Walter Bonfig[a], Nabeel J.M. Salem[a], Katrin Heiliger, Maja Hempel, Gaby Lederer, Milena Bornkamm, Karen Wieland, Peter Lohse, Stefan Burdach and Konrad Oexle*

Recurrent hypoglycemia due to growth hormone deficiency in an infant with Turner syndrome

Abstract

Background: Growth hormone (GH) deficiency may occur in Turner syndrome (TS), but infantile hypoglycemia attributable to TS with GH deficiency has not been reported before.

Objectives: We report a puzzling case of neonatal hypoglycemia due to GH deficiency in Turner syndrome. Array CGH was used to scrutinize the complex TS karyotype.

Methods: Standardized laboratory procedures.

Results: In a preterm (32 weeks) with prolonged and cholestatic jaundice, recurrent hypoglycemia occurred at the age of 1.5 months and was related to GH deficiency. There were no other endocrine or syndromic features. GH therapy was started at a usual dose of 25–30 μg/kg/day, but hypoglycemia recurred. Hepatopathy and hypogammaglobulinemia suggested X-recessive GH deficiency type 3 with non-random X-inactivation but resolved spontaneously. Nonetheless, a 45,X[75]/46,X,i(Xq)[21]/47,X,i(Xq)x2[4] TS karyotype was diagnosed with an apparent isochromosome fusion at the centromere. Upon this diagnosis, GH dose was doubled (50 μg/kg/day), and blood glucose was normalized consistently. In array CGH, the signal of Xp deviated more strongly than that of Xq, but the relation of the signals differed substantially from what the karyotype predicted. The isochromosome fusion point was relocated to Xp11.22, distal to a block of mental retardation genes that escape X-inactivation.

Conclusions: i) TS with GH deficiency should be considered as a potential differential diagnosis of hypoglycemia in infants requiring higher doses of GH. ii) While array CGH may be erroneous in quantification of TS mosaicism, it is useful in precisely delineating isochromosomes and identifying genes on them that escape X-inactivation and thus possibly affect the TS phenotype.

Keywords: array CGH; follicle-stimulating hormone; glucose; growth hormone; hypoglycemia; infant; mosaicism; preterm; sampling variance.

[a]Walter Bonfig and Nabeel J.M. Salem contributed equally to the study.

*Corresponding author: PD Dr. Konrad Oexle, Institut für Humangenetik, Klinikum rechts der Isar, Technische Universität München, Trogerstr. 32, 81675 München, Germany, Phone: +49-89-4140-6386, Fax: +49-89-4140-6382, E-mail: oexle@humangenetik.med.tum.de

Walter Bonfig: Kinderklinik, Technische Universität München, Kölner Platz 1, 80804 München, Germany

Nabeel J.M. Salem: Institut für Humangenetik, Technische Universität München, Trogerstr. 32, 81675 München, Germany; and Present address: Department of Pediatrics, Salmaniya Medical Complex, P.O. Box 12, Kingdom of Bahrain.

Katrin Heiliger: Institut für Humangenetik, Technische Universität München, Trogerstr. 32, 81675 München, Germany.

Maja Hempel: Institut für Humangenetik, Technische Universität München, Trogerstr. 32, 81675 München, Germany

Gaby Lederer: Institut für Humangenetik, Technische Universität München, Trogerstr. 32, 81675 München, Germany

Milena Bornkamm: Kinderklinik, Technische Universität München, Kölner Platz 1, 80804 München, Germany

Karen Wieland: Kinderklinik, Technische Universität München, Kölner Platz 1, 80804 München, Germany

Peter Lohse: Klinische Chemie, Großhadern, Ludwig-Maximilians-Universität München, Marchioninistr. 15, 81377 München, Germany

Stefan Burdach: Kinderklinik, Technische Universität München, Kölner Platz 1, 80804 München, Germany

Konrad Oexle: Institut für Humangenetik, Technische Universität München, Trogerstr. 32, 81675 München, Germany

Introduction

A variety of etiologies may cause neonatal hypoglycemia. These include stress factors such as sepsis, hypoxia and hypothermia, insufficient substrate availability, inborn errors of metabolism, hyperinsulinemia as in the case of islet cell hyperplasia or maternal diabetes, and deficiency of anti-insulinergic hormones as in the case of adrenal insufficiency, hypothyroidism, or growth hormone (GH) deficiency (1). We report an unusual case of recurrent hypoglycemia in a preterm with GH deficiency. The diagnostic work-up revealed Turner syndrome (TS) and GH deficiency. Array CGH was used to dissect the complex mosaic karyotype.

