

Memorial Booklet for the East Marshall Street Well Ancestors

Introduction:

When the Family Representative Council set forth its research recommendations in 2017, this project was established to address three of those goals related to DNA namely that:

DNA and microbial analysis of the Ancestral Remains should be undertaken for the specific purposes of:

- (1) reconstructing regional genetic ancestry;*
- (2) assessing molecular sex of juveniles;*
- (3) reconstructing health environments*

The information in this memorial booklet is based on both DNA and anthropological analyses of the skeletons of the ancestors from the East Marshall Street Well. The analyses provided by the Smithsonian Institution provided data concerning the minimum number of individuals in the comingled assemblage, based on counts of skeletal elements (44 adults and nine children under the age of 16 years) as well as general information on pathological conditions. VCU researchers in the Department of Forensic Science, however, have been able to apply DNA-based techniques which allowed the re-association of at least some of these bones to form 35 discrete individuals. Microbial DNA analysis of the dental calculus has provided additional information concerning the health environment of the ancestors. Both the DNA and the new anthropological analyses of these 35 individuals, have created a more complete picture of who these ancestors were. When available, we have included information concerning sex, age, ancestry of parentage, stature, handedness, phenotype (hair and eye color), and medical conditions that affected them during life – as well as how they were treated at the Medical College of Virginia (MCV) after death.

Other individuals, represented solely by skulls, are also discussed as we know that they each represent a single individual and have obtained similar information for each of them.

We have also presented general information concerning our analyses and how these results were obtained at the end of the memorial booklet.

This project was funded by:

VCU Office of the President

AAAS Future Gen Scholars grants (x3)

Wenner-Gren Global Initiatives Grant

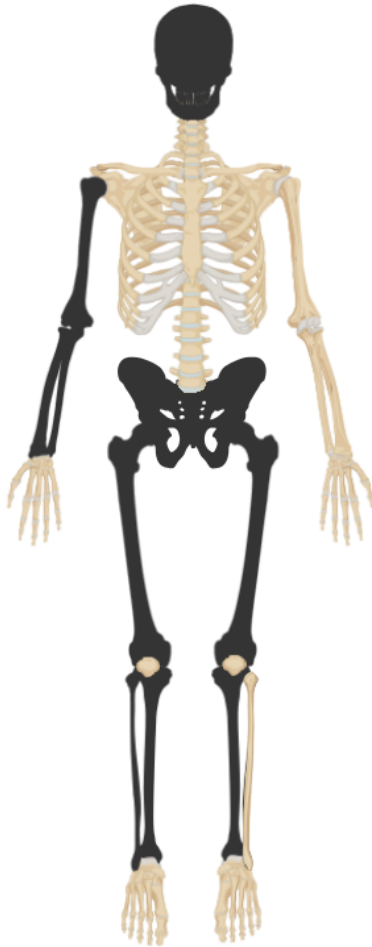
American Academy of Forensic Sciences Humanitarian and Human Rights Resource Center grant



Individual 1

Individual 1 was a female of African ancestry and her maternal lineage was of Africa origin. She had brown eyes and black hair, stood 5'5 ½" in height, and was right-handed. She was between the ages of 20-50 years of age at the time of her death. She shows evidence of Forestier's Disease, also known as Diffuse Idiopathic Skeletal Hyperostosis (DISH), which affected the joints of both shoulders and elbows, as well as those of her left knee and ankle; she shows a build-up of excess bone at the ligament attachment sites around these joints. DISH is a disease of unknown etiology that tends to affect males twice as often as females, and is most common over the age of 65 years. It usually affects the spine. She also had periodontal disease and had lost about half of her teeth before death.

After her death, her body was used for surgical practice, as indicated by saw marks, showing that medical practitioners cut through her left femur, left and right tibiae and her left fibula below the knees.



