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# The Effects of Neurofibromatosis Type 1 on Pseudoarthrosis and the Forensic Implications

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Pseudoarthrosis is the presentation of false joints or non-union, primarily in long bones. While pseudoarthrosis most often presents as the lack of union between parts of a fractured or broken bone, it is also suspected that pseudoarthrosis results from a congenital disorder of unknown origin. While the etiology is unclear, there is an association with a congenital defect in neurofibromatosis type 1 gene through the neurofibromin protein. This defect occurs during the germ line mutation of conception and is often identified during early childhood. Pseudoarthrosis is more often difficult to detect in adults as it is frequently corrected during childhood. Germ line defects along the neurofibromin protein often result in a lack of communication from the reticular activating system (RAS) molecular signaling, which, in turn, impacts skeletal osteon production. Consequently, osseous lesions may develop and lead to a lack of cellular control over osteoblast signaling in the long bones of the skeleton. Understanding the origins of congenital pseudoarthrosis and its relationship with neurofibromatosis type 1 could lead to a better understanding of both conditions. Understanding these conditions can be useful for interpreting forensic contexts. These contexts include having the histological knowledge of osteology in these diseases for identification purposes. Given that both neurofibromatosis type 1 and pseudoarthrosis are uncommon conditions, their presence may aid forensic practitioners in determining cause of death or identification of the individual. This paper reviews new advances towards understanding the root cause of pseudoarthrosis.

Neurofibromatosis type 1 (NF1) is a genetic autosomal tumor suppressor disorder (Friedman 1998; NORD 2022) that causes homozygotic loss in the production in the neurofibromin protein along the 17th chromosome. The 17th chromosome is responsible for molecular signalling and ensuring proper maintenance of cellular production to prevent overgrowth, or tumours, in the human body (Wu et al. 2006; Harbaugh et al. 2021; Koster et al. 2021). NF1 defects lead to a variety of changes and mutations to the musculoskeletal production. Mutations to the 17th chromosome during embryonic development affect the production of osteoprogenitor cells that are needed in bone

production, formation, and maintenance (Narayana Kurup and Shah 2020). Therefore, pseudoarthrosis and NF1 are likely related to a defect in the neurofibromin production. Lack of development of the osteoprogenitors would explain the presentation and development of pseudoarthrosis as a consequence of a defect in the neurofibromin protein. A defect in neurofibromin can result in the production of osteoblasts and other associated molecular bone cells, resulting in abnormal osseous lesions and ossifications, and pseudoarthrosis. If a mutation occurs in the germline embryonic development along an unknown translation point in the neurofibromin protein, enzymes may not be able to function properly, inhibiting the essential functions of the protein. Pseudoarthrosis results in non-unions of the bone, which could be mistaken for trauma on the skeleton. Between

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50–80% of individuals with congenital pseudoarthrosis also have an underlying NF1 diagnosis; however, only 5% of all patients who have an NF1 gene defect also have a presentation of pseudoarthrosis. Thus, both conditions are easily misrepresented in multiple contexts including clinical diagnoses, forensic investigation, and identification and interpretation of skeletal remains. Specifically, the misrepresentation of pseudoarthroses could result in inefficient use of time during a forensic investigation.

This paper will investigate the genomic links that associate pseudoarthrosis with neurofibromatosis. These links, supported by other studies, are being connected to propose a possible link between these two disorders. This relatively new discovery represents a potential advancement to understanding both the origins of pseudoarthrosis and why it is an indication of an individual possessing the NF1 disorder. This paper will go into depth of the genomic structure of NF1 and discuss how those factors can influence the condition of pseudoarthrosis, providing greater insight to the root cause of pseudoarthrosis, which could aid in pathological forensic interpretation.

## **Neurofibromatosis Type 1 (NF1)**

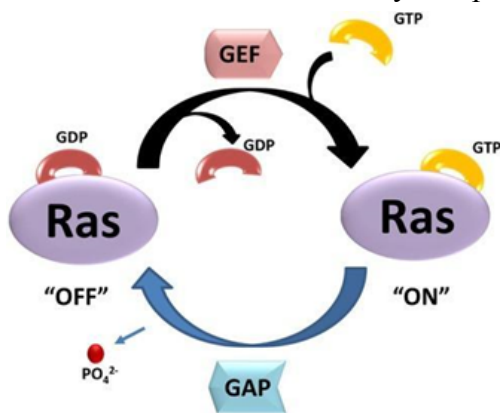
NF1 is an autosomal gene defect and mainly presents as a cutaneous and neural defect, with only 5% of those individuals affected showing presentation of pseudoarthroses fractures in the skeleton (Narayana Kurup and Shah 2020). If there is a defect occurring along chromosome 17q11.2 (17th chromosome), the NF1 gene has a mutation point within the 17th chromosome. This mutation happens during the process of cellular development during germline mutation in embryonic development of the NF1 gene (Well Lennart et al. 2021), resulting in a gene mutation

