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ARTICLE

WNT1-associated osteogenesis imperfecta with atrophic frontal lobes and arachnoid cysts

Piranit Nik Kantaputra[1,2] · Yuddhasert Sirirungruangsarn[3] · Pannee Visrutaratna[4] · Sasitorn Petcharunpaisan[5] · Bruce M. Carlson[6] · Worrachet Intachai[1] · Jutamas Sudasna[7] · Jatupol Kampuansai[8] · Prapai Dejkhamron[7]

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Abstract

A rare form of osteogenesis imperfecta (OI) caused by Wingless-type MMTV integration site family 1 (*WNT1*) mutations combines central nervous system (CNS) anomalies with the characteristic increased susceptibility to fractures. We report an additional case where arachnoid cysts extend the phenotype, and that also confirms the association of intellectual disabilities with asymmetric cerebellar hypoplasia here. Interestingly, if the cerebellum is normal in this disorder, intelligence is as well, analogous to an association with similar delays in a subset of patients with sporadic unilateral cerebellar hypoplasia. Those cases typically appear to represent vascular disruptions, and we suggest that most brain anomalies in *WNT1*-associated OI have vascular origins related to a role for *WNT1* in CNS angiogenesis. This unusual combination of benign cerebellar findings with effects on higher functions in these two situations raises the possibility that *WNT1* is involved in the pathogenesis of the associated sporadic cases as well. Finally, our patient reacted poorly to pamidronate, which appears ineffective with this form of OI, so that a lack of improvement is an indication for molecular testing that includes *WNT1*.

Introduction

Most cases of osteogenesis imperfecta (OI) with increased susceptibility to fractures are caused by mutations in *COL1A1* [MIM #120150] or *COL1A2* [MIM #120160], which encode type I collagen, the major protein component of the extracellular matrix in bone, skin, and tendon. However, a recently described form of OI with frequent midbrain and cerebellar anomalies associated with intellectual delays is caused by mutations in Wingless-type MMTV integration site family 1 (*WNT1*) [1–3].

Here, we report a patient affected with intellectual delay, left cerebellar hemisphere hypoplasia, compressed left-sided midbrain and left-sided pons, left optic disc atrophy, atrophic frontal lobes, and arachnoid cysts in the posterior fossa and right anterior temporal convexity. Arachnoid cysts, benign cerebrospinal fluid-containing cysts in the arachnoid space, appears to be a newly recognized finding associated with *WNT1* mutations. We suggest that much of the central nervous system (CNS) pathogenesis reflects vascular disruptions related to *WNT1* effects on angiogenesis [4]. With this, sporadic cases of asymmetric cerebellar hypoplasia with intellectual delays may involve *WNT1* separately from OI.

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✉ Piranit Nik Kantaputra
dentaland17@gmail.com

1 Center of Excellence in Medical Genetics Research, Chiang Mai University; Division of Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