Individual 2

Individual 2 was a female of African ancestry, with brown eyes and black hair. Additionally, her maternal lineage is of Sub-Saharan African origin, probably of Central West Africa. She stood 5'6 $\frac{3}{4}$ " in height. She was between the ages of 20-27 years of age at the time of her death. Her pelvis indicates that she had given birth, and staining on her teeth indicate that she chewed tobacco. Studies of the microbiome of the dental calculus showed that she had two different types of bacteria associated with periodontal disease. She probably carried heavy loads (such as buckets of water) with her left arm, as the bony attachment of her triceps muscle was enlarged.

After her death, her body was used for medical dissection; scalpel markings are found on both sides of her head and lower jaw, corresponding to removal of the muscles of mastication (chewing).



Individual 3

Individual 3 was a male of European ancestry. Both the maternal and paternal lineages were of Eurasian origin. He stood 5'6 ½" in height and was between the ages of 21-43 years of age at the time of his death.



Individual 4

Individual 4 was male of African ancestry, with brown eyes and black hair. Both the maternal and paternal lineages were of African origin. He stood 5'8 ½" and was 52-73 years old at the time of his death. He was right-handed and probably carried heavy loads (such as buckets of water) with his right arm, as the bony attachment of his triceps muscle was enlarged. This individual had significant periodontal disease resulting in an abscess of a left molar, dental caries and three teeth that had decayed down the roots. He also had a group of small button osteomas (benign rounded bone growths) toward the back right side of his cranium. His dental microbiome showed two bacterial genera associated with periodontal disease. His cranial bones were unusually thick, suggesting that he may have had Paget's disease.



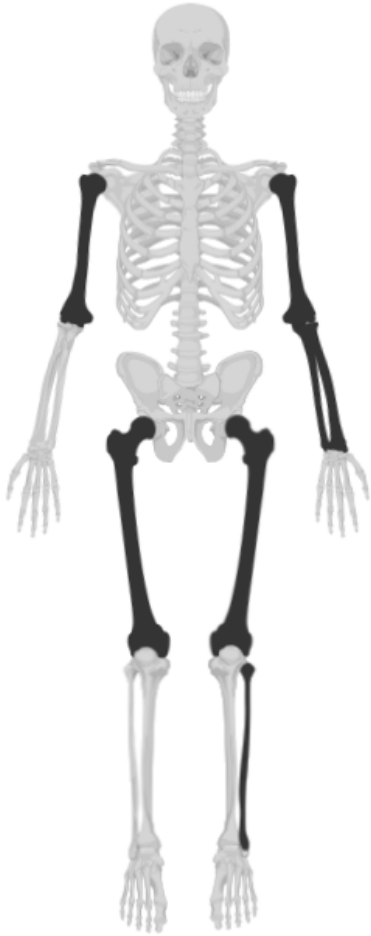
Individual 5

Individual 5 was a male of African ancestry with brown eyes and black hair. His paternal lineage was of Central West African origin. He stood 5'9" in height, was right-handed and was an adult (skeletally, over the age of 17 years) at the time of his death. His right and left elbows show signs of osteoarthritis, specifically in the radio-humeral joint, which is involved in rotation of the wrists, perhaps related to use picking crops.



Individual 6

Individual 6 was male of African ancestry, who had brown eyes and black hair. He stood 5'6 $\frac{3}{4}$ " and was between the ages of 51-71 years at the time of his death. Periodontal disease had caused him to lose all his teeth. When he died, he had a bone infection in his lower right leg, resulting in osteitis of his tibia and periostitis of the adjacent area of his right fibula.



Individual 7

Individual 7 was a male of African ancestry, who stood 5'10" and was a young adult (>17 years of age) at the time of his death. He had an inflammation of his left lower leg, which can be seen as periostitis of the fibula. After death, his body was used at the medical school for amputation practice and his left femur shows bisection in two places and an additional piece of bone cut off the shaft near the knee.