and deletion in that 17th chromosome (Koster et al. 2021). It is important to recognize the point of mutation in order to further investigate the cause of pseudoarthrosis in relation to the cause of NF1 defects and its effect on the ability of the RAS to regulate bone production. With bone disorders being an indicator of investigation for NF1, cutaneous presenting defects are more likely to be demonstrated. Phenotypical traits include dermal neurofibromas, Lisch nodules in the iris, and axillary and/or inguinal freckling (Koster et al. 2021). These phenotypic traits are often visual demonstrations of the gene defect in the overproduction of cells, resulting in a tumour-like-presentation that creates a connection between pseudoarthrosis and the neurofibromin protein.

NF1 is integral to the function of RAS-guanosine triphosphatase (GTPase) for fibroblasts and osteoprogenitor cells, which are crucial to skeletal development (Wu et al. 2006). GTPase are enzymes important for signal transduction and essential in the process of making cellular energy (Lacal 2001). The inability to send signals or interpret the molecular signalling correctly leads to the manifestation of incorrect signals being sent and the overactivation or inactivation of proteins and enzymes. The NF1 gene is encoded with 57 exons for a GTPase protein, neurofibromin, which plays an important part in cell proliferation and differentiation for the signaling of RAS and other downstream pathways (Koster et al. 2021). Patients with NF1 gene defects have shown translocations in t(1;17) and t(17; 22), deletions, and point mutations along the entire NF1 gene (Bergoug et al. 2020). The NF1 gene is responsible for encoding the neurofibromin protein, which is made up of 2818 amino acids (Bergoug et al. 2020), resulting in encoding malfunctions starting from the extracellular creation of these pathways.

## Neurofibromin

As depicted in Figure 1, neurofibromin is a GTPase protein that activates the active guanosine triphosphate (GTP) and the inactive guanosine diphosphate (GDP) (Calixto et al. 2019) molecular switches that are necessary for maintaining a normal state of inactivity for these pathways (Harbaugh et al. 2021). The main job of neurofibromin is to act as a molecular switch for the function of enzyme and protein responses in the aid of other functions, like cellular proliferation and differentiation. The neurofibromin protein is also a large encoded cytoplasmic protein (Scheffzek and Shivalingaiah 2023). Cytoplasm is important for proteins in the role of translation between the membrane, requiring RAS-GAP activity and GAP regulation of the proteins (Scheffzek and Shivalingaiah 2023). Neurofibromin is a key factor in embryonic development and contains a central GAP-related domain (GRD), but germline mutation and defects in the embryonic process



**Figure 1.** GTP and GDP have two states, active and inactive. Ras controls the state of whether it is active or inactive in state, and GAP promotes of the hydrolysis and the return to ras. GTP is the active state. GDP is the inactive state. Reproduced from Qu et al. (2019, *Frontiers in Molecular Neuroscience*), under a CC-BY license.

lead to mutation of the NF1 gene (Scheffzek and Shivalingaiah 2023). Consequently, the encoding of the NF1 gene is altered in germline, presenting the protein as folded and leaving the protein as incompatible for receiving the proper enzymes, which results in the loss of RAS-specific GTPase activating proteins (RAS-GAP) activity in one or more mechanisms for pathogenic functions (Scheffzek and Shivalingaiah 2023).

## RAS Signaling

The RAS signalling system controls and establishes molecular signaling to many proteins and cellular functions (Figure 1) including cellular proliferation, differentiation and survival (Wu et al. 2006). RAS and neurofibromin work as a partnership in the molecular extracellular signalling pathways. Studies have shown that a portion of the chain of 360 amino acids in neurofibromin are a GTPase-activating protein for RAS (Bergoug et al. 2020). RAS is a GTPase activated protein (GAP) that has a RAS-directed notion for the regulation of G proteins, which includes GTP, GDP, and the NF1 gene (Scheffzek and Shivalingaiah 2023). The protein signalling progression leads to mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK). Residing in the same RAS family, the proteins can bind directly onto the domain for activation, leading to a domino effect of translation from protein to protein (Qu et al. 2019). Normally, the common defects being presented can be visually observed through cutaneous neurofibromas and café au lait spots, which result from the over-proliferation of cellular activity as a defect from being unable to negatively regulate RAS back to being in an inactivate state, resulting in the formation of the tumors. However, it would be valuable to investigate the differentiation of cellular activity from these pathways in the over inactivation of

GDP in the NF1 gene mutation and deletion of the neurofibromin in ERK and MAPK, specifically to assess how that affects the signalling of RAS-GAP and the enzyme hydrolysis GRD for bone formation and repair. The lack of balance between active versus inactive signalling in the ERK and the inability to effectively break down, or hydrolyze, larger molecules leads to proteins and enzymes not being able to bond effectively. This means that normal signalling pathways can get blocked and disrupted in the GDP process. This has implications for the communication from pathways and translation through the cytoplasm by the proteins and enzymes used in the holistic view of ERK and MAPK extracellular signalling and response to extracellular translation.