2 Dentaland Clinic, Chiang Mai, Thailand

3 Department of Orthopaedic surgery, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

4 Department of Radiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

5 Department of Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

6 Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI, USA

7 Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

8 Division of Genetics and Molecular Biology, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

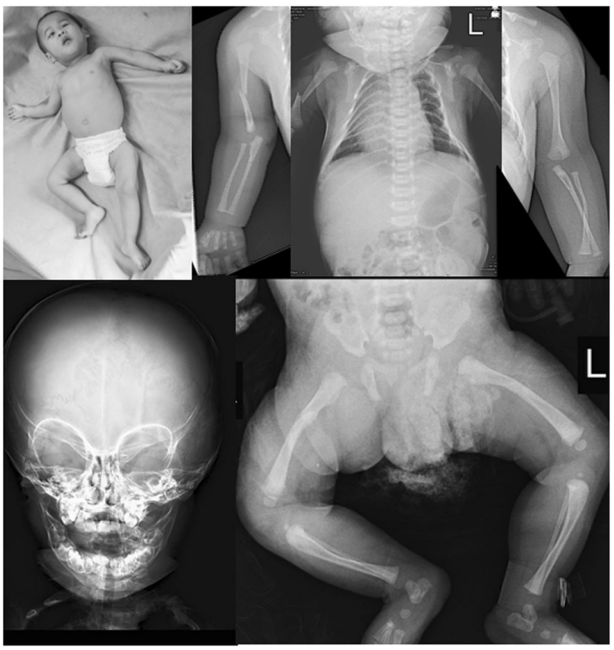

Fig. 1 A 2-year-old patient who has homozygous mutation of *WNT1*. Note left ptosis, craniotabes, and deformed right arm. Radiographs at age two years. Note generalized slender long bones and osteopenia. Multiple fractures of right humerus, left radius, right femur and ribs with callus formation. Skull radiograph shows islands of wormian bones

Finally, an absent effect from pamidronate in this form of OI [1], which differs pathogenetically from collagen related types, indicates that any nonresponsive patient should have molecular testing for *WNT1*.

Report of case

A term male newborn from an uneventful pregnancy born by cesarean section presented with multiple fractures (Fig. 1). His parents were consanguineous members of the Yao hill tribe from Thailand's Tak province. His paternal grandfather was a uncle of the maternal grandfather. Birthweight, length, and occipitofrontal circumference were 3.4 kg (50th percentile), 48 cm (3rd percentile), and 34 cm (50th percentile), respectively. At 2 months of age, his mother noticed that he could not follow an object with his left eye. Physical examination at that time revealed left microphthalmia, left ophthalmoplegia with limited ocular movements in all directions, and left optic disc atrophy with blindness in his left eye (Fig. 1). A computed tomography scan at age of one year showed left microphthalmia, mild left enophthalmos (Fig. 2a, b), left cerebellar hemisphere hypoplasia with compressed left-sided midbrain and left-sided pons (Fig. 2c–e), hypoplastic left cerebellar vermis (Fig. 2f), and an arachnoid cysts in the left posterior fossa and in the right anterior temporal convexity (Fig. 2g, h). The left ventricle was larger than the right one. Mild atrophy of frontal lobes was noted (Fig. 2i). Normal attenuation of the remaining brain parenchyma was noted. Asymmetrical small sizes of left superior, inferior, and medial rectus muscles are noted (Fig. 2a, b). Kidney ultrasonography was unremarkable.

At age 2 years, he showed normally colored sclerae, craniotabes, decreased movement of upper extremities, and a right arm deformity (Fig. 1). Developmental milestones were at the 4 to 6-month-old infant stage. Radiographic examination revealed bell-shaped thorax, multiple fractures of right humerus, left radius, right femur, and ribs with callus formation. Skull radiograph showed islands of wormian bones (Fig. 1). Laboratory included creatinine 0.2, Ca 9.0 mg/dL, PO_4 6.3 mg/dL, albumin 3.5 gm/dL, alkaline phosphatase 170 U/L, parathyroid hormone 60 pg/mL (15–65), and 25-hydroxy vitamin D = 28.8 ng/mL. Intravenous pamidronate was given at 0.5 mg/kg for 3 days every 2 month, but he continued to have bone fractures.

Mutation analysis

Mutation analysis of *WNT1* in the patient showed a homozygous single base pair duplication, of c.859dupC (RefSeq NM_005430.3, MIM164820). This variant is predicted to be functionally detrimental with a frameshift leading to premature truncation of the C-terminal part of WNT1 protein (p.His287Profs*30). The parents were heterozygous for the mutation (Supplemantary Figure S1). This variant was absent in 97 control subjects from the Yao population, in exome data of 210 non-OI Thai patients, and in the ExAC and gnomAD database.

Dual-energy X-ray absorptiometry

Since dominantly inherited early-onset osteoporosis has been reported in patients who were heterozygous for *WNT1* mutations [2], dual-energy X-ray absorptiometry was performed in both parents, and were in the expected ranges for their ages, suggesting that they did not have osteoporosis.

