Supplementary Information

A high-resolution picture of kinship practices in an Early Neolithic tomb

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SI Section 1: Osteological summary of human remains from Hazleton North

The human skeletal remains from Hazleton North (Extended Data Figure 1) were first reported on as part of the excavation report in 1990[1], and subsequently re-examined in 2016 as part of a PhD thesis on the burials within long barrows in southern Britain[2]. This summary updates the published osteological report with the findings of the 2016 thesis. Supplementary Table 1 details the genetic and osteological information together with the location of the excavated human remains found within the monument for those individuals that have been sampled for aDNA, while Supplementary Table 2 details information on all 19 individuals that are distinguishable from one another on an osteological basis, whether or not they provided aDNA for the current publication. Extended Data Table 1 supplies a summary of the key osteological data and biological relationships for each of the 35 individuals successfully sampled. As some ancient DNA samples were taken from loose teeth there is little osteological information for them, while in other cases the skeletal element sampled derived from a largely-complete skeleton (e.g. Skeleton 1). It should be noted that this is not the minimum number of individuals (MNI) identified from the site: a minimum of 41 individuals can be identified from within the tomb, of which 22 were adults, two were juveniles, ten were children between 3 and 12 years of age and seven were infants under three years of age. Eighteen individuals were excavated from the northern side of the tomb (nine adults, three children, and six infants) and 23 from the southern side (13 adults, two juveniles, seven children, and one infant). One of the adults, one child and one infant in the northern entrance were cremated. An additional eight fragments of human remains from a minimum of two individuals were located in the south quarry (from which stone was sourced to build the cairn). The individuals from the quarry would bring the total MNI from the excavations at the site as a whole to 43.

The MNI assessment for the adults was based on the presence of the right calcaneus, plus one cremated adult. The MNI for children was based on the dentition from six mandibles (two from the northern side of the tomb and four from the southern side) and one maxilla (from the southern side). The MNI of two for juveniles located in the southern side of the monument was based on the presence of two axis bones. The MNI for the infants was based on different elements of varying ages: the left ischium of a neonate (from the northern side); the mandible of a nine month old infant (from the northern side); two unfused *pars basilaris* bones from an individual aged 18 months and one aged two years and three months at death (both from the northern side); two radii from individuals aged three months old and one aged between two and a half and three years of age at death (one from the northern side and one from the southern).

The sex of the individuals for whom we successfully obtained ancient DNA was inferred from the genetic data itself. The osteological determination of sex from the assemblage was based on the right ox coxae (as the most reliable indicator of sex in the skeleton), which was only able to be identified in five males and one probable male, and three females. The aDNA identification of sex adds considerably to the number of individuals for whom biological sex can be determined, as follows:

Osteology North Side: Adults (9), juvenile (--), children (3), infants (6), male (5), female (2), **total (18).**

Genetics North Side: Adults (n/a), juvenile (n/a), children (n/a), infants (n/a), male (11), female (3), **total (14).**

Osteology South Side: Adults (13), juvenile (2), children (7), infants (1), male (8), female (1), **total (23).**

Genetics South Side: Adults (n/a), juvenile (n/a), children (n/a), infants (n/a), male (15), female (6), **total (21).**

The genetic results include seven males and three females from the north chamber, four males from the north entrance, eight males and five females from the south chamber, four males from the south passage, and three males and one female from the south entrance.

There is no reliable way to assess the original number of individuals whose remains were interred in a tomb on the basis of an osteological assessment of MNI alone. Following Monte Carlo simulations of taphonomy, Robb concluded that 'a tomb assemblage containing 50 people could equally well represent 50, 100 or 1000 original depositions'[3]. Identifying individuals using ancient DNA offers new possibilities for assessing whether the tomb population was originally similar to the minimum number of individuals or much higher. In the case of the current study, the number of individuals identified as genetically distinct is not far below the osteological MNI (see above), even though we have not been able to obtain results for all individuals identified as distinct on the basis of osteological assessment. This requires careful interpretation given the different methods used to reach these numbers. The genetic evidence comes from 17 petrous portions of the temporal bone, 45 teeth (some of which were 'loose' in the chambered areas when found; maxillary canines were preferentially selected from among these to reduce the chance of duplicate genetic results on the same individual), and four other bone elements, whereas MNI estimates drew on a wider range of skeletal elements. The genetic sample does not necessarily cover all of the individuals identified as distinct on the basis of osteology *and* includes individuals whose remains might not be present among those used to calculate the osteological MNI (particularly where 'loose' teeth were sampled). Nonetheless, the comparison suggests the genetic sample provides good coverage of the number of individuals whose remains survived within the tomb: in particular, there are genetic results for 21 individuals from the south side for which osteological analysis suggests an MNI of 23. The real number of individuals is likely higher than 23, but since the 21 distinct genetic individuals were identified from 39 samples yielding successful results from the south side, including loose teeth, it is possible that the actual number of individuals whose remains are present in the tomb overall may be not significantly higher than the MNI: that is, tens rather than hundreds. Analysis of a later tomb in Iberia suggests that only teeth survived from an early phase of use while the surviving bones derived from later individuals[4]. In such a case a calculation of MNI based on bone would

overlook earlier individuals, but in that particular tomb there was a significant span of time between the dates obtained from teeth and those from bone. Although the three aDNA samples from first generation individuals at Hazleton North were all from teeth, skeletal remains do exist for two of them, and while we have not carried out an equivalent analysis to Aranda Jiménez et al., there is no apparent gap between the dates from teeth and those from bone (SI section 4). There is no clear indication that the chambered areas were used prior to the introduction of the individuals assessed in this study, and, indeed, the combination of the excavator's assessment of the sequence of site construction and use, the chronological models, and the patterning we have identified in the placement of individuals from the two branches of the lineage on either side of the monument, suggests that a previous use of the monument for remains that have decayed completely or have been removed is unlikely.

Mortuary transformation and taphonomy

Mortuary practices vary slightly on either side of the tomb, but discrete deposits of bones from specific individuals are evident in both cases. These do not seem to have been significantly disturbed following deposition: although the excavators' analysis of this was limited, conjoining bones from the same individual were all kept within the same compartments in the north chambered area, and a similar picture is evident in the southern chambered area where only a few bones from the south chamber conjoined or were paired with bones in the south passage or entrance[1]. Among the sampled remains, skeletal elements belonging to SC3m, SC6m and SC8m were found both in the south chamber and in the south passage, while the predominance of bones from SE4m were found at the rear of the entrance with one in the passage, suggesting some further displacement of remains within the south chambered area. There are no recorded instances of bones from the south chambered area combining with those from the north chambered area, and we detected no genetic duplicates between the south and north chambered areas, suggesting that remains were not moved between the two burial zones.

Fifty-one bone elements from more than five individuals in the north chambered area had been gnawed by canids when the bone was fresh, attested by helical fractures, longitudinal fractures, gnaw marks on the diaphyses, some crenulated edges (especially on the ribs), and tooth furrows[5–7]. This suggests that some individuals were left exposed to the elements where the bodies were scavenged (excarnation). Most of the affected bone elements could not be assigned to specific individuals, but osteologically defined individuals A, C, G and H (i.e. NE2m(A), NC2f(C), NC5m(G) and NC6m(H)), all from the northern chambered area, bear clear signs of canid gnawing (e.g. Extended Data Figure 2). Scavenging by canids was not identified in the original osteological report[8].

Individual G (NC5m), a child of between three and four years of age at death, exhibited not only gnawing by canids on the distal end of the right humerus but also signs of weathering on the bone, suggesting a period of exposure to heat, cold, wet or dry environments[9]. Individual C (NCf2), a young female aged less than 24 years at death, exhibited extensive gnawing to all of

the long bones present and to four of the left tarsals. Furthermore, the right humerus had been extensively chewed at both ends and on the shaft, and exhibited a helical fracture on the proximal end. The right tibia exhibited extensive damage to the proximal end in the form of crenulated edges, and clear signs of chewing and puncture marks were present on the right femoral head together with gnawing of the greater and lesser trochanters. Significantly, all of the long bones were weathered and match the description of those detailed in Stage 1 of Behrensmeyer's scheme[9], equating to a period of exposure of between a few days, and up to three years. While all of the limbs are present, the proximal and distal ends of some long bones were chewed including the right femoral head which must therefore have been disarticulated from the pelvis. Research[10] on the effects of human remains subjected to scavenging by dogs suggests that if this stage of disarticulation has been reached, a period of not more than a year (between two and eleven months) has passed since the body was exposed to faunal activity. Thus, we can infer a period in which canids had access to the remains before they were collected and deposited in the tomb (access to the tomb was blocked by stone slabs which were presumable replaced each time a set of remains was added[1]). Only three bones showing signs of canid gnawing, representing at least two individuals (an adult and a child), were excavated from the southern chambered area (adult rib 4805 and clavicle 10499 in the entrance, and child's tibia 11438 from the chamber).