## Genomics

The hydrolysis of GTP is an important function of the GTPase in the cellular processes and the regulation of proteins (Calixto et al. 2019). In this process, the use of ERK being transducers of MAPK help in regulation and differentiation of osteoblasts and osteoclasts in the formation and reabsorption of bone (Greenblatt et al. 2022). RAS and GTPases have an important function of extracellular signalling in sending extracellular cues to ERK for the process of cellular proliferation, differentiation, growth, and survival, which includes osteogenic cells (Lavoie et al. 2020).

These pathways, ERK and MAPK, are important for bone formation, and loss of function could result in defects with differentiation of skeletal progenitors in skeletal mineralization and the osteoblastic descendants (Greenblatt et al. 2022). ERK and MAPK are also important in the role of pathogenic mechanisms in the stimulation and activation of proteins, like neurofibromin, and can contribute to the defect in

the NF1 gene (Greenblatt 2022). The NF1 protein negatively regulates the pathways of ERK and MAPK through GTPase activity (Greenblatt 2022). Deficiency of the cells responsible for bone formation by the NF1 gene directly effects the ERK and RAS relationship, leading to an increase of the endogenous inhibition of the mineralization of bone, and resulting in a deficiency in the skeletal system (Greenblatt 2022).

Fibroblasts are also an important part of the bone matrix and with ERK. The main characteristic that fibroblasts are reliant on is the platelet-derived growth factor (PDGF) for signaling and functions from polypeptides A-D (Plikus et al. 2021), which are essential for the G homozygous polypeptides in the neurofibromin protein. PDGF engage in receptors and activate a downstream signal in the extracellular matrix, including RAS and MAPK, which is expressed most commonly as a progenitor cell for multiple mesenchymal descendants, including, fibroblasts (Plikus et al. 2021). Loss of this function could result in an inability to maintain homeostasis as PDGF is one of the main signalling pathways for fibroblast regulation and stem cell self-renewal proliferation (Plikus et al. 2021).

## Pseudoarthrosis

Pseudoarthrosis is commonly known as the lack of union, or the inability for a fracture to heal, in the long bones of the body that leads to progressive deformities starting in early childhood (Narayana Kurup and Shah 2020). Pseudoarthrosis presents as pathogenic variants and mutations specifically of the bone and its inability to commence the healing process and signalling, sometimes even with medical interventions. The inability of the bone to heal effectively indicates a defect, an extracellular miscommunication, or a mutation in germline

development of the osteoblasts, fibroblasts, and descendants to respond to signals sent to initiate healing processes. The normal process for bone and fracture healing begins with a hematoma that forms and surrounds the part of the bone that has been damaged, creating what would look like a protective shield encasing the injured area. Hematomas are essential for the secretion of the growth factor of macrophages for initiating osteoclasts signalling (Aiyer 2022). Next, blood vessels extend medially towards each other to repair blood flow. Vascularization is important for the bone to grow and connect the bony ends together. In this stage, the granulation tissue is important for the process of proliferation of osteoprogenitor cells (Pountos et al. 2018). Simultaneously, trabecular bone is being deposited by osteoblasts. Osteoblasts are consequently differentiated into osteocytes as they die, constituting the bone matrix of osteoid for the blood vessels to flow through. This process creates a bony callus in place of the hematoma, while the trabeculae is resorbed into the matrix and calcified to the point of being converted and reworked into cortical woven bone. At this stage, the bone is then remodelled by osteoclasts and vascularized by the blood vessels. In this process, if the osteoblasts are not being differentiated and osteoprogenitor cells are not being proliferated, the healing process would not be able to commence the structural repair of the osteoid. This malfunction of the osteoprogenitor cells provides evidence of congenital defects like NF1 or pseudoarthrosis.

