPOP database v4/R13 [37].

2.3. Data analyses - autosomal STRs

2.3.1. Removal of highly related individuals based on autosomal STRs

To avoid biases resulting from the inclusion of highly related individuals, we used the Familias software v.3.3.1 [38,39] to identify pairs of individuals related at 1st or 2nd degree level. We chose the Blind Search Module with the extended stepwise mutation model and the following parameter settings: rate (integer mutations) = 0.001, range = 0.1 (one step being 10 times more likely than two steps), and rate 2 (fractional mutations) = 0.000005. Pairs of individuals with estimated kinship coefficient above 0.125 were flagged as first-degree relationships or closely related second-degree relationships [40,41]. From the resulting set of closely related individuals, we successively removed individuals until no close relationships remained. We excluded individuals that occurred in multiple pairs first in order to keep the remaining dataset as large as possible.

2.3.2. Within-population genetic diversity and forensic parameters

We computed within-population summary statistics including observed heterozygosity (Ho), expected (He) heterozygosity, and mean number of alleles per location, using the STR Analysis for Forensics (STRAF) online tool v2.1.5 [42] (Table 2). In addition, we estimated forensically relevant parameters using the same software (STRAF v2.1.5). These included the polymorphism information content (PIC) to assess the information content of the markers; the typical paternity index (TPI) to assess the markers' suitability for paternity testing; the match probability (PM) to estimate the likelihood of two randomly selected individuals sharing the same genotype. Exact tests of Hardy-Weinberg equilibrium (HWE) and Linkage disequilibrium (LD) were carried out with Arlequin v.3.5.2.2 [43]. The presence of null-alleles was tested using the program ML-NullFreq [44]. To correct for multiple comparisons, the p values in the HWE, LD, and null alleles tests were adjusted with the Benjamini-Hochberg correction [45].

2.3.3. Simulation of a Swiss reference population

We generated an idealized Swiss reference population (Ref-Pop) by simulating genotypes of 1000 individuals from the Swiss autosomal STR dataset generated by Zieger et al. [46]. This study reports allele frequencies for the 23 autosomal STRs typed in our study based on 1198 individuals of Swiss nationality aged between 30 and 50 years old, and speaking one of the four national languages (Swiss German, French, Italian, and Romansh). For each of the 23 STRs, we randomly sampled two alleles (with replacement) from the allele frequency distribution using a custom Python script. The frequency of simulated genotypes was compared to the original frequencies published in Zieger et al. [47]. The

Table 2
Estimates of within-population genetic diversity using 23 autosomal STRs.

Group/Community	2 *N*	H_o	H_e	F_{IS}	F_{IS} *interval*	MNA	Null-alleles
non-Walser in Lower Valais (Unteres Rhonetal)	72	0.814	0.785	−0.04	[−0.08, 0.01]	8.609	-
Walser-homeland	146	0.775	0.788	0.02	[−0.01, 0.04]	9.304	-
Lötschental	54	0.768	0.765	0.00	[−0.05, 0.04]	7.478	-
Oberes Rhonetal	40	0.780	0.774	−0.01	[−0.06, 0.04]	7.739	-
Vispertal	36	0.783	0.777	−0.01	[−0.07, 0.05]	7.652	-
Törbel	16	0.772	0.725	−0.07	[−0.14, 0.01]	6.087	**D5S818 (0.0115)**
Walser	332	0.784	0.787	0.00	[−0.02, 0.02]	10.696	-
Gressoney	32	0.766	0.733	−0.05	[−0.09, −0.02]	6.696	-
Bosco Gurin	42	0.782	0.779	−0.01	[−0.05, 0.03]	8.130	-
Rheinwald	52	0.786	0.777	−0.01	[−0.06, 0.03]	8.391	-
Vals	44	0.762	0.750	−0.01	[−0.06, 0.03]	7.478	-
Safiental	50	0.779	0.770	−0.02	[−0.07, 0.04]	7.826	**Penta E (0.0345)**
Avers	22	0.791	0.746	−0.05	[−0.11, 0.01]	6.739	**D2S441 (0.0299)**
Furna	40	0.809	0.777	−0.04	[−0.08, −0.01]	7.913	-
Valzeina	50	0.799	0.800	−0.04	[−0.08, 0.01]	7.565	-
non-Walser in Grisons	128	0.810	0.789	−0.03	[−0.06, 0.00]	9.783	-
Lugnez	14	0.839	0.760	−0.11	[−0.03, −0.18]	5.870	-
Thusis	54	0.794	0.780	−0.05	[−0.07, 0.03]	8.435	-
Savognin	60	0.817	0.778	−0.02	[−0.09, −0.02]	8.652	-
All	678	0.790	0.767	−0.03	[−0.04, 0.02]	12.130	-

2 *N*: number of chromosomes; H_o: observed heterozygosity (average across all loci); H_e: expected heterozygosity (average across all loci); F_{IS}: mean inbreeding coefficient across STR loci; F_{IS} interval: mean inbreeding coefficient ± 2 standard errors; MNA: mean number of alleles; Null-alleles: presence of null alleles and the adjusted *p* values in brackets (only significant values post Benjamini-Hochberg correction are listed).

allele frequency deviations across all markers were within 2% of the original values. Subsequently, we applied the criteria described in Section 2.3.1 to remove related individuals from the simulated dataset. From the remaining individuals, we randomly selected a subset for further analyses; this subset comprised the same number of individuals as the number of individuals included from Walser-homeland and Walser communities in this study (after removal of closely related individuals).

2.3.4. Genetic relationship among populations and between-population differentiation

To explore population structure, we used STRUCTURE v2.3.4 [47], with 100,000 burn-in and 100,000 MCMC cycles with 30 iterations. We let the number of ancestral components *K* vary from 1 to 6 and performed analyses both including and excluding the simulated Ref-Pop. These analyses employed STRUCTURE's admixture model and the correlated-allele-frequencies model [48]. We visualized and assessed the STRUCTURE results using the software pong v1.4.9 [49]. In addition, we investigated the partitioning of the total genetic variation across three hierarchical levels; i) among groups (Walser-homeland, Walser, and non-Walser in Grisons), ii) among populations within groups, and iii) between populations, through an analysis of molecular variance (AMOVA) using Arlequin v3.5.2.2 [43].

