Williams-Beuren Syndrome Hypercalcemia: Is TRPC3 a Novel Mediator in Calcium Homeostasis?

abstract
Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder associated with hypercalcemia of unknown origin. This syndrome results from the deletion of contiguous genes on chromosome 7, including the general transcription factor III gene. The general transcription factor III gene encodes TFII-I, which suppresses cell-surface accumulation of transient receptor potential C3 (TRPC3) channels, involved in calcium transport in lymphocytes. We describe the case of a patient with WBS with hypercalcemia associated with abnormal TRPC3 expression. Analysis of peripheral lymphocytes revealed a sharp increase in TRPC3 expression, compared with control patients. To investigate the potential role of TRPC3 in calcium homeostasis, we performed specific immunostaining on the intestine and the kidney, major calcium-regulating tissues. We provide the first demonstration that TRPC3 is expressed in normal digestive epithelium and renal tubules in control patients, and overexpressed in the intestine in the patient with WBS. Taken together, these data suggest that calcium metabolism abnormalities observed in WBS may be attributable to TFII-I haploinsufficiency and subsequent TRPC3 overexpression, thereby increasing both digestive and renal calcium absorption. This original observation prompts further investigation of TRPC3 as a novel actor of calcium homeostasis. Pediatrics 2012;129:e1626–e1630

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KEY WORDS
Williams-Beuren syndrome, calcium

ABBREVIATIONS
IIH—idiopathic infantile hypercalcemia
TRP (C3,C6,V5,V6)—transient receptor potential (C3,C6,V5,V6)
WBS—Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS) is a multisystem disorder resulting from the deletion of 26 to 28 contiguous genes in the 7q11.23 region of chromosome 7. Its estimated prevalence is 1 in 7500. The main clinical findings are a distinctive face and body shape; connective tissue and cardiovascular malformations, including blood vessel narrowing attributable to media and intima hyperplasia, mitral valve prolapse, supra-aortic vascular stenosis; and a characteristic cognitive and emotional profile, including moderate mental retardation and visuospatial deficit, in contrast to strong verbal abilities and friendly personality. The relative contribution of each gene deleted to WBS phenotype is mainly hypothetical, with the exception of the elastin gene, to which haploinsufficiency is related to cardiovascular abnormalities. WBS may also be complicated by hypercalcemia. The first description of the syndrome, defined a few years later by Williams and Beuren, may have been reported by Dr S.E. Schlesinger in 1956, who described the association of infantile hypercalcemia and unusual facies. Hypercalcemia is mild and inconstantly reported, but ionized calcium is rarely performed in published studies. Interestingly, hypercalcemia is more severe during infancy and resolves with aging. The mechanism of hypercalcemia does not result from increased levels of parathyroid hormone or vitamin D; moreover, there is no evidence for increased bone demineralization when compared with other developmental disabilities. Hypercalcuria seems particularly common in WBS, and nephrocalcinosis has been described in a series of patients who underwent renal ultrasonography. Although the precise mechanism for WBS-associated hypercalcemia remains unclear, these data strongly suggest an increased digestive absorption of calcium. An increased sensitivity to calcitriol has been suspected but remains unproven.

Williams-Beuren 7q11.23 deletion includes the general transcription factor III gene, which encodes TFII-I, a multifunctional transcription factor. TFII-I is expressed in brain and the general transcription factor III gene is 1 of the main genes responsible for the WBS neurocognitive profile. TFII-I also acts outside the nucleus of human B lymphocytes as a negative regulator of calcium entry by suppressing surface accumulation of transient receptor potential C3 (TRPC3) channels. Recent experimental studies identified that TRPC3 is also expressed in rat kidneys and suggested that TRPC3 could be involved in renal calcium reabsorption in addition to the classic channels TRPV5 and TRPV6. We are not aware of studies about expression of TRPC3 in intestinal epithelial cells.

We hypothesized that (1) TRPC3 is expressed in human digestive epithelium and renal tubules and (2) TRPC3 is overexpressed in patients with WBS because of reduced TFII-I expression, which may explain calcium metabolism disorders associated with this syndrome.