While the definitive cause of pseudoarthrosis is unknown, there may be a link to an underlying congenital NF1 gene defect. The information stated in section three, Genomics, explains the base of the importance of development in osteoprogenitor cells. When the osteoprogenitor cells have a defect or are disrupted by a disorder,

the functions of osteons become inhibited. Since the osteoclasts are signaled by the red blood cells once the hematoma is formed, the damaged bone with dead osteon gets removed. The disorder inhibits the production of osteoprogenitor cells due to a lack of signalling from the 17th chromosome, which would enable the deposition of new bone and remodelling after the fracture has occurred. Further research is required to definitively confirm the link between the neurofibromin protein, NF1, and pseudoarthrosis. In this case, pseudoarthrosis would be a congenital defect along the 17th chromosome, similar to NF1, due to the link between bone deposits and the lack of communication with RAS signalling for those bone related molecular signals.

When bone lacks the ability to create reunion naturally through its osteoprogenitor cells, interventions are usually needed to aid in the process of healing. These interventions include surgical implantations of bone fragments, plates, and screws to hold the bone in place, rods through the intramedullary to support functional healing, and bone grafts to enhance osteogenic healing (Narayana Kurup and Shah 2020). Pseudoarthrosis is most commonly found in long bones but does on occasion present in other skeletal areas and bones. The non-long bone presentations are less common but do happen and are signs of non-union and false joint presentations.

The non-union of pseudoarthroses fractures and breaks is called “false joints.” The appearance of a joint forms in the musculoskeletal system when the break results in one bone being positioned on top of the other. The resulting hematoma forms a joint capsule due to the lack of healing and malformation in the bone. In the normal healing process, a hematoma is formed. The hematoma is signaled from blood

pathways, sending blood monocytes and macrophages to the site of the break or fracture (The Histology Guide n.d.). Due to the inability for the osteoprogenitor cells to respond and create an osteoid for the osteoclasts, the bone is then remodeled and resorbed at the bony ends, leaving a space open where the hematoma will sit and be removed by the osteoclasts. This creates a joint-like space in between the two ends. Since proper healing could not transpire, the bone then becomes avascular near the ends where the bone had broken and been remodelled. The lack of support needed by long bones leads to the appearance of malformation and bowing .

A congenital disorder similar to pseudoarthrosis, campomelic dysplasia, is also observed in the 17th chromosome (Kwok et al. 1995). The reason this is relevant is due to the link of having a point mutation along the 17th chromosome, like the neurofibromin. This is significant because it could be an indication linking the point of mutations together to display similar skeletal defects along similar deletion and mutation points with similar presenting defects.

### **Pseudoarthrosis and NF1 Connections**

The connection between pseudoarthrosis and NF1 is currently under investigation (NORD 2022; Narayana Kurup and Shah 2020; Koster et al. 2021; Harbaugh et al. 2021). NF1 can cause bone dysplasia that ultimately leads to the inability to heal, or pseudoarthrosis (McCoy et al. 2016, 330). Although the cause is unknown, it can be linked to a molecular signaling pathway deficiency caused by a germline mutation of the neurofibromin protein. This protein is linked with controlling the signals and being associated with precursors for osteogenesis. Early on in embryonic development, both NF1 and pseudoarthrosis are already present, which confirms the origin of these conditions being

mutated. The link between the two shows a trickling effect from the NF1 gene having mutations in the neurofibromin protein. This results in the inability for osteoprogenitor cells to function properly in RAS signalling through RAS-GAP and GTPase activating proteins. The consequent defects from this would reduce their ability to correctly transmit signals for proper osteoblast production from mesenchymal cells and osteoclast differentiation, with a final result of pseudoarthroses of the bones. The inhibition of RAS signaling from the beginning process of ERK and MAPK means that the signals that initiate cellular proliferation and differentiation are non-existent. This leads to osteoblasts and osteoblastic descendants unable to obtain the signals to start the healing process. Osteoblasts secrete bone matrix by releasing proteins, like type 1 collagen and osteocalcin. The osteoblasts then die and become differentiated from mesenchymal progenitor cells into osteocytes which then become embedded into the bone matrix (Papaioannou et al. 2016). Bone matrix begins formation early in embryonic development as the mesenchymal stem cells start forming and osteogenesis commences (Breeland et al. 2022). Osteoblasts are also responsible for the regulation of the osteoclasts and a decreased activity of the osteoclasts can result in osteopetrosis (Breeland et al. 2022). The impact of the NF1 neurofibromin protein mutation on RAS signalling causes decreased activity. This event leads to mutations and defects in bone formation because osteoblasts are unable to fulfill their roles due to a lack of signalling and miscommunication from the molecular pathways as a result of the defect of the neurofibromin protein.