Discussion

Our patient had severe OI and structural brain anomalies with a homozygous single base pair duplication c.859dupC (p.His287Profs*30) mutation in *WNT1* and his consanguineous parents were heterozygous for the mutation. The same mutation has been reported in three members of a consanguineous Turkish family with moderately severe

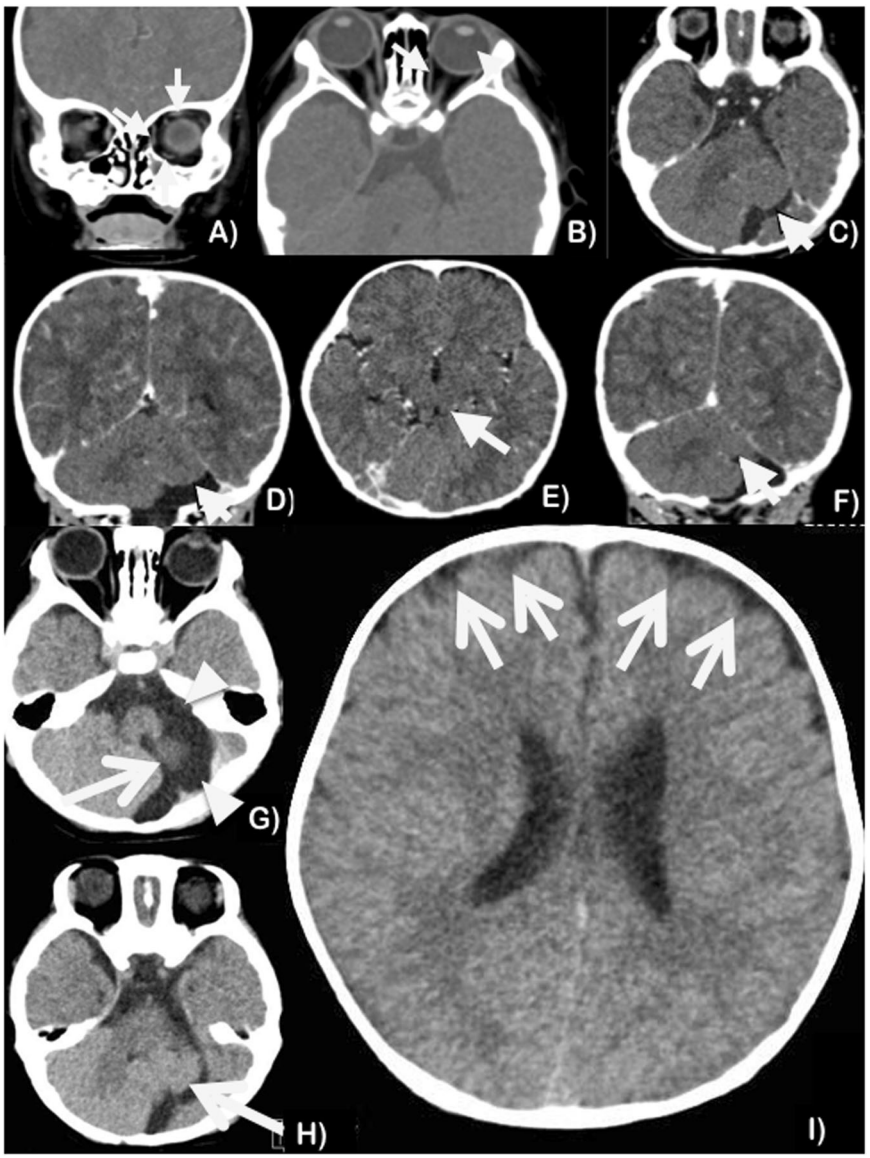

Fig. 2 **a**, **b** Coronal and axial CT images of orbits at age of one year. Asymmetrical small size of left superior, inferior, and medial rectus muscles (arrows). Relatively small anteroposterior diameter and more posterior position of left eye globe with left enophthalmos (arrow head). **c**–**f** Axial and coronal CT images of the brain. Asymmetrical small size of **c**, **d** left cerebellum (arrowhead). **e** left side of midbrain (arrow). **f** Cerebellar vermis (arrow). **g**, **h** Left micropthalmia, left mild enophthalmos, and hypoplastic left cerebellar hemisphere. Compressed left-sided midbrain and left-sided pons. Arachnoid cysts in the left posterior fossa and right anterior temporal convexity (arrowheads). **i** Mild atrophy of frontal lobes (arrows). Left ventricle is larger than the right one

