GROWTH HORMONE DYNAMICS AMONG CHILDREN WITH MIXED GONADAL DYSGENESIS (45,X/46,XY)

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Abstract

Context. Learn the growth hormone dynamics and discuss the issues of growth hormone therapy in subjects with 45,X/46,XY.

Objective. To study the growth hormone dynamics in children with 45,X/46,XY karyotyping and mixed gonadal dysgenesis (MGD).

Design. Descriptive clinical study.

Participants. Five subjects with karyotype 45,X / 46,XY with or without genital ambiguity and somatic features of SHOX haploinsufficiency.

Interventions. Growth hormone dynamic study and gonadectomy.

Main outcome. IGF-1, peak GH levels, Turner's stigmata and histology of gonadal tissue.

Results. Five cases of MGD with both male and female phenotype were studied. IGF-1 levels and GH levels showed both features of growth hormone deficiency and growth hormone insensitivity. One study subject has gonadal germ cell tumour (dysgerminoma). We discuss here the issues regarding the GH therapy in MGD subjects.

Conclusion. Growth deceleration in MGD subjects is partly due to defective growth hormone secretion and partly due to growth hormone insensitivity. MGD subjects are at high risk for occurrence of gonadal tumours. Gonadectomy or biopsy of underlying dysgenetic gonads is essential prior GH therapy. Close surveillance for second neoplasm is to be considered in subjects with history of gonadal tumors prior starting GH for short stature.

Keywords: Mixed gonadal dysgenesis, Growth hormone, Second neoplasm, Gonadal Germ cell tumours.

INTRODUCTION

Mixed gonadal dysgenesis (MGD) or 45,X/46,XY mosaicism is a rare condition which presents as a spectrum of phenotype ranging from women with or without Turner's stigmata to individuals with ambiguous genitals, and rarely normal males (1). The clinical presentation includes ambiguous genitals

at birth, delayed puberty, virilisation at puberty in women or infertility. Turner's stigmata include linear growth failure, neck webbing, lymphedema, early sensorineural hearing loss, distinctive congenital cardiovascular, skeletal, digital, autoimmune disease and renal anomalies (2). Internal ducts may range from the presence of Mullerian ducts or/and wolffian ducts and gonads show normal testis to streak gonads. MGD arises due to late anaphase lag during mitosis in the zygote (3). Y chromosomal abnormalities such as ring chromosomes may also lead to mosaicism due to meiotic instability. The 45,X/46,XY mosaic has a worldwide incidence rate ranging 1.5 – 1.7/10,000 conceptions (1,4). Growth hormone dynamics has been studied by a few authors, yet no concrete evidence regarding GH-IGF-1 levels among MGD has been elaborated and no specific guidelines to manage growth issues in these subjects are available. Here we present a case series on growth hormone dynamics among MGD and its management issues.

MATERIALS AND METHOD

The study was conducted in the Department of Endocrinology, Government Medical College, Thiruvananthapuram, India between October 2015 and April 2020 after gathering consent from study subjects and approval from the ethics committee of the institute. Five children with MGD were available during the study period. MGD was diagnosed based on genital phenotype, gonadal phenotype and mosaic karyotype. Genital phenotype ranged from female external genitalia or mild clitoromegaly through all the stages of ambiguous genitalia to hypospadias or a normal penis. Ambiguity in external genitalia was scored by external masculinization score (EMS). EMS is an objective score to measure the degree of virilisation of genitals and takes into account features such as presence or absence of scrotal fusion, micropenis and the location

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of each gonad and urethral meatus (5). Gonadal phenotype included streak gonads through dysgenetic testis to normal testis and was confirmed following gonadectomy and histological examination. All subjects had gonadectomy done within one year following the diagnosis of MGD. All gonadal specimens were tissue fixed with 10% formalin for 24 hours, followed by paraffin wax embedding and preparation of slides of 4 micrometre thickness. Hematoxylin- Eosin stainings of the samples were done and classified as normal testis, dysgenetic testis, ovary, streak, combination of these or gonadal germ cell tumours. All specimens were subjected to immunohistochemistry (IHC) staining. IHC staining used to stain GCT included octamer binding transcription factor 3/4 (Oct 4), placental alkaline phosphatase (PLAP), alpha-fetoprotein when gonadal tumour was confirmed histologically (6). Karyotype included were 45,X/46,XY or 45,X/47,XXY or 45,X/47,XYY. Karyotyping was studied from the 30 cells of peripheral blood lymphocyte. 100 cells karyotyping was done if the 30 cells study was negative for the Y chromosome despite having a strong clinical suspicion of the presence of the Y chromosome. In our case series, all cases had a karyotype 45,X/46,XY. There were no specific Y chromosomal abnormalities detected in this case series. Presence of Y chromosomal abnormalities is associated with infertility (7).