Methods

Blood parameter analyses were done according to standard procedures, including the glucose oxidase test for glucose determination. DNA extraction and gene sequencing also followed standardized protocols. The same was true for conventional cytogenetics (430 GTG bands). The lymphocyte culture did not extend longer than – one to two generations, so that in vitro shifting of the mosaic distribution was minimalized. Copy number variation (CNV) was detected by array CGH (Roche NimbleGen CGX-12 array with 135 K 60-mer oligonucleotide probes; NimbleGen, Madison, WI, USA). As a control, a female in-house sample was used that was shown previously to be free of pathological CNVs. This sample resulted in a lower noise level compared to a pooled control sample. Preparation, labeling, hybridization, and scanning were performed according to the NimbleGen Arrays User Guide version 2.4. For data analysis, Genoglyphix software was applied (build version 2.6-11.ion; Signature Genomics, Spokane, WA, USA).

Results

Case report

A preterm twin B baby was delivered by cesarean section at a gestational age of 31+6 weeks with a pH of 7.33, APGAR values of 6/7/8, length of 36.0 cm (<P3), weight of 1070 g (P7), head circumference of 26.3 cm (P6), and prolonged cholestatic jaundice. During the first days after birth, CRP increased but returned to normal levels after iv antibiotic treatment. At the age of 1.5 months, the infant presented with recurrent non-ketotic hypoglycemia (blood glucose <2.5 mmol/L with a minimum of 1.8 mmol/L and a necessary glucose substitution of 19 mg/kg/min). Free fatty acids were not elevated during hypoglycemia, suggesting hyperinsulinism or panhypopituitarism (cortisol and/or GH deficiency)

as differential diagnoses. However, the diagnostic work-up during hypoglycemia (Table 1) reproducibly revealed adequately suppressed insulin and C-peptide levels and normal rise in cortisol concentration (>20 µg/dL), while GH concentrations were low (<7 µg/L). IGF-1 and IGF-BP3 were also low (i.e., below the limit of detection), further supporting the diagnosis of GH deficiency. TSH and thyroid hormones were normal (TSH 2.5 µU/mL, freeT4 15.5 pmol/L, and freeT3 4.8 pmol/L). Plasma lactate was normal, and there was no evidence of metabolic disease. Elevated liver enzyme activities (GOT 123 U/L, GPT 86 U/L, GGT 345 U/L, AP 640 U/L) indicated hepatopathy, but no infectious cause was detectable. Further diagnostic work-up revealed hypogammaglobulinemia (IgG<40 mg/dL).

On cerebral MRI, the location and structure of the pituitary gland were normal. The infant did not have dysmorphic features, and no cardiac or renal malformations were detectable by echocardiography and abdominal ultrasound, respectively.

GH therapy was started with a conventional dose of 25–30 µg/kg/day, and blood glucose levels rose slightly, but still, intermittent mild hypoglycemia recurred.

The coincidence of GH deficiency and hypogammaglobulinemia reminded us of X chromosomal recessive, isolated GH deficiency type 3 (OMIM #307200), which may be symptomatic in females due to non-random X-inactivation (2). Therefore, we analyzed the genes *ELF4* and *BTK* as well as the karyotype. While the gene sequences were normal, TS was diagnosed with a 45,X[75]/46,X,i(Xq)[21]/47,X,i(Xq) x2[4] karyotype (Figure 1). Upon this diagnosis at the age of 3 months, GH substitution was further increased to a supraphysiological "Turner" dose of 50 µg/kg/day. Subsequently, blood glucose levels completely normalized as if a lever had been moved. Follicle-stimulating hormone (FSH), which was not significantly elevated initially (LH 0.6 U/L, FSH 2.5 U/L),