OI [1], one with an undescribed brain malformation and delayed intellectual development. Eyes, hearing, and teeth were unremarkable. A heterozygous father had early-onset osteoporosis, but BMD of other parents were in the lower normal range. Functional study demonstrated failure of the mutant protein to activate the WNT-regulated β-catenin signaling cascade [1].

Frequent CNS malformations distinguish the *WNT1* phenotype from that of classic OI [5], and the arachnoid cysts in our patient extend the phenotypic spectrum here. However, other CNS malformations may have different origins. In particular, our patient confirms an unusual dichotomy, with typical asymmetric cerebellar malformations associated with significant intellectual delays, versus a normal cerebellum with normal intelligence, even with the same molecular findings.

WNT1 and vascular disruption in CNS

There is a part of the broader question of the origins of brain anomalies that are absent in all other known forms of OI. Despite an interesting case for *WNT1* developmental contributions to CNS findings [1], there are indications that *WNT1* developmental effect are responsible for a vascular/disruptive mechanism. As Aldinger et al. (2016) noted that "The most unexpected feature is the asymmetry seen in several patients." Asymmetric cerebellar hypoplasia with cerebellar clefts has been reported as an isolated anomaly presumed to be caused by prenatal posterior fossa or cerebellar hemorrhage [5]. Such striking asymmetry is rare among known genetic types of cerebellar hypoplasia. Potential involvement of *WNT1* is justified by known relationships with several specific mechanisms based on

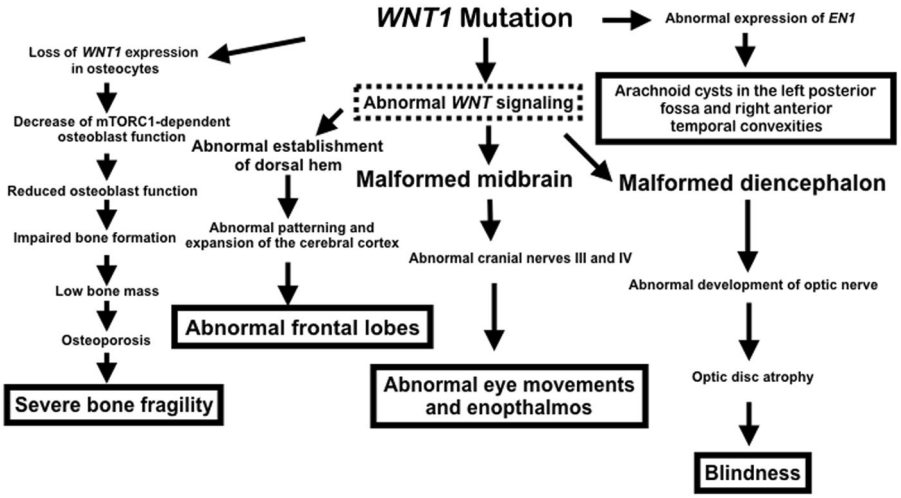

Fig. 3 Flowchart demonstrates the consequences of *WNT1* mutations (Supplemental Fig. 1S): electropherograms of the variant. The patient has a homozygous single base pair duplication of c.859dupC. The parents are heterozygous for the mutation

canonical/β-catenin signaling: (1) Midbrain–hindbrain boundary morphogenesis [6]. (2) A specific role for CNS angiogenesis [4] (Fig. 3). (3) Brain inflammation especially related to hemorrhagic by-products [7]. Besides asymmetry of *WNT1*-associated cerebellar anomalies, the *WNT10B*-associated split hand-foot malformation is usually asymmetric [8]. It is hypothesized that WNT signaling evidently plays an important role in the mirror image morphogenesis of human organs or at least hands, feet, and cerebellar hemisphere.