Remains deposited in the northern chambered area were more likely to have been exposed to the elements than in the south: 51 bone fragments in the north versus 3 in the south. Cremated remains from at least three individuals — one adult of unknown sex, one child of unknown age and one infant of unknown age — were concentrated at the north entrance, where over 187 fragments were recovered. By contrast, only 26 fragments of cremated bone were found in the south chambered area[8]. Some differences are therefore detectable in the treatment of human remains prior to deposition in the north compared with the south chambered area.

The range of treatments of human remains at Hazleton North is not particularly unusual in the context of other Early Neolithic sites in southern Britain, although these display a notable diversity in mortuary practices[11]. The introduction of intact or almost intact corpses into chambers is now suspected for many Cotswolds tombs, with varying degrees of disturbance or deliberate movement of the remains during successive activity within chambers, including the placement of further human remains. Evidence of canid gnawing is known from a few sites, most notably Adlestrop in the Cotswolds, where they are interpreted as evidence of the exposure of remains prior to selective inclusion in the chamber[12]. Cremated remains are attested in small numbers at several long barrows[2,11]. Tool marks from cutting flesh from bones or deliberately disarticulating body parts was not detected at Hazleton North but is known elsewhere, including Adlestrop[13]. Finally, the remains of the Early Neolithic dead were not only placed in stone chambered tombs in southern Britain. They are also found in wooden chambers covered by earthen mounds, in caves, in pit graves, in the ditches of causewayed enclosures, and occasionally in features associated with occupation[11].

Health, pathology and trauma

Six individuals suffered from conditions suggesting periods of poor nutrition. Four children suffered from scurvy, including SC6m and SC9f, both born into the core lineage in the third and fourth generation, and two not yet sampled for aDNA (an infant from the north entrance and a nine-month old from the north chamber). Scurvy is caused by a lack of vitamin C in the diet, which is crucial to combat infections, allow the normal development of all bodily tissues, in particular collagen, and to facilitate iron absorption[14]. SC6m and SC9f did not survive past six and nine years of age respectively. Two adults suffered from both cribra orbitalia (CO) and porotic hyperostosis (PH), which are conditions considered to be non-specific indicators of physiological stress. One was SC10f (Individual viii), who was not born into the lineage and for which we have no evidence of bearing lineage children; another was NC2f (Individual C), whose first-generation union with NC1m was key to the foundation of a maternal sub-lineage within the patriline. These conditions are usually found in children, and where evidence of the lesions caused by CO or PH are found in adults it is a relic of childhood[15–17]. Lack of vital nutrients, such as vitamin B12, vitamin B9, and Vitamin C (in the case of CO), together with a lack of animal protein in the mother's diet, are all passed on in breastmilk causing megaloblastic anaemia in small children. Furthermore, poor sanitary living conditions and weaning foods lacking in sufficient dietary value contribute to the development of these conditions[16]. The presence of scurvy in four individuals is significant, as this condition is rarely reported in assemblages of this period. Cuthbert's study of human remains from 42 Neolithic long barrows in southern Britain found that three sites had evidence of scurvy, and that 4 out of 6 (67%) of the cases were from Hazleton North[2]; an additional possible case of scurvy was noted at West Tump long barrow, Gloucestershire by Smith and Brickley[13]. The prevalence of CO and PH at Hazleton North was low compared to other sites of the same period[2].

The cases of poor nutrition need to be set alongside Carbon and Nitrogen stable isotope analyses on 22 human femurs from the tomb (16 from adults, 6 from subadults) which indicate that all sampled individuals consumed a very high level of animal protein consistent with a meat-rich diet [18], and a proteomic analysis of dental calculus on four individuals from the tomb which suggests the ingestion of processed bovine milk products[19]. This suggests that diets in this community were generally similar to those in the wider region, where animal protein formed a substantial dietary contribution and there is good evidence for the use of dairy products[20].

The assemblage as a whole exhibited a high prevalence of dental disease. Three adults detailed in Supplementary Table 1 exhibited evidence of periodontal disease (PD), a condition caused by the inflammation of the soft tissues surrounding the tooth, forming a pocket where bacteria proliferate[21]. Without treatment, the alveolar bone will become infected and reabsorption occurs, culminating in the loss of the affected tooth[22]. Two further individuals also had PD but were not tested for aDNA. PD is the most common cause of ante-mortem tooth loss (AMTL)[23], a condition that was exhibited by six individuals sampled for aDNA analysis (Supplementary

Table 1). A further six individuals in the assemblage suffered from ATML. Attrition (wearing) of the dentition was evident in eight individuals and is generally associated with advancing old age, a coarse diet, or technological activities[24]. Nine other individuals who are not featured in Supplementary Table 1 also suffered from attrition. Dental abscesses were exhibited by four individuals and are caused by bacteria entering the pulp cavity of a tooth, causing inflammation and a build-up of pus. When the pressure in the jaw becomes excessive, a hole forms, allowing the pus to escape[25]. A further seven individuals from the tomb also suffered from dental abscesses. Calculus, dental plaque that is not removed and becomes mineralized[26], was present on eight individuals for whom we definitely obtained genetic data (listed in Supplementary Table 1) and on a further five individuals and reflects lack of dental hygiene. Dental caries were present on three loose teeth excavated from the monument, but could not be assigned to a particular individual. The incidence of these particular dental pathologies is greater at Hazleton North than other long barrows from southern Britain[2].

At least six individuals exhibited evidence of osteoarthritis (OA). Four mature adult male individuals from Supplementary Table 1 exhibited signs of the condition. OA only affects the synovial joints of the skeleton, where the degeneration of the articular cartilage results in subchondral changes which affect the efficacy of the joint[8]. Two individuals in particular had widespread osteoarthritis. One had OA of the left and right sternoclavicular joints, the spine, left and right shoulder and left wrist, and the other had OA of the spine, right hip, right knee and right foot. The other two individuals did not have as many joints affected by the condition: one had OA of the left temporomandibular joint, and the other had OA of the right shoulder and spine. OA was also evident on further individuals from the monument. An adult female (Individual F), had the condition in the right sternoclavicular joint, both shoulders, the spine and left foot. Several disarticulated bones, which could not be allocated to any particular individual, also exhibited osteoarthritic changes: another case of OA of the temporomandibular joint; 4 vertebrae; six carpals and three finger bones; two further cases of OA of the hip; and one more of the knee. The prevalence of osteoarthritis in the assemblage as a whole is high compared to other sites of a comparable nature[2]. Evidence of OA was found in some individuals covering all joints across the assemblage except for the elbow. The difference is notable when comparing the Hazleton North remains with those from West Kennet, which has a similar MNI of 42 yet has far less evidence of the disease. The aetiology of OA is unclear but may be linked to age, activity, obesity, or trauma, among other factors[21].

The re-analysis of the assemblage confirmed the diagnosis of Diffuse Idiopathic Skeletal Hyperostosis (DISH) for one individual, together with evidence of septic arthritis in the left foot. Both conditions are associated with individuals who also have diabetes[27,28]. Another individual exhibited two lesions on the anterior surface of the left humerus which may be indicative of a benign cartilaginous tumour called a chondroblastoma, but a differential diagnosis could include a simple cist or tuberculosis. He also exhibited a lesion on the left navicular, a circulatory disorder condition called osteochondritis dissecans (OD) which only affects synovial joints, eroding a small area of the subchondral bone and the overlying cartilage[29]. Three further

examples of the condition were found on three disarticulated bones (a right trapezium, an axis, and the glenoid fossa of a left scapula), that could not be assigned to a particular individual. The presence of at least three individuals with OD is high compared to other contemporary sites in southern Britain: one case was found at Haddenham long barrow, Cambridgeshire, and at least one at Rodmarton long barrow in the Cotswolds[2].

There were four cases of infectious disease among the assemblage, one of which came from an individual for whom we obtained aDNA (SE4m(D)). Three disarticulated lower limb bones (a right fibula, a right tibia and a left femur) from the north chamber all exhibited periosteal new bone formation on the diaphysis. Periosteal new bone formation is caused by inflammation of the periosteal membrane that covers the outside of the bone, resulting in an area of new woven bone that will eventually remodel into hardened lamellar bone[30]. However, the presence of periosteal new bone on an element may not always be an indication of an infectious process, but can also be linked to trauma, cancer, tearing events, or stretching[31,32]. A possible case of a viral infection was detected in Individual D (SE4m). This individual exhibited signs of poliomyelitis, a virus that is spread inter-personally by infected faeces and which causes permanent limb paralysis[33,34]. His skeleton has a noticeably more gracile forearm on the right side than on the left, and the limb has suffered disuse atrophy due to the underdevelopment of the muscles.

