retardation, hypotonia, acquired microcephaly, dysmorphic face, and HCC. *FOXG1* encodes forkhead box protein G1, which plays important roles in the establishment of the regional subdivision of the developing brain and development of the telencephalon, and its defect causes a congenital variant of Rett syndrome (RTTCV, MIM #613454) [1]. RTTCV is characterized by severe neurodevelopmental impairments including psychomotor regression, hypotonia, progressive microcephaly, and HCC, which are comparable to the manifestations of our patient.

Case of a submicroscopic 14q12 deletion, involving regulatory elements of *FOXG1*, with the coding region of *FOXG1* being unaffected, is extremely rare. In addition to a patient reported by Kortum et al. [2], a recently published paper by Allou et al. [3] reported three 14q12 deletions that do not include the *FOXG1* coding region and were associated with phenotypes similar to patients with *FOXG1* mutations. Allou et al. mapped a putative long-range *FOXG1* regulatory element in a 0.43 Mb DNA segment encompassing the *PRKD1* locus. Using fibroblast cells established from these three patients, with increased expression level of *FOXG1*, they also showed that a cis-acting regulatory sequence, acting as a silencer, was located more than 0.6 Mb away from *FOXG1* (Fig. 3). Subsequently, Ellaway et al. reported three additional patients with severe intellectual impairment, early-onset developmental delay, postnatal microcephaly, and hypotonia, harboring 14q12 deletions that do not include the *FOXG1* coding region [4]. As is in our case, expression level of *FOXG1* in two of three patients report by Ellaway et al. was decreased. These finding strongly suggests that both over- and underdosage of FOXG1 are associated with similar phenotype, overlapping between RTTCV with *FOXG1* mutations and 14q12 microdeletion, not including the coding region of *FOXG1*. Interestingly, one patient with a 4.5 Mb duplication including *FOXG1* showed *FOXG1* downregulation [3,5]. This finding supports at the molecular level the phenotypic similarities between deletion and duplication cases.

Of the eight deleted genes in our case, whereas seven genes have been implicated in any known Copy Number Variations, the remaining gene *PRKD1* encodes a serine/threonine kinase that regulates a variety of cellular functions. In rodent model, PRKD1 are strongly expressed in the ventricular layer of the neocortex, striatum, septum, choroid plexus, and superior colliculus of mouse embryo [6], supporting developmental roles in central nervous system. Notably, all reported deletions distal to *FOXG1* also affect *PRKD1*. To determine whether a defect in the regulatory elements of *FOXG1* is sufficient for disease onset or whether *PRKD1* is responsible for a clinical condition overlapping the FOXG1/RTTCV, further studies or cases with intragenic mutations in *PRKD1* may be required. A number of CNVs in the general population are susceptibility alleles for common neuropsychiatric disorders, so some caution should be also paid to the 7 genes, which have been considered phenotypically neutral.

In summary, we identified 2.0 Mb of a novel deletion on chromosome 14q12, involving 8 genes and putative regulatory elements of *FOXG1* by array CGH in a patient with severe neurodevelopmental defects. Our finding provides additional evidence that not only over-dosage of FOXG1 as previously mentioned, under-dosage of FOXG1 is also associated with phenotype, overlapping between RTTCV with *FOXG1* mutations and 14q12 microdeletion, not including the coding region of *FOXG1*. Though the gene dosage of *FOXG1* appears to be critical for the normal development of brain, the complex mechanism of its regulation of gene expression remains to be elucidated. To correlate genotype to phenotype, clinical characterization of further deletion cases and saturation of the deletion map of this region is necessary to accomplish this task.

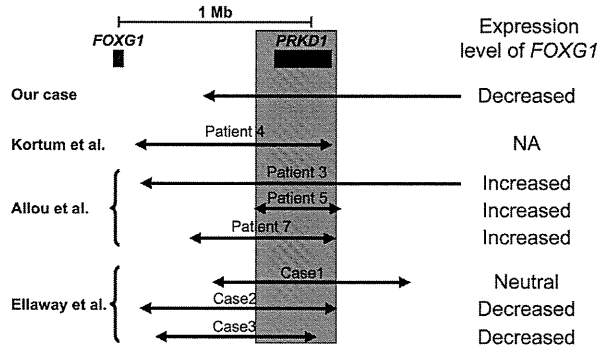

Fig. 3. Overview of the deletion in our patient and 7 previously reported deletions involving distal to *FOXG1*. Red shaded area indicates the putative long-range *FOXG1* regulatory element in a 0.43 Mb DNA segment encompassing the *PRKD1* locus mapped by Allou et al. Expression levels of *FOXG1* are listed on the right. NA, not analyzed.

Conflict of interest

The authors have nothing to declare.

Acknowledgments

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RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

A Recurrent Mutation in the 5′-UTR of *IFITM5* Causes Osteogenesis Imperfecta Type V

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TO THE EDITOR:

Osteogenesis imperfecta (OI) comprises a heterogeneous group of connective tissue disorders characterized by fragile bones with susceptibility to fractures. Recent investigations have revealed that OI is caused not only by mutations in collagen type I genes, but also in genes responsible for the post-translational processing of type I procollagen, which lead to the molecular-genetic division of OI into 11 types [Forlino et al., 2011]. Among them, type V OI (OI-V: OMIM #610967) is a variant of OI inherited as an autosomal dominant trait and has distinctive clinical and histologic manifestations [Glorieux et al., 2000]. Affected individuals develop ossifications of the interosseous membrane of the forearm, resulting in elbow restriction and dislocation. Other hallmarks include hypertrophic callus formation and a radiodense metaphyseal band of the long bones invariably seen in younger patients. On histological examination, a characteristic mesh-like pattern of lamellation is seen with polarized light microscopy. The causative gene remained unknown until recently. However, two groups have identified a single recurrent heterozygous mutation of c.-14C > T in the 5′-UTR of the gene encoding interferon-induced transmembrane protein 5 (*IFITM5*) in several families transmitted with complete co-segregation [Cho et al., 2012; Semler et al., 2012]. We report here the clinical and radiological manifestations in two affected individuals with the recurrent mutation.

Patient 1: A 5-year-old Japanese boy, was the first child of nonconsanguineous healthy parents. He was born at term after uneventful pregnancy and delivery. Birth weight, length, and OFC were 2,300 g (below 3rd centile), 46.9 cm (3rd–10th centile), and 32.0 cm (3rd–10th centile), respectively. At age 12 months, soon after he started walking, he suffered a fracture of the right tibia. Subsequently, fractures frequently occurred after mild trauma (four fractures between 1 and 4 years of age). He was referred for further assessment at age 4 years 10 months. His height was 96.8 cm (10th centile) and weight 13.0 kg (3rd–10th centile). The sclerae were white and dentition was normal. There was no joint laxity, skin hyperelasticity, or hearing impairment.

Radiological examination (Fig. 1A–F) showed generalized osteopenia and a narrow thorax with slender ribs. The ulnae appeared thin, and there was hyperostosis at the attachment of the interosseous membrane, radial bowing, and anteriorly dislocation of the proximal radii. Cortical hyperostosis was also noted at the lateral aspect of the proximal femoral shafts. The distal fibulae were medially bowed along with mild cortical thickening. Metaphyseal sclerosis of the long bones was evident particularly in the distal femur and distal radius. Calvarial wormian bones were not seen. The bone mineral density (BMD) of the lumbar spine (L2–L4) was