One of the diagnostic characteristics for the NF1 gene is thinning of the cortical bone (Harbaugh et al. 2021). Neurofibromin is also a main driver in pathogenic trait defects, and 30% of those pathogenic variants are caused by a

splicing in the mRNA sequencing, which results from a shortened, or truncated, neurofibromin protein (Koster et al. 2021). Defects present in the differentiated bone cells and osteoprogenitor cells can result in bone fragility, abnormal skeletal development, and lower threshold for withstanding mechanical stress on the skeletal system (Wu et al. 2006). Biomechanical signaling is key in the formation and maintenance of the bone matrix. Thus, resulting malfunctions to maintenance of the osteoprogenitor cells can predetermine cellular production and growth from the embryonic development and result in detrimental and unstable bone matrix maintenance. This would directly affect the ability for the bone to function properly and inhibit the ability for biomechanical signals to be sent for bone repair. NF1 and pseudoarthrosis share many similarities, including the congenital defects that occur. Both result from defects to the neurofibromin protein during initial cellular formation, from conception to embryonic growth and development.

Fibroblasts secrete collagen protein and other materials that support and maintain the bone matrix from as early as the embryonic development (Plikus et al. 2021). Along the 17th chromosome, a defect can occur that directly impacts the body's ability to send RAS signals, convert GDP into GTP through hydrolysis and RAS-GAP to stimulate a downstream signal to the enzymes and cells. This is needed for osteoblast proliferation, and consequently, to begin collagen secretion for the trabecular bone repair process of a break or fracture. When the RAS pathway is being mutated, the proper signals are not being translated to respond in the appropriate way. The defect in neurofibromin sets off a chain reaction from the embryonic level. This includes defects to the management of cell differentiation, proliferation, and survival. When

mutated, the wrong signals get translated through the membrane, meaning the correct cellular response is not reciprocated by the extracellular level.

### *Germline*

During sexual reproduction, germ cells consisting of the sperm and egg cells are created as the specialized sex cells that carry the genomes from both parents, making up the germ lines in that individual (Hurle 2023). Both pseudoarthrosis and NF1 begin as a germline mutation which happens during the embryonic development. Specifically, pseudoarthrosis shows cellular linkage to being an extracellular defect stemming from a mutation in the neurofibromin protein and the lack of enzyme catalytic in the production of RAS-GAP and GRD. This process can be traced back to the formation of the initial osteoprogenitors for mesenchymal cells, osteoblasts, and osteoblastic descendants. Starting with the neurofibromin, there appears to be an unknown point of mutation that reverses the common characteristic of overactivation in the RAS signalling to becoming an under-reactive response, due to a lack of signaling. This response, or lack thereof, indicates a common link to the germline mutations that have resulted in defects in the ability of signaling and interpretation in the molecular pathways, transduction, and translation.

Endochondral development resulting from the germline and germ cells is where most signaling pathways start forming (Breeland et al. 2022). Osteochondroprogenitor cells are mesenchymal stem cells (Breeland et al. 2022) and are the main source for osteoblast differentiation and new osteoblast proliferation for deposition (The Histology Guide n.d.). Issues with the deposition of the bone matrix can be linked to the germline deletions and mutations

debilitating those functions; however, the osteoclasts are not responsive to the same mutation as they are not impacted by osteoprogenitor cells but by blood monocytes and macrophages for the removal and break down of the bone matrix (The Histology Guide). The production of osteoclasts for the maintenance of the bone matrix still being translated explains the presentation of pseudoarthrosis and the bone being resorbed as it normally would. Without the signals being sent by pathways due to the mutation in embryonic development, there would be no osteoblasts being sent to the bone matrix to be deposited into osteocytes to make up the bone matrix, or osteoid, to be then resorbed and maintained by the osteoclasts.

### **Forensic Relevance and the Potential for Misinterpretation**

Understanding the molecular genomics of pseudoarthrosis is important for the correct pathological identification of non-unions in forensic cases. The phenotypic results of defects in the genetic code can be highly influential in the course of a person's life, and in some cases, could even affect the manner of death. Understanding the osteological implications of congenital defects is essential for forensic identification of osseous non-union, defects in presentation, and pathological versus traumatic indications and markers. In Canada specifically, it is beneficial for criminal cases and investigation when forensic scientists understand osteological disorders. Currently, the Royal Canadian Mounted Police (RCMP) only have three forensic laboratories across Canada and use the Forensic Science and Identification Services (FS&IS) as a key component to crime scene investigations and solving crimes (Canadian Society of Forensic Science 2023; RCMP 2018). Having a forensic anthropologist full time with a forensic scientist

