Copy Number Variants in Short Children Born Small for Gestational Age

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Key Words

Short stature · Small for gestational age · Growth · Copy number variations · Single nucleotide polymorphism array · Genetics

Abstract

Background/Aims: In addition to genome-wide association studies (GWAS), height-associated genes may be uncovered by studying individuals with extreme short or tall stature.

Methods: Genome-wide analysis for copy number variants (CNVs), using single nucleotide polymorphism (SNP) arrays, was performed in 49 index cases born small for gestational age with persistent short stature. Segregation analysis was performed, and genes in CNVs were compared with information from GWAS, gene expression in rodents' growth plates, and published information. **Results:** CNVs were detected in 13 cases. In 5 children a known cause of short stature was found: UPD7, UPD14, a duplication of the *SHOX* enhancer region, an *IGF1R* deletion, and a 22q11.21 deletion. In the remaining 8 cases, potential pathogenic CNVs were detected, either de novo (n = 1), segregating (n = 2), or not segregating

with short stature (n = 5). Bioinformatic analysis of the de novo and segregating CNVs suggested that *HOXD4*, *AGPS*, *PDE11A*, *OSBPL6*, *PRKRA* and *PLEKHA3*, and possibly *DGKB* and *TNFRSF11B* are potential candidate genes. A *SERPINA7* or *NRK* defect may be associated with an X-linked form of short stature. **Conclusion:** SNP arrays detected 5 known causes of short stature with prenatal onset and suggested several potential candidate genes.

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Introduction

Height, in childhood as well as in adulthood, is predominantly determined by genetic factors, and is considered a classic polygenic trait. It is now clear that, according to genome-wide association studies (GWAS), the number of loci associated with adult height is at least 180 [1]. The majority of the genes located in or close to these loci identified by GWAS have not been associated with severe growth disorders.

In the diagnostic process of a child with short stature, the clinician should start with a thorough medical history and physical examination, followed by targeted or screening laboratory and radiologic investigations, and in many cases tests to estimate growth hormone (GH) secretory status [2]. If physical characteristics are suspected for a congenital syndrome, consultation of a clinical geneticist is usually indicated [3]. This can lead to the suspicion of a specific disorder, which can then be confirmed with an appropriate genetic test (the candidate gene approach). In case of a low circulating IGF-I, specific genetic tests can be performed to establish the diagnosis [4, 5]. However, in most cases the clinician ends up with no diagnosis and classifies the condition under the term idiopathic short stature (ISS) or persistent short stature after being born small for gestational age (SGA) of unknown origin, depending on birth size [6]. In such cases, one can consider to perform a genome-wide analysis for copy number variants (CNVs) using single nucleotide polymorphism (SNP) arrays [7–9].

SNP array analysis is a powerful tool to detect CNVs. A major advantage of SNP arrays in comparison to array-based comparative genomic hybridization is that it offers SNP genotyping, which enables the detection of copy-number neutral chromosomal aberrations such as uniparental disomies (UPD) and loss of heterozygosity (LOH). UPD can result in rare recessive disorders, or developmental problems due to the effects of genomic imprinting. An even more powerful tool which is expected to be widely available in the coming years is whole-exome sequencing [10–12]. With this technique small CNVs and point mutations in coding regions of the genome can be detected.

In two previous papers from our group [5, 13] we have described the results of a candidate gene approach in children with short stature, either associated with a low birth size (SGA) or with a normal birth size (ISS) [14]. In a third paper we described the results of a genome-wide analysis for CNVs using SNP arrays in children with short stature [7]. Three studies on genome-wide association analysis of CNVs and stature showed that children with short stature had a greater global burden of lower frequency and rare deletions and a greater average CNV length than controls [8, 9, 15]. In the latter two reports, 10–16% of patients with short stature carried a disease-causing CNV [8, 9]. These observations suggest that CNVs might contribute to genetic variation in stature in the general population.

In the present paper we describe the results of a genome-wide analysis for CNVs in short children born SGA, in a further effort to identify novel gene variants associated with short stature.