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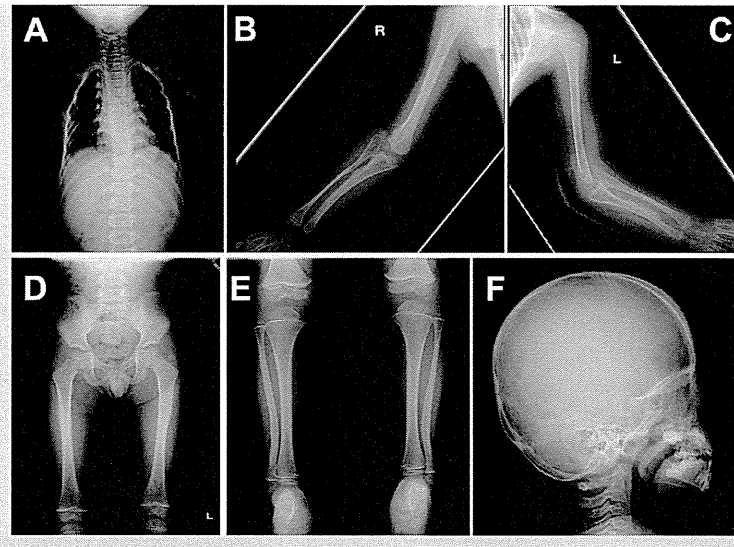
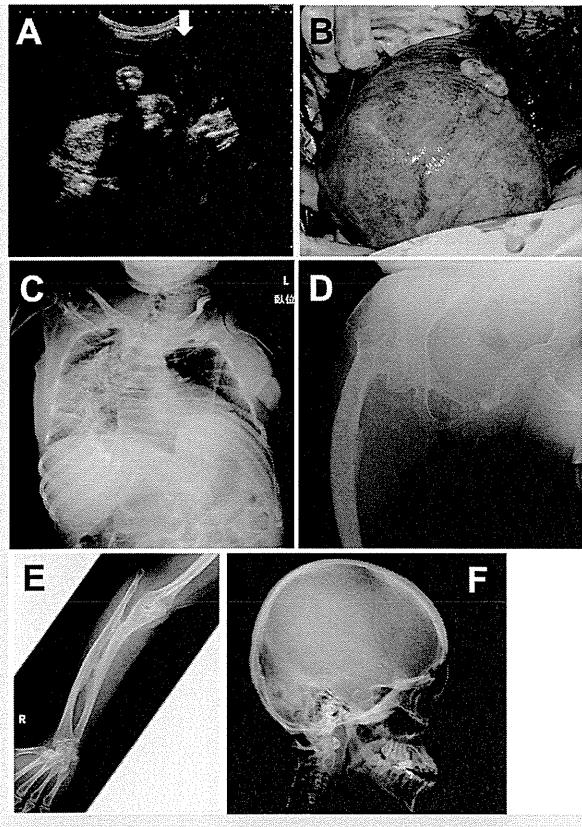


FIG. 1. Radiographs of Patient 1 at age 4 years 10 months. A: The thorax is narrow and ribs are slender. B,C: The ulnae appeared thin, and there is hyperostosis at the attachment of the interosseous membrane, radial bowing, and anteriorly dislocation of the proximal radii. Metaphyseal sclerosis is evident in the distal radii. D,E: Cortical hyperostosis is noted at the lateral aspect of the proximal femoral shafts. The distal fibulae are medially bowed along with mild cortical thickening. Metaphyseal sclerosis of the long bones was evident particularly in the distal femur. F: Calvarial wormian bones are not seen.



0.383 gm/cm^2 (Z-score of −3.4) (we used BMD reference data [del Rio et al., 1994] in Spanish children). Pamidronate treatment was initiated.

Patient 2: A 30-year-old Japanese woman, was born to healthy parents. The details of the clinical course in childhood were not available. She was said to have experienced the first fracture at age 7 years, and then suffered over 20 fractures, some of which required surgical correction. She developed progressive deformities of the extremities and scoliosis, and was consequently wheelchair bound. Adult height was 142.0 cm (below 3rd centile). She had white sclerae and normal dentition. She had no hearing impairment. At age 28 years, she gave birth to her first healthy child at 30 weeks of gestation by cesarean. The baby was a girl with birth weight of 1,332 g (appropriate for gestational age). The delivery was

FIG. 2. Ultrasonography and postpartum radiological examination of Patient 2. A: Ultrasonography at 28 weeks of gestation. Note a focal thinning and bulging at the upper segment of uterine wall (arrow). B: The head of the fetus is visible through the thin, almost transparent uterine wall. C–F: Postpartum radiological examination of Patient 2. C,D: Note severe thoracic scoliosis with thin ribs, acetabular protrusion, and generalized osteopenia of the axial skeleton. The tubular bones are slim along with metaphyseal osteopenia and diaphyseal cortical thickening. E: Abnormal ossifications are seen at the radioulnar interosseous membrane with proximal radial dislocation. Hyperostosis is noted at the medial aspect of the proximal radial shafts. F: No wormian bones are found.

complicated by incomplete uterine rupture. Ultrasonography at 28 weeks of gestation had shown a focal thinning and bulging at the upper segment of uterine wall (Fig. 2A). During the cesarean, the head of the fetus was visible through the thin, almost transparent uterine wall (Fig. 2B).

Postpartum radiological examination in the mother (Fig. 2C–F) demonstrated severe thoracic scoliosis with thin ribs, acetabular protrusion, and generalized osteopenia of the axial skeleton. The tubular bones were slim along with metaphyseal osteopenia and diaphyseal cortical thickening. Abnormal ossifications were seen at the radioulnar interosseous membrane with proximal radial dislocation. Hyperostosis was also noted at the medial aspect of the proximal radial shafts. No wormian bones were found. Quantitative heel ultrasound (QUS) showed her reduced bone quality (Z-score of −4.01, as measured by Speed of Sound).

After genetic counseling, we obtained written informed consent from the patient or parents for molecular studies, which were approved by the Institutional Review Board of the Keio University School of Medicine. Genomic DNAs were extracted from peripheral blood of the patients and parents by using standard techniques. We analyzed all coding exons and flanking introns of *IFITM5*, *COL1A1*, *COL1A2*, *LEPRE1*, *CRTAP*, *PPIB*, *FKBP10*, *SERPINF1*, *SERPINH1*, and *SP7* by using PCR and direct sequencing. Deletion or duplication involving *COL1A1* and *COL1A2* was checked by multiplex ligation-dependent probe amplification (MLPA) analyses (SALSA MLPA KIT P271, P272; MRC-Holland, Amsterdam, The Netherlands). We found a recurrent c.-14C > T mutation in the 5′-UTR of *IFITM5* in both patients. The mutation in Patient 1 was confirmed to be de novo. Other molecular analyses yielded normal results.

In conclusion, we validated the recently reported association between OI-V and the recurrent *IFITM5* mutation. However, further investigation is required to ascertain if the mutation is the exclusive cause of OI-V. Apart from the presence of hyperostosis, the skeletal manifestations in Patient 1 were mild to moderate, while those in Patient 2 were severe. The condition warrants a guarded prognosis because progressive bone deformity commonly ensues with age. It is important to note that the delivery in Patient 2 was complicated by uterine rupture despite absence of other extraosseous connective tissue abnormalities.

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ORIGINAL

Antenatal management of recurrent fetal goitrous hyperthyroidism associated with fetal cardiac failure in a pregnant woman with persistent high levels of thyroid-stimulating hormone receptor antibody after ablative therapy

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Abstract. High titer of maternal thyroid-stimulating hormone receptor antibody (TRAb) in patients with Graves' disease could cause fetal hyperthyroidism during pregnancy. Clinical features of fetal hyperthyroidism include tachycardia, goiter, growth restriction, advanced bone maturation, cardiomegaly, and fetal death. The recognition and treatment of fetal hyperthyroidism are believed to be important to optimize growth and intellectual development in affected fetuses. We herein report a case of fetal treatment in two successive siblings showing *in utero* hyperthyroid status in a woman with a history of ablative treatment for Graves' disease. The fetuses were considered in hyperthyroid status based on high levels of maternal TRAb, a goiter, and persistent tachycardia. In particular, cardiac failure was observed in the second fetus. With intrauterine treatment using potassium iodine and propylthiouracil, fetal cardiac function improved. A high level of TRAb was detected in the both neonates. To the best of our knowledge, this is the first report on the changes of fetal cardiac function in response to fetal treatment in two siblings showing *in utero* hyperthyroid status. This case report illustrates the impact of prenatal medication via the maternal circulation for fetal hyperthyroidism and cardiac failure.

Key words: Graves' disease, Fetal hyperthyroidism, Fetal goiter, Prenatal diagnosis, Fetal therapy

ANTIBODIES to the thyroid-stimulating hormone receptor (TRAb) , like other IgG, do not cross the placenta until 16 weeks and, therefore, could not play a role in early thyroid embryogenesis [1]. However, after 17 weeks gestation, since TRAb cross the placenta and fetal thyroid gland also develops, patients with Graves' disease showing high titer of TRAb might have adverse impact on fetal and neonatal outcomes including hyperthyroidism, non-immune fetal hydrops, and growth restriction [2]. We herein described a case of successful management of fetal cardiac failure complicated by fetal goitrous hyperthyroidism associated with persistent high levels of maternal TRAb in a pregnant woman with Graves' disease in hypothyroid status treated with thyroid hormone replacement after subtotal thyroidectomy and radioiodine therapy. Our experience would be significant information on perinatal management in women with Graves' disease presenting high levels of TRAb.