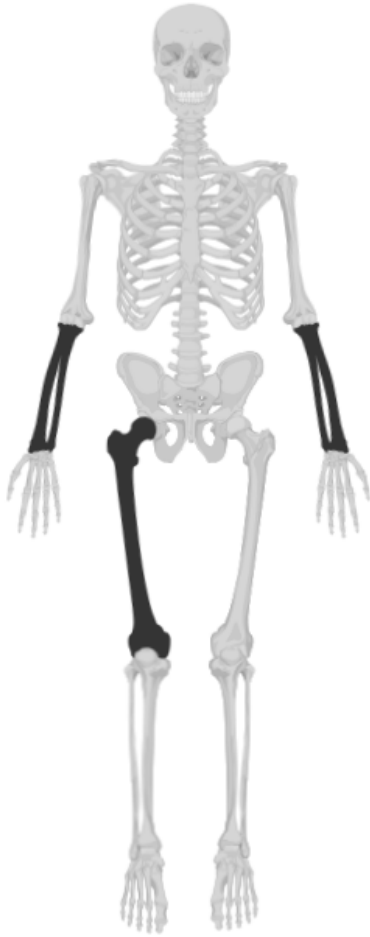
Individual 8

Individual 8 was a male of African ancestry, who had brown eyes and black hair. His maternal lineage was of African origin. He stood 5'7 ½" and was between the ages of 38-60 at the time of his death. He was right-handed. His teeth show that he was a habitual pipe-smoker, as there are facets worn into his teeth to hold the pipe in three different positions (two on the left and one on the right side of his mouth). He had osteoarthritis of the right elbow, specifically in the radio-humeral joint which is involved in rotation of the wrist, perhaps related to use picking crops.



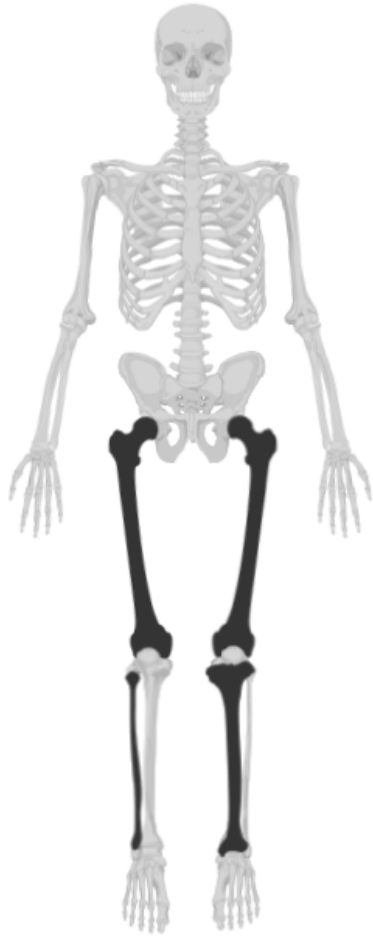
Individual 9

Individual 9 is a female of mixed ancestry, African and European. She stood 5'4 ½" and was an adult (skeletally >14 years of age) at the time of her death. She had osteoarthritis in her left knee.



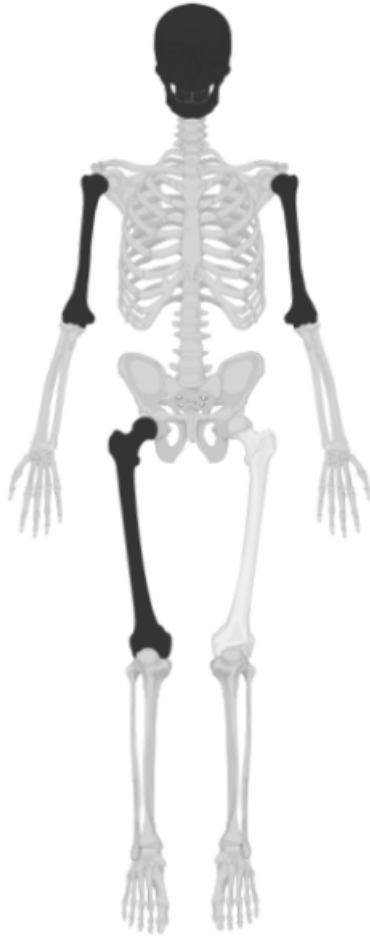
Individual 10

Individual 10 is a male of African ancestry, with black hair. He stood 5'11" and was an adult (skeletally, over the age of 17 years) at the time of his death. He had large biceps muscle insertions and osteoarthritis of the humero-ulnar joints of both elbows, indicating he engaged in a lot of heavy lifting like a biceps curl).