Subjects and Methods

Patients

Patients were selected from the Tübingen fraction ($n = 199$) of the NESTEGG cohort, consisting of children with short stature (height after the age of 3 years ≤ -2 SDS according to Swiss references [16] that are representative for this South-West German population) who presented between 2002 and 2005 at the Tübingen Pediatric Endocrinology Outpatient Clinic. Selection criteria included a low birth weight and/or length (SGA) of unknown etiology; availability of sufficient good quality DNA, and absence of additional abnormalities at medical history, physical examination, biochemical investigations, and radiography of the hand and wrist, except for dysmorphic features or skeletal variants in some cases. Very preterm birth (<30 weeks of gestation) and complicated postnatal period (ventilation >72 h) were exclusion criteria, as well as diseases with potentially negative effects on growth (asthma, chronic diarrhea, celiac disease, endocrine disorders including GH deficiency, congenital malformations, known syndromes or chromosomal aberrations). Developmental delay and mild dysmorphic features were not considered exclusion criteria.

SGA was defined as a birth weight (BW) and/or birth length (BL) <-2 SDS [17]. Birth data were obtained from the preventive healthcare check-up booklet that every German child receives after birth. 49 subjects complied with these criteria. BW and BL were available for all patients, birth head circumference (BHC) in 40 of them.

At inclusion into the study, auxological measurements and laboratory analysis were performed. Height, head circumference (HC) [16], body mass index (BMI) [18], and serum IGF-I and IGFBP-3 levels [19, 20] were expressed as SDS for age and gender. For children under GH treatment the last height measurement before start of therapy was used. Clinical data on 47 of these children were described previously [21], as well as the results of *IGF1*, *IGF1R* and *SHOX* mutation analysis [13]. In the latter publication the clinical data of cases I.3 and I.4 were presented, but in order to provide the reader with a full picture of the diagnostic yield of SNP arrays we include these patients also in the present paper. Informed consent was obtained from parents and if appropriate from the patient. The study was approved by the medical ethics committee of the University of Tübingen.

Genetic Analysis

Genomic DNA was extracted from peripheral venous blood samples [22]. Concentrations were measured using a Nanodrop® ND-1000 spectrophotometer (Isogen Life Science, De Meern, The Netherlands). SNP array analysis was performed using the Affymetrix GeneChip® Human Mapping 262K *NspI* array (Affymetrix, Santa Clara, Calif., USA), containing 262,262 25-mer oligonucleotides. An amount of 250 ng DNA was processed according to the manufacturer's protocol. SNP copy numbers were assessed using the software program CNAG (Copy Number Analyzer for GeneChip®) version 2.0 and 3.0 [23].

Evaluation of CNVs

Analysis was performed of deletions of at least five adjacent SNPs and a minimum region of 150 kb and duplications of at least seven adjacent SNPs and a minimum region of 200 kb [24]. The rationale of this approach was to minimize the number of false-

positive findings. The detected CNVs were categorized into four groups: (I) known pathogenic CNVs (known microdeletion or microduplication syndrome or UPD); (II) potentially pathogenic CNVs, not described in the Database of Genomic Variants (DGV; The Centre of Applied Genomics, The Hospital for Sick Children, Toronto, Ont., Canada, <http://projectsc.tcag.ca/variation/>); (III) CNVs not described in the DGV, but not containing any protein-coding genes, and (IV) known polymorphic CNVs described in the DGV or observed in our in-house reference set, whereby at least 3 individuals must have been reported with the same rearrangement. All type III and IV CNVs were excluded from further analysis. The type II CNVs were further assessed with Ensembl release 71 (April 2013; Wellcome Trust Genome Campus, Hinxton, Cambridge, UK, <http://www.ensembl.org>) for gene content and DECIPHER (Wellcome Trust Genome Campus) for similar patients. The possible function of microRNAs in the CNVs was evaluated using two specific databases (miRBase and miRTarBase) [25, 26] and PubMed.