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Materials and Methods

Measurements of thyroid hormone and TRAb

TSH, free T4 (fT4), and free T3 (fT3) were measured using an electro chemiluminescence immunoassay (ECLIA) with the Cobas ECLIA kits (Roche Diagnostics, Japan). The values were interpreted according to gestational or postnatal age [3]. TRAb were measured

by ECLIA with third-generation assay using anti TSH receptor monoclonal antibody (M22) (Elecsys anti-TSH receptor assay (Roche Diagnostics GmbH, Penzberg, Germany)) [4]. A positive result was defined as an antibody titer greater than or equal to 2.0 IU/L.

Assessment of fetal cardiac function

ACUSON Antares (Siemens Medical Solutions, Mountain View, CA, USA) ultrasound system equipped with a CH6-2 transducer (2-6 MHz) was used to evaluate fetal cardiac function. M-mode sonography or cardiotocography tracings were used commonly to measure baseline fetal heart rate. Current international guidelines recommend for the normal fetal heart rate baseline different ranges of 110 to 160 bpm. If the baseline rate is greater than 160 bpm, it is termed tachycardia [5]. The preload indexes (PLIs) and cardiothoracic area ratios (CTARs) were analyzed on the second fetus showing *in utero* hyperthyroid status. CTARs was defined as the ratio of the cardiac area to the thoracic area in the four-chamber view of the heart in diastole [6]. Less than 35 % CTAR is normal regardless of gestational age, whereas cardiomegaly was defined as CTAR greater than or equal to 35 %. The inferior vena cava (IVC) waveform of human fetuses has a pulsatile pattern comprising 3 phases, namely, reversed flow during atrial contraction (A), early forward flow coinciding with atrial diastole and ventricular systole (Sf), and late forward flow coinciding with ventricular diastole (Df). The PLIs-IVC is the ratio of the peak velocity of A to the peak velocity of Sf, and the value of this parameter increases under high preload conditions. In normal fetuses, PLIs-IVC values range from 0 to 0.37 and have no relation with gestational age [7].

Case Report

A 30-year-old-Japanese woman, gravida 1, para 0, was referred to the high-risk prenatal care unit in our hospital because of fetal tachycardia at 23 weeks of gestation. Obstetric ultrasound examination revealed an anterior fetal neck mass, which was bilobed, symmetrical, solid, and measuring 17 × 9 mm (Fig. 1). The fetal heart rate was around 170 bpm. No other structural abnormalities were noted and the amniotic fluid volume was within normal range. The mother had a history of Graves' disease and received medical therapy, followed by subtotal thyroidectomy and radioiodine therapy. As a result of those treatments, she developed hypothyroidism and levothyroxine (LT4) replacement was started. Until the mid-second trimester, she had been euthyroid with LT4 replacement therapy, although high titer of TRAb persisted. It was noted that she showed marked bilateral periorbital edema and exophthalmos. Her thyroid functions at 23 weeks of gestation were as follows: TRAb 381 IU/L [manufacture reference range: <2.0 IU/L], TSH 0.09 μU/mL [manufacture reference range: 0.3-4.5 μU/mL], free thyroxine (fT4) 1.7 ng/dL [manufacture reference range: 0.7-1.8 ng/dL], free triiodothyronine (fT3) 2.7 pg/mL [manufacture reference range: 2.0-4.5 pg/mL] (Table 1a). Based on these clinical findings, the fetus was diagnosed as having hyper-

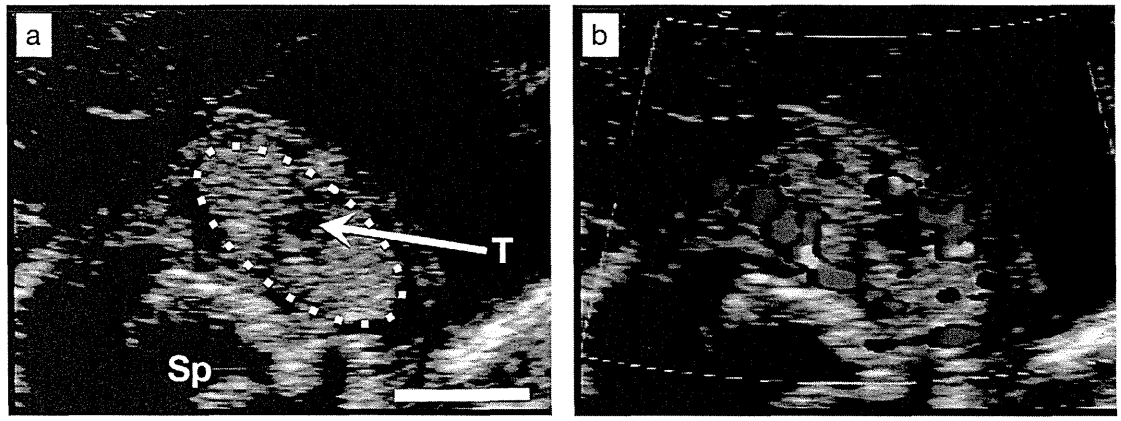

Fig. 1 Sonographic features of a fetal goitor
a. Transverse view of the fetal neck at 23 weeks gestation showing the thyroid mass located within the region of the ellipse. The trachea (T) is shown in the middle of the mass. Sp=spine. Bar=10mm
b. Color-flow Doppler imaging demonstrating the mass surrounded by abundant blood flow.

thyroidism. Because she had a history of eruption with use of methimazole (MMI), oral maternal potassium iodide (KI) (50 mg/day) and propylthiouracil (PTU: 150 mg/day) was initiated as *in utero* treatment for fetal hyperthyroidism. Since fetal heart rate didn't dropped and remained between 160 and 180 bpm, the dosage of PTU gradually increased up to 300mg at 34 weeks of gestation. During the follow-up, fetal growth restriction, defined as sonographic estimated fetal weight < -1.5 SD, became remarkable as pregnancy progressed, however, fetus did not develop cardiac failure and the heart rate was within normal range (141 bpm) after 35 weeks of gestation. At 36 weeks of gestation, a male neonate weighing 1,966 g was delivered by cesarean section because of possible dystocia due to enlarged fetal thyroid gland and non-reassuring fetal status. His Apgar scores at 1 min and 5 min were 8 and 9, respectively. On the day of birth, his thyroid gland was obviously enlarged and blood examination showed suppressed level of TSH (TRAb 445.0 IU/L [normal: <2.0IU/L], TSH 0.01 μU/mL [normal: 1.0-20.0 μU/mL], fT4 0.9 ng/dL [normal: 2.0-5.0 ng/dL], fT3 4.3 pg/mL [normal: 2.0-6.1 pg/mL], TSAb 2470 % [normal: <180 %]) (Table 1b) [reference ranges for neo-

Table 1a Maternal Thyroid Status

	Gestational weeks	TRAb [IU/L] (<2.0)	TSH [μU/mL]	fT4 [ng/dL] (0.7-1.8)	fT3 [pg/mL] (2.0-4.5)	Maternal treatment (/day)		
						LT4	PTU	KI
1st pregnancy	pre gestation	–	4.40 (0.3-4.5)	1.00	–	100μg	–	–
	23	381.0	0.09 (0.2-3.0)	1.7	2.7	150μg	150mg	50mg
	36	397.0	1.07 (0.3-3.0)	1.3	2.1	150μg	300mg	50mg
	postpartum 1 month	424.0	0.21 (0.3-4.5)	1.5	3.2	100μg	–	–
2nd pregnancy	pre gestation	230.0	4.16 (0.3-4.5)	1.7	1.8	100μg	–	–
	10	230.0	0.09 (0.1-2.5)	1.7	2.7	150μg	–	–
	21	223.0	0.10 (0.2-3.0)	1.8	3.6	150μg	300mg	50mg
	34	221.0	1.92 (0.3-3.0)	1.5	2.3	150μg	300mg	100mg
	postpartum 1 month	223.0	0.30 (0.3-4.5)	1.9	3.5	100μg	–	–