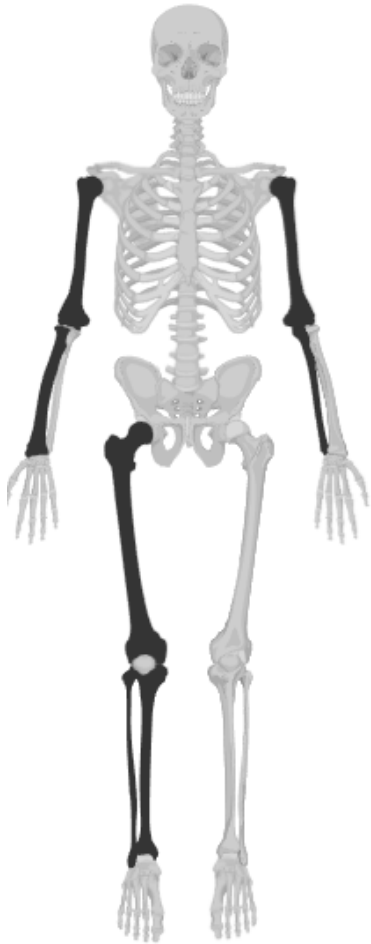


Individual 11

Individual 11 is a male of European ancestry, with brown eyes and brown/dark brown hair. His paternal lineage was of European origin. He stood 5'4 ½" and was an adult (skeletally over the age of 16 years) at the time of his death.

**Individual 12**

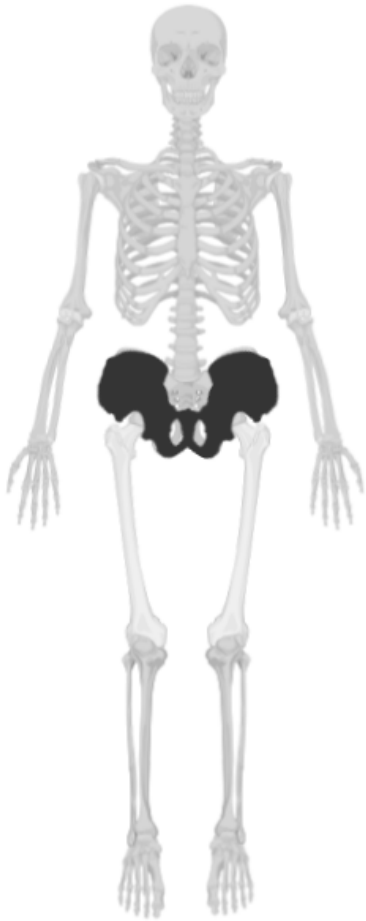
Individual 12 is a male of African ancestry, with brown eyes and black hair. His paternal lineage was of Central West African origin. He stood 5'5 ½" in height and was between the ages of 64-84 years at the time of his death. He had lost nearly all his teeth due to periodontal disease and had an arthritic right knee.



Individual 13

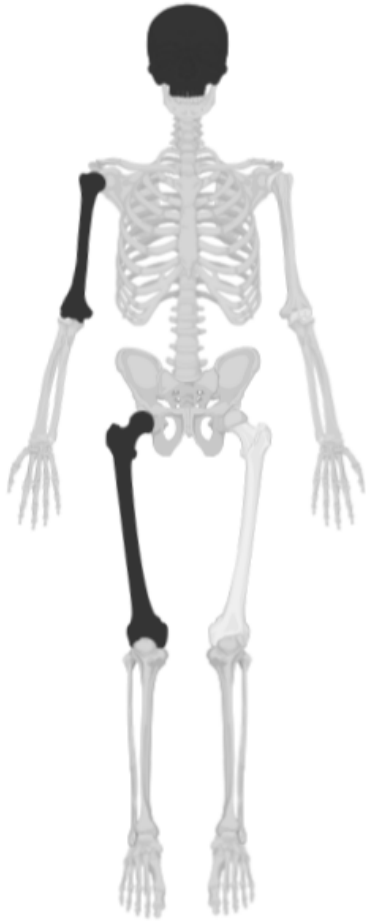
Individual 13 is a male of African ancestry, who stood 5'9 $\frac{3}{4}$ " and was an adult (skeletally, over the age 20 years) at the time of his death. He probably carried heavy loads (such as buckets of water) with his left arm, as the bony attachment of his triceps muscle was enlarged.