Validation of CNVs

Multiplex ligation-dependent probe amplification (MLPA) analysis was used to validate CNVs encompassing *SHOX* and *IGF1R* genes, using the SALSA MLPA probe mix P018C *SHOX* and the SALSA MLPA probe mix P217 *IGF1R*, respectively, according to the manufacturer's instructions (MRC-Holland, Amsterdam, The Netherlands). Amplification products were identified and quantified by capillary electrophoresis on an ABI 3130 genetic analyzer (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands). Analysis was performed using the GeneMarker[®] genotyping software (SoftGenetics, State College, Pa., USA). Thresholds for deletions and duplications were set at 0.75 and 1.25, respectively [27]. If parents were available, segregation analysis was performed by SNP array or MLPA analysis.

Bioinformatics

For all type II CNVs we used three approaches. First, we assessed whether they were located in one of the chromosomal regions associated with height in GWAS [1], and whether the genes in the deleted or duplicated regions were known to be associated with short stature in the Online Mendelian Inheritance in Man (OMIM) [28] or Gene Reference into Function (GeneRIF; <http://www.ncbi.nlm.nih.gov/gene>) database. In addition, genes were mapped to biological pathways in the Gene Ontology [29] and ConsensusPath database [30] to see whether they were involved in growth. To facilitate the process of retrieving gene-specific information from databases and integrating the results we used an automated workflow that had been developed in-house.

Second, the rodent homologues were checked for three criteria: (1) higher expression in 1-week-old mouse growth plate than in 1-week-old mouse lung, kidney, and heart; (2) spatial regulation: significant difference between zones in the 1-week-old rat growth plate, and (3) temporal regulation: significant difference between 3 and 12 weeks of age in the rat growth plate using previously established mRNA expression profiles [7, 31, 32].

Third, associations were investigated for mouse growth plate-related phenotypes. For details, see Lui et al. [32] and Van Duyvenvoorde et al. [7].

Results

Uniparental Disomy

In 2 cases a UPD was found that is known to be associated with short stature. Clinical details are presented in tables 1 and 2.

Case I.1 showed LOH of chromosome 7 (UPD) of maternal origin (matUPD7), one of the known causes of Silver-Russell syndrome. Minor anomalies that segregate with the genotype were a prominent forehead and bone deformities (late dentition and clubfeet).

Case I.2 presented with LOH of chromosome 14, inherited from his mother (matUPD14). Anomalies that have previously been described in maternal UPD14 were also seen in this case, including bone deformities (kyphosis, wedge-shaped vertebra Th2) and precocious puberty.

Type I CNVs

In 3 cases a type I CNV was observed, including a duplication of the *SHOX* enhancer region, an *IGF1R* deletion, and a 22q11.21 deletion (tables 1, 2).

In case I.3, a boy with severe intrauterine growth retardation, and subsequent proportionate growth failure and microcephaly in childhood, a duplication in Xp22.32, 177 Kb downstream of the *SHOX* gene, was detected (PAR probes 3–12 with MLPA analysis), as we reported previously [13]. The minimal duplication only contains the *SHOX* enhancer region, no known protein-coding genes, but the maximal duplicated region contains five protein-coding genes. The duplication was found to be also present in the mother who had a marginally low BW and a normal height (table 2). Sequence analysis of *SHOX*, *IGF1R* and *IGF1*, as well as MLPA for *IGF1R* showed no additional abnormalities.

In case I.4, also reported previously [13], a terminal 4.11-Mb deletion of the long arm of chromosome 15, including the *IGF1R* gene, was found. Subsequent MLPA analysis showed a de novo heterozygous deletion of all probes of *IGF1R* gene (exon 1–21) including the two flanking probes located 2.0 and 2.8 Mb downstream of *IGF1R*.

Patient I.5 showed a maternally inherited 22q11.21 deletion of 2.52 Mb (velocardiofacial or DiGeorge syndrome, MIM #192430). Maternal height was in the low-normal range.