To quantify the between-population genetic differentiation among the studied communities, we computed pairwise F_{ST} values using Arlequin v3.5.2.2 [43], applying the Benjamini-Hochberg correction for multiple hypothesis testing. Based on the pairwise F_{ST} values, we constructed a Neighbor-Joining (NJ) tree with PHYLIP v3.698 [50] and visualized the tree using the Interactive Tree Of Life (iTOL) v5 [51]. We set negative branch lengths to zero and added the absolute length of the deleted branches to the respective adjacent branch length so that the total distance between an adjacent pair of terminal nodes remained unaffected [52]. Subsequently, using the same pairwise values we generated a Multidimensional Scaling (MDS) plot using a custom Python script. Negative pairwise F_{ST} values were set to zero.

2.4. Data analyses - uniparental markers

2.4.1. Y-STRs

To investigate the genetic relationship among the Y-chromosomal haplotypes, data from the Y-STRs typed for all male individuals in our cohort (after exclusion of closely related individuals) were used to construct a median-joining (MJ) network. We used NETWORK software v10 [53] and Network Publisher v2.1.2.5 to compute and visualize an MJ network, respectively. At the DYS389II locus, the repeat count was determined by calculating the difference in repeat numbers between the two amplification products obtained, i.e., repeats at DYS389II minus repeats at DYS389I. For locus DYS389I the number of repeats was used unchanged.

We calculated the R_{ST} distances between the newly reported dataset and a panel of representative populations from Switzerland [54,55], Italy [56–59] and Germany [60,61], using the AMOVA tool in the Y-Chromosome STR Haplotype Reference Database (YHRD) [62]. We used the R_{ST} distances obtained to construct an NJ tree and visualize the relative genetic proximity between populations, using custom scripts in R as well as the R package ape [63]. For the population-based analysis, a minimum sample size of 4 was required.

Haplotype similarities within and between pairs of populations was assessed by analyzing the number of shared identical and similar haplotypes, using custom R scripts. For this analysis, the DYS389 locus was treated as mentioned above, and locus DYS385 was excluded due to the duplicated nature of this locus [64]. BRUVO distances were used to estimate pairwise haplotype distances. In addition to identical haplotypes, we examined haplotypes with distances less than or equal to thresholds corresponding to the 0.001 and 0.005 quantile probabilities in the BRUVO distance distribution. This data-driven approach allows for an objective classification of haplotypes based on their similarity.

2.4.2. mtDNA

We analyzed the mtDNA haplogroup distribution in a subset of 48 individuals from the Walser and Walser-homeland communities (4 individuals randomly chosen from each community) and compared it with mtDNA datasets from Swiss n = 200, Austrian n = 273 [65], Italian n = 395 [66], and general European populations n = 10,911 [67]. The Swiss dataset, consisting of 200 individuals, was derived from an internal dataset at our institute (manuscript in preparation). The other datasets were obtained from studies aiming to characterize the Austrian, Italian and general European mtDNA diversity patterns [65–67]. These studies focused on the control region of the mtDNA. To enable a comparison across the different studies, and the different haplogroup levels

reported, we classified all results into the following major haplogroups: R0, J, T, U (including K), N1 (including I), N2 (including W), X, M, L, and grouped all minor haplogroups under 'Other'.

Individual counts per haplogroup for each group were compared using Fisher's exact test to assess the statistical significance of differences in haplogroup distribution. To account for multiple testing, we applied the Benjamini-Hochberg correction to control the family-wise error rate. Statistical analyses were performed using R software, and a p value of 0.05 was considered significant after correction.

3. Results

3.1. Removal of related individuals

In total, 432 samples had sufficient DNA quality and quantity for the successful typing of autosomal markers (PowerPlex Fusion 6 C). Our analyses of relatedness revealed 3 pairs of identical individuals, 34 putative parent-offspring pairs, and 33 putative full-sibling pairs. In addition, among the pairs with kinship coefficients above 0.125, the threshold for close relatedness, we inferred 93 pairs of half-siblings or grandparent–grandchild, or aunt/uncle–niece/nephew (for further details refer to Supplementary Figure S1 and Table S3). After a parsimonious deletion of one individual from each closely related pair, we obtained a final dataset comprising 339 individuals (193 males and 146 females). Details on quality filtering and individuals removed are provided in Supplementary Figure S2. All males in the final dataset were typed for Y-STRs. For mtDNA sequencing, we selected 4 individuals from each Walser-homeland and Walser community (excluding non-Walser), which resulted in a total of 48 samples. An overview of the markers typed for each individual is provided in Supplementary Table S1. The data has been quality controlled by STRidER [68], YHRD [62], and EMPOP [37].

3.2. Within-population genetic diversity using 23 autosomal STRs

Our analyses of within-population genetic diversity yielded average observed heterozygosities (H_o) ranging from 0.762 (Vals) to 0.839 (Lugnez), while expected heterozygosities (H_e) ranged from 0.776 (Vals) to 0.828 (Lugnez) (Table 2). The inbreeding coefficient F_{IS}, i.e. the mean difference between H_e and H_o relative to H_e across loci, deviated from zero only for the Walser communities Furna and Gressoney, and the non-Walser communities Lugnez and Savognin. In all four cases, there was a slight excess of observed versus expected heterozygosity (Table 2), and thus no indication of inbreeding at the community level. All autosomal markers were in HWE in all populations.

The mean number of alleles per STR varied from 5.870 (Lugnez) to 8.652 (Savognin). Additionally, no marker pair was in linkage disequilibrium (LD). Using the maximum likelihood estimation tool implemented in ML-NullFreq, we inferred null alleles for marker D2S441 in Avers (p value = 0.0299), marker Penta E in Safiental (p value = 0.0345), and marker D5S818 in Törbel (p value = 0.0115; Table 2). As these null alleles occurred for different markers in different communities, we chose to retain all markers for subsequent analyses. Furthermore, we observed three novel alleles that have not been previously reported in European populations (see STRidER v3/R2): SE33 - 30.3, Penta D - 17, and CSF1PO - 7. Allele frequencies (across Walser-homeland and Walser samples) and forensic parameter estimates are reported in Supplementary Table S4.

3.3. Genetic relationship among populations and between-population differentiation

The STRUCTURE analyses did not reveal any prominent differences among the 16 communities, or between these and Ref-Pop (see Supplementary Figure S3 A and B). The AMOVA for the forensic autosomal STR markers showed that most of the genetic variation (99.29%, p value < 0.00001) was found within communities, whereas 0.67% of the genetic variation was explained by differences among communities within groups (p value < 0.00001). There was no evidence for genetic variation among groups (0.04%, p value = 0.26574; Table 3).