Two individuals had minor congenital disorders of the vertebral column, which is formed from three different structures. One adult from the south chamber had Klippel-Feil Syndrome, where two vertebrae fuse together (the second and third cervical vertebrae in this case)[35]. This condition is not life-threatening but may reduce the length of the neck and impair movement[36]. Individual D (SE4m, who may also have suffered from polio) had a unilateral and unsymmetrical sacralization of the fifth lumbar vertebra. This condition occurs when the fifth lumbar vertebra is fused to the sacrum, thereby reducing the lumbar spine by one vertebral body and increasing the height of the sacrum[37]. The fact that the fifth lumbar vertebra was unfused on the right side of the sacrum would have caused the bone to lean to the left, thereby causing the spine to twist (scoliosis). No vertebrae were recovered from this individual to confirm this, but the association of scoliosis with poliomyelitis has been attested[38]. Reported cases of poliomyelitis for the Neolithic period are very rare and only one other example of the disease has been described for an individual found at a causewayed enclosure at Cissbury, West Sussex[39]. Only one other example of sacralization of the fifth lumbar vertebrae has been noted among Cotswold long barrows, at Lanhill[2].

Individuals A, D, F and Skeletons 1 and 2 (i.e., NE2m(A), SE4m(D), NC3f(F), NE4m(1) and NE1m(2)) had more severe pathology than others and may have been visibly and physically more frail than others: Individual A may have moved awkwardly due to DISH and septic arthritis in his foot; Individual D may have had polio and scoliosis of the spine, resulting in awkward movement; Individual F had severe OA in both shoulders, the spine, one sternoclavicular joint and the foot, and may have limped; Skeleton 1 had OA of the shoulder and spine, and a healed fracture of the fibula that may have caused a limp; and Skeleton 2 had widespread OA of the

spine and right lower limb, which would likely have affected movement. There is evidence of OA of the hip and knee at two other Neolithic sites which may have affected the movement of those suffering from the disease, but likely not to the extent of those from Hazleton. Similarly, two other individuals from different sites had evidence of a fractured fibula, but the individuals from Hazleton North appear to have had more serious and extensive conditions, which would likely have affected their range of movement[2].

Traumata are evident on the remains of at least two individuals in Supplementary Table 1. One adult male had healing facial fractures[40] at the time of death, injuries which are often the result of assault[41]. Another adult male had a healed fracture to the left distal fibula, an injury associated with twisting and/or abduction[42]. A further individual from the assemblage, represented only by a right fibula, suffered a fracture to the neck and an avulsion fracture to the styloid process of the proximal shaft, resulting in remodelling and flattening of the head. Individual D had a well healed fracture to the right forearm (a parry fracture), often sustained by a direct blow to the forearm[43]. One example of a vertebral fracture was found, often sustained following vertical compression[44]. Three instances of soft tissue injury were found. A right fibula from the assemblage exhibited myositis ossificans traumatica. This condition occurs when a tendon or muscle attachment is injured, and the resulting haematoma calcifies then eventually ossifies, leaving an easily identifiable unorganised bony mass on the bone[17]. This condition was also found on a disarticulated left fifth metatarsal of an adult. A cortical defect was detected on a disarticulated left humerus of an adult, and is characterised by deep grooves on the proximal end of the bone where powerful muscles attach. This condition may be caused by several different actions such as repetitive stress on the muscles and trauma[45]. Whilst the fractures mentioned were not life-threatening (or uncommon), only two may have sustained trauma due to inter-personal violence, whilst the other cases were likely caused by accidents. The lack of evidence for trauma caused by inter-personal violence at Hazleton North is somewhat unusual: there is evidence of such trauma from one or more individuals from many of the Early Neolithic monuments in southern Britain, including perimortem blunt force trauma to the cranium, healed cranial trauma, and arrowheads lodged in bones[2,11,13,46,47].

Spatial summary

The analysis of the human remains from Hazleton North reveal some differential mortuary treatments prior to deposition in the two sides of the tomb: individuals who were subjected to excarnation and cremation were predominately placed within the northern side of the monument. Those who suffered from the more severe health conditions (DISH, septic arthritis, widespread OA and other joint problems) were in the northern side of the monument with the exception of individual D (limb atrophy, possible polio, parry fracture) whose remains were placed in the south entrance where it met the passage. The majority of these were individuals who had lived into older adulthood. Four of seven cases of nutritional deficiency (57%) were interred within the northern side of the tomb but the majority of individuals with dental disease were placed within

the southern side which included a larger number of individuals overall. In general, there is no evidence for systematic differences in health among those buried on either side of the monument.

SI Section 2: Genetic analysis of biological relatedness and family tree reconstruction

2.1. Introduction

We took advantage of the high-quality genome-wide data generated for most individuals to study their biological relationships. We recovered information from the autosomes (chromosomes 1–22), which provide rich information about ancestry as they are a mosaic of DNA segments inherited from ancestors across the whole family tree. Therefore, our data allowed us to determine relationships among individuals through all lines of descent, unlike ancient DNA methodologies that predated the advent of Next-Generation technologies that only recover mitochondrial and/or Y-chromosome information and therefore only infer strictly matrilineal and patrilineal relationships, respectively. Our goal was to identify a unique family tree whose topology fits all types of genomic and anthropological evidence available for the Hazleton North individuals, while discarding all the other possible tree topologies that might be considered for relationships between these individuals. A summary of the process is as follows:

-*Section 2.2:* For each pair of individuals, we estimated the relatedness coefficients *r* that represents the fraction of the genome shared between 2 individuals. In the case of pairs identified as first-degree relatives (*r*~0.5), we also determined the type of relation (parent-offspring or siblings).

-*Section 2.3:* Using the pairwise degrees of relationship between all individuals, we followed a triangulation procedure to discard non-fitting tree topologies. To aid this process, we also incorporated information regarding the type of first-degree relationships, the mitochondrial and Y-chromosome lineages, the genetic evidence for inbreeding, and the age-at-death as determined through anthropological analysis. We arrived at two possible tree solutions fitting all the aforementioned pieces of evidence (Fig. 1c and Extended Data Fig. 4).

-*Section 2.4:* To disambiguate between the two possible tree topologies, we studied the co-localization of break points of shared DNA segments that inform about recombination events between the maternal and paternal chromosomes. This allowed us to obtain a unique family pedigree relating most of the Hazleton North individuals (Fig. 1c).

-*Section 2.5:* To further evaluate the validity of the proposed family tree structure, we used three lines of genetic evidence (not used in *sections 2.3* and *2.4*): 1) X-chromosome information which is completely independent from autosomal data, 2) number of shared segments between first and second-degree pairs, and 3) the software *NgsRelate* v.2[48] that estimates biological kinship using a different method as compared to that used in *section 2.2*.

2.2. Estimation of pairwise relatedness coefficients (r)

We began by estimating relatedness coefficients *r* that represents the fraction of the genome shared between 2 individuals. We estimated pairwise allelic mismatch rates in the autosomes[49–51] for each pair of libraries (n=156) deriving from 66 different samples, randomly sampling one DNA sequence at each '1240k' polymorphic position and masking the two terminal nucleotides

of each sequence to reduce the effects of post-mortem deamination. We then computed relatedness coefficients *r* for each pair (Supplementary Table 4):

$$r = 1 - (2*(x-(b/2))/b)$$

with *x* being the mismatch rate of the pair under analysis and *b* the mismatch rate expected for two unrelated individuals from the same population. We also computed 95% confidence intervals using block jackknife standard errors over 5 Megabase (Mb) blocks[52].

To estimate the constant *b*, we used genomic data from 53 Neolithic individuals from England, Scotland and Wales from previous publications[53,54]. This set (Supplementary Table 3) includes only individuals with the same type of data as our Hazleton North individuals (captured data and UDG-treated); individuals not identified as close relatives to others in previous publications; and individuals lacking recent Mesolithic hunter-gatherer admixture. This ensures that this set represents individuals with very similar ancestry background as Hazleton North individuals (Extended Data Fig. 9a). We then computed allelic mismatch rates for all pairwise comparisons between the 53 Neolithic individuals from Britain (1,378 pairs) and also comparisons between these 53 individuals and Hazleton samples (3,498 pairs; using only one library per sample). Including the comparisons between Hazleton samples (2,145 pairs; using only one library per sample) yields a total of 7021 comparisons, of which 4,528 had more than 100,000 overlapping SNPs. We computed the median mismatch rate among this set of 4,528 pairs (of which 1,280 are Hazleton-Hazleton pairs) and obtained a value of 0.2504 that we used to represent *b*, the value expected for unrelated pairs. Even if there were plenty of related pairs among the Hazleton-Hazleton pairs and plausibly some among Hazleton-Other Sites pairs (although close relatives across sites are extremely rare in the ancient DNA literature) or among the 53 Neolithic individuals from Britain that went undetected in previous publications, by using the median value we ensured that the lower mismatch rate in these related pairs had minimal impact on the estimate of mismatch rate in unrelated pairs. For these related pairs to have an impact in the median value, they would need to account for at least half of the 4,528 comparisons and this would imply more than a thousand closely related pairs across sites around Britain, which is exceptionally unlikely. Computing the median value using only across-site comparisons yielded a very similar value of 0.2507, and only for Hazleton-Hazleton pairs yields a slightly lower value of 0.2488, which could be affected by the presence of a large number of closely related pairs in Hazleton (see below). Therefore, we keep for analysis the value obtained using all comparisons (0.2504).