After his death, his body was used for surgical practice, as indicated by saw marks, showing that medical practitioners cut through his right tibia and fibula below the knee.



Individual 14

Individual 14 is a male child (<10 years of age) of African ancestry, who had brown eyes and black hair.



Individual 15

Individual 15 is a female of African ancestry, who had brown eyes and black hair. She stood 5'3 ½" in height and was between the ages of 52-66 years at the time of her death. She had periodontal disease and temporo-mandibular joint (involved in chewing) osteoarthritis. Studies of the dental calculus microbiome indicated the presence of four different types of bacteria that are closely associated with periodontal disease.



Individual 16

Individual 16 is a young male of African ancestry, with brown eyes and black hair. Both the maternal and paternal lineages are of Central West African origin. He was 19-29 years of age at the time of his death. He had significant dental issues, including: five teeth with untreated caries, one tooth represented by only the root, and heavy dental calculus indicating poor oral hygiene and periodontal disease.



Individual 17

Individual 17 is a young adult male of mixed African and European ancestry, with brown eyes and dark brown/black hair. His paternal lineage was of Central West African origin. He was 26-36 years of age with dark brown hair and brown eye.



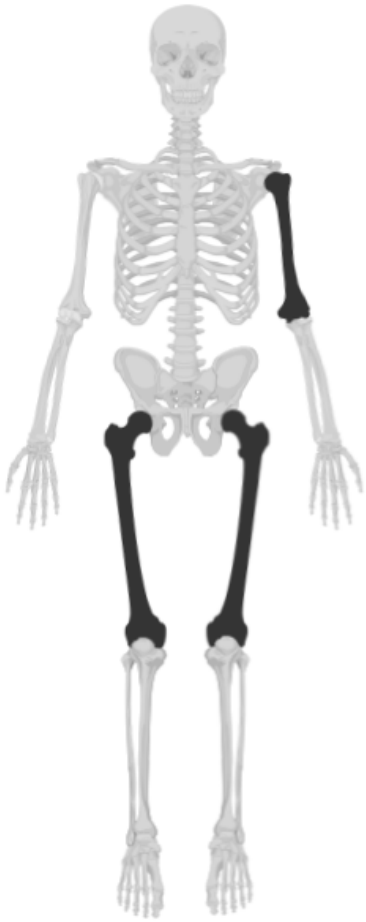
Individual 18

Individual 18 is an older adult female, who shows evidence of Paget's Disease (a condition involving increased bone production with no known cause), but it is uncommon in individuals under the age of 50 years.



Individual 19

Individual 19 is 53-73 year old female of African ancestry who had brown eyes and black hair. During her lifetime, she had small fractures to her right nasal and forehead that were well-healed at the time of her death.



Individual 20

Individual 20 is a child of African ancestry who was 10.5-11.5 years of age at the time of their death.



Individual 21

Individual 21 is a 37-57 year old female of African ancestry, with dark brown/black hair. Her maternal lineage was of African origin. She was slightly built, but had strong neck muscles, probably due to carrying baskets on the top of her head or using a carrying strap over her forehead.



Individual 22

Individual 22 is a female of African ancestry. She was 28-49 years of age at the time of her death. She had quite worn teeth, and both her tooth loss and dental microbiome indicates that she suffered from periodontal disease (three different bacteria were present that are associated with periodontal disease).