would prove beneficial to the criminal investigation process from the amount of training forensic anthropologists endure in both the cultural and forensic components to become certified. The current process for criminal investigation in Canada is reliant on the RCMP or national police agency and the services they deem appropriate for the investigation needing to be conducted through the Forensic Assessment Centre (FAC) (Canadian Society of Forensic Science 2023). Problems can arise from not having a forensic anthropologist, including the loss of the initial observations and interpretations, which are crucial for understanding the taphonomy, environmental exposures, and the relation of each item in understanding and applying a cultural context towards the scene. Understanding how congenital defects alter standard bone morphology is essential in forensic science to assess the etiology of lack of union, defects in presentation, and signs of pathological versus traumatic changes to bone. Having a clear understanding of these methods helps in gaining the most accurate possibility of the postmortem interval (PMI) of an individual. Pseudoarthrosis and NF1 gene mutations are already uncommon, so the identification based on bone pathogenic deformities could aid in identification features and processes. Often times, forensic scientists, forensic pathologists, and forensic anthropologists have to recreate a scenario to better understand the trauma and impact on the skeletal remains. In doing this, understanding the full histological history of an individual can help in processing the correct scenarios and potential time frames in which an injury or pathological deformation presented on the bone. Historically, forensic scientists have focused on macroscopic identifying features, but integrating microscopic and molecular analysis can improve the investigation of pathologic alterations in the bone

matrix in relation to decomposition (Hale and Ross 2023:2).

Additionally, the uncommon nature of NF1 and pseudoarthrosis may narrow the pool of individuals for identification in a forensic context when only skeletal remains are available for analysis. Understanding pseudoarthrosis at a cellular and molecular level also allows for interpretation and understanding of the cause of non-union. This can lead to a better understanding of the histological differences between perimortem fractures and pseudoarthrosis. Histological analysis provides insights into the degree of cellular differentiation and proliferation in the bones as an indication of when the trauma happened. However, if the individual had pseudoarthrosis, the histological profile of the skeleton would be misleading, showing no signs of healing and a lack of union in the bone matrix.

The presentation of pseudoarthrosis in juvenile bones could complicate analyses since most congenital defects are clinically identified in utero through genetic testing or by their presentation upon birth and into early childhood. Congenital defects happen during the germline development and embryonic dispositions of mesenchymal stem cells and progenitor cells. Forensic implications that could arise from congenital anomalies in the skeleton could lead to misdiagnosis, which could potentially be an inhibitor for investigations, or present as traumas in the juvenile skeletal system as the union process is trying to differentiate and proliferate the cells for the union. Non-union of bones from pseudoarthrosis could also be presented as potential ossification defects in the growing skeletal structure from the extracellular signaling pathways being unable to communicate to cells the job they are supposed to be doing.

Pseudoarthrosis can lead to an easy bone break, which can result in being mistaken for trauma, like the Monteggia fracture, which is the most similar in appearance to pseudoarthrosis. The Monteggia fracture is commonly found in children as a result from falling (Gaillard 2023). This, in contrast to a pseudarthroses break, fracture, or dislocation can look quite similar and lead to misinterpretations. Specifically, this could implicate malicious reasoning to the break, such as abuse or defensive wounds. In reality, the individual would have experienced pathogenic trauma to the bone that did not heal due to the bone's inability to create a union.

Nightstick fractures are another break that is specifically caused by trauma and linked with blunt force trauma that could be mistaken with pseudoarthrosis (Davis and Kane 2022). Nightstick fractures obtained their name from trauma inflicted by police truncheons, resulting in direct trauma to the forearm while holding a defensive position above or at the face level (Davis and Kane 2022). Nightstick fractures and pseudoarthrosis share the main characteristic of non-union. Nightstick fractures have a 5% non-union rate when intramedullary fixtures are required (Davis and Kane 2022). Although this is a low rate, there is a small possibility of misrepresentation and consequent misinterpretation. The presentation of these two breaks can be seen as trauma, either malicious or accidental. When the presentation of pseudoarthrosis as an uncommon pathogenic variant becomes apparent, it could be mistaken for a perimortem break; the presentation of the non-union and lack of evidence for healing mirrors perimortem trauma, which can lead to forensic misinterpretations of the pathogenic condition as trauma.