Using b=0.2504, we computed relatedness coefficients for all pairs (n=12,090) of Hazleton libraries (Supplementary Table 4). A total of 105 pairs of libraries stemming from 44 pairs of samples had relatedness coefficients larger than 0.85, indicating that they share their entire genome and that they derived from the same individual. This is not surprising given that human remains in both chambers were commingled and given the large number of samples we analyzed. To increase resolution in the kinship analysis, we merged the data from samples deriving from the same individual (as well as data from libraries deriving from the same sample), keeping 35

unique individuals for further analysis. We gave a unique identifier to each of these 35 individuals (Supplementary Table 1) based on their burial location and genetic sex (e.g., NC1m = male individual 1 from the north chamber), and use this identifier through the supplementary materials and main text.

We recomputed the mismatch rates and relatedness coefficients *r* on the merged dataset and annotated degrees of relationship (Supplementary Table 5 and Extended Data Fig. 2). Following a similar approach as in Monroy Kuhn *et al.* 2018[55], we used cutoffs lying halfway between the expected relatedness coefficients for different degrees of genetic relationships: 1 for identical twins or samples deriving from the same individuals, 0.5 for first-degree relationships (parent-offspring and siblings), 0.25 for second-degree relationships (grandparent-grandchild, uncle/aunt-nephew/niece, half-siblings, double cousins), 0.125 for third-degree relatives (first cousins, great-grandparent-great-grandchild, half uncle/aunt-nephew/niece, etc) and 0.0625 for fourth-degree relationships. The cutoffs are the following:

-We annotated a pair as first-degree biological relatives if the 95% confidence interval of their relatedness coefficient overlapped the range (0.375-0.75].

-We annotated a pair as second-degree biological relatives if the 95% confidence interval of their relatedness coefficient overlapped the range (0.1875–0.375].

-We annotated a pair as third-degree biological relatives if the 95% confidence interval of their relatedness coefficient overlapped the range (0.09375–0.1875].

-We annotated a pair as fourth-degree or more distant biological relatives if the 95% confidence interval of their relatedness coefficient overlapped the range (0-0.09375].

-If the 95% confidence interval of the relatedness coefficient of a given pair overlapped more than one of these ranges, we annotated multiple degrees of relationships as possible for this pair.

-We annotated a pair as biologically unrelated within resolution if the 95% confidence interval of their relatedness coefficient overlapped 0.

A total of 8 individuals did not yield any close biological kin relationship to other Hazleton North individuals within the limits of our resolution (Extended Data Fig. 2). The remaining 27 individuals were connected through close biological kinship relationships and were part of a large family.

Additionally, we determined the type of relationship (siblings or parent-offspring) connecting first-degree relatives based on uniparental markers (mtDNA and Y-chromosome) and the DNA sharing along the chromosomes: biological siblings present ~25% of the genome consistent with two chromosomes being identical by descent (IBD2), ~25% of the genome consistent with zero chromosomes being identical by descent (IBD0) and ~50% of the genome consistent with one chromosome being identical by descent (IBD1), whereas parent-offspring pairs share one chromosome across all the autosomal chromosomes. To analyse DNA sharing patterns along the chromosomes, we computed allelic mismatch rates patterns across sliding windows of 20 Mb,

moving by 1 Mb each step (Supplementary Table 6), and visually identified the presence or absence of regions with 0 or 2 chromosomes sharing for each first-degree relative pair with sufficient coverage. We illustrate this approach in Extended Data Fig. 3a and annotate the type of relationship for each first-degree pair (Supplementary Table 5).

2.3. Family tree reconstruction

In this section, we attempt to reconstruct the family tree relating 27 individuals from Hazleton North using the pairwise degrees of genetic relatedness (Extended Data Fig. 2) through a process of triangulation that allows us to discard most tree topologies relating these individuals. To aid this process, we also incorporated information regarding:

- The types of first-degree relationships (Supplementary Table 5).
- The mtDNA and Y-chromosome lineages transmitted through maternal and paternal lines (Supplementary Table 1).
- Genetic sex (Supplementary Table 1).
- Presence or absence of runs of homozygosity (ROH) indicative of inbreeding (see Methods section and Extended Data Fig. 9b).
- Age-at-death as determined through osteological analysis (Supplementary Table 1).

In what follows, we start by working out the biological relationships among different sets of individuals.

2.3.1. Tree topology relating males NC1m, SC2m, SP1m, SC3m, NC4m, NE2m

The core of the family is formed by 6 males (NC1m, SC2m, SP1m, SC3m, NC4m, NE2m) who are all either first- or second-degree relatives and who all have different mitochondrial lineages, with the exception of **SC2m** and **SP1m** who share the same maternal lineage. Genetic data shows that:

1) **SC2m** are **SP1m** are first-degree relatives (Extended Data Fig. 2) via a **sibling** relationship (Supplementary Table 5), and both are first-degree relatives of male **NC1m** (Extended Data Fig. 2) with different mitochondrial lineages, who can only be their **father**.
2) **NC4m** is a first-degree relative of male **NC1m** (Extended Data Fig. 2) with a different mitochondrial lineage, who can be only be **NC4m's father** because if **NC1m** were **NC4m**'s son, **NC2f** (as mother of **NC4m**; see section 2.3.3) would be grandmother of **NC1m** and therefore his second-degree relative, but **NC2f** is clearly unrelated to **NC1m** (Extended Data Fig. 2).
3) **NE2m** is a first-degree relative of male **NC1m** (Extended Data Fig. 2) with a different mitochondrial lineage, who can only be **NE2m's father** because if **NC1m** were **NE2m's** son, **NC3f** (as mother of **NE2m;** see section 2.3.4) would be grandmother of **NC1m** and

therefore his second-degree relative, but **NC3f** is clearly unrelated to **NC1m** (Extended Data Fig. 2).

4) **NC4m** and **NE2m** are second-degree relatives of each other and both also second-degree relatives of brothers **SC2m** and **SP1m** (Extended Data Fig. 2). Since they all are sons of **NC1m, NC4m** and **NE2m, they** must be **paternal half-brothers** and also **paternal half-brothers** of **SC2m** and **SP1m.**

5) **SC3m** is a first-degree relative of male **NC1m** and second-degree relative of **SC2m**, **SP1m, NC4m and NE2m** (Extended Data Fig. 2). Thus, **SC3m** can be either **NC1m's father** and **paternal grandfather** of **SC2m**, **SP1m, NC4m** and **NE2m,** or **NC1m's son** and **half-brother** of **SC2m**, **SP1m, NC4m** and **NE2m.**

Conclusions: Male **NC1m** is the **father** of **brothers SC2m** and **SP1m, father** of **NC4m** with a **different woman, father of NE2m** with a yet **different woman** and either **father** of **SC3m** with yet a **different woman** or **SC3m's son.**

2.3.2. Tree topology relating males SC2m, SP1m, SC6m, SC7m, NC9m and female SC1f

Genetic data shows that:

1) Female **SC1f** is a first-degree relative of brothers **SC2m** and **SP1m** (Extended Data Fig. 2) related to them through a **parent-offspring** relationship (Supplementary Table 5) and sharing with them the same mitochondrial lineage. Also, she is not related to **SC2m**'s and **SP1m**'s father **NC1m**. Thus, **SC1f** can only be **SC2m's** and **SP1m's mother.**

2) Males **SC6m** and **SC7m** are first-degree relatives (Extended Data Fig. 2) via a **sibling** relationship (Supplementary Table 5). They are both second-degree relatives of brothers **SC2m-SP1m**, and both second-degree relatives of **SC2m**'s and **SP1m's** parents **SC1f** and **NC1m** (Extended Data Fig. 2). With these constraints, the only possible topology is one where **SC6m** and **SC7m** are indeed **siblings, grandsons** of **SC1f** and **NC1m** and **nephews** of brothers **SC2m-SP1m**.

3) Male **NC9m** is a second-degree relative of **SC2m** with a different mitochondrial lineage, and also a third-degree relative of **SC2m's** brother **SP1m** and parents **SC1f-NC1m (Extended Data Fig. 2).** This allows us to discard **NC9m** as **SC2m**'s half-brother, nephew, double cousin, grandfather or uncle, as all these scenarios predict a second-degree relation between **NC9m** and **SP1m** and either first-degree, second-degree or no relationship between **NC9m** and both **SC1f** and **NC1m.** Thus, the only possible relationship is **SC2m** as paternal **grandfather** of **NC9m; he cannot be the** maternal grandfather of **NC9m**, because he would then also be the maternal grandfather of **NC7f-SP3m-NC8m**, which he is clearly not since he is not their second-degree relative.

Conclusions: **NC1m** and **SC1f** are **parents** of brothers **SC2m-SP1m** and **grandparents** of brothers **SC6m-SC7m** through an unsampled brother of **SC2m-SP1m**. **SC2m** is **NC9m's** paternal **grandfather.**

2.3.3. Tree topology relating female NC2f and males NC4m, SE1m and SP2m

Genomic data shows that:

1) **NC4m** and **NC2f** are first-degree relatives (Extended Data Fig. 2) with a parent-offspring relationship (Supplementary Table 5) and with the same mitochondrial lineage. **NC2f** can only be **NC4m's mother** because if **NC2f** were **NC4m**'s daughter, she would be granddaughter of **NC1m**, but **NC2f** is clearly **not related** to **NC1m** (Extended Data Fig. 2).