Her cranium exhibits a single 28 mm long cut mark from a heavy, sharp object on her left frontal bone (forehead) that was inflicted around the time of her death.

**Individual 23**

Individual 23 is a 26-49 year old male of European ancestry, who had blue eyes and blond/dark blond hair. His paternal lineage was of Eurasian origin.

After death, his body was used for medical dissection; he is represented only by the top of his skull, which had been sawed apart to remove his brain for anatomical studies.



Individual 24

Individual 24 is a young adult male of African ancestry with brown eyes and dark brown/black hair. Both the maternal and paternal lineages are of Central West African origin.

After death, his body was used for medical dissection; he is represented only by the top of his skull, which had been sawed apart to remove his brain for anatomical studies. Scalpel cuts are visible up the midline of his frontal bone (forehead). His cause of death is homicide, related to a gunshot injury to the back left of his head, which shows no signs of healing. There is a medical trephination (neurological burr hole) adjacent to the gunshot entrance defect, indicating that surgical intervention was attempted, probably to relieve intracranial bleeding and pressure from brain swelling.



Individual 25

Individual 25 is a young adult of African ancestry.

After death, this individual was used for medical dissection and is represented only by the top of the skull, which had been sawed apart to remove the brain for anatomical studies. Scalpel marks are present all around the saw cut.



Individual 26

Individual 26 is a young teenager/older child, based on the very thin bones of the skull and open cranial sutures. DNA informs us that this child was of African ancestry



Individual 27

There is little information available for Individual 27. After death, this individual was used for medical dissection and is represented only by approximately half of the top of the skull, which had been sawed apart to remove the brain for anatomical studies.



Individual 28

Individual 28 is a 29-50 year old male of African ancestry. He is represented only by his skull. He had only a single tooth remaining in his mouth, although two others are represented by root fragments still in the sockets. Studies of the dental calculus microbiome indicated the presence of four different types of bacteria that are closely associated with periodontal disease, smoking and obesity.



Individual 29

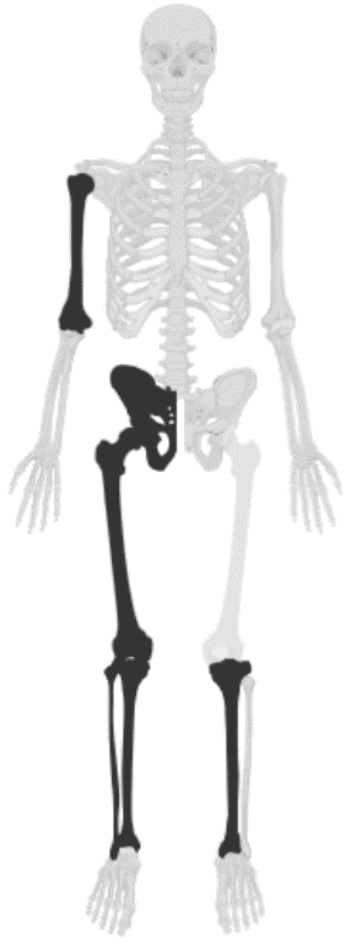
Individual 29 is 28-52 years of age and is represented by only a fragmentary cranial vault. This individual showed signs of temporomandibular osteoarthritis on the left side, which likely caused discomfort when chewing.



Individual 30

Individual 30 is a 37-58 year old male of African ancestry. He is represented only by his skull and most of his teeth, three of which had caries. Studies of the dental calculus microbiome indicated the presence of two different types of bacteria that are closely associated with periodontal disease and tonsillitis.

After his death, he was used for medical dissection, where the left side of his lower jaw was removed.



Individual 31

Individual 33 is a child of African ancestry who was 11-12 years of age at the time of their death.



Individual 32

Individual 32 is a 30-51 year old male of African ancestry. He had brown eyes and dark brown hair. His teeth show evidence of enamel hypoplasia, which indicates that he experienced non-specific environmental stressors at various times during his dental development (from before birth through early childhood). Studies of the dental calculus microbiome indicated the presence of two different types of bacteria, one of which is associated with periodontal disease. The bacterium that causes tuberculosis was also present, indicating it is likely he had the disease at the time of his death.