Parameter	Conventional unit	SI unit	Normal range
Glucose*	32/40 mg/dL	1.8/2.2 mmol/L	>2.5 mmol/L
hGH*	2.7/3.2 µg/L	0.126/0.149 nmol/L	>0.465 nmol/L
Cortisol*	22/23.2 µg/dL	607/640 nmol/L	>552 nmol/L
Insulin*	<2/<2 µU/mL	<14.4/<14.4 pmol/L	<14.4 pmol/L
C-peptide*	<0.2/<0.2 mmol/L	<0.2/<0.2 mmol/L	<0.2 mmol/L
Free fatty acids*	0.2/0.4 mmol/L	0.2/0.4 mmol/L	<1.5 mmol/L
β-Hydroxybutyrate*	0.03/0.05 mmol/L	0.03/0.05 mmol/L	<1 mmol/L
IGF-1	<25 ng/mL	<3.3 nmol/L	5.4–41 nmol/L
IGF-BP3	<0.5 µg/mL	<0.5 µg/mL	0.6–2.9 µg/mL
TSH	2.5 µU/mL	2.5 µU/mL	0.7–11 µU/mL
Free T4	1.2 ng/dL	15.5 pmol/L	11.8–25.6 pmol/L
Free T3	3.1 pg/mL	4.8 pmol/L	3.3–8.5 pmol/L

Table 1 Laboratory parameters during episodes of symptomatic hypoglycemia at the age of 1.5–2 months.
For parameters with asterisks, values of two representative episodes are shown (episode 1/episode 2).
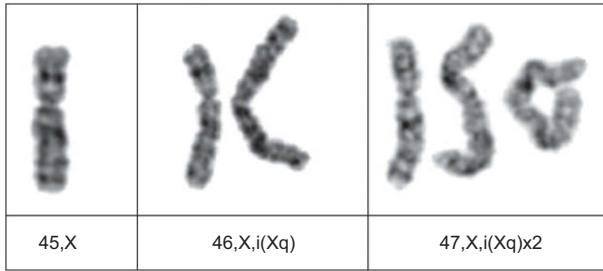
| 45,X | 46,X,i(Xq) | 47,X,i(Xq)x2 |

Figure 1 X chromosomes from the three different types of metaphases observed in the patient's lymphocytes.

rose to a level of 80 U/L at the age of 3 months. The hepatopathy started to resolve gradually after the commencement of GH therapy, and liver parameters were normal at the age of 7.5 months. At that time, the immunoglobulins also had increased to a normal level. Thereafter, at three monthly follow-ups, hepatic parameters and blood glucose concentrations stayed in the normal range. At the age of 1 year, the twice daily GH supplementation could be switched to a once daily administration with stable glucose concentrations.

Array CGH

While conventional cytogenetics located the isochromosome fusion point to the centromere, array CGH (Figure 2) located it to Xp11.22 (51.9 Mb, hg19, Feb 2009) with a distance of 6 Mb from the centromere. In array CGH, the final data is calculated as the binary logarithm of the ratio of patient DNA hybridization intensity to control DNA hybridization intensity. With one copy in the Xp arm of the patient's DNA and two copies in the control DNA, this yields $\log_2(1/2)=-1$, in the ideal case. The measured value (i.e., average of 2816 probes±SD of this mean) was -0.8 ± 0.0021 (Figure 2). For the Xq arm, the mosaic karyotype of the patient predicted a copy number

ratio of $(1\times75+3\times21+5\times4)/200=0.79$ with $\log_2(0.79)=-0.34$. The measured value was -0.5 ± 0.0016 (average±SD of 4619 probes). As the relation of measured and predicted value at Xq $(-0.5<-0.34)$ was inverse to the relation at Xp $(-0.8>-1)$, we concluded that an evaluation of the mosaic distribution by array CGH would underestimate the number of cells with i(Xq) isochromosomes. Sampling variance in conventional chromosome analysis, which may have affected the prediction at Xq (as opposed to Xp), could partially explain this difference but was unlikely to explain it entirely as the difference was more than twice the standard error (=0.058) in the prediction of the $\log_2$(copy number ratio) at Xq.

The standard error of the prediction at Xq was calculated as follows: estimated mean and variance of the Xq copy number per cell in the sample of 100 cells were 1.58 and 1.16, respectively. For the calculation of the copy number ratio, the random variable "Xq copy number" was divided by a constant of 2 (assuming that there is no sampling variance in the control). Thus, mean and variance of the Xq copy number ratio were 1.58/2=0.79 and $1.16/2^2=0.29$, respectively. The mean copy number ratio taken from 100 cells, thus, was estimated as $\mu_r=0.79$ having a variance of $\sigma_r^2=0.29/100=0.0029$. The variance of the mean log-transformed copy number ratio then was calculated as $\sigma_t^2\approx\sigma_r^2/\mu_r^2$ (3). This finally yielded the standard error of $\sigma_t\approx0.058$. (For simplicity this calculation ignored the fact that the sampled cells have gone through one to two divisions in culture so that some clones may have been sampled twice.)