The association with intellectual disabilities can be seen with sporadic unilateral cerebellar hypoplasia with apparent prenatal hemorrhagic disruptions. This is not universal, but affects a subset of patients with no other apparent etiology. While this group is undoubtedly etiologically heterogeneous [9, 10], these observations support a disruptive rather than a developmental origin for *WNT1*-associated OI (Fig. 3). This also raises the possibility that *WNT1* without OI may be involved with these sporadic cerebellar issues.

Wnt1 is an important regulator of brain development [11]. *Wnt1*$^{-/-}$ mice are lethal at the neonatal period as a result of agenesis of the midbrain and cerebellum [11]. The spontaneous occurring *Wnt1* mutant "swaying"(*Sw*) mice that lived to adulthood were affected with ataxia and hypertonia as a result of malformations of the anterior regions of the cerebellum and hypoplasia of midbrain structures [12, 13].

LIM homeobox transcription factor 1 beta (Lmx1b) and *Wnt1* are expressed at the posterior border of the midbrain. Lmx1b activates *Wnt1* signaling, a ventralizing signal, which regulates midbrain and anterior hindbrain regionalization [6]. A role of Wnt1 signaling in development of CNS is to interact with Fgf8, a fundamental protein involved in development of the forebrain, midbrain, and cerebellum, in the isthmus organizer in order to maintain the expression of *En1* in the developing midbrain and anterior hindbrain [14]. Mice lacking *En1* fail to develop most of the tectum and cerebellum [15, 16]. Maintenance of *En1* in the developing midbrain and hindbrain is vital for normal brain development [17]. Even though *WNT1* is essential for brain development, some patients with *WNT1*-associated OI had no structural brain anomalies [18, 19]. The absence of *WNT1* might have been compensated by other *WNT* genes (genetic redundancy).

Mammalian brain neurons synthetizing the neurotransmitter dopamine exert important functions including execution of motor, affective, cognitive, motivational, and rewarding behaviors. *Wnt1* has been demonstrated to regulate the genetic networks required for development of midbrain dopaminergic neurons. *Wnt1*$^{-/-}$, *swaying* mutant mice had severe reduction of dopaminergic neurons in midbrain [20]. This partly explains why our patient had those dopamine-associated behaviors.

WNT1 mutation and arachnoid cysts

Arachnoid cysts in the left posterior fossa and right anterior temporal convexities have not previously been found in patients who were affected with *WNT1*-associated OI. These cysts were likely the results of abnormal expression of *En1* secondary to *WNT1* mutation because transgenic mice that ectopically expressed *En1* in the developing brain under control of the *Wnt1* enhancer also had cystic malformations in the posterior cerebellar vermis [21] (Fig. 3).

WNT1 mutation and atrophic frontal lobes

Atrophic frontal lobes found in our patient is very rare and has been reported only once [22]. Maldevelopment of the frontal lobes in the patients with *WNT1* mutations implies the role of *WNT1* in development of frontal lobes. In early brain development, Wnts are involved in telencephalic

dorsalization. Wnts play an important role in establishment of the dorsal hem, a medial signaling center that is involved in the patterning and expansion of the cerebral cortex [23] (Fig. 3). However our patient's frontal lobe atrophy could also be secondary to pressure effects from hemorrhage.