Growth hormone dynamic study. All the five cases underwent a growth hormone dynamic study. The growth hormone dynamics study was indicated as the children had clinical significant severe short stature (as per the IAP 2015 growth reference charts). All study subjects underwent an anterior pituitary functional assessment which includes T4, TSH, 8 am cortisol, FSH and LH levels. IGF-1 levels and GH stimulation were done on two separate occasions with clonidine (0.15mg/m²) and glucagon (0.03mg/kg). The stimulation tests are done after an overnight fast and blood samples for GH are taken at the basal (zero) hour and at 30, 60, 90 and 120 minutes. Growth hormone deficiency is defined when IGF-1 is below the normal range for age and pubertal status and a two peak provocative GH test done on two separate days is below 10 ng/mL in an individual with growth failure after exclusion of other causes. Growth hormone insensitivity is characterized by low serum IGF-1 levels for age and pubertal status in the presence of normal or increased production of GH (basal GH >5 ng/mL or stimulated GH >15 ng/mL) in the presence of growth failure. The growth hormone dynamics and peak GH levels obtained in each provocative test are

shown (Table 1). Table 2 shows the endocrine tests of the study subjects.

hCG stimulation test. This was done to find the presence of an underlying functioning testicular tissue. Secondly, the test also helps us to distinguish the close differential diagnosis of androgen biosynthetic defects. The test includes a baseline blood sample collection in fasting for total testosterone. hCG is given deep intramuscular for three consecutive days in a dose based on the age of the patient (age < 1 year 500 IU per day hCG, age 1-12 years 1000 IU per day and age >12 years 1500 IU per day). Blood samples are collected 24 hours after the third dose for total testosterone, dihydrotestosterone and androstenedione. A value of two to eight times the basal value suggests the presence of underlying testicular tissue (8). Ratio of dihydrotestosterone: testosterone <10 and testosterone to androstenedione <0.8 is suggestive of 5 alpha reductase enzyme deficiency and 17 β hydroxysteroid dehydrogenase enzyme deficiency respectively.

CASE SERIES

Case 1

A 5-year-old child reared as a girl brought with ambiguous genitalia since birth and no features of salt wasting and hyperpigmentation and non-contributory family and developmental history. The child had short stature 94 cm (- 2.8 SD) with a mid parental height of 164 cm (75th centile for a girl adult height and 25th centile if raised as a boy). She had normal weight, normal blood pressure and short neck with multiple nevi, Prader Score 2 (phallus like structure of 4 cm length with blind vaginal opening and a perineal urethral opening and no palpable gonads anywhere) and SMR A0B1P1. Physical examinations were normal. Ultrasound revealed a hypoplastic uterus with no gonads visualized and unilateral aplastic kidney. There was no evidence of hypocortisolemia (8 am cortisol 14.08 mcg/dL) or elevated level of 17-OH-progesterone (0.4ng/mL). Pooled FSH and LH levels were 2.66mIU/mL and 0.1mIU/mL, TSH: 2.36 mIU/L, T4: 134.5 nmol/L(58 - 140 nmol/L), total testosterone: 2.5 ng/dL. Post hCG stimulation showed total testosterone levels 267 ng/dL, dihydrotestosterone (DHT): 155pg/mL, androstenedione:0.3 ng/mL suggesting functional testicular tissue and ruled out 5alpha reductase deficiency. IGF-1 was low for chronological age and sex [40 ng/mL (50-286)]. The peak GH levels on two separate occasions after clonidine stimulation and glucagon stimulation were 22 and 18 ng/mL,

respectively. Bone age was 4 years, according to Tanner Whitehouse 3 scoring. Karyotyping (100 cells) revealed 45,X [90]/46,XY[10]. Laparoscopy showed gonad in the right ovarian fossa and left streak gonad with bilateral fallopian tubes and hypoplastic uterus. Genitoscopy showed normal vagina and cervix. Cystoscopy showed normal urethra and urinary bladder. A biopsy from gonads showed seminiferous tubules with Leydig cells in the right gonad with streak ovary on the left side. The child was rehabilitated to lead a life as a girl. Growth hormone was planned at a dose of 0.375mg/kg per week considering underlying GH insensitivity but denied by parents.