We estimated pairwise F_{ST} to quantify genetic differentiation between pairs of communities. Pairwise F_{ST} ranged from 0% to 3.2% (Fig. 2A). The highest values were obtained for pairs involving the Walser-homeland community of Lötschental, and the Walser communities of Vals and Gressoney. These values remained statistically significant even after adjustments for multiple comparisons using the Benjamini-Hochberg (BH) correction. We recomputed the AMOVA analyses without these three communities and found that the proportion of variance attributed to differences among communities (within groups) was no longer significant (p value = 0.05238), indicating that Lötschental, Vals, and Gressoney have an important influence.

The communities of Törbel and Avers were also associated with relatively high F_{ST} values (from 0.3% to 2.7%). These values were statistically non-significant after correction for multiple testing, except in the pairwise analyses involving Lötschental, Vals, and Gressoney, and the pairwise F_{ST} for Törbel and Avers (p value = 0.042). It is worth noting that these two, Avers and Törbel, are comparatively remote communities, but also had smaller sample sizes, which may have influenced the significance as well as the overall generalizability of these results.

To visualize the genetic relationships among the communities, we constructed an NJ tree based on the pairwise F_{ST} values (Fig. 2B). In this tree, the branches leading to the communities of Lötschental, Vals, Gressoney, and Törbel are comparatively long. This result suggests that these populations diverged from the other communities by genetic drift. Notably, Avers, Safiental, and Vals clustered together, which is consistent with historical records suggesting that Vals and Safiental were founded from Rheinwald [19]. Moreover, the branch leading to the Avers community originates from the branch leading to Safiental and Vals. This result suggests that Avers might also have been founded from Rheinwald, instead of having been founded directly from Upper Valais (Walser-homeland) via a separate putative southern migration route (see Fig. 1). The relatively high genetic differentiation between Avers and the two communities of Törbel, and Vispertal, further argues against the southern putative migration route and in favor of a higher genetic relatedness between the Avers and the Rheinwald and its descendants. Additionally, the non-Walser community of Savognin in Grisons showed unexpected genetic similarities to Törbel, a community within the Walser-homeland. Törbel appeared closely related to Bosco Gurin but distinct from Vispertal, despite the geographical proximity of Törbel and Vispertal. The close relationship between Törbel and Bosco Gurin is surprising given that the two communities are not located along a common established or putative migration route (Fig. 1).

Further visualization of the pairwise F_{ST} values on an MDS plot further accentuates the distinctiveness of Avers, Gressoney, Lötschental, Törbel, and Vals, identifying them as communities that have putatively experienced genetic drift (Fig. 2C). Gressoney, Lötschental and Törbel are geographically close to each other, but nevertheless topographically isolated. The Avers and Vals communities are both geographically remote from the Walser-homeland as well as located in the upper part of comparatively isolated mountain valleys.

3.4. Uniparental markers

We examined uniparental markers, including whole mtDNA sequences as well as 23 Y-STR markers and a set of 5 G-haplogroup-defining Y-SNP markers. We scanned for signatures of population structure in the 23 Y-STR markers through the construction of a median-joining network of individual haplotypes (see Supplementary Table S5 for the haplogroup assignments). From the 193 total haplotypes typed, eleven haplotypes were excluded due to the presence of partial repeats (microvariants, e.g., 19.2) or missing allelic information. The median-

Table 3
Analysis of molecular variance (AMOVA) among groups (Walser-homeland, Walser, and non-Walser in Grisons), among communities within these groups, and within individual communities.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance	*p* value
Among groups	2	25.375	0.00332	0.04%	0.26574
Among communities within groups	12	139.536	0.06244	0.67%	< 0.00001
Within communities	591	5429.760	9.19583	99.29%	< 0.00001

joining network (Fig. 3) showed that the individual haplotypes (circles) were distributed across the different regions, without indications for geographical structuring or association with Walser/non-Walser (Figs. 3A and 3B). However, as expected, the haplotypes did cluster according to their associated haplogroups (Fig. 3C).

Haplogroups were determined for all male individuals except for seven (accounting for 3.8% of the dataset), for which inconsistent haplogroup predictions were observed with NEVGEN and Whit Athey's predictors (see Methods 2.3.5.1 for details). Incongruencies between haplogroup predictors may arise due to several factors, including the number of markers included, the underlying database employed, and the computational models used. Across all communities, a total of 11 haplogroups were identified (Table 4). The most prevalent Y-STR haplogroups were R1b and G2a, accounting for 52.8% and 13.2% of all haplogroups, respectively. The most noteworthy distinction between Walser-homeland and the Walser groups, was that the R1a haplogroup was absent in Walser-homeland but accounted for 6.2% in the Walser. Furthermore, the E1b1b haplogroup was observed in 11.1% of the male individuals in the Walser-homeland communities but in less than 4.9% in the Walser communities. The Q haplogroup was only found in one individual among the non-Walser in Grisons.

Overall, the G2a haplogroup was found in 24 male individuals. The G2a haplogroup was frequent in the early Anatolian farmers as well as in Neolithic Europe, having been observed in the Tyrolean Bronze Age iceman Ötzi [69,70]. Over time, this lineage became rare, with certain sub-lineages remaining in high frequencies in specific regions, for instance Sardinia, which still retains a strong early Neolithic genetic ancestry [71]. To explore the frequencies of G2a in our samples, as also done by Berger et al. [29], and to more thoroughly analyze the subclades of the G2a haplogroup, we focused on 5 G-haplogroup specific Y-SNPs (see Methods 2.2.4). Supplementary Table S6 provides results of the Y-SNP typing. One sample from Vals (ID 10007) was excluded due to insufficient DNA material. Our analysis revealed that 19 (82.6%) individuals belonged to subclade G-L497 and the remaining 4 individuals to subclade G-L32 (17.4%). None of our cohort members shared the exact same subclade as Ötzi (G-L91).

We additionally reconstructed a Y-STR NJ tree including data from Switzerland, Germany and Italy available in the forensic YHRD database [62] and based on R_{ST} distances (Supplementary Figure S4). Similar to the NJ tree based on autosomal STRs, the NJ tree based on Y-STRs showed relatively long branches for Lötschental and Törbel. Notably, unlike the autosomal STR tree, the Y-STR tree also showed long branches for the communities of Safiental, Bosco Gurin and Oberes Rhonetal. Other communities appeared genetically close either to other German- or French-speaking Swiss populations, to Ticino and northern Italian populations, or to southern German populations.