2) **SE1m** and **NC2f** are first-degree relatives (Extended Data Fig. 2) with a **parent-offspring relationship** (Supplementary Table 5). **SE1m** is also a second- or third-degree relative of **NC4m** (Extended Data Fig. 2). Given that **NC4m**'s, **SE1m**'s and **NC2f**'s mtDNA lineage U8b1b is rare in Neolithic Britain (only one other individual out of 82 published Neolithic individuals (1.2%) belongs to this maternal lineage, I2935 from Scotland[53]), and that we did not find any sign of genetic inbreeding in NC2f (see Methods section and Extended Data Fig. 9b), **SE1m** as a father of **NC2f** is very unlikely because fathers very rarely share their mtDNA lineage with their daughters in outbred populations, even more so when the mtDNA lineage is rare. Thus, we conclude that **SE1m** is **most likely** to be the **son** of **NC2f** and **maternal half-brother** of **NC4m**.

3) **SE1m** and **SP2m** are first-degree relatives (Extended Data Fig. 2) with a **father-son** relationship based on different mtDNA lineages (Supplementary Table 1). Given that **NC4m** and **SE1m** are maternal half-brothers sharing mother **NC2f**, **SP2m cannot** be the father of **SE1m** because **SP2m** would in this case not be biological relative of **NC2f** and **NC4m**, which is contradicted by the data. Specifically, **SP2m** is clearly second-degree relative of **NC2f** and third or more distant relative of **NC4m,** which means that the order of the relationship is **SE1m** as the father and **SP2m** as the son (Extended Data Fig. 2).

4) **NC1m** and **SE1m** are **fourth-degree or more distant** relatives (Extended Data Fig. 2), **more likely fifth** given their relatedness coefficient of 0.027 (Supplementary Table 1). Since **SE1m**'s mother **NC2f** is not related to **NC1m**, **SE1m**'s relation to **NC1m** must run through **SE1m**'s father, who was likely **NC1m**'s **fourth-degree relative** (one degree closer than SE1m-NC1m). This agrees with **SE1m** and **NC1m** sharing the same Y-chromosome lineage (Supplementary Table 1).

Conclusions: **NC2f** and **NC1m** are the **parents of NC4m. SE1m** is **most likely** the **son** of **NC2f**, **father** of **SP2m** and **maternal half-brother** of **NC4m. SE1m's** unsampled father **U1m** is **likely** a **fourth-degree** relative of **NC1m.**

2.3.4. Tree topology relating NC3f, NE2m, NE1m, SC5m, NC7f, SP3m, NC8m and NC6m

Genomic data shows that:

1) **NE2m** and **NC3f** are **first-degree relatives** (Extended Data Fig. 2) with a parent-offspring relationship (Supplementary Table 5) and with the same mitochondrial lineage. **NC3f** can only be **NE2m's mother** because if **NC3f** were **NE2m's** daughter, she would be granddaughter of **NC1m**, but **NC3f** is clearly **not related** to **NC1m** (Extended Data Fig. 2).

2) **NC7f, SP3m and NC8m** are all **first-degree relatives** (Extended Data Fig. 2) with the same mitochondrial lineage, and they are also **NE2m**'s **first-degree relatives** (Extended Data Fig. 2). Since **NE2m** belongs to a different mitochondrial lineage (Supplementary Table 1), he can only be the **father** of **siblings NC7f, SP3m and NC8m.**

3) **SC5m** is also a **first-degree relative** of **NE2m** (Extended Data Fig. 2) again with a different maternal lineage to that of **NE2m** and **NC7f-SP3m-NC8m.** Thus, **SC5m** can only be **NE2m's son** and **paternal half-brother** of **NC7f-SP3m-NC8m**.

4) **NE1m** and **NC3f** are first-degree relatives (Extended Data Fig. 2) with a **parent-offspring relationship** (Supplementary Table 5) and sharing the same maternal lineage. **NE1m** is also a second-degree relative of **NE2m** (Extended Data Fig. 2). Given that **NE1m's**, **NC3f's** and **NE2m's** mtDNA lineage K1a3a1 is rare in Neolithic Britain (only one other individual out of 82 published Neolithic individuals (1.2%) belongs to this maternal lineage, I0518 from Northampton, England[53]), **NE1m** as a father of **NC3f** is very unlikely because fathers very rarely share their mtDNA lineage with their daughters in outbred populations, even more so when the mtDNA lineage is rare. Thus, we conclude that **NE1m** is **most likely** the **son** of **NC3f** and **maternal half-brother** of **NE2m**.

5) **NC9m** is a second-degree relative of siblings **NC7f, SP3m and NC8m** (Extended Data Fig. 2) sharing the same mtDNA lineage. The most likely scenario is that **NC7f-SP3m-NC8m's** mother (unsampled woman U6f) is also the **mother** of **NC9m** with a different male (**SC2m**'s unsampled son U11m) who is third-degree relative of **NC7f-SP3m-NC8m's** father **NE2m**. Having woman U6f as a sister of **NC9m** (and **NC9m** as a maternal uncle of **NC7f-SP3m-NC8m**) predicts that **NC7f-SP3m-NC8m's** parents are fourth-degree relatives and that **NC7f, SP3m and NC8m** have several long runs of homozygosity. The expected length of ROH would be intermediate between that characteristic of offspring of first cousins (third-degree) and offspring of second cousins (fifth-degree) (see Extended data Fig. 9b), but these individuals clearly lack any long ROH (Extended data Fig. 9b) making this scenario **very unlikely**. Having **NC9m** as double cousin of **NC7f-SP3m-NC8m** is **impossible** because **NC9m** is not a second-degree relative of **NC3f** (Extended Data Fig. 2).

6) **NC1m** and **NE1m** are **fourth-degree or more distant** relatives (Extended Data Fig. 2), **more likely fourth-degree** given their relatedness coefficient of 0.062 (Supplementary Table 1). Since **NE1m**'s mother **NC3f** is not related to **NC1m (Extended Data Fig. 2)**, **NE1m**'s relation to **NC1m** must run through **NE1m**'s father, who was likely **NC1m**'s **third-degree relative** (one degree closer than NE1m-NC1m). This agrees with **NE1m** and **NC1m** sharing the same Y-chromosome lineage (Supplementary Table 1).

7) **NE1m** and **SE1m** are **fourth-degree or more distant** relatives (Extended Data Fig. 2), **more likely fifth-degree** given their relatedness coefficient of 0.038 (Supplementary Table 1). Since **SE1m**'s mother **NC2f** is not related to **NE1m's** mother **NC3f (Extended Data Fig. 2)**, **SE1m**'s relation to **NE1m** must run through their **fathers**, who were **likely third-degree relatives** (two degrees closer than their sons' pairwise relationship). This agrees with **SE1m** and **NE1m** sharing the same Y-chromosome lineage (Supplementary Table 1).

8) **NC6m** is **NC3f's second- or third-degree relative, more likely second-degree** given their relatedness coefficient of 0.22 (Supplementary Table 1). **NC6m** is also **third-degree or more distant** relative of **NC3f's** sons **NE2m** and **NE2m, more likely third-degree** given their relatedness coefficient of 0.13 (Supplementary Table 1). Depending on which generation **NC6m** is placed, we could have:

- **NC3f** as niece of **NC6m,** daughter of NC6m's brother.
- **NC3f** as paternal half-sister of **NC6m.**
- **NC3f** as paternal aunt of **NC6m.**
- **NC3f** as paternal grandmother of **NC6m,** through a reproductive union between **NC3f** and a different male (not **U2m** or **NC1m**).

Given that there are different topologies relating **NC6m** with his close relatives, we connect them in the trees with dotted lines without implying any specific topology.

Conclusions: **NC3f** and **NC1m** are the **parents** of **NE2m. NE2m** is the **father** of **siblings NC7f-SP3m-NC8m** and also the **father** of **SC5m** with a **different woman. NE1m** is **most likely** the **son** of **NC3f**, and **maternal half-brother** of **NC4m. NC9m** is **most likely the maternal half-brother** of siblings **NC7f-SP3m-NC8m. NE1m's** unsampled father **U2m** is **likely** a **third-degree** relative of both **NC1m** and **SE1m's** unsampled father **U1m.**

2.3.5. Tree topology relating SC3m, SC4f, SE3m, SC8m, SC9f, SP4m, NC5m and SE2m

Genomic data shows that:

1) **SC4f** and **SE3m** are **first-degree** relatives (Extended Data Fig. 2) with a **parent-offspring** relation (Supplementary Table 1). Given that **SC4f**'s and **SE3m**'s mtDNA lineage K1d is rare in Neolithic Britain (no other individual belongs to this maternal

lineage), **SE3m** as a father of **SC4f** is **very unlikely** because fathers very rarely share their mtDNA lineage with their daughters in outbred populations, even more so when the mtDNA lineage is rare. Thus, we conclude that **SC4f** is **most likely** the mother of **SE3m.**

2) **SC8m** and **SC9f** are **first-degree** relatives (Extended Data Fig. 2) with a **sibling** relationship (Supplementary Table 1).