Normal range is shown in parenthesis.
The mother having a history of Graves' disease and received medical therapy was euthyroid status with levothyroxine replacement therapy, however, high titer of TRAb was persisted through two times of gestational period. TSH, thyroid-stimulating hormone (thyrotropin); TRAb, TSH receptor antibody; fT4, free thyroxine; fT3, free triodothyronine; LT4, levothyroxine; PTU, propylthiouracil; KI, potassium iodide

Table 1b Neonatal Thyroid Statuses

	thyroid status of each sibling	TRAb [IU/L]	TSH [μU/mL]	fT4 [ng/dL]	fT3 [pg/mL]
1st pregnancy	day 0	445.0 (<2.0)	0.01 (1.0-20.0)	0.9 (2.0-5.0)	4.3 (2.0-6.1)
	day 36	–	<0.01 (0.5-6.5)	0.8 (0.9-2.2)	3.8 (2.4-5.6)
	2 years	<0.3 (<2.0)	0.23 (0.5-4.5)	1.1 (0.7-2.0)	3.2 (2.3-6.6)
2nd pregnancy	day 0	120.0 (<2.0)	0.01 (1.0-20.0)	1.4 (2.0-5.0)	4.9 (2.0-6.1)
	day 15	–	<0.01 (0.5-6.5)	0.7 (0.9-2.2)	4.2 (2.4-5.6)
	8 months	0.6 (<2.0)	<0.01 (0.5-4.5)	1.7 (0.7-2.0)	4.0 (2.3-6.6)

Normal range is shown in parenthesis.
Laboratory examination of both neonates showed hyperthyroid status, and MMI was initiated immediately after birth. Levothyroxine replacement was implemented on Day 36 of 1st neonates and on Day 15 of 2nd neonates because the neonates showed central hypothyroidism. TSH, thyroid-stimulating hormone (thyrotropin); TRAb, TSH receptor antibody; fT4, free thyroxine; fT3, free triodothyronine

natal thyroid status according to Kliegman *et al.* [8]]. The neonatal hyperthyroidism manifested with tachycardia (140-180 bpm), prompting administration of 2 mg of MMI immediately after birth. In addition, on Day 17, 4 mg of atenolol was also started for persistent tachycardia. Thereafter, MMI and atenolol were discontinued at 8 weeks of age. LT4 replacement was implemented on Day 36 because of central hypothyroidism and the infant received LT4 replacement therapy until 2 years of age, when the hypothyroidic state resolved [9]. Follow-up examination at the age of two showed the failure to thrive. He weighted 8.2 kg (< 5th percentile) and was 77 cm tall (<5th percentile).

Two years after the first delivery, she became pregnant again. When first seen at 10 weeks of gestation, she was euthyroid under the LT4 replacement therapy, as follows: TRAb 230.0 IU/L, TSH 0.09 μU/mL, fT4 1.7 ng/dL, fT3 2.7 pg/mL (Table 1a). Obstetric ultrasound showed appropriate-for-date fetus with no abnormalities and the pregnancy course was uneventful. At 21 weeks of gestation, however, the fetus developed baseline tachycardia over 170 bpm. Fetal ultrasound examination revealed an enlarged thyroid gland (15 × 12 mm), pericardial effusion and cardiomegaly (Fig. 2a). Additionally, at 22 weeks of gestation, CTAR and PLI of the inferior vena cava were 53 % and 0.94, respectively. Taken together, the fetus was diagnosed to have *in utero* cardiac failure associated with hyperthyroidism. Based on the clinical course in the first pregnancy, oral maternal potassium iodide (50mg/day) and PTU (300 mg/day) were initiated (Fig. 3). At 24 weeks of gestation, pericardial effusion resolved. In addition, the CTAR as well as the PLI gradually improved to 46% and 0.72 after the initiation of fetal treatment. Since the PLI was still elevated and persistent fetal tachycardia (160 bpm) was seen at 30 weeks of gestation, oral maternal potassium iodide increased to 100 mg/day. Finally, fetal tachycardia and cardiomegaly resolved at the 34 weeks gestation (Fig. 2b), however, the enlarged thyroid gland was still present (Fig. 4) and the circumference of the thyroid was 9.8cm [normal: 3.7~6.0 cm] [reference ranges for fetal thyroid size according to Ranzini AC *et al.* [10]]. Subsequently, because preterm premature rapture of the membrane occurred at 35 weeks gestation, the mother delivered a 2034-g female neonate by repeated cesarean, with Apgar scores of 8 and 9 at 1 and 5 min, respectively. Laboratory examination on Day 0 showed neonatal subclinical hyperthyroidism (TRAb 120.0 IU/L, TSH 0.01 μU/mL, fT4 1.4 ng/dL, fT3 4.9 pg/mL) (Table 1b). Moreover, because the electrocardiogram of the baby after birth showed persistent tachycardia and accelerated maturation of the femoral ossification center was seen, MMI was initiated immediately after birth. Similar to her sibling, LT4 replacement was performed for possible central hypothyroidism. At 7 months of age, the neonate showed growth and gross motor developmental delays. She weighted 5.7 kg (< 5th percentile) and was 62 cm tall (< 5th percentile), and she wasn't able to sit without support and to crawl.

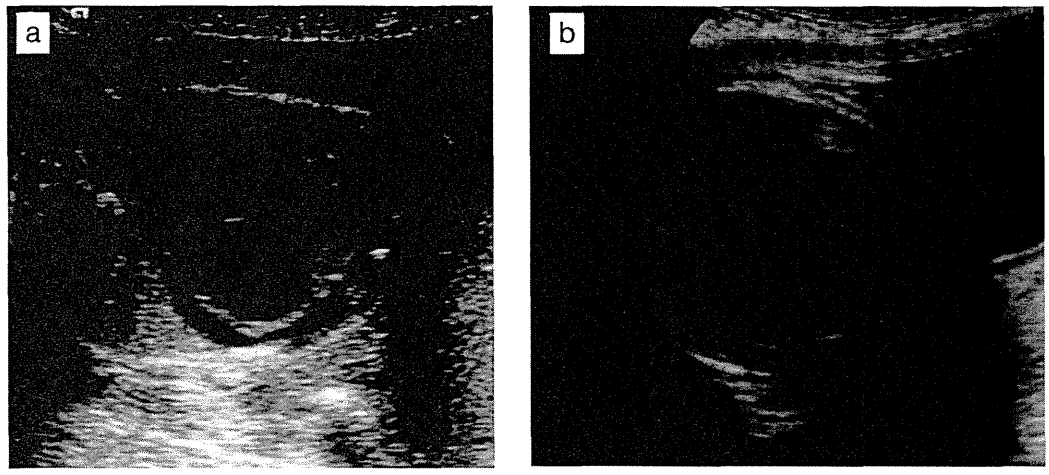

Fig. 2 Sonographic cross-sectional view of fetal thorax
a. Note fetal cardiomegaly with pericardial effusion at 21 weeks gestation.
b. Note cardiomegaly resolved at 34 weeks gestation by *in utero* treatment.