Our analyses of identical haplotypes (Supplementary Figure S5 A) showed the highest number of identical parental ancestries for Safiental (with one pair and one triplet of identical haplotypes), followed by Vals and Savognin (one pair each). Identical haplotypes were shared between Oberes Rhonetal, Unteres Rhonetal, and Lötschental. In addition, similar shared haplotypes (based on quantile distribution of BRUVO distances; Supplementary Figure S5 B and C) were recorded for the populations in the west: the Walser-homeland and the non-Walser community of Unteres Rhonetal. Sharing of similar haplotypes was also found between Valzeina and Furna, Valzeina and Rheinwald, and Rheinwald and Vals, confirming connections among the Walser for the shortest distance similarity (0.001 probability over the quantile distribution, Supplementary Figure S5 B). Less similar haplotypes (0.005 probability, Supplementary Figure S5 C) highlight further connections for Oberes Rhonetal and other Walser and non-Walser groups, and confirm connections between Walser and Walser-homeland.

For the maternal lineages, we first compared haplogroup frequencies of our newly generated sample against other Swiss and European comparative groups (Supplementary Table S7). Our analysis revealed, overall, similar patterns in the distribution of mtDNA haplogroups across all groups (p value = 0.077, Fig. 4), in line with findings from previous studies indicating no obvious geographical clustering in Europe [72]. As expected, haplogroup R, and its sub-haplogroups R0, T and U, were the most frequent across all groups, with R0 found in approximately half of all sequenced individuals. R0 includes further sub-haplogroups that are particularly frequent in Europe, like haplogroup H, and HV, as well as less frequent ones like R0a'b and V. We explored the frequency of R0 sub-haplogroups for the Walser + Walser-homeland and Swiss groups, for which we have whole mtDNA genomes (Supplementary Figure S6). H1 was the most common branch in the Swiss group, while basal H* and H5 were the most common branches in the Walser + Walser-homeland group.

In addition to sub-haplogroup R0, haplogroup T and U were represented at high frequency across all groups: for haplogroup T frequencies were similar and in the range of 13.2–14.6% of individuals, while for haplogroup U slightly lower frequencies were observed in the Walser + Walser-homeland (16.7%) compared to the Swiss (24.0%), Austrian (28.22%), Italian (21.8%) and European (21.6%) datasets. For two haplogroups, much larger differences were observed. For instance, haplogroup J is far less common in the Walser + Walser-homeland than in the rest of the groups (2.1% versus 7.9-10.5%). The most salient difference observed was for haplogroup N2 (here represented only by sub-haplogroup W), found in 10.4% in the Walser + Walser-homeland but only 2.0% or less in other groups.

Within sub-haplogroup W, we found sequences pertaining to haplogroup W6 (classified using EMPOP) among five Walser individuals from Vals and Furna (out of 48 individuals examined). Specifically, two individuals from Vals shared the same W6 haplotype, and differed from each other due to a point heteroplasmy in one of them. Interestingly, this W6 haplotype has three additional mutations not found in other W6 haplotypes in EMPOP. This possibly represents an unreported branch of W6. In the remaining three individuals we observed haplogroup W6a; two of them (from Furna) differed only in the presence of a heteroplasmy, while the third individual from Vals lacked a mutation found in the others. In the Italian and Austrian populations, where Walser communities were also established, only four individuals out of 395 in Italy [66] and four out of 273 in Austria [65] belonged to haplogroup W. Of these, only one individual from Austria, based on the mutations in the control region, might pertain to haplogroup W6, but the assignment is uncertain due to the presence of four extra mutations. Similarly, among the Swiss dataset, four individuals out of 200 were assigned to haplogroup W, though all belonged to sub-haplogroup W1, with no W6 observed.