After his death, he was used for medical dissection, where his right zygomatic arch was removed.

**Individual 33**

Individual 33 is an older adult male of African ancestry who stood 5/8.5" in height. He had severe osteoarthritis of his right elbow, which deformed the entire joint.



Individual 34

Individual 34 is an adult male of African ancestry, who stood 5'9" in height. He suffered from syphilis, showing the typical deformity of both lower legs (sabre shin tibia), which was confirmed in the bones' microbiome by the presence of *Treponema sp.* bacteria, which transmit the disease. Sabre shin tibiae can result from congenital syphilis, which was transmitted from the mother to the fetus prior to birth. His bone microbiome also shows the presence of the bacterium *Clostridium tetani*, which causes the disease tetanus. Whilst easily preventable today with vaccination, tetanus vaccines were not developed until 1924, and this disease may have caused the individual's death.



Individual 35

Individual 35 is an adult male of African ancestry. He suffered from syphilis, showing the typical deformity of his left lower leg (sabre shin tibia) and his cranium displayed caries sicca lesions, which are also typical of tertiary syphilis. The infection was confirmed in the bones' microbiome by the presence of *Treponema sp.* bacteria, which transmit the disease. Sabre shin tibiae can result from congenital syphilis, which was transmitted from the mother to the fetus prior to birth. Tertiary syphilis can take 2-50 years to develop.

After death, this individual was used for medical dissection and his cranium was sawed apart to remove the brain for anatomical studies. Scalpel marks are present on both the cranium and mandible, contiguous between the two.

The Research Process

The East Marshall Street Well Project's Forensic Anthropology and DNA Laboratory was able to achieve the results for the 35 Individuals as outlined below.

Anthropological Analysis

The anthropologists at the Smithsonian Institution published the results of their initial research (Owsley and Bruwelheide 2012a, 2012b) into the Ancestral remains from the East Marshall Street Well (EMSW) and concluded that there was a minimum of 53 individuals, 44 adults and nine children under the age of 18 years. Their analysis covered aspects of ancestry and pathology for the East Marshall Street Well assemblage in a generalized manner, but they did not have the resources to re-associate these remains and relate these broad findings to specific Individuals. DNA re-association of the commingled remains together with new anthropological analyses allowed the team to provide a unique skeletal biography for 35 of these people. Pathological conditions on individual bones, noted by the Smithsonian anthropologists, are now given context as it is possible to discuss a complete individual's life history.

Genetic Reassociation of Commingled Bone Elements

The analysis of specific genetic markers, such as those on autosomes (non-sex chromosomes), make it possible to compare DNA profiles from different bone elements to determine if they belong to the same individual. The EMSW remains underwent a DNA analysis workflow designed to target specific genetic markers that are optimized for complex and degraded samples. Due to the highly degraded state of the human remains from the EMSW, not all bone elements could undergo genetic reassociation and not all tested samples yielded results. Based on the available genetic data, including individual profiles not associated with other bone elements, it was concluded that the remains represented a minimum of 43 adult individuals and three children.

Only 35 individuals were discussed in this memorial booklet, i.e. fewer than the total number of individuals noted above. This is because for only those 35 could we be certain that either (1) we had put together several bones to form that individual, or (2) we had a skull, which certainly represented only one individual. Whilst there were additional DNA profiles of single bones that did not match any other DNA profiles, it was not possible to provide further information about these single bones. Therefore, the total number of "DNA-signatures" of individuals was 43 adults and three children. This is close to what the Smithsonian originally estimated of 44 adults and nine children. Of course, there are likely more individuals, whose remains yielded no DNA, and there may be others still unrecovered from the Well itself.