Discussion

To our knowledge, Turner syndrome (TS), i.e., mosaic or non-mosaic states with only one normal X chromosome in
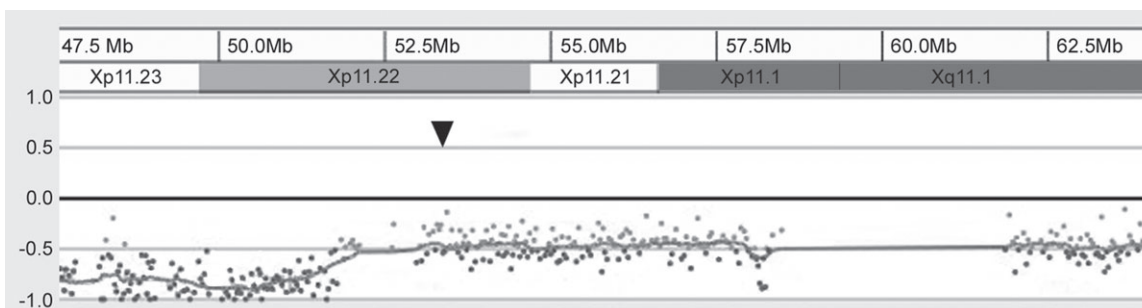

Figure 2 Array CGH result of the region between Xp11.23 and Xq11.1 with a shift of the average signal (i.e., line fitted to the signal distribution) from −0.8 to −0.5 within band Xp11.22 indicating the fusion point of the isochromosome at or somewhat distal to the physical position 51.9 Mb (hg19). A short segment in the isochromosome that has only one copy (i.e., two separate fusion points at short distance of each other) cannot be excluded. The position of the genes *KDM5C*, *IQSEC2*, *SMC1*, *RIBC1* that escape X-inactivation is indicated by the arrowhead. The centromere corresponds to the region between Xp11.1 and Xq11.1 that is not covered by array CGH probes.

girls, has not been related before to infantile hypoglycemia due to GH deficiency (GHD), although TS patients are known to be GH resistant, necessitating supraphysiological GH doses to treat their short stature (4).

Alkhayyat et al. (5) reported on hypoglycemia in a mosaic TS child with ring and marker chromosomes. Hypoglycemia in their patient resulted from hyperinsulinism, however, while our patient had normal insulin and normal C-peptide levels, i.e., no evidence of hyperinsulinism (Table 1). Prematurity and IUGR were considered as additional causes of glucose dysregulation in our patient, but lost explanatory value as the infant grew older. GH deficiency was diagnosed according to diagnostic guidelines. Neonatal GHD is defined by having low IGF-BP3 concentrations and GH concentrations below 7 μg/L during hypoglycemia as proposed recently by (6).

Hyperglycemia due to insulin resistance is a well-known feature in older children and adults with TS (7). In contrast, our infantile TS patient had hypoglycemia due to GHD. In fact, we cannot determine yet, whether our patient would present with hypoglycemic episodes again, if GH treatment was reduced and finally stopped. Thus, a transient tendency to hypoglycemia in early infancy may previously have gone undetected in TS patients. To assess this possibility, glucose and GH should be examined in a larger cohort of TS infants.

Treatment of hypoglycemia in our patient required substitution of supraphysiological doses of GH. This reminded us of the relative GH resistance in TS patients who are short in stature (4). However, the evaluation of the metabolic GH response in infantile TS patients also should be based on a larger cohort of such patients.

The combination of hypoglycemia and transient cholestatic hepatopathy in our patient was reminiscent of panhypopituitarism. However, apart from GH, all other pituitary hormones were normal in our patient and remained normal during follow-up, so far. Pohlenz et al. (8) and Xinias et al. (9) reported on the rare cases of male infants with isolated GH deficiency, hypoglycemia, and cholestasis. As in our patient, the signs of liver disease improved spontaneously. Pohlenz et al. (8) observed that glucose levels also normalized during the second year of life, even before GH replacement therapy was started. Incidentally, it should be mentioned that hepatopathy is frequent in TS, but not in patients younger than 7 years of age (10).