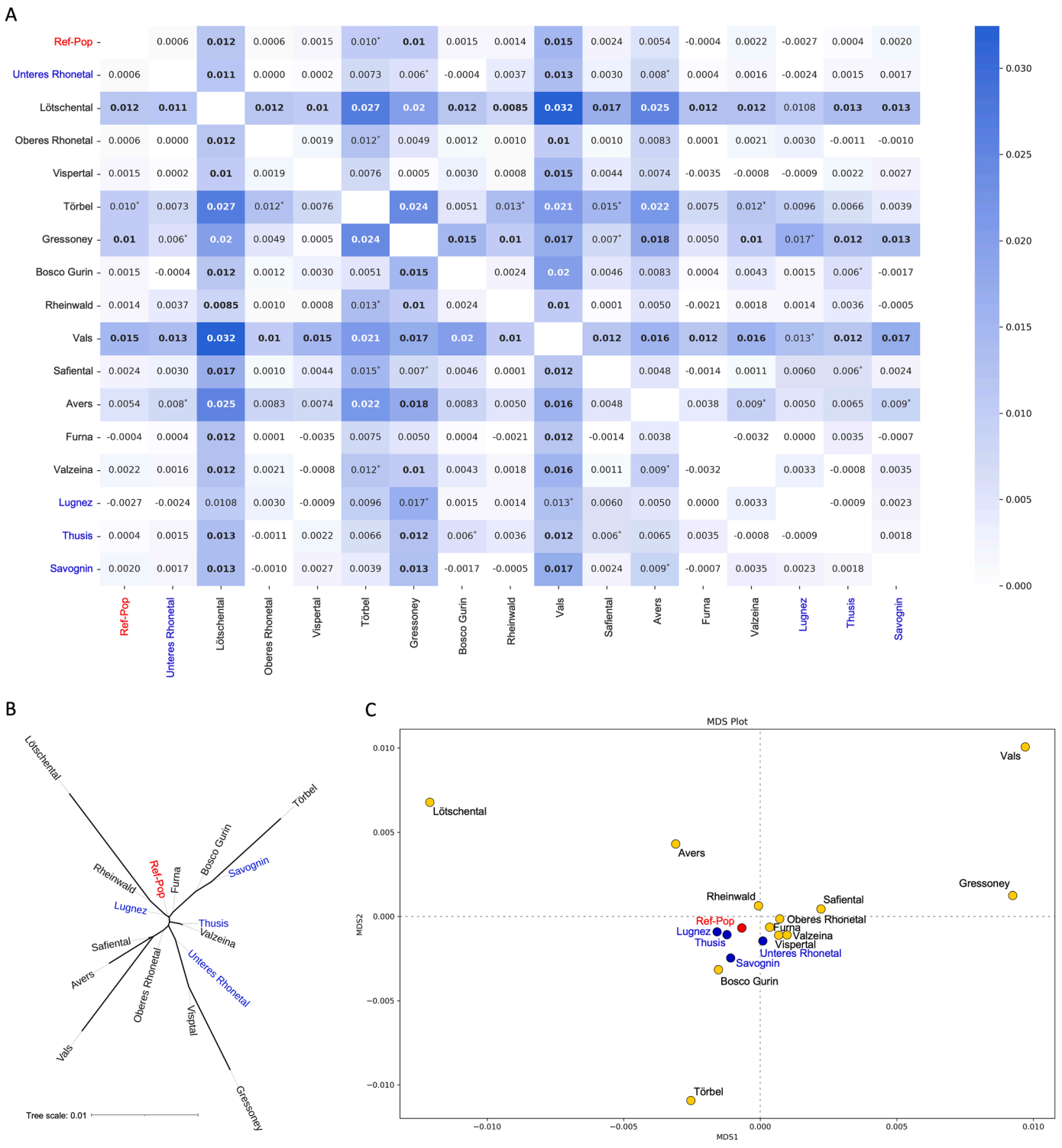

Fig. 2. : Genetic relationships among Walser-homeland, Walser, and non-Walser communities. The analyses included individuals from four Walser-homeland and eight Walser communities (black font, yellow circles), four non-Walser communities (blue), and an idealized Swiss Reference Population (Ref-Pop in red). (A) Heatmap of pairwise F_{ST} values estimated with Arlequin. FST values with *p* values that were significant before correcting for multiple testing are indicated with asterisk (*). Those that remained significant after the Benjamini-Hochberg correction are given in bold. (B) Neighbor Joining (NJ) tree based on linearized pairwise F_{ST}. (C) Visualization of results from Multidimensional Scaling (MDS) applied to linearized pairwise F_{ST} and constrained to two dimensions.

4. Discussion

We investigated the population genetic structure of Walser-homeland, Walser, and non-Walser communities in the European Alps. To our knowledge, this is the first study to examine autosomal microsatellites as well as Y-chromosome and mtDNA markers comprehensively in this geographic area. Our results for the autosomal markers show that the Walser communities and their homeland communities in Upper Valais exhibit low to moderate genetic differentiation from each other, to other non-Walser communities, and to an idealized Swiss reference population (Ref-Pop). The Walser-homeland communities Lötschental and Törbel, and the Walser communities Avers,

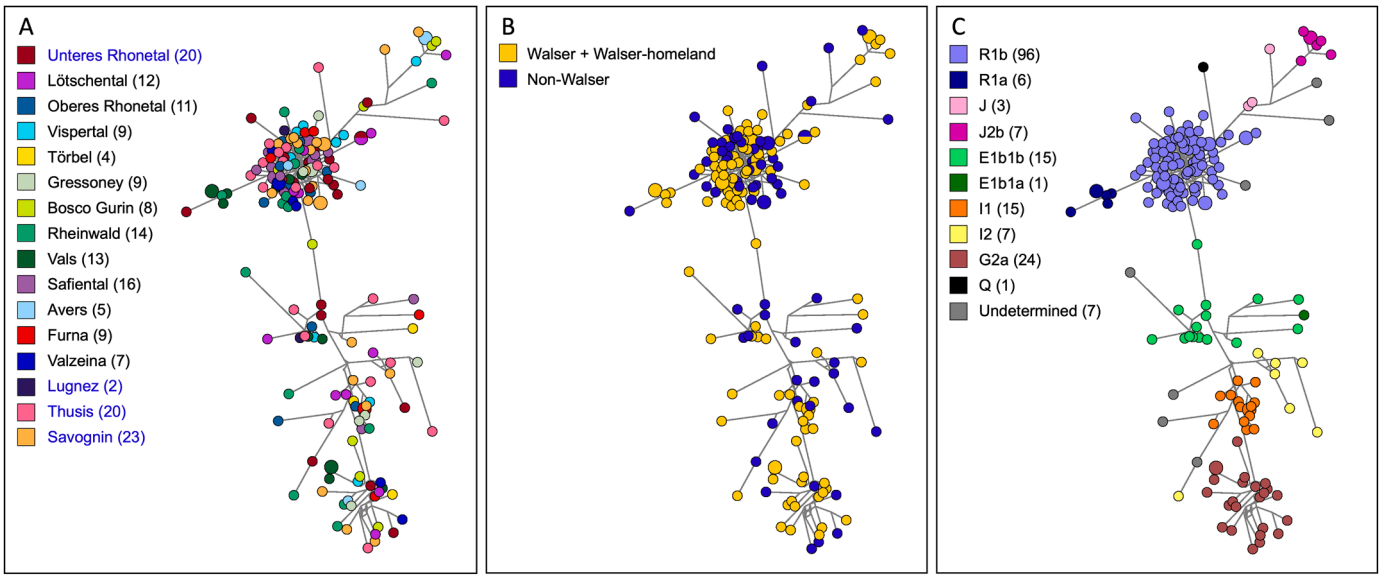

Fig. 3. : Visualization of evolutionary relationship among Y-chromosomal haplotypes as Median-Joining (MJ) networks. Each haplotype is coloured by either the male individual's geographical origin (A), associations with the Walser + Walser-homeland or the non-Walser (B), or genetic haplogroup (C).

Table 4
Distribution of Y-chromosomal haplogroups across five groups of communities.

Haplogroup	non-Walser in Lower Valais (n = 20)	Walser-homeland (n = 36)	Walser (n = 81)	non-Walser in Grisons (n = 45)	All (n = 182)
R1b	60.0%	52.8%	49.4%	55.6%	52.8%
R1a	5.0%	0.0%	6.2%	0.0%	3.3%
J	5.0%	0.0%	1.2%	2.2%	1.7%
J2b	0.0%	5.6%	4.9%	2.2%	3.9%
E1b1b	10.0%	11.1%	4.9%	11.1%	8.2%
E1b1a	0.0%	0.0%	1.2%	0.0%	0.6%
I1	0.0%	13.9%	7.4%	8.9%	8.2%
I2	5.0%	2.8%	2.5%	6.7%	3.9%
G2a	10.0%	11.1%	17.3%	8.9%	13.2%
Q	0.0%	0.0%	0.0%	2.2%	0.6%
Undetermined	5.0%	2.8%	4.9%	2.2%	3.9%

non-Walser communities in Lower Valais (Unteres Rhonetal), Walser-homeland communities (Oberes Rhonetal, Lötschental, Vispertal, Törbel); Walser communities (Gressoney, Bosco Gurin, Rheinwald, Vals, Safiental, Avers, Furna, Valzeina); non-Walser communities in Grisons (Lugenz, Thusis, Savognin), and all communities combined.