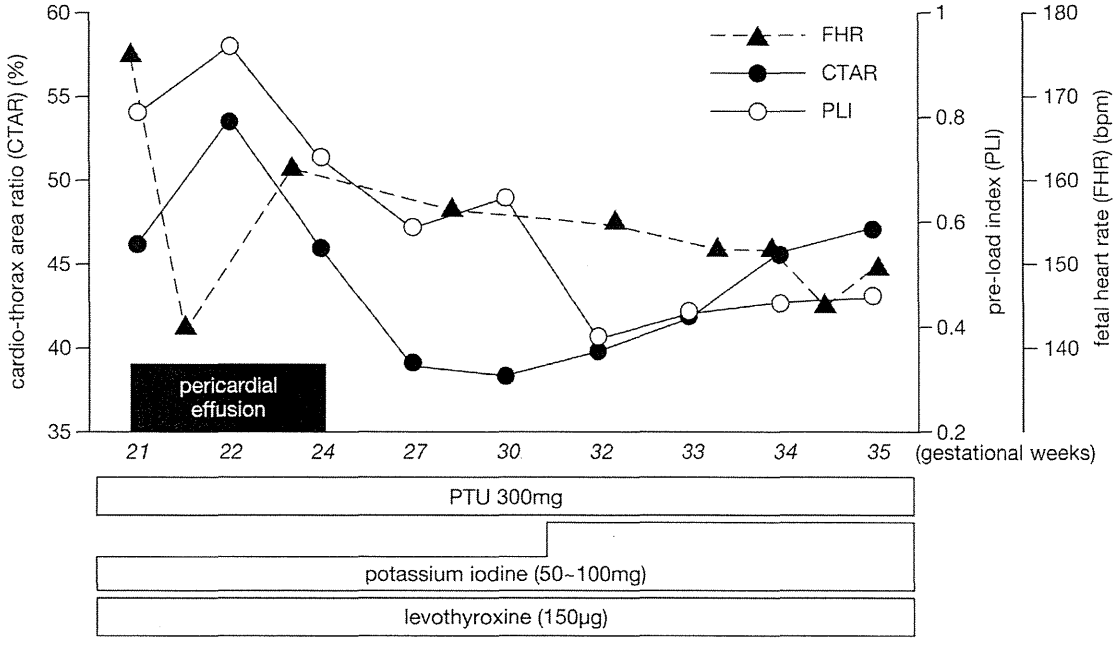

Fig. 3 Changes in sonographic measurements for fetal cardiac function
The cardio-thorax area ratio (CTAR) and pre-load index (PLI) of the inferior vena cava improved after the initiation of *in utero* treatment. Potassium iodide was increased up to 100 mg/day because of persistent fetal tachycardia.

Fig. 4 Fetal magnetic resonance imaging
Note the enlarged thyroid gland showing heterogeneous intensity on the T2-weighted imaging (arrow).

Discussion

In our case, the mother with a history of Graves' disease showed persistent high levels of maternal TRAb, although she was in a hypothyroidic state being treated with LT4 replacement therapy after subtotal thyroidectomy and radioiodine therapy. Our case was significant in that 1) fetal hyperthyroidism was diagnosed based on the clinical features, 2) fetal treatment using oral maternal anti-thyroid medicines was effective for improving cardiac failure, 3) maternal TRAb contributed to the development of fetal and neonatal hyperthyroidism.

Cordocentesis allows direct and accurate evaluation of the fetal endocrine status, however, most concern is the procedure-related complications including transient bleeding at puncture site, transient fetal bradycardia, chorioamnionitis, and cord hematoma [11]. In this case, high serum levels of maternal TRAb values as well as fetal sonographic features led us to the diagnosis of fetal hyperthyroidism, which was confirmed after birth. Therefore, fetal hyperthyroidism could be diagnosed without the invasive procedures (i.e. cordocentesis). In particular, the key feature for the suspicion of fetal hyperthyroidism might be persistent tachycardia in women with Graves' disease, as was shown in previous reports [12].

Fetal treatment using oral maternal potassium iodide and PTU was performed in our patient. Especially, PTU is preferred to MMI during the first trimester because the latter has been associated with aplasia cutis congenital and other malformation [12, 13]. In our case, the patient received PTU because of a history of allergic symptoms to MMI. Potassium iodide acutely inhibits hormonal secretion within hours in hyperthyroid

patients [14]. The reason for our choice of potassium iodide in combination with PTU was that 1) iodine has few side effects, 2) iodine has prompt therapeutic effects, and 3) the urgent treatment was needed for fetus in hyperthyroid status [15, 16].

Our report demonstrated changes in fetal cardiac function in response to oral maternal anti-thyroid medicine. Based on the indices (*i.e.* PLI and CTAR), we have adjusted the dosage of potassium iodide and PTU during treatment. In pregnant patients with active Graves' hyperthyroidism, it is well established that fetuses can be maintained euthyroid by keeping maternal FT4 in the upper normal to mildly thyrotoxic range for pregnant women [17]. However, there has been no safe and reliable method of determining fetal thyroid function due to the lack of monitoring maternal thyroid function. Morine *et al.* have shown the usefulness of sonographic measurements to gain insights into the pathophysiology of cardiac failure associated with fetal goitrous hypothyroidism [18]. Our results suggest both PLI and CTAR could contribute to the appropriate follow-up in fetal hyperthyroidism. In this case, although fetal cardiac functions were well controlled in both fetuses, the both neonates showed developmental delay at the age of two years and 7 months, respectively. The reason for this discrepancy remained unknown, but could be due to the association with fetal hyperthyroidism or other abnormalities, since both fetuses already showed growth restriction *in utero*. The clinical features of fetal hyperthyroidism were less severe in the first fetus than in the second, but higher levels of maternal and neonatal TRAb were noted in the first pregnancy. Reports of outcomes in siblings born to a mother with Graves' disease after total thyroidectomy or radioiodine treatment are limited. Hamada *et al.* demonstrated that the occurrence of neonatal hyperthyroidism is not only due to high levels of TRAb before pregnancy, but also to TRAb not decreasing during pregnancy [19]. Additionally, fetal thyroid function might depend on the balance between the transplacental passage of maternal TRAb and thyroid inhibiting antithyroid drugs [20]. Thus, it seems difficult to predict fetal and/or neonatal outcome in Graves' disease with high levels of TRAb.

In conclusion, when managing pregnant women with complicated Graves' disease, it should be remembered that two patients are being treated: the mother and the fetus. Especially, women with history of surgery or radioiodine therapy are at risk for fetal hyperthyroidism. To the best of our knowledge, this is the first report on the changes of fetal cardiac function in response to the fetal treatment in two siblings showing *in utero* hyperthyroid status. The fetal heart rate and sonographic parameters for cardiac function could be useful in indicating the onset of hyperthyroidism and monitoring a response to therapy.

Disclosure Statement

The authors declare that no competing financial interests exist.

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Assessment of user-friendliness of the Norditropin FlexPro for pediatric patients treated with recombinant human growth hormone: results of an open-label user survey

Abstract

Objective: To assess the user-friendliness of the Norditropin FlexPro (Novo Nordisk A/S, Bagsvaerd, Denmark), a newly developed growth hormone (GH) injection pen.

Methods: This study consisted of a single-center, single-arm, open-label, questionnaire based survey of patients undergoing GH treatment for diverse indications who were scheduled to switch from the formerly used device Norditropin NordiFlex (Novo Nordisk A/S) to the FlexPro and evaluate each device's usability.

Results: A total of 82 patients participated in the study. Compared to the NordiFlex, the FlexPro was regarded as easier to grip, easier to hold during injection, and more stable during injection. The degree of pain at insertion perceived by the patient and the patient's fear of injection were significantly decreased when using the FlexPro, and 81% of the respondents selected the FlexPro as the more preferable device.

Conclusions: The FlexPro was more user-friendly than the NordiFlex. Perception of pain at insertion and fear of injection were lessened by using the FlexPro.

Keywords: adherence; growth hormone; injection device; pain.

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Introduction

Recombinant human growth hormone (GH) is used in the treatment of a wide range of causes of growth retardation in children, including GH deficiency (GHD), Turner syndrome (TS), and short children born small for gestational age (1–3). In Japan, achondroplasia and hypochondroplasia are also the targets of GH treatment (4). Regardless of etiology, earlier initiation of GH treatment is recommended to obtain a better height outcome (5–8). However, a significant association between insufficient adherence to the treatment regimen and poor treatment outcome has been demonstrated by several studies (9–14). In fact, we often witness insufficient adherence, or even discontinuation of treatment, on either a short- or long-term basis. In addition to poor height outcome, insufficient adherence may result in the waste of health care resources (12). The reason for the high incidence of non-adherence to GH therapy may be multifactorial (9, 11–13). First, GH treatment requires daily subcutaneous self-injections, which may be cumbersome and/or painful, and may lead to a fear of injections. Second, GH therapy requires a long treatment period. Third, skipping GH injections does not necessarily immediately lead to serious health problems.

To promote patients' adherence, it has been suggested that an injection device should be user-friendly (12, 14–16). Accordingly, features enhancing simple handling and an easier injection procedure have been pursued by the manufacturers of GH injection devices.

In this study, we assessed the user-friendliness aspects for pediatric patients of the Norditropin FlexPro (Novo Nordisk A/S, Bagsvaerd, Denmark), one of the newly developed GH injection pens (17), by comparing it to the preceding product, Norditropin NordiFlex (Novo Nordisk A/S) (18).