Varying levels of endogenous GH secretion may be another reason why hypoglycemia is not observed in every TS infant. Some authors assume that girls with TS are not usually GH deficient [reviewed in (11)]. On the other hand, abnormal GH secretion in TS patients has been documented (12, 13). According to Schmitt et al. (12), GH deficiency might be more frequent in TS patients with isochromosomes. In adult TS patients, Gravholt et al. (14) could not show an intrinsic abnormality of GH secretion after correction for lean body mass (LBM) and physical fitness. However, the same group (15) later showed that GH secretion is impaired in TS girls (aged 9.5 to 14.8 years) even after the correction for LBM.

Our patient did not present with typical signs of neonatal TS, such as congenital lymphedema, redundant nuchal skin, or heart defect. Indeed, the phenotype of TS is highly variable. This is partly due to the variability of the karyotype, which is 45,X in half of the patients, mosaic in one third, and structurally abnormal in the rest (7). Thus, for instance, none of the 26 patients with 45,X/46,X,i(Xq) mosaicism, but 65% of the non-mosaic 45,X-patients reported by Sybert and McCauley (7), had edema.

Without typical signs, the diagnosis in our patient resulted from indirect suspicion. Besides patients having elevated FSH levels as in hypergonadotropic hypogonadism, this suspicion resulted from the patients coincidentally having both GH deficiency and hypogammaglobulinemia, which occurs in X-chromosomal recessive GH deficiency type 3 (OMIM #307200). However, while the diagnosis of TS was confirmed, the sequences of the two genes (*BTK*, *ELF4*), which have been discussed as the cause of GHD type 3 (2, 16), were normal. Moreover, the hypogammaglobulinemia, which may have been related to the patient's prematurity (17), resolved spontaneously. The conventional karyotype was 45,X[75]/46,X,i(Xq)[21]/47,X,i(Xq)x2[4]. Similar mosaic karyotypes with an isochromosome i(Xq) consisting of two q-arms have been observed in 8% of the TS patients (7). The isochromosome fusion point in our patient appeared to reside within the centromere (Figure 1). However, when we analyzed the patient's DNA by array CGH (Figure 2), the fusion point was found to be at Xp11.22, i.e., at physical position 51.9 Mb (hg19). Previous studies [recently reviewed in (18)] have shown that most isochromosomes in TS are isodicentric, that is, idic(X)(p11). The segment of p11 involved varies from one case to another. Precise identification of the fusion point could be relevant for investigations on the genotype-phenotype relation as the isochromosome may contain proximal Xp genes that escape inactivation and thus may influence the phenotype. The fusion point in our patient was located on the telomeric side of a block of four such genes (19) at position 53.2–53.5 Mb (hg19). Three of these genes (*KDM5C*, *IQSEC2*, *SMC1*) have been involved with syndromic and non-syndromic forms of mental retardation. Symptomatic carriers of loss-of-function mutations in *KDM5C* and *IQSEC2* have been observed (20, 21), so that

gene dose may possibly have some effect. Incidentally, an intragenic in-frame deletion of *SMC1* causing Cornelia de Lange syndrome type 2 (CDLS2) has been observed in a patient with 45,X/46,XX TS mosaicism (22). The mutation effect in CDLS2 is assumed to be dominant-negative, however. Scott et al. (18) presented three other idic(X)(p11) cases whose fusion points were such that the above-mentioned block of genes was included in the isochromosome. However, the authors did not remark and thus may have missed the special relevance of these genes.

Isochromosome mosaicism allowed us to highlight the problem of array CGH quantification of mosaicism in a specific way, i.e., on two different levels but both from the same blood sampling. The step size at the Xp11.22 fusion site did not correspond to the prediction derived from the karyotype. Sampling variance in karyotyping could not sufficiently explain this deviation. Array CGH thus may not be reliable enough for exact quantification of mosaicism. Incidentally, in the case of 45,X/46,Xi(Xq) mosaics with 50% mosaicism, array CGH may actually only detect the monosomy at Xp. Hence, in the examination of complex TS karyotypes, a combination of array CGH and conventional cytogenetics appears to be optimal.

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