Gressoney, and Vals appeared genetically most differentiated from the ensemble of the remaining communities. These findings suggest the importance of local historical and geographical factors affecting communities differentially. Our analyses of Y-haplogroups and mtDNA haplogroups did not indicate stratification by geography or association with the Walser. However, amongst the Walser, we found an mtDNA haplogroup that is otherwise rare in Central Europe.

Our population genetic analyses with STRUCTURE did not reveal distinction among the communities examined, or between these and the Ref-Pop. Furthermore, the AMOVA results revealed that most of the overall genetic variation was explained by variation within individual communities rather than among groups, a pattern expected when gene flow or population size hinder genetic drift, or when populations have only recently diverged. Differentiation among communities within groups accounted for a small but statistically significant proportion of genetic variance, and it was driven by the communities of Lötschental, Vals, and Gressoney. In line with this finding, our F_{ST} pairwise analyses showed that these three communities had relatively high F_{ST} values that remained significant after correction for multiple testing, suggesting distinct allelic frequencies.

The genetic differentiation of Lötschental could be attributed to genetic isolation, influenced by its remote location, economic autarchy, and limited accessibility until very recent times. Additionally, linguistic evidence suggests that Lötschental was settled earlier by a linguistically, and potentially genetically, distinct Germanic population via the Lötschen and Gemmi passes, as opposed to the eastern Upper Valais (Goms), where the settlement occurred later through the Haslital and Grimsel pass [73].

For Vals, geographical remoteness as well as historical and sociocultural factors may have contributed to relatively high genetic differentiation through genetic drift. Notably, recurrent natural disasters, such as floods and avalanches, have historically plagued this area and might have caused population bottlenecks [74]. Furthermore, differences in religious affiliations between Vals and other Walser communities could have played a role in the genetic isolation of Vals. As reported in Zinsli [14], Vals maintained its Catholic identity post-Reformation: their religious confession might have posed a critical barrier to inter-marriages with individuals from the adjacent and reformed Grisons communities, such as Safiental and Rheinwald.

Gressoney is geographically remote from the Walser homeland, located within the Italian-speaking region of the Aosta Valley, and distant from other Walser communities. Gressoney's geographical context might have contributed to its genetic distinctiveness. Interestingly, Boattini et al. [25] conducted a study on endogamy in a region of the Aosta valley and including three Walser communities (Gressoney-la-Trinité, Gressoney-Saint-Jean, and Issime), as well as the Romance-speaking community of Gaby. Their analysis of matrimonial records from 1838 to 1938 revealed that the Walser communities had lower endogamy rates compared to the Romance community, indicating a higher level of genetic exchange with neighboring populations. This suggests that within the region, the Gressoney communities are 'linguistic but not genetic isolates".

Avers and Törbel are both located at relatively high elevations (1960 and 1506 m above sea level [masl], respectively) and have the second and third lowest census size (n = 140 and n = ~500, respectively; see Table 1). Törbel, situated within the Walser-homeland, was historically difficult to reach before the road construction from the valley to the village in 1940. This factor might have, through genetic drift, contributed to the relatively high genetic divergence that we observed at both

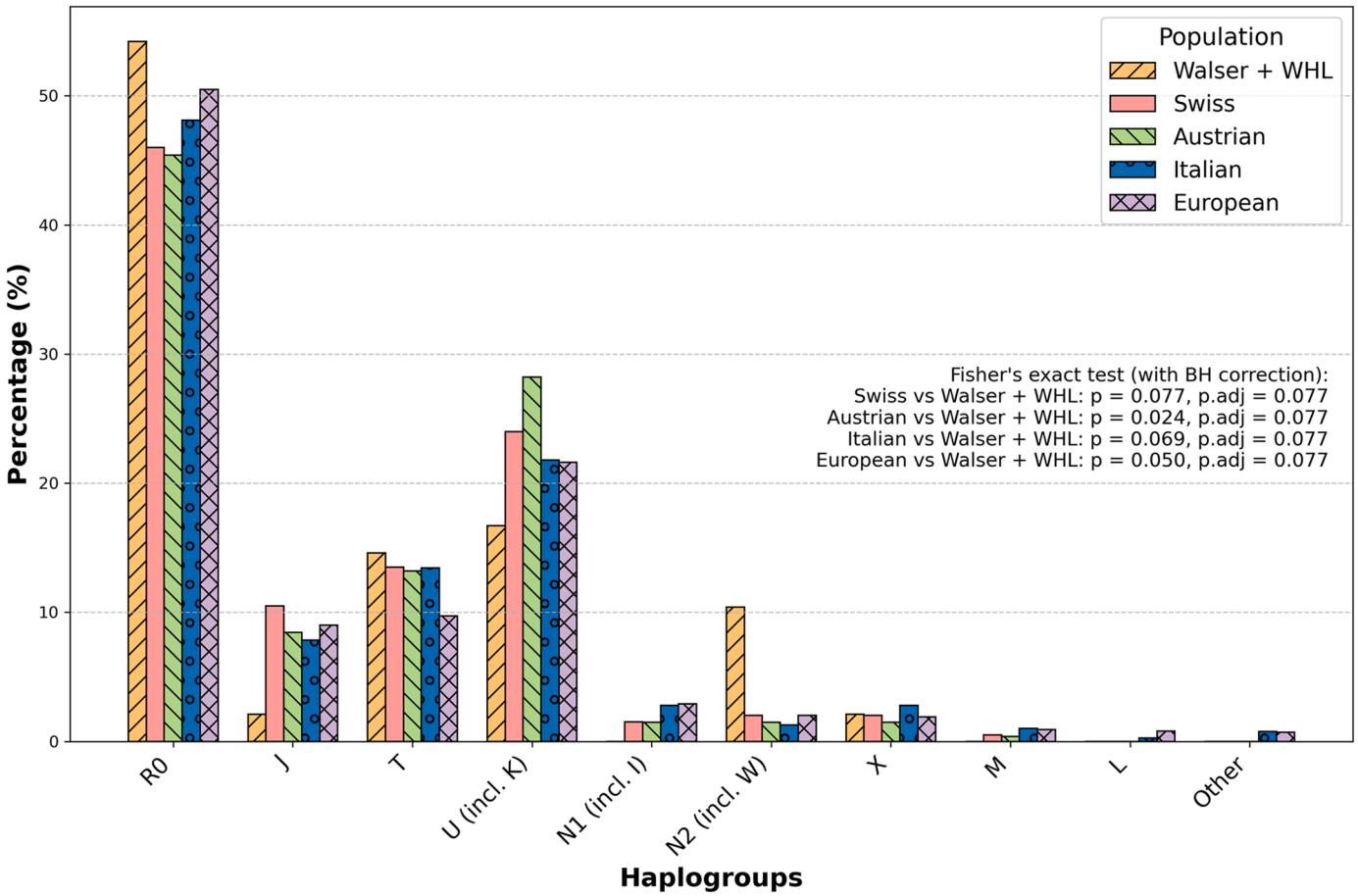

Fig. 4. : Comparison of mtDNA haplogroup frequencies across five populations: Walser + Walser-homeland (WHL) communities, Swiss (study in preparation), Austrian [65], Italian [66], and European [67]. The percentages of haplogroups (x-axis) are shown for each group. Fisher's exact test was used to assess the significance of haplogroup frequency differences between Walser + Walser-homeland and the other four groups. Test results are indicated below the legend, with 'p' representing the *p* value before multiple testing correction, and 'p.adj' the corrected *p* value.