WNT1 mutation and ineffective pamidronate treatment

The bone fragility caused by *WNT1* mutations is predominantly related to impaired bone formation, cortical bone thinning, reduced trabecular density, reduced bone mass, and subsequent reduced structural properties. However, bone fragility in collagen gene-associated OI is the result of altered mechanical properties of collagen together with hypermineralization of bone matrix [24]. *WNT1* is expressed in osteocytes, the former osteoblasts that are differentiated and embedded in mineralized bone matrix. Osteocytes play an important role in bone homeostasis by regulating osteoblasts and osteoclasts. WNT1-associated OI and osteoporosis are caused in part by the decrease of mechanistic target of rapamycin complex 1 (mTORC1)-dependent osteoblast function as a result of loss of WNT1 signaling in osteocytes [13]. Overexpression of *Wnt1* in osteocytes markedly increases bone formation. Mice with targeted inactivation of *Wnt1* in osteocytes showed the skeletal phenotype similar to that of animals with global *Wnt1* deficiency, including poor bone formation, severe low-bone mass, and spontaneous bone fractures. Inactivation or overexpression of *Wnt1* do not have a major effect on bone resorption. The production of WNT1 in osteocytes is crucial for the regulation of osteoblast function via mTORC1 signaling during bone homeostasis [13].

Brittle bone in *WNT1*-associated OI is caused in part by reduced osteoblast function as a result of loss of WNT1 signaling from osteocytes to osteoblast [13]. Thus *WNT1*-associated OI is a disease of impaired bone formation, not bone resorption (Fig. 3). This explains why the anti-bone resorptive approach by pamidronate treatment was not effective in our patients and other patients affected with *WNT1*-associated OI [18, 24]. Therefore the treatment for these patients should aim to "inhibit the inhibition" of bone formation. In normal situation sclerostin, an osteocyte-secreted protein, decreases WNT signaling and subsequently inhibits bone formation by interacting with LPR5, a co-receptor of WNT ligand and thereby prevents the binding of WNT ligands to LPR5. That is why anti-sclerostin antibody treatment has been hypothesized to be an effective treatment for these patients [25]. mTORC1 signaling mediates the effect of WNT1 signaling in osteoblasts, thus increased mTORC1 signaling in osteocytes of *swaying* mice has been shown to increase bone mass and prevent spontaneous bone fractures [13]. Since pamidronate does not seem to be effective in this form of OI [1], which differs pathogenetically from collagen related types. Therefore any patient that seems unresponsive should have molecular testing that includes *WNT1*.

WNT1 and eye anomalies

Left optic disc atrophy in our patient is most likely the result of abnormal midbrain development because optic disc or optic nerve head is the beginning of optic nerve, which is derived from an out-pouching of diencephalon. We would wonder if the optic disc atrophy diagnosed in our patient actually represents a related optic nerve hypoplasia. Hypoplasia of the optic chiasma has been reported in a patient with *WNT1* mutation (case II-6) [3]. Dorso-ventral patterning in the diencephalon is regulated by Bmp4-activated *Wnt1* expression and its downstream cascade of primary dorsalizing signals in the diencephalon [6]. Abnormal oculomotor and trochlear nerves and midbrain malformation have also been demonstrated in $En1^{-/-}$ mice [15]. Enophthalmos and abnormal eye movement secondary to extraocular muscles found in our patients and other patients affected with WNT1-associated OI [5], might have been the consequence of abnormal oculomotor and trochlear cranial nerves which originate from the malformed midbrain (Fig. 3).

Conclusion

We report a patient who carried a homozygous *WNT1* mutation. CNS malformations consist of hypoplasia of left cerebellar hemisphere, compressed left-sided midbrain and left-sided pons, hypoplastic ocular muscles, and left optic disc atrophy. Arachnoid cysts and atrophic frontal lobes are to be newly recognized findings associated with *WNT1* mutations. Most brain anomalies in *WNT1*-associated OI appear to have vascular origins related to a role for *WNT1* in CNS angiogenesis. Asymmetric cerebellar malformations are frequently associated with significant intellectual delays. Clinical phenotypes are explained in relation to the roles of *WNT1* in bone and CNS development. Pamidronate appears ineffective with this form of OI, a lack of response to treatment with cyclic intravenous bisphosphonate of the osteoporosis in a patient with OI should be an indication for molecular testing that includes *WNT1*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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