Case 2

A 16-year-old adolescent presented with delayed puberty. The patient has a history of ambiguous genitalia noticed since birth and was reared as a male child. There were no clinical features too suggestive of congenital adrenal hyperplasia (CAH). The patient was evaluated for the concern of the ambiguous genitalia at 2 years old age by a pediatrician and underwent left orchidopexy for an undescendent left sided testis. The old records gave a description of external genitalia as penile hypospadias, empty scrotum left side and gonad palpable on the right side of labio-scrotum folds. The surgical notes of orchidopexy with left inguinal exploration testis on examination (also confirmed by histology) with fallopian tube in the left inguinal sac. A 30 cell Karyotyping sent showed 46,XY. At 3 years the patient underwent a second surgery for a right strangulated inguinal hernia. The sac showed a small gonad which on histology showed atrophic testis. At 6 years, parents noticed a poor gain in height compared to peers with poor scholastic particularly in mathematical skills. He underwent a laparoscopic exploration at 8 years and found to have an infantile uterus. At presentation he had a short neck, widely spaced nipples, pectus excavatum, short 4th metacarpal, wide carrying angle, short stature with mesomelia (height measured 134 cm = - 4.5 SD, MPH 169 cm (97th centile for a girl adult height and 50th centile if raised as a boy) and normal blood pressure. Bone age was 12 years. External genital examination showed a phallus like structure of 7 cm length with a glans like formation, incomplete fusion of labio-scrotal folds, urethral opening at the perineum and no vaginal opening (External masculinization score 0/12), SMR A0P2B1 and normal system examination. There was no evidence of hypocortisolemia (8 am cortisol 15.08 mcg/dL) or elevated level of 17-OH-progesterone (0.8

ng/mL). Pooled FSH and LH levels were 55.4 mIU/mL and 4.9 mIU/mL, TSH: 12.41mIU/L (0.4- 4), T4: 56.5 nmol/L (58 - 140 nmol/L), antiTPO 400 IU/mL (<40 IU), total testosterone: 18ng/dL and estradiol was <5 pg/mL. Post hCG stimulation showed no further elevation of total testosterone levels (21 ng/dL). DHT and A levels were 83 pg/mL and 2.55ng/mL respectively. Thyroxine replacement was given and IGF-1 done later was found to be low for age and sex [110 ng/mL (226 – 900)]. The peak GH levels after clonidine and glucagon stimulation were 4 ng/mL and 5 ng/mL respectively. Bone age was 12 years, according to Tanner Whitehouse 3 scoring MRI Pelvis showed a tubular structure in the left posterior lateral aspect of urinary bladder (probably uterus) with its lower end reaching the level of the urogenital diaphragm on the left lateral aspect of the posterior urethra. Ovaries, prostate and testis not visualized. The presence of Turner's stigmata prompts us to search for the presence of 45,X cells and hence a 100 cell karyotyping was sent. 100 cell karyotyping showed a karyotype of 45,X[80]/46,XY[20]. Patient and parents wished to continue as male. Surgical correction for hypospadias was done. Growth hormone was initiated at a dose of 0.18 mg/kg per week considering underlying GH deficiency.

Case 3

A 15-year-old reared as a female, born out of non-consanguineous marriage, presented with complaints of genital ambiguity noticed at birth presented with primary amenorrhea. There was no history to suggest CAH or cyclical abdominal pain. USG showed a small uterus and no gonads. There was proportionate short stature 132 cm (- 4.1 SD) with normal weight for age and blood pressure. Turner's stigmata in the form of wide carrying angle, multiple nevi, webbing necking and madelung deformity was found. Genital examination revealed a single perineal opening, phallic length of 5 cm, well-formed glans, well-formed scrotal sac and no gonads palpable anywhere. Prader staging was 3/5, SMR was P1B2. She had an ejection systolic murmur in left second intercostal space. Her 8 am cortisol was 17.9 mcg/dL and 17-OH-progesterone was 0.4 ng/mL. Pooled FSH and LH levels were 60 mIU/mL and 34 mIU/mL, TSH: 36 mIU/L, T4: 40.5 nmol/L, Anti TPO 1200 IU/mL (<40IU), total testosterone: 280ng/dL and estradiol was 10 pg/mL. Thyroxine hormone replacement was given and normalized. Post hCG stimulation showed several fold elevation of total testosterone levels (690