3) **SC4f** and **SC3m** are not close relatives but they are both second-degree relatives of siblings **SC8m-SC9f** and first degree relatives of **SE2m (Extended Data Fig. 2)**. Therefore, **SC4f** and **SC3m** are **SE2m**'s parents and **paternal grandparents** of siblings **SC8m-SC9f**. We **confirm** this scenario by comparing allelic mismatch rates along the chromosomes (Supplementary Table 6) between **SC9f** and **SC4f**/**SC5m**. In regions of the genome where **SC9f** is consistent with 1 chromosome being shared with **SC4f**, **SC9f** does not share any chromosome with **SC3m**, and vice-versa (Extended Data Fig. 3b). This is the expected pattern when comparing an individual with his two paternal grandparents (or maternal grandparents) because either the father's paternal chromosome or the father's maternal chromosome is inherited at a given location of the genome, but never both at the same time.

4) **SP4m** is a third-degree relative of **SC4f**, a third-degree or more distant relative of **SC3m** and a second- or third-degree relative of **SE2m** (Extended Data Fig. 2), more likely third-degree given their relatedness coefficient of 0.15. Since **SC4f** and **SC3m** are not themselves related, **SP4m** must be their **descendant** through a sibling of **SE3m**, specifically their great-grandson either through two male steps or one male and one female (more likely two male steps given that **SP4m** and **SC3m** share the same Y-chromosome lineage).

5) **SE2m** is a first degree relative of **SC3m**, **SC4f**, **SC8m** and **SC9f** (Extended Data Fig. 2), and thus he can only be the father of **SC8m** and **SC9f**, and the son of **SC3m** and **SC4f**.

6) **NC5m** is a third or more distant relative of **SP4m** (Extended Data Fig. 2), most likely fourth relative given their relatedness coefficient of 0.077 (Supplementary Table 1). They also share the same mitochondrial lineage. One possibility is that **NC5m** is **SP4m**'s half-grand-uncle, a half-brother of his maternal grandmother. However, since there are other possible topologies relating these two individuals such as brother of his great-grandmother or son of his maternal female cousin (less likely given a relatively early radiocarbon date for NC5m), we connect them in the trees with a dotted line without implying any specific topology.

Conclusions: **SC4f** and **SC3m** are the **parents** of **SE2m**, who is the **father** of **siblings SC8m** and **SC9f. SE3m** is **most likely** the **son** of **SC4f** with a **different male (not SC3m). SP4m** is the **great-grandson** of **SC4f** and **SC3m** through a different son (not **SE2m**). **NC5m** is **likely** a **maternal fourth-degree** relative of **SP4m.**

In summary, we have retained two main possible tree topologies relating individuals in this large family. They differ based on whether SC3m is NC1m's son (Tree in Fig. 1c) or father (Extended Data Fig. 4).

2.4. Disambiguation between the two possible tree topologies

In this section, we compare the location of IBD segment breakpoints, that is, points where an IBD segments begins or ends, between different pairs of individuals.

Specifically, we compare IBD breakpoint locations between **SC3m-SC2m**, **SC3m-SP1m**, **SC3m-NC4m** and **SC3m-NE2m**. Recombination events in the paternal gamete (within NC4m's father's testis) that eventually led to **NC4m** will produce a change in the paternal chromosome that is inherited by **NC4m** (from inheriting the paternal grandmother's chromosome to inheriting the paternal grandfather's chromosome or vice-versa).

In the **tree in Fig. 1c**, since **SC3m** is **paternal half-brother** of **SC2m**, **SP1m**, **NC4m** and **NE2m**, recombination events in **SC3m**'s gamete will **break IBD segments** between SC3m and his four half-brothers at **the exact same position**, and we will therefore observe that in several locations of the genome (where these recombination events in SC3m occurred), the allelic mismatch rate between SC3m and each of his four half-brothers **SC2m** will change from 0 chromosome shared to one chromosome shared or vice-versa.

In contrast, in **the alternative tree** (Extended Data Fig. 4), since **SC3m** is the **paternal grandfather** of **SC2m**, **SP1m**, **NC4m** and **NE2m**, recombination events in **SC3m**'s gametes (within his mother's and father's bodies) would determine what combination of paternal and maternal chromosomes he inherited, but would be invisible when comparing mismatch rates between **SC3m** to each of his grandsons because we would not be able to tell whether a chromosomal segment shared between **SC3m** and one of his grandsons is derived from **SC5**'s maternal or paternal chromosome. The recombination events that **would** be visible when comparing **SC3m** to each of his grandsons are the ones happening in the gametes leading to each of his grandsons (within **SC3m**'s son's testis). However, the recombination events in **SC2m's** gamete would **produce a change** in the allelic mismatch rate between **SC2m** and **SC3m**, from 0 chromosome shared to one chromosome shared or vice-versa, but they **will not be observed when looking at the sharing pattern between SC3m and each of his other three grandsons** (see Extended Data Fig. 5 for the rationale behind this approach). The same logic applies to the recombination events in the gametes leading to **SP1m**, **NE2m** or **NC4m**. In this scenario, the **only possibility** for observing a **change in the allelic mismatch rate patterns at the same location** when comparing **SC3m-SC2m**, **SC3m-SP1m**, **SC3m-NC4m** and **SC3m-NE2m** pairs is the occurrence of **four independent recombination events at the same genomic location**, one in each of the gametes leading to **SC2m**, **SP1m**, **NC4m** and **NE2m**, **which is extremely unlikely**. Thus, if we detected s**everal cases of IBD breaking points at the same genomic locations** for **SC3m-SC2m**, **SC3m-SP1m**, **SC3m-NC4m** and **SC3m-NE2m** comparisons, this

would strongly support **the tree in Fig. 1c** (**SC3m**, **SC2m**. **SP1m**, **NC4m** and **NE2m** as paternal half-brothers) over **tree in Extended Data Fig. 4** (**SC3m** as their paternal grandfather).

Indeed, we observe such cases, for instance in chromosome 3 (Extended Data Fig. 5; Supplementary Table 6) where we detect two IBD break points between **SC3m-NE2m** at 130 Mb and 175 Mb. These two break points are detected at the exact same locations in **SC3m-SC2m**, **SC3m-SP1m** and **SC3m-NC4m** comparisons. If **SC3m** is a **paternal half-brother** of **NE2m**, **SC2m**, **SP1m** and **NC4m** (**Fig. 1c**), this is easily explainable **by two recombination events in SC3m's gamete** at chromosome 3, one at 130 Mb and other at 180 Mb (Extended Data Figure 5). If **SC3m** is the paternal grandfather of **NE2m**, **SC2m**, **SP1m** and **NC4m**, we **would need two recombination** events at 130 Mb and 175 Mb in **NE2m**'s gamete to produce this pattern, **two independent recombination events** at the same locations in **SC2m**'s gamete, **two independent recombination events** at the same locations in **SP1m**'s gamete and **two independent recombination events** at the same locations in **NC4m**'s gamete (Extended Data Figure 5). This scenario is **extremely unlikely** and therefore we keep **one feasible tree** in which **SC3m is paternal half-brother of NE2m, SC2m, SP1m and NC4m (Fig. 1c).**

2.5. Evaluating the validity of the proposed family pedigree with other lines of evidence.

In this section, we report on three independent lines of evidence (not used in previous sections) that we used to validate the family tree in Fig 1c.

2.5.1. X-chromosome information

In previous sections, we have reached a unique tree structure using exclusively genomic data from the autosomes, Y-chromosome and mitochondrial genome. If this tree structure is correct, it should also be consistent with the X-chromosome data.

For female-female and female-male comparisons, we computed pairwise mismatch rates and relatedness coefficients on the X-chromosome (Supplementary Table 5) following the same formula as in **section 2.2.** For male-male comparisons, we adjusted the formula as follows:

$$r = 1 - (x/b)$$

to account for the fact that males have one X-chromosome as compared to two sets of autosomes, and that two samples from the same male individual would yield 0 mismatch rate on the X-chromosome as compared to b/2 on the autosomes (when comparing two samples from the same individual in the autosomes, the same homologous chromosome will be sampled only half of the time).

We again estimated the mismatch rate value expected for unrelated pairs b using the median value of all comparisons between Hazleton North individuals and the set of 53 Neolithic individuals from Britain. Restricting to comparisons with more than 5,000 overlapping SNPs, we obtained a value of 0.1978.

We plotted relatedness coefficients in the X-chromosome for first and second-degree pairs (Extended Data Fig. 6a), grouping these pairs based on whether they are expected to share X-chromosome DNA according to the tree structure proposed in the previous section (Fig. 1c):

-The following relationships in the proposed tree are not expected to share DNA in the X-chromosome: Father-son, grandchild-paternal grandfather, paternal half-brothers, paternal half siblings (male-female) and nephew-paternal uncle.