Biparental (nuclear) DNA Analysis

To study biparental ancestry, DNA inherited from both parents was analyzed, focusing on specific genetic markers found on autosomes (non-sex chromosomes) in the cell's nucleus. These markers were chosen because they show distinct genetic patterns among populations from different continental origins. By

comparing this genetic information to reference data from populations worldwide, it was possible to uncover clues about an individual's genetic origins. This analysis often revealed a mix of genetic contributions, reflecting the complex history of human migrations and interactions.

For the EMSW ancestral remains, comparisons with African, European, and Native American populations showed that most individuals had a predominant African continental ancestry. European genetic input was also detected, as two individuals displayed mixed African and European ancestry, while three others showed primarily European ancestry.

mtDNA Analysis (maternal lineage)

To study maternal ancestry, specific genetic markers found on the mitochondrial DNA were targeted. This genetic information is passed down from mother to sons and daughters and remains relatively unchanged across generations, making them ideal for tracing maternal lineages. By comparing the mitochondrial DNA profiles of EMSW Individuals to reference populations, it was possible to identify patterns of genetic inheritance and determine maternal origins.

The analysis of mitochondrial DNA markers EMSW remains, revealed the presence of maternal lineages traceable to Central West Africa. Additionally, maternal lineages with less geographic specificity from both Europe and Africa were also identified among the EMSW ancestors.

Y-STR DNA Analysis (paternal lineage)

To study paternal ancestry, specific genetic markers found on the Y chromosome are targeted. This genetic information is passed down from father to son and remains relatively unchanged across generations, making them ideal for tracing paternal lineages. By comparing the Y-chromosomal profiles of EMSW Individuals to reference populations, it was possible to identify patterns of genetic inheritance and determine paternal origins.

The analysis of Y chromosome markers on the EMSW remains, revealed the presence of paternal lineages traceable to Central West Africa. Additionally, paternal lineages with less geographic specificity from both Europe and Africa were also identified among the EMSW ancestors.

16S DNA Analysis (the microbiome)

Bacteria associated with dental calculus (tartar/plaque) on teeth or with bones showing diagnostic criteria of certain diseases can be determined by DNA sequencing of a small portion of 16S ribosomal DNA (16S rDNA). To identify bacteria, DNA sequences obtained from bone and tooth samples were compared with known reference sequences. In this study, the bacterial structure of 17 individuals were successfully determined. Disease-causing bacteria (e.g., *Mycobacteria* sp. (tuberculosis), *Clostridium tetani* (Tetanus), *Treponema* sp. (Syphilis), *Streptococcus* sp. (dental caries and other infections, etc.) was present in several individuals. Although the presence of these bacteria does not indicate cause of death, it did provide greater insight into both the health conditions and living environment of the individuals recovered from EMSW.

Ancestry of Children

Mitochondrial DNA analysis was used for the three juvenile individuals (Individuals 14, 20 and 31) since nuclear DNA analysis did not produce much information about them. Information from the mitochondrial DNA helped infer geographic ancestry and provided leads towards individualization, which was later confirmed with anthropology. DNA was isolated from the juvenile remains, including DNA found in the mitochondria. Mitochondrial DNA is passed from the mother to all her children; therefore, siblings or maternal cousins have the same mitochondrial DNA sequence. Specific regions of the mitochondrial DNA were targeted and compared to reference sample databases of individuals with known ancestry. This helped to determine the likely area from which that mitochondrial lineage originated geographically. Samples with similar sequences were compared to one another anthropologically to determine if they could be from the same juvenile individual, making sure that all bones represented the same growth stage (i.e. age).

References Cited

Owsley D. W., and Bruwelheide K. (2012a). Artifacts and Commingled Skeletal Remains from a Well on the Medical College of Virginia Campus: Introduction. Richmond, VA: Department of Anthropology, National Museum of Natural History, Smithsonian Institution.

Owsley D. W., and Bruwelheide K. (2012b). Artifacts and Commingled Skeletal Remains from a Well on the Medical College of Virginia Campus: Human Skeletal Remains from Archeological Site 44HE814. Richmond, VA: Department of Anthropology, National Museum of Natural History, Smithsonian Institution.

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