ng/dL). DHT and A levels were 200 pg/mL and 1.39 ng/mL respectively. IGF-1 96 ng/mL (230 – 900) and peak GH was 7ng/mL and 5 ng/mL with clonidine and glucagon stimulation (after normalizing TSH). Bone age was 12 years, according to Tanner Whitehouse 3 scoring. 2-D echo revealed bicuspid aortic valve. Diagnostic laparoscopy showed uterus with right sided fallopian tube with gonads in ovarian fossa. Left inguinal region showed a small gonad. Both gonads were removed; right gonad showed testicular tissue and left gonad showed streak. 100 cell karyotyping showed 45,X[90]/46XY[10]. Patient denied growth hormone therapy and she wanted to continue as female and was started on oral estrogen.

Case 4

A 6 year old child reared as a girl child was brought with concern of no increase in height, noticed from 6 months of age and no features of CAH. On examination the child had short stature 84cm (- 5.5 SD) with a MPH of 167 cm (75th centile for a girl adult height and 50th centile if raised as a boy). The child had normal weight, normal blood pressure, short neck and wide carrying angle. External genitalia which showed normal female genital organs with SMR were A0P1B1. Physical examinations were normal. Karyotyping done showed 45,X. MRI abdomen showed streaky uterus tissue in the retrovesical region measuring 3.5 cm in sagittal dimension and one cm in transverse diameter. Another structure beneath the urinary bladder was seen measuring 1x 1 cm possibility of prostate. There was no evidence of hypocortisolemia (8 am cortisol 20 mcg/dL) or elevated level of 17-OH-progesterone

(0.8ng/mL). Pooled FSH and LH levels were 1.4 mIU/mL and LH 0.1 mIU/mL, TSH: 3.1 mIU/L, T4: 110.5 nmol/L (58 - 140 nmol/L), total testosterone: 2.5 ng/dL. Post hCG stimulation was done in view of the presence of prostate showed total testosterone levels 267 ng/dL, DHT: 155pg/mL, A:0.3ng/mL indicating functioning testicular tissue without any evidence of 17- beta hydroxylase or 5 alpha reductase deficiency. IGF-1 was normal for chronological age and sex [40 ng/mL (56-298)]. The peak GH levels after clonidine and glucagon stimulation were 22 ng/mL and 16 ng/mL. Bone age was 4 years, according to Tanner Whitehouse 3 scoring. The presence of prostate and an elevation of testosterone after hCG stimulation prompted us for the search of the Y chromosome. 100 cell karyotyping revealed 45,X[90]/46XY[10]. The child underwent bilateral gonadectomy and rehabilitated her to lead a life as a girl. Laparoscopy showed gonads on either side of the ovarian fossa. Gonadectomy specimen showed right sided dysgenetic testicular tissue and left sided streak testis. Growth hormone was initiated at a dose of 0.375mg/kg per week.

Case 5

A 6-year-old girl child's parents took medical attention due to short stature. On examination the child had pathological short stature (height 90 cm, -4 SD) with a MPH of 169 cm (75th centile for a girl adult height and 50th centile if raised as a boy). External genitalia which showed normal female genital organs with SMR were A0P1B1. Ultrasound of pelvis revealed the uterus with no gonads. Karyotyping showed 45,X[60]/46,XY[40] in 100 cell study. Laparoscopy

Table 1. The growth hormone dynamics of children with 45,X/46,XY (MGD)

Cases	IGF-1 levels (ng/mL)	GH (Basal) (ng/mL)	Peak GH levels at first provocative test (ng/mL) – Clonidine stimulation	Peak GH levels at second provocative test (ng/mL)- Glucagon stimulation	GH therapy and dosage used
1	40 (50-286)	6	22	18	Denied
2	110 (226-900)	3	4	5	0.18mg/kg per week
3	96 (230-900)	2	7	5	Denied
4	40 (50-298)	8	22	16	0.375mg/kg per week
5	33(52-297)	4	5	6	0.18mg/kg per week