-The following relationships in the proposed tree must share DNA in the X-chromosome: mother-son and father-daughter (r=1 as the males in these pairs share their whole X-chromosome with the females), and paternal grandmother-granddaughter (r=0.5 as fathers pass their entire X-chromosome from their mothers directly to their daughters).

-The following relationships in the proposed tree can (but will not necessarily) share DNA in the X-chromosome: Brothers, brother-sister, maternal half-brothers, maternal half siblings (male-female) and niece-paternal uncle. All these pairs can have a r coefficient in the X-chromosome between 0 to 1.

We found that X-chromosome sharing patterns perfectly fit the proposed tree structure (Extended Data Fig. 6a).

2.5.2. Number of IBD segments shared for second-degree relatives

Second-degree relatives share 25% of their genomes, but the number of chromosomal segments shared from a very recent common ancestor varies depending on the type of relationship due to the different number of meioses separating both individuals. Grandparent-grandchildren relations are separated by two meioses (although only the one in the father is visible in our data when comparing grandparents and their grandchildren), while avuncular relationships (uncle/aunt-nephew/niece) are separated by three meioses, resulting in more recombination events splitting up shared DNA segments. As a consequence, avuncular relationships show a higher number of shorter IBD segments as compared to grandparent-grandchildren relationships[56]. Half-siblings are separated by two meioses, but since recombination rate in females is higher than in males[57], paternal half-siblings resemble grandparent-grandchildren relationships in the number of IBD segments shared, while maternal half-siblings resemble avuncular relationships.

For each first- or second-degree pair with more than 100,000 overlapping SNPs, we computed allelic mismatch rate values across sliding windows of 20 Mb, moving by 1 Mb each step (Supplementary Table 6). We plotted these values along the chromosomes and visually identified contiguous regions where the allelic mismatch rate is consistent with one shared chromosome. For example, at chromosome two we count two IBD segments between SC9f and his grandfather SC3m, and one IBD segment between SC9f and his grandmother SC4f (Extended Data Fig. 3b). We annotated in Supplementary Table 5 the number of such segments identified for each first and second-degree relative pair. In the future, algorithms recovering the haplotype sequences

through imputation and phasing in ancient DNA capture data will allow a more accurate detection of IBD segments and thus a more accurate estimation of the number of IBD segments.

We next plotted the number of IBD segments for first- and second-degree relationships (Extended Data Fig. 6b), again grouping the pairs according to their type of relationship in the proposed tree (Fig. 1c). We recover the expected pattern of a higher number of IBD segments in avuncular and maternal half-sibling pairs as compared to grandparent-grandchild and paternal half-sibling pairs, adding further support to the proposed tree structure. Furthermore, these data add further evidence supporting the placement of NE1m and SE1m as maternal half-brothers of NE2m and NC4m, respectively (40 and 30 shared segments), rather than as their maternal grandfathers.

2.5.3. Concordance with *NgsRelate* kinship estimates

In this section, we replicated our results using the software *NgsRelate* v.2[48] that estimates biological kinship using genotype likelihoods and population allele frequencies to estimate Cotterman coefficients k0, k1 and k2, which correspond to the probability of sharing 0, 1 and 2 alleles in IBD. From these coefficients, the software computes the Theta coefficient (θ) which is equivalent to the relatedness coefficient *r*.

To run *NgsRelate*, we first created genotype likelihoods directly from the bam alignment files using ANGSD[58]. We included Hazleton North individuals as well as the set of 53 Neolithic individuals from other sites in Britain. We then ran *NgsRelate* providing as input the genotype likelihood file and allele frequencies estimated only on the Neolithic set from Britain, to avoid possible bias in allele frequencies stemming from the presence of a high number of closely related individuals at Hazleton North.

Pairwise coefficients computed with *NgsRelate* are included in Supplementary Table 5. To visualize the correspondence between the two methodologies, we plotted for each pair the Theta coefficient (θ) and the relatedness coefficient (*r*) from section 2.2 (Extended Data Fig 7a). We observe a striking correlation between both estimates and a good correspondence between the theta coefficients and the degrees of relationship in the proposed tree structure. We also plotted Cotterman coefficients k0 and k2 for first and second-degree pairs (Extended Data Fig 7b), again showing a good correspondence with their type of relation in the proposed tree in Fig. 1c. As expected, parent-offspring pairs have k0 and k2 values close to 0, sibling relationships have k0 and k2 close to 0.25, and second-degree pairs have k0 values close to 0.50 and k2 values close to 0 (with the exception of some pairs that are related through both their maternal and paternal lines and are therefore expected to present slightly elevated k2 values).

Conclusion

Based on the previous sections, we conclude that the tree in Fig. 1c is the only one strongly supported by all lines of evidence. We therefore use this tree across the paper, but also highlight

that the main findings about the social organization of the group at Hazleton do not significantly change (Supplementary Table 7) when using the alternative tree (Extended Data Fig 4).

SI Section 3: Statistical testing of kinship patterns

Taking advantage of the large pedigree reconstructed in previous sections, we statistically tested several pattern/rules of social organization in the human group buried at Hazleton (Supplementary Table 7).

We designed the following tests:

1) Sex bias among the individuals buried at Hazleton

We tested whether the number of genetic males and females buried at Hazleton was significantly different (Supplementary Table 7).

Result: The number of males buried at Hazleton was significantly higher than the number of females, both in all individuals and in individuals from the large pedigree. **This implies a deliberate sex bias against the burial of women at Hazleton**.

2) Patrilineality versus matrilineality

We tested whether there was a significant difference in the number of patrilineal and matrilineal genealogical transmissions between the founding male NC1m and his descendants (both biological and through adoption) in generations 3–5. Our strategy for counting genealogical transmissions was as follows:

- To establish whether a genealogical transmission between a first-generation individual and one of his or her descendants runs through a male or a female, we need at least one generation in between the two. Thus, we only consider descendants in generations 3–5.

- Males who do not biologically descend from NC1m but who are sons of women reproducing with him or his sons (SE1m, NE1m and SE3m) were treated in the same way as their maternal-half-brothers.

- If a genealogical transmission has already been traversed when analysing a different descendant of NC1m, we do not count this transmission again. For example, siblings SC8m and SC9f are both connected with NC1m through two male transmissions (U13m and SC3m). However, the U13m to NC1m connection running through SC3m is shared by both SC8m and SC9f and thus we count only 3 male transmissions connecting SC8m and SC9f with NC1m.

Result: Inclusion in the Hazleton North tomb for lineage members is **strictly patrilineal**, with all 15 genealogical transmissions between the founding male NC1m and his descendants running through male individuals (Supplementary Table 7).

3) Sex bias among adult offspring

We tested whether there was a significant sex bias among the adult offspring of all reproductive unions in the pedigree (including the ones between females and males not descending from the founding male NC1m), either including missing individuals who we

know reached adulthood because they have descendants buried in the tomb, or without including these individuals.

Result: There is a **complete absence** (0 females versus 14 males) of adult females descending from reproductive unions in the pedigree (Supplementary Table 7). This strongly suggests either that female descendants left the Hazleton community as they reached reproductive age and were buried elsewhere, or that they were given a different type of mortuary treatment which did not result in the inclusion of their remains in the tomb and potentially did not result in the archaeological survival and recovery of their remains.

4) Association between female lineages and burial location

We tested whether there was a significant association between the female sub-lineage each individual belonged to and burial location in the north or south side of the tomb. Four female sub-lineages are evident in the tree: NC2f, NC3f, SC1f and U3f. Individuals were included in a female sub-lineage if they descended from that female or if they reproduced with a descendant of that female (the founding females themselves are included as well). NC9m can be either included in SC1f's sub-lineage as great-grandson of SC1f or in NC3f's sub-lineage as step-son of NC3f's son. NC9m was buried in the north chamber together with two of his maternal half-siblings (themselves NC3f's grandchildren), and not with his closest paternal relatives (all members of SC1f's lineage) who were all buried in the southern chambered area. This suggests that he was viewed as a member of NC3f's lineage, and we thus considered him as member of NC3f's sub-lineage for this analysis. Considering NC9m as a member of SC1f's lineage (P=0.009318) or removing this individual for this analysis (P=0.002392) still yields a significant association between female sub-lineages and burial in the north or south of the tomb.

Result: We find a **significant association between** female lineages and burial location (Supplementary Table 7), with members of females SC1f's and U3f's sub-lineages **being exclusively buried in the south** chambered area and members of females NC2f's and NC3f's lineages preferentially in the north chambered area.

5) Temporal signal in the burial location of members of females NC2f's and NC3f's lineages

As explained below (SI section 4), the collapse of the north passage prevented continued use of the north chamber and passage some time during the period 3660–3630 cal. BC, and this may have played a factor in the shift in deposition of some individuals descended from NC2f and NC3f.