CLINICAL REPORT AND RESULTS

We describe the case of a patient born in 1960 and affected by a 7q11.23 deletion identified by fluorescence in situ hybridization, responsible for WBS. Persistent mild hypercalcemia (ionized calcium: 1.31–1.35 mmol/L; N: 1.14–1.31 mmol/L) and hypercalciuria (urinary calcium excretion: 8.0–9.3 mmol per day) were present since childhood. Oral calcium load tests performed in the 1980s revealed increased serum calcium level, from 2.35 mmol/L after calcium-free diet to 2.64 mmol/L after oral calcium load in 1989 (N: 2.16–2.52 mmol/L), and increased urinary calcium excretion after load (urinary calcium/creatinine ratio 8 after-before load: 1.3 mmol/mmol; N <0.6 mmol/mmol). Taken together, these results suggest both increased digestive calcium absorption and renal calcium handling as seen in primary hyperparathyroidism, but parathyroid hormone level was appropriately suppressed during calcium load, from 50 to 13 pg/mL (N: 15–55 pg/mL). Vitamin D metabolites were within normal ranges (25 [OH]–D3: 22.5 ng/mL; N: 7–30 ng/mL and 1,25(OH)2-D3: 32.7 pg/mL; N: 17–67 pg/mL). Bone remodeling markers (osteocalcin, bone alkaline phosphatase, urinary deoxypyridinoline) and bone mineral density were normal. Interestingly, proteinuria of glomerular origin had persisted since 1982 (0.92–1.5 g per day). Renal function was normal during the first decades but decreased after 2000 (creatinine clearance 102 mL/min/1.73m2 in 1988, 84 mL/min/1.73 m2 in 2000, and 68 mL/min/1.73m2 in 2008). Urine sediment was normal. Unfortunately, no renal biopsy was performed. The patient had also been treated for hypertension since 1988, with nicardipine and ramipril alternatively. In 2001, a gastrointestinal endoscopy, including colon biopsies, was performed for atypical abdominal pain. Intestinal biopsies did not reveal any pathologic process and abdominal pain resolved rapidly. An abdominal tomodensitometry was performed at that time, without evidence for nephrocalcinosis.

Blood samples taken in routine practice from the patient with WBS and normal healthy subjects were used after written consent according to French legislation. They were depleted of erythrocytes and centrifuged to select mononuclear cells by density gradient on Lymphoprep (AbCys SA, Paris, France). The monocytes were isolated by adhesion. The expression of TRPC3 and TRPC6 was assessed by confocal microscopy (primary antibodies ab51560 and ab12249 respectively, Abcam [Cambridge, United Kingdom], ab12249 respectively, Abcam [Cambridge, United Kingdom]). Alexa fluor anti-rabbit secondary antibody, Invitrogen (Carlsbad, CA)). TRPC3 expression was dramatically increased in peripheral lymphocytes collected from the patients with WBS but minimal in...
FIGURE 1
TRPC3 overexpression in WBS. A, TRPC3 immunostaining of distal tubules in a control kidney (renal biopsy) (original magnification ×400). B, TRPC3 immunostaining of a normal colic biopsy: TRPC3 is expressed by lymphocytes infiltrating mucosa (arrow), and, to a lesser extent, in epithelial cells (*) (original magnification ×400). C and D, TRPC3 immunostaining of a colic biopsy from a healthy subject and a patient with WBS, respectively. Biopsies were fixed and immunostained in similar conditions and counterstained with hematoxylin. TRPC3 is overexpressed in both lymphocytes and epithelial cells from

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control subjects (confocal immunofluorescence, Fig 1H and 1G, respectively). TRPC6 expression shares similarities with TRPC3 expression and was used as a control. Its expression was very faint in lymphocytes from both the patient with WBS and control subjects (not shown).