Table 2. The endocrine test of children with 45,X/46,XY (MGD)

Cases	FSH (mIU/mL)	LH (mIU/mL)	Basal total testosterone (ng/dL)	17-OHP (ng/mL)	Basal serum cortisol (mcg/dL)	hCG stimulated total testosterone (ng/dL)	hCG stimulated A (ng/mL)	hCG stimulated DHT (pg/mL)
1	2.66	0.1	2.5	0.4	14.08	267	0.3	155
2	55.4	4.9	18	0.8	15.08	21	2.55	83
3	60	34	280	0.4	17.9	690	1.39	200
4	1.4	0.1	2.5	0.8	20	267	0.3	155
5	2.4	0.2	2.5	0.5	25	72	0.8	100

examination showed an enlarged gonad in the right ovarian fossa with uterus and fallopian tubes on both sides. Left gonad was visualised over the left broad ligament. Histologic examination of the right showed dysgerminoma in the right gonadal tissue and streak gonad in the left side. IHC stained for OCT 3 and 4, PLAP and inhibin. Gonadectomy was done due to the presence of the Y chromosome. There was no evidence of metastasis and tumour markers like β -hCG, α -fetoproteins and serum lactate dehydrogenase were normal after surgery. To the parental concern of short stature the child underwent investigation for short stature and found to have growth hormone deficiency (IGF-1 were 33 ng/mL (52-297) and peak GH levels were 5 ng/mL and 6 ng/mL with clonidine and glucagon stimulation). Bone age was 3 years, according to Tanner Whitehouse 3 scoring. There was no evidence of hypocortisolemia (8 am cortisol 25 mcg/dL) or elevated level of 17-OH-progesterone (0.5 ng/mL). Pooled FSH and LH levels were 2.4 mIU/mL and LH 0.2 mIU/mL, TSH: 2.4 mIU/L, T4: 100 nmol/L (58 - 140 nmol/L), total testosterone: 2.5 ng/dL. Growth hormone was planned for the patient and was given based on multi-disciplinary decision. Growth hormone was planned at a dose of 0.18 mg/kg per week.

DISCUSSION

Here we present five cases of MGD where all patients presented with Turner stigmata (like short stature, mesomelia, Madelung deformity, wide carrying angle). Karyotyping of all five individuals had a predominant 45 X cell population as seen in the peripheral blood lymphocytes (Case 1: 90%, Case 2: 80%, Case 3: 90%, Case 4: 90% and Case 5 60%). Growth failure was documented in most cases at an age of 5 to 6 years, though it may occur earlier. The primary cited causation being SHOX haploinsufficiency. SHOX gene is located in pseudoautosomal region (PAR1) of sex chromosomes (both X and Y) and is one of the major growth regulating genes in humans. SHOX protein controls chondrocytes apoptosis and proliferation by regulating multiple target genes, including BNP, Fgfr3, Agcl and ctgf (9). SHOX abnormalities have been associated with short stature and various skeletal abnormalities. Growth failure is evident from the first year of life among SHOX haploinsufficiency (10). The most characteristic clinical finding of SHOX haploinsufficiency is madelung deformity and mesomelic short stature (11). There are no studies highlighting the frequency of

SHOX haploinsufficiency among MGD. We believe the effects of 45, X related SHOX haploinsufficiency do contribute to short stature which is well documented in Turner's syndrome and non-Turner's SHOX haploinsufficiency.