Patients and methods

Study design

This study was a single-center, single-arm, open-label, questionnaire based survey conducted between 2010 and 2011 at the 419 bed Kanagawa Children's Medical Center, which is one of the largest children's hospitals in Japan, neighboring the Tokyo Metropolitan area.

Pediatric patients who satisfied both of the following criteria were consecutively invited to participate in this survey: i) were currently using the NordiFlex for GH treatment of any indication; and ii) were scheduled to switch to the FlexPro. Originally, at the introduction of GH treatment, the NordiFlex was chosen among all the available GH injection devises either by an attending physician's recommendation or by the patient's own preference. Participants were asked to complete a 16-item questionnaire twice to assess the NordiFlex and the FlexPro (Figure 1). The questionnaire regarding the NordiFlex was provided at the time of switching from NordiFlex to FlexPro, and the FlexPro questionnaire was distributed 12 weeks after the switch. The questionnaire was completed at home by whoever was actually injecting the GH, either the patient or by a caregiver. The questionnaire was then collected at the next outpatient visit. No additional clinical interventions, examinations, or follow-up visits were required for this study.

The questionnaire consisted of four sections: i) evaluation of the design of the pen (three items: pen's weight, pen's size, and the degree of easiness to grip); ii) evaluation of the pen's handling ease (nine items regarding ease of the following: attaching needle, reading the scale on the pen, setting and correcting the dose, dialing up the dose, reverse-dialing, holding the pen during injection, pushing the button, finishing delivery (confidence of full delivery), and stability during injection; iii) questions about pain and fear of injection (three items: pain at insertion perceived by the patient, pain during injection perceived by the patient, and the patient's fear of injection); iv) overall satisfaction with the pen (one item). In addition, in the last part of the second questionnaire, the respondent was asked which pen was preferable. In most question items, respondents were instructed to rate their assessment using a five category ordinal response scale (5=best, 3=unsure, 1=worst) (Figure 1). There were no rewards given to the patients for participating in the study and they were guaranteed that they would be at no disadvantage if they decided not to take part.

This study was reviewed and approved by an ethics committee of the Kanagawa Children's Medical Center. Written informed consent was obtained from all participating patients or their caregivers.

Norditropin FlexPro

The Norditropin FlexPro is a pre-filled, multi-dose, disposable pen containing a liquid GH preparation (17). It is an evolution model of the Norditropin NordiFlex (18), which was one of the most widely used pre-filled GH pens. Compared with the NordiFlex, the FlexPro is 17 mm shorter in length and 4 mm thicker in diameter. It was anticipated that this shape would improve ease of handling the pen and stability during injection, especially for pediatric patients, who have smaller hands. In addition, with its new crafted spring, the FlexPro needs a 4-fold lower injection trigger force than the NordiFlex (19). The FlexPro has been marketed in Denmark, the Netherlands, the USA, Germany, Switzerland, Austria, Australia, and Japan since 2010.

Data analysis

Standard descriptive statistics were used to summarize the characteristics of the patients. Responses rated as 4 and 5 were treated as positive responses, whereas those rated as 1 and 2 were treated as

Please provide your impression about Norditropin FlexPro.	
Evaluation of the pen's design (should be scored by the person who is actually injecting GH)	☺ very good ·····☻ average ····· ☹ very bad
Is pen's weight appropriate?	5 · 4 · 3 · 2 · 1
Is pen's size appropriate?	5 · 4 · 3 · 2 · 1
How did you feel about the easiness to grip?	5 · 4 · 3 · 2 · 1
Evaluation of the handling ease of the pen (should be scored by the person who is actually injecting GH)	☺ very good ·····☻ average ····· ☹ very bad
Is it easy to attach the needle?	5 · 4 · 3 · 2 · 1
Is it easy to read the scale on the pen?	5 · 4 · 3 · 2 · 1
Is it easy to set and correct the GH dose?	5 · 4 · 3 · 2 · 1
Questions about pain and fear of injection (should be scored by the patient himself or herself)	☺ no pain/no fear···☻ painful/fearful··☹ much painful/much fearful
How do you feel in insertion?	5 · 4 · 3 · 2 · 1
How do you feel during injection?	5 · 4 · 3 · 2 · 1
Is injection fearful for you?	5 · 4 · 3 · 2 · 1

Figure 1 A sample portion of the questionnaire.

negative. The response distribution between the two pens was compared using the Wilcoxon signed-rank test. Responses to the questions about pain and fear of injection were compared according to the difference of needle brand [BD Micro-Fine plus 31G 5 mm (Nippon Becton Dickinson Co., Ltd, Tokyo, Japan) vs. NanoPass 33G 5 mm (Terumo Corp., Tokyo, Japan)] using the Wilcoxon rank sum test.

Statistical tests were set as two-tailed, with a level of significance of 0.05. The above calculations were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Of the 108 patients who were consecutively invited to participate in this survey, 82 completed the questionnaires twice, that is, both before and after switching from the NordiFlex to the FlexPro. The clinical characteristics of the patients are summarized in Table 1. The mean age of the patients was 11.1 years, and 30.5% of patients were younger than 10 years. GHD was the most prevalent indication (42.7%) for GH treatment. When the patients with TS were excluded, the sex distribution of the participants was almost even. The rate of self-injection reported in the first questionnaire (when using the NordiFlex) was 34.1%, and 43.9% in the second questionnaire (after switching to the FlexPro). Regarding self-reported adherence, the proportions of 'complete adherence' responses were 53.7% on the first, and 63.0% on the second questionnaire. In contrast, 'lower than 70% adherence' was reported in 11.0% and 11.1% of the first and second questionnaires, respectively.

Table 1 Patient characteristics (n=82).

Characteristic	Number (%)
Age group	
1–4 years	3 (3.7)
5–9 years	22 (26.8)
10–14 years	43 (52.4)
15 or older	14 (17.1)
Sex	
Male	38 (46.3)
Female	44 (53.7)
Indication	
GHD	35 (42.7) (M16, F19)
SGA	24 (29.3) (M15, F9)
ACH/HCH	15 (18.3) (M7, F8)
TS	8 (9.8)
Administrator of GH injections	
NordiFlex	
Patients (self-injection)	28 (34.1)
Caregivers	54 (65.9)
FlexPro	
Patients (self-injection)	36 (43.9)
Caregivers	46 (56.1)
Needle used in injections[a]	
BD Micro-Fine plus 31G 5 mm	53 (64.6)
NanoPass 33G 5 mm	29 (35.4)

[a]In a given patient, the same brand of needle was used during the switch of the pen. GHD, growth hormone deficiency; SGA, small for gestational age; ACH, achondroplasia; HCH, hypochondroplasia; TS, Turner syndrome; M, male; F, female.

Evaluations of the design of the pen

Positive responses (rated as 4 and 5) to questions about the pen's weight, pen's size, and the degree of easiness to grip all significantly increased after the switch to the FlexPro. Regarding 'pen's weight' and 'pen's size', an approximately 20% increase in positive responses occurred [for 'weight', from 47.6% to 64.6% (p=0.0195); and for 'size' from 35.4% to 58.5% (p<0.0001)]. Positive responses to 'easiness to grip' doubled after switching to the FlexPro [from 28.0% to 56.1% (p=0.0024)], whereas negative responses decreased only slightly (from 12.2% to 11.0%).

Evaluation of the ease of handling of the pen (Figure 2)

For the FlexPro, approximately 70% of respondents expressed positive evaluations in 'reading the scale on the pen' (74.4%), 'setting and correcting the dose' (69.5%), 'dialing up the dose' (69.1%), and 'pushing the button' (67.1%). The positive responses for the FlexPro was about 60% for 'attaching needle' (61.0%), 'holding the pen during injection' (56.3%), 'finishing delivery' (56.3%), and 'stability during injecting' (56.8%).

In comparison to the responses regarding the NordiFlex, an approximately 20% increase in positive responses occurred, with statistical significance, for all items except 'dialing up the dose' and 'reverse-dialing'. In particular, positive responses almost doubled for 'holding the pen during injection' (from 28.8% to 56.3%) and 'stability during injection' (from 25.9% to 56.8%). Positive responses more than tripled for 'pushing the button' (from 22.0% to 67.1%). 'Reverse-dialing' was the only item for which positive responses decreased for the FlexPro compared to the NordiFlex (from 65.4% to 34.6%).