We tested whether the burial location among members of females NC2f's and NC3f's sub-lineages changed over time from occurring preferentially in the north to occurring preferentially in the south. To that end, we divide the members of these two sub-lineages into groups based on the generation they belong to and their age of death:

- A group with a likely earlier date of death: members of generation 1 (NC2f and NC3f), members of generation 2 who died as young adults (NC4m), and members of generation 3 who died as infants or young children (NC7f and NC8m).

- A group with a likely later date of death: members of generation 2 who died as old adults and members of generations 3-4 who died as teenagers or as adults.

Result: We found a **significant temporal pattern** (Supplementary Table 7), with individuals in the first group being buried exclusively in the north chamber, and individuals in the second group being buried in other spaces outside of the north chamber with the exception of NC9m. This suggests that the south vs north duality in burial location between members of females SC1f's and U3f's lineages and members of females NC2f's and NC3f's lineages was broken due to the collapse of the north chamber, rather than through renegotiation of kinship and burial rules.

SI Section 4: Comparison of generational reconstruction with Bayesian modelling of radiocarbon dates from Hazleton North

Here we consider the implications of the family tree presented in Fig 1c in comparison with the Bayesian model of burials from Hazleton North presented by Meadows *et al*.[59]

In a comparative anthropological analysis, Fenner concluded that the reproductive interval between generations averages at least 20 years for women and at least 28 for men for diverse societies[60]. If this is the case, then conservatively the five generations detected in the genetic analysis in Figure 1 span at least four intergenerational intervals and thus at least 80 years. This counts the space between births whereas radiocarbon dating, and thus the Bayesian model of radiocarbon dates at Hazleton North set out by Meadows[59], calculates the dates at which each death occurred. The overall timespan for the Bayesian model is 15–75 years for the 'first phase' of tomb use, from which all of the dated samples derive, then a hiatus, then a few further burials after 3515 cal. BC. We also have new dates for ten individuals from four of the five generations of the main lineage identified by the aDNA analysis, and since the generations are continuous, we conclude their deaths all occurred in or prior to this first phase of activity. At first glance this seems to suggest the genetic model of generations is not consistent with the Bayesian model, but a closer analysis suggests that both are compatible if either (a) the Bayesian model dates only the first three generations of the lineage or (b) different rates of reproduction applied in one or both sets of sub-lineages. We explore this below, but note that the new dates obtained on individuals from the north chamber are consistent with the existing Bayesian model, while some of those from the southern chambered area suggest that the period of use modelled for that area needs to be extended slightly later. All the dates used by Meadows *et al.*[59] were on samples taken from human femora, so are directly comparable with one another and likely to relate to the formation of bone within the last ten years of life, but some of them have a wide range of deviation (e.g. ±70 years). Their modelling used the IntCal04 calibration curve. The new dates are from petrous (in the case of SC9f and SC6m only) or teeth, have only a ±25 year range of deviation, and have been calibrated to 2 sigma using OxCal v4.4.2 and the IntCal20 calibration curve. Petrous bone does not remodel after early childhood, while teeth form in utero, in infancy and in childhood, depending on which tooth is dated[61]. During this time period the two calibration curves IntCAl04 and IntCAl20 are almost identical, so it is not inappropriate to jointly discuss dates obtained based on the different calibration curves. All the dates in italics below are from Meadows et al.[59]'s model 1:

Generation 1: NC2f(C) has provided two radiocarbon dates: 3950–3630 cal. BC, which was modelled to a date of death within the period *3685–3640 cal. BC* by Meadows *et al.*, and 3761–3637 cal. BC (this paper). This individual died aged 17–25, so could have been born as early as 3720 cal. BC and still fit the Bayesian model. Her remains were exposed to the elements and scavengers, so may even have been located outside the chamber prior to tomb construction. There are other dated remains which, on the basis of the Bayesian modelling, would likely derive from individuals living in generation 1, particularly femurs 11035 and 9554 in the south

chamber, which derive from an adult woman who died *c. 3685–3640 cal. BC*; we cannot associate her with an aDNA sample at present.

Generation 2: NC4m, a son of NC2f(C), has now been dated to 3774–3650 cal. BC: he died aged 17-25. NE1m(2) died *3655–3630 cal. BC* aged 45+, and so could have been born as early as 3700+ cal BC to as late as 3675+ cal. BC. NE2m(A) died *3650–3620 cal. BC* aged 23–57, and could have been born in the last decade or so of the 3700s or as late as 3643 cal. BC. These two half-brothers were entombed after the collapse of the walling at the junction of the North entrance and North passage. This collapse is dated in Meadows *et al.* model to *3660–3630 cal. BC, probably 3640s,* on the basis that it physically and chronologically separates the placement of the dated individuals in the north chamber, including NC2f(C), and these two individuals. Our results are still compatible with that model. The passage collapse prevented further access to the north chamber, which may also explain why several subsequent individuals from this sub-lineage were buried in the southern passage or southern entrance rather than joining their lineage predecessors in the northern chambered area (see SI section 3). NC5m(G), who died in infancy, also likely lived during this generation but died before NE1m(2) and NE2m(A), and his remains were placed in the north chamber. NE4m(1), who was not a biological lineage member, could also be contemporary with this generation or with generation 3 (he died *3645–3615 cal. BC* aged c. 40). SE4m(D), who was not biologically related to the main lineage, died in adulthood *3685–3635 cal. BC* so may also have been a contemporary of generation 2 or 3.

Generation 3: SC5m(E) died *3680–3625 cal. BC* aged 9–15, and was the son of NE2m(A) who Meadows *et al.*[59]'s model suggests died *3650–3620 cal. BC* aged 23–57. The son therefore likely died before his father, and was likely born as early as 3695 cal. BC or as late as 3634 cal. BC. SP2m(vi) died within the period 3632–3380 cal. BC, aged 25-35 years old. There are some other Bayesian modelled dates that would fall within this generation, notably two dated femurs from the southern chamber.

Generation 4: Siblings SC8m and SC9f died 3624–3374 aged 23–35 and 3632–3380 cal. BC aged 6-9 respectively. It is possible that no individuals from this generation were dated in previous studies, but we note that a femur with the bone number 7835 was included in the Meadows *et al.*[59] analysis, modelled at *3640–3615 cal. BC*, and considered to be later than the rest of the activity in the southern chambered area. The generation 3 results discussed above suggest there was no hiatus in activity, and that deposition in the southern chambered area continued later than that model suggested.

Generation 5: We only have one individual from this generation and it does not seem to have been dated by Meadows *et al.*[59], so this generation lies outside their model. He remains undated.

NC5m(G): The death of NC5m(G) aged 3–4 circa *3685–3640 cal. BC* suggests he was a member of generation 2, or possibly generation 3. He was likely a fourth-degree relative of SP4m from generation 5, sharing the same mitochondrial lineage, and potentially his great grand-uncle (brother of his great-grandmother through the maternal line). From a genetic perspective he could also have lived in generation 3 as the maternal half-brother of the maternal grandmother of

SP4m, in generation 4 as the maternal cousin of the mother of SP4m, or in generation 5 as maternal half-cousin of SP4m, although the Bayesian model suggests these are less likely. The remains of NC5m(G) were gnawed, indicating excarnation, and were placed in a discrete pile, so may have spent some time outside the tomb.

The collapse of the northern passage would have cut off access to the north chamber. It might be argued that this influenced where the 'southern branch' of the lineage could place their dead, affecting our conclusion that choice of north versus south side of the tomb related to maternal sub-lineages. While we cannot model with precision whether SC2m, SP1m, SC4f and SC3m and some of their offspring died prior to or after the collapse of the northern passage, it is notable that no individuals descending from SC1f or U3f were ever buried in the north side of the tomb, including the north entrance which clearly remained open for use.

Meadows *et al.*[59] suggested the use of the southern chambered area ended in the 3640s, but all of our four new dates from generation 3 and 4 samples have start dates later than 3641 cal. BC at 2 sigma. It is possible that Meadows *et al.*[59] were missing samples from generations 4 and 5, so their model dates the peak period of tomb use but underestimates the tail end of this activity. The Bayesian model and the lineage tree are technically compatible, therefore, but the estimated overall timespan in the Bayesian model would be too short if it does not include any samples from generations 4 and 5, at a time when we suggest tomb use was dwindling.

Meadows *et al.*[59] model the construction of the tomb to within the period *3695–3650 cal*. BC, and note that some of the disarticulated remains might have been those of 'ancestors' who had died before the tomb was completed, though they felt there was not strong archaeological evidence for this[59]. Cuthbert's osteological analysis suggests that some of the remains in the north chamber were exposed to scavengers and/or the weathering prior to being placed in the tomb. While this might result from the introduction of remains from those who had died some time before tomb construction, it could potentially also result from a repeated mortuary practice whereby the bodies of the dead were exposed to the elements or stored somewhere less well-sealed than the tomb chambers prior to being installed in the tomb. It is attested in several generations.

Finally, it is worth noting that Fenner's data on reproductive intervals is based on a survey of westernized industrialized societies and hunter-gatherers. It is possible that the adults buried at Hazleton North reproduced more frequently than in the communities considered by Fenner, in which case this interval may have been lower and a greater number of generations would fit within the Bayesian modelled timespan.

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