autosomal and Y-chromosomal markers. Avers is geographically remote from the Walser-homeland, and the demographic history of the Avers community involved not only a bottleneck associated with the migration out of Upper Valais, but also one associated with the foundation of the community from Rheinwald (Fig. 1, [19]). This series of bottlenecks may have contributed to higher levels of genetic drift. Interestingly, although geographical remoteness, topographical barriers, and low population size might explain the high levels of genetic differentiation for Avers, Gressoney, Lötschental, Törbel, and Vals, these factors do not always seem to be associated with high genetic differentiation. For instance, Bosco Gurin does not display particularly high levels of differentiation, despite its geographical remoteness, relatively high elevation (located at 1506 masl and thus 353 higher than Vals), and the fact that its census estimate around the time of sample collection was only 65 individuals compared to 929 for Vals.

Based on our autosomal marker results, the community of Avers appeared to be genetically more closely related to the communities of Rheinwald and Safiental, as opposed to the communities of Törbel, Vispertal, and Gressoney. It is historically well documented that Rheinwald was established from the Pomatt valley in Italy and that Safiental was founded from Rheinwald [75] (Fig. 1). Historical evidence about the origin of Avers, however, has been much more scattered [76]. Fibicher [19] reported two hypotheses: one indicating a putative migration route from the Vispertäler via Italy directly to the Avers valley, and one suggesting a colonization via the previously existing Rheinwald community. The latter hypothesis of Avers as a 'daughter' colony of Rheinwald has long been favored based on linguistic similarities [76]. However, Bundi [77] and, later, Weber [78] reviewed historical evidence supporting a third hypothesis: that Avers was also colonized via the Pomatt valley, but independently from Rheinwald. This hypothesis places Avers as a 'sister' colony of the Rheinwald and is compatible with linguistic evidence suggesting close ties between Rheinwald and Avers. Our population genetic results are in line with the linguistic evidence supporting high affinity of Avers with Rheinwald. However, the resolution of our population genetic analyses is not sufficient to distinguish between the 'daughter' and the 'sister' hypotheses. Disentangling these two scenarios will likely require larger numbers of samples and/or genetic markers.

For the remaining communities, we observed comparatively low pairwise F_{ST} and thus low genetic differentiation of individual communities from the ensemble of the other communities. The branching patterns in the NJ tree based on autosomal markers partially reflected, but were not fully consistent with, the putative trajectories of the Walser migrations (Fig. 1 vs. Fig. 2b). The deviations could reflect gene flow facilitated by various factors. First, the abandonment of high-altitude settlements during the Little Ice Age (early 14th to the mid-19th century CE) and the influence of the Industrial Revolution with its peak between 1850 and 1900 potentially leading to increased connectivity and genetic exchange among (remote) communities and likely played a role in shaping current patterns of genetic structure. Second, the Walser communities were connected culturally among themselves and with their homeland in Upper Valais [25]. Thus, it is probable that some genetic exchange limited the genetic differentiation of the Walser-homeland and Walser communities. Third, even though the

Walser most likely married within the Walser communities for economic and cultural reasons [79], marriages with non-Walser have also been documented [14]. Such marriages might have contributed to the gene pool of the sampled Walser communities.

Uniparental markers indicated an overall lack of population structure across the individual Walser-homeland and Walser communities. A substantial amount of similar Y-chromosomal haplotypes across nearby and distant populations indicates a very recent population divergence of the investigated communities. The Y-chromosomal haplogroups observed are in line with those typically observed in Central Europe [80]. As in other Central European populations, the R1b haplogroup occurs at a high frequency among the Walser. This haplogroup was brought to Europe with the expansion of the Yamnaya out of the Pontic-Caspian steppe [9,81–83]. Interestingly, the second most prevalent haplogroup in the communities we investigated was the G2a haplogroup, which was particularly frequent in Walser communities (~17%). While ancient genetic data reveal a high frequency of the G-haplogroup in prehistoric populations of Central Europe, levels below 10% are routinely reported today. Two exceptions are Corsica and Sardinia, where the G-haplogroup is found is found at relatively high frequencies 21.7% [84] and 14.3% [85], respectively, attributed to the diffusion of Neolithic farmers from Asia. In Corsica, the most prominent G sub-haplogroup is G-L91, which is also that of the Tyrolean iceman mummy Ötzi. In the Walser, in contrast, we did not detect this sub-haplogroup; the most frequent sub-haplogroup was G-L497, a group also found to occur at high frequencies (75.56%) in Alpine communities of Tyrol [29].

In our analysis of mtDNA haplogroups, one of the most striking findings was the significantly higher frequency of haplogroup W in the Walser and Walser-homeland (WHL) communities (10.4%), compared to other populations where it was found at or below 2.0%. Within this haplogroup, we identified sub-haplogroups W6 and W6a. Haplogroup W6 is typically scarce in Europe, occurring at frequencies of less than 1% except in specific regions like Estonia, while it has a higher prevalence in the Caucasus and Levant regions (~5.3%) [86]. The presence of basal W6 as well as W3 and W4 lineages in Anatolia and South Caucasus supports these regions as sources of origin [87]. Amongst the Walser, it is possible that this haplogroup is a relic of previous migration events, or alternatively, that it has been recently introduced into the Walser or their recent ancestors. Interestingly, W6 and W6a were only detected in the Grisons communities of Vals and Furna, suggesting that gene flow rather than founder effects from the initial settlers may have introduced these haplogroups into the Walser. Nonetheless other scenarios cannot be excluded, such as the survival of the W6 haplogroup only in a few specific communities, perhaps through genetic isolation and drift, which would have influenced the chances of loss or fixation of rare haplogroups.