Questions about pain and fear of injection

For the FlexPro, more than two-thirds of patients (whether self-injecting or not) responded positively regarding 'pain

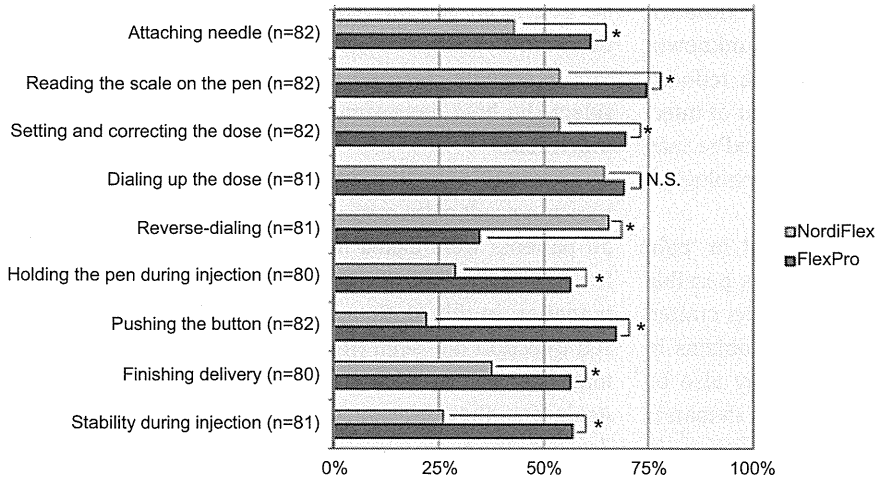

Figure 2 Cumulative positive answers (%) for evaluation of the ease of handling the pens. An asterisk indicates statistically significant differences in patients' ratings between NordiFlex and FlexPro. N.S., not significant.

at insertion', 'pain during injection', and 'patient's fear of injection' (70.2%, 66.7%, and 70.8%, respectively). Furthermore, after switching to the FlexPro, positive responses significantly increased for 'pain at insertion' and 'patient's fear of injection' (Figure 3).

The difference of needle brand did not influence the responses to the above questions. The incidence of positive response to 'pain at insertion' was not statistically significantly different between users of BD Micro-Fine plus 31G 5 mm and those of NanoPass 33G 5 mm, when using either the NordiFlex (58.0% vs. 51.2%; p=0.5926) or the FlexPro (74.0% vs. 63%; p=0.6841). No statistically significant differences were found regarding 'pain during injection' or 'fear of injection' (data not shown).

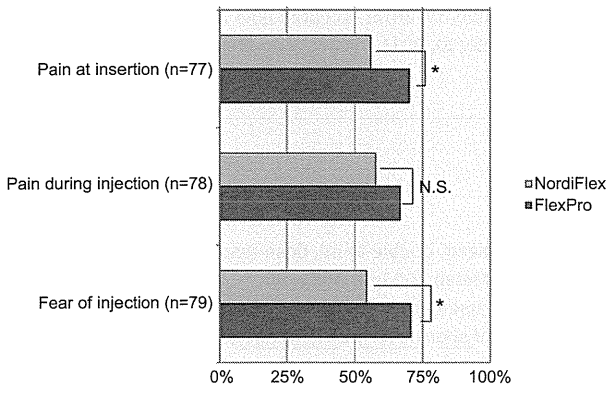

Figure 3 Cumulative positive answers (which indicate presence of little or no pain or fear) (%). An asterisk indicates statistically significant differences in patients' ratings between NordiFlex and FlexPro. N.S., not significant.

Overall satisfaction and device preference

Regarding overall device satisfaction, a significantly higher number of respondents expressed satisfaction with the FlexPro than the NordiFlex (50.0% vs. 82.7%, respectively; p=0.0001). To the question of which pen was preferable, the majority of respondents selected the FlexPro compared to the NordiFlex (81% vs. 19%, respectively).

Discussion

This study demonstrated that the FlexPro was rated better than the NordiFlex in nearly all aspects concerning user-friendliness (Figure 2).

One of the reasons for this improvement may be the pen's shape. The shorter and thicker design seemed to improve the ease of grip, which may be directly related to the significant increase of positive responses to 'holding the pen during injection' and to 'stability during injection'.

The reduced injection trigger force achieved by the new crafted spring may be another reason for improved user-friendliness, considering the 3-fold increase of positive responses to the question in 'pushing the button'. Another simple study assessing usability of the FlexPro vs. the NordiFlex by multinational patients (n=50) demonstrated that 80% of the patients preferred the FlexPro over the NordiFlex (20).

In addition to improved ease in handling, responses to 'pain at insertion perceived by the patient' and those to 'the patient's fear of injection' showed a favorable

trend, irrespective of the needle thickness used. Although the precise reason for this improvement is unknown, increased ease of grip may be relevant to the reduced insertion pain, and thus, to the reduction of fear of injections. In addition, the shorter length of the FlexPro may be less intimidating, resulting in a positive psychological impact.

In contrast, improvement was not evident in 'pain during injection perceived by the patient'. It is possible that an increased injection speed caused by the crafted spring was detrimental in this respect. The problems in 'dialing up the dose' and 'reverse-dialing' may also be as a result of the crafted spring system, which demands increased effort to go against the force of the spring. In addition, an unpleasant mechanical sound is generated by reverse-dialing. A sustained and vigorous effort to develop an even better device is needed.

From this study, it is impossible to estimate the contribution of use of the FlexPro to the improved treatment adherence. However, it was reported that the ease of use of a drug-delivery device was associated with greater patient adherence to treatment of GHD and diabetes (13–16, 21, 22). In addition, pain and fear of injections have been shown to be associated with poor adherence (9, 14, 21, 22). In these respects, the FlexPro may have the potential to improve adherence; assessing this topic is an objective of a planned future study.

In addition to progress in the design and function of pens, a needle insertion accessory has been also used, which was expected to reduce needle fear (18, 20). Additionally, an electrically controlled auto-injection device is an alternative method of improving user-friendliness (13, 23, 24). Although such devices present some difficulties for learning to operate, and may not be preferable for some patients (25), they could be attractive for others (26). In addition, the fact that the personal injection history is recorded automatically by the device may be beneficial for increasing adherence (13). Currently there are a variety of methods for GH delivery. It is important to provide complete information to patients, and to select the best device/method of GH injection for each individual.

An important limitation of this study is a potential bias as a result of the non-cross-over design. First, because all patients were asked to change pens, they may have felt that the newer FlexPro must be better. Second, participation in this study may be relevant to the increased self-injection rate seen (from 34% to 44%), which in turn may have led to a positive impression of the FlexPro. Aside from these biases, the question remains as to which device features had more important roles in determining patient preference. It should also be noted that certain important aspects influencing patient preference might not have been captured by our questionnaire.

In conclusion, the FlexPro was found to be more user-friendly than the NordiFlex. In addition, perception of pain at insertion and fear of injection were decreased in patients using the FlexPro.

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Conflict of interest statement

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Case Report

Association Between Graves' Disease and Renal Coloboma Syndrome: A Case Report

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Abstract. Renal coloboma syndrome is an autosomal dominant condition characterized by renal lesions and optic nerve abnormalities. We report an 11-yr-old Japanese girl with familial renal coloboma syndrome, who also had Graves' disease. Four affected family members had a previously reported heterozygous mutation (c.76dupG, p.Val26Glyfs*28) in the *PAX2* gene. We hypothesized that *PAX2* mutations may increase the risk of autoimmune diseases through alterations of human ß-defensin 1 expression.

Key words: renal coloboma syndrome, *PAX2,* mutation, Graves' disease

Introduction

Renal coloboma syndrome (OMIM#120330) is an autosomal dominant condition characterized by renal lesions and optic nerve abnormalities (1). Renal manifestations include renal hypoplasia, enuresis due to urinary concentrating defect, multicystic dysplastic kidney, and renal insufficiency. Ocular manifestations include optic nerve dysplasia, scleral staphyloma, optic nerve cyst and retinal detachment. More than 170 affected individuals and 80 families have been reported to date (2).

PAX2 is the only gene known to cause renal coloboma syndrome. Genotype-phenotype correlation is not evident among *PAX2* mutation carriers. Optic nerve abnormalities and renal lesions are variably manifested by patients with *PAX2* mutations, even within the same family (2, 3). While several nonrenal or non-ophthalmological features, such as hearing impairment, central nervous system anomalies, developmental delay, cardiac defects, abnormality of the hand, and ligamentous laxity, have been reported (4), there is no report documenting autoimmune diseases or thyroid diseases.