## Conclusion

The presentation of pseudoarthrosis in general is still yet to be fully understood by pathologists. The link between NF1 and pseudoarthrosis can be seen in the 17th chromosome in the mutation of the gene of the protein neurofibromin. Along the chromosome, certain defects produce different mutations depending on the point of mutation along that same chromosome. This explains why only 5% of patients diagnosed with NF1 gene defect have pseudoarthrosis, whereas the 50–80% of patients that are diagnosed with congenital pseudoarthrosis have an underlying NF1 gene mutation. What NF1 and pseudoarthrosis share in common is a defect in the neurofibromin protein and the consequent inhibition of RAS signalling in molecular pathways. This inadvertently leads to osteogenesis and osteoprogenitor cells being impacted by the germline mutation along the amino acid chain of the protein in the NF1 gene. This paper supports the connection between pseudoarthrosis and the germline neurofibromin mutation in the 17th chromosome. This link can be observed along the neurofibromin protein being unable to maintain homeostasis in the RAS-GAP functions which control osteoprogenitor cells. Understanding the genomics behind the cause of pseudoarthrosis can lead to a better understanding both of the condition for medico-legal investigations, and knowledge behind different bone formations and lack thereof. Presentation of pseudoarthrosis can present similarly to trauma breaks and fractures, and differentiation of their pathogenic presentations would aid in understanding skeletal trauma.

## References Cited

Aiyer, Amiethab. 2022. Fracture Healing. *Orthobullets*. <https://www.orthobullets.com/basic-science/9009/fracture-healing>

*COMPASS* (2023) Volume 3, Issue 2, Pages 161–171

- Bergoug, Mohammed, Michel Doudeau, Fabienne Godin, Christine Mosrin, Béatrice Vallée, and Hélène Bénédicti. 2020. Neurofibromin structure, functions and regulation. *Cells* 9(11): 2365. <https://doi.org/10.3390/cells9112365>
- Breeland, Grant, Margaret Sinkler, and Ritesh G. Menezes. 2022. Embryology, bone ossification. In *StatPearls [Internet]*. Treasure Island, FL: StatPearls.
- Calixto, Ana R., Cátia Moreira, Anna Pabis, Carsen Kötting, Klaus Gerwent, Till Rudack, and Shina C. L. Kamerlin. 2019. GTP hydrolysis without an active site base: a unifying mechanism for Ras and related GTPases. *Journal of the American Chemical Society* 141(27):10684–10701. <https://doi.org/10.1021/jacs.9b03193>
- Canadian Society of Forensic Science. 2023. Royal Canadian Mounted Police Forensic Laboratory Services. <https://www.csfs.ca/resources/government-labs-in-canada/rcmp-forensic-laboratory-services/>
- Davis, Donald D., and Steven M. Kane. 2022. Nightstick fractures. In *StatPearls [Internet]*. Treasure Island, StatPearls. <https://www.ncbi.nlm.nih.gov/books/NBK555951/>
- Friedman, J. M. 1998. Neurofibromatosis 1. In *GeneReviews*. Margaret P. Adam, Jerry Feldman, Ghayda M. Mirzaa, Roberta A. Pagon, Stephanie E. Wallace, Lora J. H. Bean, Karen W. Gripp, and Anne Amemiya, eds. Seattle: University of Washington Press.
- Gaillard, Frank., Joshua Yap, Henry Knipe, Amanda Er, Yuranga Weerakkody, Ian Bickle, and Amir Mahmud. 2023. Monteggia fracture-dislocation. *Radiopaedia*.
- Greenblatt, Matthew, Jae-Hyuck Shim, Seoyeon Bok, and Jung-Min Kim. 2022. The extracellular signal-regulated kinase mitogen-activated protein kinase pathway in osteoblasts. *Journal of Bone Metabolism* 29(1):1–15. <https://doi.org/10.11005/jbm.2022.29.1.1>
- Hale, Amanda R., and Ann H. Ross. 2023. Investigating the timing and extent of juvenile and fetal bone diagenesis in a temperate environment. *Biology* 12(3):403. <https://doi.org/10.3390/biology12030403>
- Harbaugh, Kimberly, Neel T. Patel, and Elias Rizk. 2021. Genetics of nerve tumors. In *Diagnostic assessment and treatment of peripheral nerve tumours*. Fernando Guedes, Eric L. Zager, Debora Garozzo, Lukas Rasculic, and Mariano Socolovsky, eds. Pp. 17–27. Cham, Switzerland: Springer.
- Hurle, Belen. 2023. Germline. Talking glossary of genomic and genetic terms. National Human Genome Research Institute. <https://www.genome.gov/genetics-glossary/germ-line>
- Koster, R., R. D. Brandão, D. Tserpelis, C. E. P. van Roozendaal, C. N. van Oosterhoud, K. B. M. Claes, A. D. C. Paulussen, et al. 2021. Pathogenic neurofibromatosis type 1 (NF1) RNA splicing resolved by targeted RNAseq. *npj Genomic Medicine* 6:95. <https://doi.org/10.1038/s41525-021-00258-w>