Overall, our findings suggest that, at the time of sampling, most of the Walser-homeland and Walser communities did not appear to be markedly differentiated from the ensemble of the other communities, except for Avers, Gressoney, Lötschental, Törbel, and Vals, which exhibited moderate to strong genetic differentiation. This pattern mirrors findings from other studies of other Alpine populations that have historically occupied geographically similar regions and experienced comparable environmental influences. Specifically, the research conducted by Capocasa et al. [88] in Italian isolates and Thomas et al. [89] in Ladin communities generally found low levels of genetic differentiation among the populations they investigated, except for a few outliers. In the case of Capocasa et al. [88], the exceptions were Sappada and Luserna, while Thomas et al. [89] identified two Ladin populations as being distinct. This differentiation was similarly attributed to various factors including geographic isolation, linguistic barriers, or small population sizes.

The genetic patterns identified within the Walser communities differ from those observed in other populations that have undergone recent range expansions. One example is that of the French-Quebec population [90,91], who are largely descendants of the approximately 8500 founder immigrants, mostly of French origin in the 17th century. This population experienced bottlenecks and serial founder effects, leading to reduced genetic variation and increased differentiation from their French ancestors. In the French-Quebec communities, the effects of genetic drift remain more discernible and traceable to the present day. In contrast, our results indicate more nuanced genetic patterns among the Walser people, possibly reflecting not only older range expansions but also possibly more complex demographic processes.

Several limitations might affect our results. First, the population genetic inferences presented here are based on genetic samples from the 1980s, a relatively recent period. It is conceivable that genetic differentiation of the Walser and Walser-homeland communities was higher in the past, particularly before the development of infrastructure. We hypothesize that the genetic structure of these locations has also changed significantly today, given the increased connectivity and opportunities for genetic exchange since the time of sampling. However, it is also possible that the Walser were never entirely genetically isolated, except in very remote and hard-to-reach locations. Research into cultural practices reveals that some Walser communities prioritized practical considerations over cultural restrictions in selecting partners. For example, in Obermutten, men frequently married maids (often of Italian origin), who came there for work (Erwyn Wyss, personal communication). Additionally, the community switched to Protestantism when it became evident that this would expedite the building of a local church, demonstrating pragmatism over strong cultural attachment (Erwyn Wyss, personal communication). It is nonetheless challenging to make conclusive statements about changes in genetic structure over time without access to historical samples.

Second, the limited size of our sample collection, particularly for certain groups, may have impacted our results. For example, in Lugnez, Avers, and Törbel, we could only type fewer than 15 individuals. These small sample sizes might have influenced the significance of null-allele tests, notably in Avers and Törbel, where one significant marker was identified, and also contributed to a lack of significant F_{ST} p values. Additionally, the limited set of analyzed mtDNA samples (48 in total) may have contributed to the lack of detected diversity, as certain lineages present in the Walser population might not have been captured.

Third, while autosomal STRs continue to be useful tools in forensic population genetic studies, it is important to acknowledge a possible limitation of the STR markers used for the pairwise F_{ST} calculations. These markers, chosen primarily based on criteria useful for individual identification, may underestimate the overall level of genetic differentiation between populations [92]. This limitation underscores the need for harnessing the power of genome-wide SNP data, which is expected to facilitate more in-depth analyses. For example, such data should allow for a more accurate assessment of phylogenetic relationships, the exploration of admixture events, the inference of divergence times, and detection of a possible increase in the frequencies of deleterious variants. Overall, future studies exploring whole genome SNP data or microarray SNP data should enable a more comprehensive understanding of the evolutionary processes that have shaped genetic structure in the Walser-homeland and Walser communities, as well as other populations of the European Alps and their historical relationships.

The detection of recent local-scale range expansions may be more challenging compared to large global expansions such as the out-of-Africa migrations. The latter has resulted in enduring signatures despite the passage of millennia. However, at finer, local scales and when considering more recent range expansions, the genetic footprints may be eroded with even small levels of gene flow [93]. While it's important to acknowledge that the markers utilized in this study may not allow for unequivocal conclusions concerning the detection of the medieval Walser migration, there is also a possibility that the signatures of population expansion have been erased by subsequent gene flow, whether this occurred since the time of settlement or was spurred by the increased connectivity brought out through infrastructure development.

However, it's important to state that our study did not formally investigate gene flow mechanisms. Therefore, while gene flow could have contributed to the observed genetic similarities, further research is needed to explicitly test this hypothesis and understand the underlying processes. Furthermore, the demographic growth of the Walser communities and the number of generations that have elapsed since their founding event may have impacted the genetic signals of the medieval Walser expansion, presenting an intriguing avenue for future research.

5. Conclusion

Our study provides insights into the genetic diversity and differentiation of Walser-homeland, Walser, and non-Walser communities in the Swiss Alps based on genetic data sampled in the 1980s. Our findings portray a complex picture, with variation in the extent of genetic differentiation observed, compatible with the range expansion of the Walser from their homeland. Communities that are geographically or topographically remote from the homeland and from other communities showed higher genetic differentiation, likely as a consequence of genetic drift. The genetic similarity among the other communities might be explained by the relatively short divergence time, coupled with cultural and economic exchange among the Walser and Walser-homeland communities. Small sample sizes and the use of small numbers of markers likely limit the resolution of our analyses. Future studies on the population genetic structure and evolutionary past of populations in the European Alps should benefit from genome-wide data. We expect that such studies will provide greater depth and resolution to our understanding of how demographic processes shaped the contemporary genetic structure of the Walser communities.

CRediT authorship contribution statement

Simon Aeschbacher: Writing – review & editing, Methodology, Conceptualization. **Natasha Arora:** Methodology, Writing – review & editing, Supervision, Project administration, Conceptualization. **Joëlle Schneider:** Formal analysis, Data curation. **Cordula Haas:** Writing – review & editing, Project administration, Conceptualization. **Peter Resutik:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Michael Krützen:** Writing – review & editing, Resources, Project administration. **Chiara Barbieri:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Corinne Moser:** Formal analysis. **Mario Gysi:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Magnus Dehli Vigeland:** Writing – review & editing, Methodology. **Adelgunde Kratzer:** Resources, Funding acquisition, Conceptualization. **Mathias Currat:** Writing – review & editing, Conceptualization. **Paul Widmer:** Writing – review & editing, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT v3.5 and v4.0 in order to improve the legibility and flow of the text without influencing the content. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2024.103206.

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