Here, we report a patient with Graves' disease and a familial *PAX2* mutation, and discuss the potential relationship between

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Table 1 Renal and ocular manifestations of the patients

Patient, gender and age	Renal manifestations					Ocular manifestations	Associated findings	Other diseases
	Renal size	Enuresis	Onset of proteinuria	Initiation of hemodialysis	Current Scr (mg/dL)	Fundus		
I-2 F/53 yr[a]	Not available	Not available	29 yr	32 yr	9.64[b]	Bilateral retinal detachment	None	Breast cancer
II-3 M/43 yr	Not available	(+)	10 yr	18 yr[c]	1.40	Bilateral optic nerve dysplasia, left retinal detachment	None	None
II-4 F/37 yr	Bilateral hypoplasia	(+)	12 yr	(–)	1.27	Normal	None	Obesity, hypertension, hyperlipidemia
III-3 Unknown/ abortus[d]	Bilateral hypoplasia	ND	ND	(–)	ND	ND	Oligohydramnios	ND
III-4 F/11 yr	Normal	(+)	9 yr	(–)	0.60	Bilateral optic nerve dysplasia	Oligohydramnios, strabismus, nystagmus	Graves' disease, bronchial asthma
III-7 M/2 yr	Bilateral hypoplasia	ND	(–)	(–)	0.41	Normal	Oligohydramnios	None

F, female; M, male; Scr, serum creatinine concentration. [a] She died at 53 yr of age. [b] At the initiation of hemodialysis. [c] He had a kidney transplant at 19 yr of age. [d] The family elected to terminate the pregnancy at 20 wk of gestation.

Graves' disease and *PAX2* mutations.

Case Report

Family pedigree is shown in Fig. 1A. Clinical information of the family members is summarized in Table 1. We describe clinical pictures of three affected family members.

III-4

The proband is an 11-yr-old girl. She is the second child of non-consanguineous Japanese parents. Serial prenatal ultrasound examinations showed no renal anomalies, although oligohydramnios was noted. She was delivered vaginally at 39 wk of gestation. Her birth weight was 2,752 g (−0.6 SD), length was, 47.5 cm (−0.6 SD), and head circumference was, 31.0 cm (−1.6 SD).

At 4 mo of age, internal strabismus and horizontal nystagmus were noticed, and she was referred to our hospital. Ocular fundus examination showed bilateral optic nerve dysplasia (Fig. 2A, B). She has received patching treatment and worn glasses for myopia.

Nocturnal enuresis continued from birth. At 6 yr of age, diurnal enuresis developed. Lifestyle guidance, water restriction and alarm treatment did not result in a favorable response. At 9 yr of age, her kidneys were normal in size and structure according to an ultrasound examination. The Fishberg concentration test revealed that the maximal urine osmolality was 752 mOsm/kg with elevated antidiuretic hormone concentrations (antidiuretic hormone concentration, 8.7 pg/mL, at a serum sodium concentration of 136 mmol/L), suggestive of a mild urinary concentrating defect. A 24-h specimen of urine contained 0.33 g of protein, suggestive of mild proteinuria. Her blood pressure was within normal limits. Renal biopsy

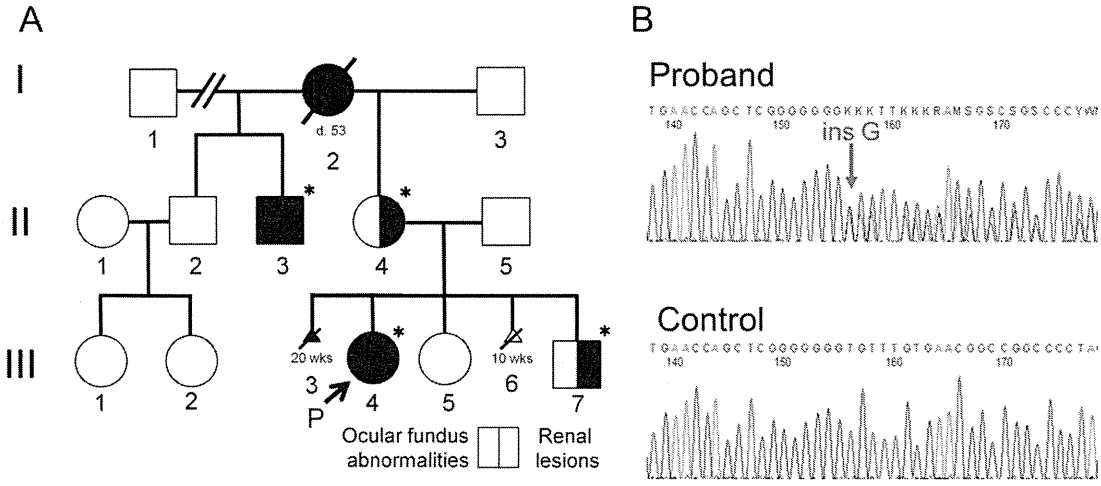

Fig. 1. A: Pedigree of the family. Family members who underwent the genetic testing are marked with an asterisk. B: Sequencing of exon 2 of the *PAX2* gene.

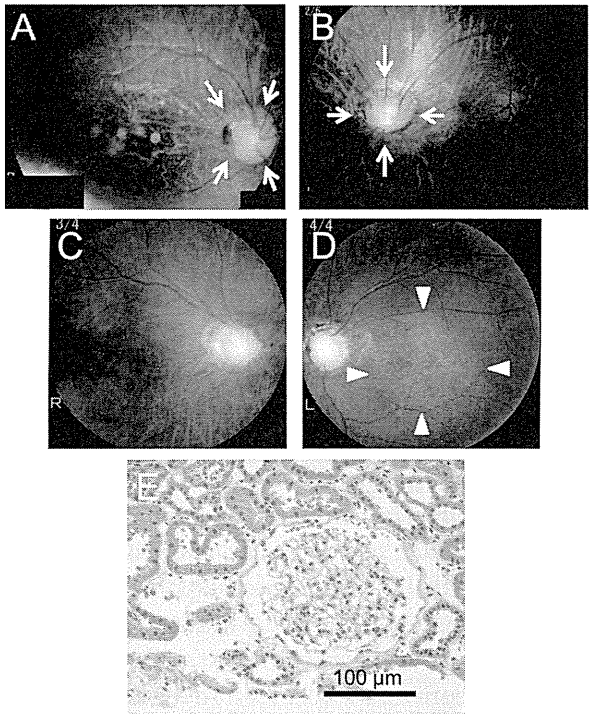

Fig. 2. A–D: Ophthalmological examination of the patients. Right (A) and left (B) eye of the proband (III-4). Bilateral optic discs were wide and deeply excavated (*surrounded by arrows*), suggestive of optic nerve dysplasia, so called "morning glory" variant. Right (C) and left (D) eye of patient II-3. Macular degeneration of his left eye (*surrounded by arrowheads*) and bilateral optic disc dysplasia were shown. E: Renal biopsy from patient II-4 at 26 yr of age. The glomerulus exhibits mesangial cell proliferation slightly. Hematoxylin and eosin stain.

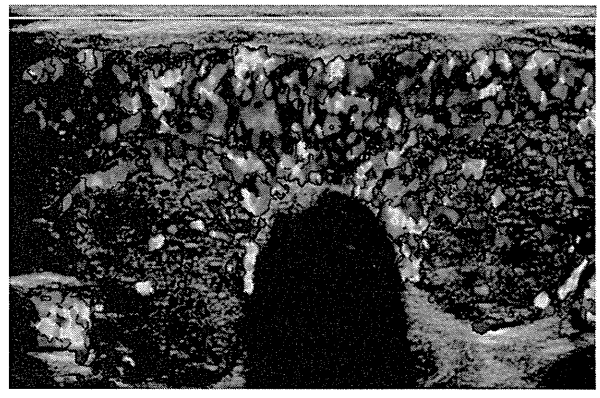

Fig. 3. Color Doppler ultrasound imaging of the thyroid in the proband (III-4). Ultrasonography revealed an enlarged thyroid with hypervascularity and heterogeneously low echogenicity.