Using immunohistochemistry, TRPC3 and TRPC6 expression was assessed, in human colonic and renal biopsies (polyclonal primary antibodies ab51560 and ab12249, respectively, Abcam, 1/400). We observed that TRPC3 is expressed in the distal part of kidney tubules and to a lesser extent in digestive epithelium from colic biopsies (Fig 1A and B, respectively). The examination of the intestinal biopsies revealed a high TRPC3 expression in intestinal epithelial cells (and lymphocytes from mucosa) in biopsies from the patients with WBS patient but only a mild expression of TRPC3 in 3 normal control biopsies (Fig 1D and 1C, respectively). TRPC6 expression was restricted to unidentified cells in intestine and did not increase in the patient with WBS (Fig 1E and F).

DISCUSSION

WBS hypercalcemia has been a mystery for decades, but the gene(s) involved in calcium disorders remain unidentified. Interestingly, idiopathic infantile hypercalcemia (IIH) is another rare syndrome of unknown origin that shares similarities with calcium disorders observed in WBS: hypercalcemia is caused by digestive calcium hyperabsorption without involvement of parathyroid hormone or vitamin D metabolites, hypercalcemia resolves during infancy, and resulting hypercalciuria is sometimes responsible for nephrocalcinosis. Two genes included in the 7q11.23 deletion, CLDN3 and CLDN4, encode tight junction proteins claudin 3 and 4, respectively. Because members of the claudin family are involved in paracellular calcium absorption in kidney tubules and intestine, Lameris et al19 hypothesized that mutations of CLDN3 or CLDN4 could explain IIH or WBS hypercalcemia but they did not identify genetic abnormalities in CLDN3, CLDN4, or in the TRPV6 gene (encoding the TRP channel responsible for transcellular calcium intestinal absorption) in patients with IIH. Recently, Schlingmann et al20 identified the vitamin D–metabolizing enzyme CYP24A1 as responsible for severe familial forms of IIH, but the gene is located on chromosome 20 and therefore cannot explain WBS hypercalcemia.

We hypothesized that the TFII-I gene deletion may explain the WBS phenotype because it acts as a negative regulator of TRPC3 expression in human B lymphocytes. In rats, Goel et al16–18 recently described that TRPC3 is localized at the apical membrane of the principal cells in collecting ducts, and that activation of TRPC3 leads to net transepithelial apical-to-basolateral Ca (2+) flux. TRPC3 expression at the apical membrane is regulated by vasopressin.17,18 These results suggest that TRPC3 is a new candidate for renal calcium handling in the most distal part of the nephron, of which regulation could be complementary to paracellular calcium absorption in the ascending limb of the loop of Henle and transcellular absorption of calcium through TRPV5 in the distal tubule. We confirm that TRPC3 is highly expressed in collecting duct cells in human kidney biopsies, suggesting that TRPC3 may be involved in human physiology.

Whether TRPC3 plays a role in intestinal calcium absorption has not been determined. Its expression is weak in the normal colic biopsies that we analyzed, contrasting with high expression in epithelial cells from biopsies of patients with WBS. Calcium intestinal absorption predominates in the ileal part of the small intestine. We could not analyze TRPC3 expression in the ileum for obvious reasons, but TRPC3 was overexpressed in at least 2 tissues in our patient (lymphocytes and intestine), suggesting that all cellular types expressing TRPC3 may be involved. TRPC6 shares distribution and structural similarities with TRPC3, but its expression did not increase in biopsies from patients with WBS, suggesting that TRPC3 overexpression is specific.

Taking advantage of this exceptional observation, we show that TRPC3 is expressed in normal human kidney tubules and digestive epithelial cells, and specifically overexpressed in intestine and lymphocytes in a patient affected by WBS. TRPC3, therefore, may contribute to the calcium metabolism abnormalities frequently observed in this syndrome, by increasing both gastrointestinal and renal calcium absorption. This observation prompts further investigation, especially in a series of patients with WBS. Specifically, it would be of interest to test whether TRPC3 expression is increased in kidneys from patients with WBS who underwent renal biopsy. In addition to TRPV5 and TRPV6, we suggest that TRPC3 may be a novel actor of calcium homeostasis.

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