Short stature has been an integral part of patients with MGD as discussed above. GH and IGF-1 levels estimations have been done in a few studies and found to be similar to patients with TS showing GH deficiency in some subjects and in other subsets GH insensitivity (12). Subject with 45,X/46,XY treated with GH has shown improvement in linear growth. However, there have been no concrete studies which have studied the GH-IGF-1 levels in depth. Growth hormone studies among these subjects in our case series showed both growth hormones deficiency and insensitivity (other causes of short stature were excluded) Table 1. Short stature despite low IGF-1 and GH levels (both the basal and peak GH in all cases) were suggestive of growth hormone insensitivity or resistance. There have been no previous studies that have documented completely the growth hormone axis function among MGD. A few authors have shown normal to low normal IGF-1 levels among 45,X/46,XY mosaicism though in these study baseline and stimulated GH levels were not well documented (13,14). These studies which have shown a normal to low IGF-1 levels were demonstrated in MGD with male phenotypes. We found that growth hormone dynamics among MGD was similar to that of TS subjects. Studies in TS subjects have shown reduced GH secretions, normal GH levels, reduced free IGF-1, increased proteolysis of IGFBP-3 and IGF-1 resistance (15,16). Early growth failure and biochemical data suggesting both growth hormone deficiency and insensitivity among MGD makes it necessary for growth hormone therapy.

The GH dose required for MGD subjects is yet another question here. As already known the dose required for GH therapy among TS patients is higher than in the GH deficient population (17). Supraphysiologic doses are advised in TS patients to overcome the subtle defects of GH-IGF1 axis seen. Considering the presence of SHOX haploinsufficiency and GH resistance in MGD subjects as evident from our series, we advise to give a supraphysiological dose of GH to patients with MGD similar to TS to have an adequate growth response. Previous studies on the effect of GH therapy among MGD also had a significant growth response to higher doses (0.33-0.5 mg/kg per week) (18,19). It is also worth mentioning that certain patients with MGD, who have growth

hormone deficiency in GH stimulation, perhaps benefit with a lower GH dose. One study done in 2004 showed an improvement in height among MGD subjects on physiological GH dose (0.19-0.33 mg/kg per week) (20). Hence it would be prudent to confirm the GH status and decide the dose of GH therapy among MGD subjects. The postulate made has a limitation that our study has not learned the effects of growth with GH therapy in precise doses and these study subjects are currently under follow-up. The time of starting GH therapy in MGD subjects would be soon as growth failure becomes evident clinically (20).

Two cases had autoimmune hypothyroidism in our series. Autoimmune disease is associated with TS and SHOX haploinsufficiency (21). Individuals with TS have an increased rate of development of several autoimmune disorders, including thyroiditis, hyperthyroidism and hypothyroidism, celiac disease and type 1 diabetes mellitus, alopecia areata, juvenile rheumatoid arthritis, uveitis and inflammatory bowel disease (22). While the genetic basis for the predisposition is not known, there is a decrease in the CD4-CD8 lymphocyte ratio, suggesting an immune alteration that may predispose to autoimmunity. Girls and women with TS face a lifelong heavy burden of congenital and acquired cardiovascular disease, with increased mortality and morbidity. Congenital heart disease occurs in approximately 50% of girls with TS, including a high incidence of bicuspid aortic valve, coarctation of the aorta and an aortopathy that can lead to rare but often fatal dissection or rupture of the thoracic aorta (20). One patient (case 3) had bicuspid aortic valve. Left sided heart disease known to exist with TS and similar picture is seen in MGD cases. Case 1 also had a unilateral renal aplasia which is also described in TS. One patient had dysgerminoma (Case 5) and underwent complete resection. There was no metastasis evident. The issue we faced here was the concern for a second neoplasm (22). It is known that GH therapy can rarely lead to a second neoplasm due to its mitogenic and antiapoptotic effect of GH and IGF-1 (23). The concept of secondary neoplasm is also still a controversy. However, in our case 5 we started GH after making sure that the patient was in remission and by explaining to him the need for intensive surveillance for a second neoplasm. We also emphasize on the GH therapy to be considered only after gonadectomy in MGD subjects as the tumour risk is as high as 15-18 %. All study subjects underwent an IHC staining for the presence of an underlying germ cell tumour arising from the gonad. The routine IHC staining done was to rule out the presence of asymptomatic germ cell tumour.

In conclusion, GH dynamics in MGD includes both GH deficient and resistance state. GH dynamics should be considered in cases of MGD with short stature, or at the point of any clinical evidence of growth failure. GH dosage should be based on the GH dynamics results. Early gonadectomy followed by GH therapy in MGD subject is what we advise on managing short stature considering the possibility of underlying gonadal tumours in a dysgenetic gonad. Intensive surveillance is needed for a second neoplasm in MGD subjects who had a prior history of gonadal tumours and are on growth hormone therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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