- Kwok, Cheni, Polly A. Weller, Silvana Guioli, Jamie W. Foster, Sahar Mansour, Orsetta Zuffardi, Hope H. Punnett, et al. 1995. Mutations in SOX9, the gene responsible for campomelic dysplasia and autosomal sex reversal. *American Journal of Human Genetics* 57(5):1028–1036.
- Lacal, Juan Carlos. 2001. GTPase. In *Encyclopedic reference of cancer*. Manfred Schwab, ed. Pp. 375–378. Berlin: Springer. [https://doi.org/10.1007/3-540-30683-8\\_688](https://doi.org/10.1007/3-540-30683-8_688)
- Lavoie, Hugo, Jessica Gagnon, and Marc Therrien. 2020. ERK signalling: a master regulator of cell behaviour, life and fate. *Nature Reviews Molecular Cell Biology* 21:607–632. <https://doi.org/10.1038/s41580-020-0255-7>
- McCoy, Garrett, Joel Joyce, Donald Basel, and Dawn H. Siegel. 2016. Pseudoarthrosis of the ulna in neurofibromatosis type 1. *Journal of Pediatrics* 177:330. <https://doi.org/10.1016/j.jpeds.2016.06.054>
- Narayana Kurup, J. K., and Hitesh H. Shah. 2020. Congenital pseudoarthrosis of the radius in Neurofibromatosis Type 1: an entity not to be missed! *Journal of Orthopaedics* 22:427–430. <https://doi.org/10.1016/j.jor.2020.09.013>
- National Organization for Rare Disorders (NORD). 2022. Neurofibromatosis 1. <https://rarediseases.org/rare-diseases/neurofibromatosis-type-1-nf1/>
- Papaioannou, Garyfallia, Fatemeh Mirzamohammadi, and Tatsuya Kobayashi. 2016. Ras signaling regulates osteoprogenitor cell proliferation and bone formation. *Cell Death and Disease* 7:e2405. <https://doi.org/10.1038/cddis.2016.314>
- Plikus, Maksim V., Xiaojie Wang, Sarthak Sinha, Elvira Forte, Sean M. Thompson, Erica L. Herzog, Ryan R. Driskell, Nadia Rosenthal, Jeff Biernaskie, and Valerie Horsley. 2021. Fibroblasts: origins, definitions, and functions in health and disease. *Cell* 184(15):3852–3872. <https://doi.org/10.1016/j.cell.2021.06.024>
- Pountos, Ippokratis, and Peter V. Giannoudis. 2018. Fracture healing: back to basics and latest advances. In *Fracture reduction and fixation techniques: upper extremities*. Peter V. Giannoudis, ed. Pp. 3–17. Cham, Switzerland: Springer. [https://doi.org/10.1007/978-3-319-68628-8\\_1](https://doi.org/10.1007/978-3-319-68628-8_1)
- Qu, Liang, Chao Pan, Shi-Ming He, Bing Lang, Guo-Dong, Gao, Xue-Lian Wang, and Yuan Wang. 2019. The Ras superfamily of small GTPases in non-neoplastic cerebral diseases. *Frontiers in Molecular Neuroscience* 12:121. <https://doi.org/10.3389/fnmol.2019.00121>
- Royal Canadian Mounted Police (RCMP). 2018. Forensic Science and Identification Services. <https://www.rcmp-grc.gc.ca/fsis-ssji/index-eng.htm>
- Scheffzek, Klaus and Giridhar Shivalingaiah. 2019. Ras-specific GTPase-activating proteins—structures, mechanisms, and interactions. *Cold Spring Harbor Perspectives in Medicine* 9(3):a031500. <https://doi.org/10.1101/cshperspect.a031500>
- The Histology Guide. n.d. Bone: types of cell found in bone. Cartilage, bone and ossification. Faculty of Biologocial Sciences, University of Leeds. [https://www.histology.leeds.ac.uk/bone/bone\\_cell\\_types.php](https://www.histology.leeds.ac.uk/bone/bone_cell_types.php)
- Well, Lennart, Kimberly Döbel, Lan Kluwe, Peter Bannas, Said Farschtschi, Gerhard Adam, Victor-Felix Mautner, Johannes Salamon. 2021. Genotype-phenotype correlation in neurofibromatosis type-1: *NF1* whole gene deletions lead to high tumor-burden and increased tumor-growth. *PLOS Genetics* 17(5): e1009517. <https://doi.org/10.1371/journal.pgen.1009517>
- Wu, Xiaohua, Selina A. Estwick, Shi Chen, Menggang Yu, Wenyu Ming, Todd D. Nebesio, Yan Li, et al. 2006. Neurofibromin plays a critical role in modulating osteoblast differentiation of mesenchymal stem/progenitor cells. *Human Molecular Genetics* 15(19):2837–2845. <https://doi.org/10.1093/hmg/ddl208>