Type II CNVs

Type II CNVs were found in 8 unrelated cases (16.7%). Clinical and biochemical data are shown in table 3. Segregation analysis could be performed for all cases, which

Table 1. UPDs and type I CNVs

ID M/F	Ht SDS	Karyotype (ISCN 2009) Start–end	Size, kb	Protein-coding genes ^a and miRNAs	Clinical details
<i>UPD</i>					
I.1 M	–2.8	mat UPD7	N/A	N/A	Prominent forehead, bone deformities (clubfeet, late dentition)
I.2 M	–1.8 ^b	matUPD14	N/A	N/A	Minor facial abnormalities (triangular face), clinodactyly, typical bone deformities (kyphosis, wedge-shaped vertebrae Th2), precocious puberty
<i>Type I CNVs</i>					
I.3 M ^c	–2.8	arr Xp22.33 (682,101–1,522,908)×3 mat	840.8	<i>CRLF2</i> , <i>CSF2RA</i> , <i>IL3RA</i> , <i>SLC25A6</i> and part of <i>ASMTL</i> ; including the <i>SHOX</i> enhancer region (PAR3-12); MIR3690	No signs of Leri-Weill syndrome
I.4 F ^d	–4.6	arr 15q26.2q26.3 (98,276,327–102,531,392)×1 dn	4,255.1	23 protein-coding genes; from <i>ARRDC4</i> to <i>OR4F15</i> , including <i>IGF1R</i> and <i>ADAMTS17</i> ; MIR4714	Microcephaly, relatively short right leg, short philtrum, malrotation of right ear
I.5 M	–3.0	arr 22q11.21 (18,640,301–21,928,915)×1 mat	3,288.6	54 protein-coding genes; from part of <i>USP18</i> to part of <i>UBE2L3</i> and 6 miRNAs; contains the typical ~3 Mb 22q11 deletion (DECIPHER: 22:19009792–21452445)	Small deeply set ears, small nostrils, small mouth, hair swirls, shawl scrotum, epicanthus, syndactyly of toes (II–III), joint hypermobility

mat = Maternally inherited; dn = de novo; N/A = not applicable.

^a For CNVs containing ≤5 protein-coding genes, all protein-coding genes are shown. For CNVs containing ≥6 protein-coding genes, the number, and the first and last protein-coding gene is given. ^b Height SDS was <–2 SDS from 0 to 6 years. ^c Previously published [13] as case 2. ^d Previously published [13] as case 7.

Table 2. Auxologic and biochemical features of patients with UPDs and type I CNVs

ID, M/F	Age, years	BW SDS	BL SDS	BHC SDS	Height SDS	BMI SDS	HC SDS	Pat height SDS	Mat height SDS	IGF-I SDS	IGFBP-3 SDS	BA/CA
I.1, M	2.78	–2.5	–0.3	–0.5	–2.8	–1.9	0.4	–0.1	0.8	–3.8	–0.1	
I.2, M	6.77	–3.5	–2.2	–2.7	–1.8	–2.2	–2.0	2.3	0.0			5.5/6.8
I.3, M	7.47	–3.2	–2.7	–2.2	–2.8	–3.75	–3.3	–0.9	0.2	–2.6	–1.2	6.0/7.5
I.4, F	3.29	–1.7	–2.3	–1.8	–4.6	–1.0	–4.8	–0.2	–1.5	0.6	2.6	2.0/3.3
I.5, M	7.41	–2.3	–2.0	–1.1	–3.0	–0.7	–2.7	–1.7	–1.3	–2.8	–1.5	

ID = Identifier; BW = birth weight; BL = birth length; BHC = birth head circumference; BMI = body mass index; HC = head circumference; Pat = paternal; Mat = maternal; BA/CA = bone age/chronological age; M = male; F = female.

led to 1 de novo CNV and 2 CNVs inherited from a parent with short stature. Genetic details and the arguments for and against possible pathogenicity of the CNV for short stature are shown in table 4. The results of the comparison with gene expression profiles in rodent growth plates are shown in online supplementary table 1 (see www.karger.com/doi/10.1159/000367712 for all online suppl. material).

Bioinformatic analysis of the genes within the only de novo type II CNV identified six potential candidate genes (*HOXD4*, *AGPS*, *PDE11A*, *OSBPL6*, *PRKRA* and

PLEKHA3) and one microRNA (MIR10B) for which either associations with skeletal pathology in mice (*Hoxd4*) or humans (*AGPS*) were found, associations with height in GWAS (*PDE11A*) or differential expression profiles in rodents (*Osblp6*, *Plekha3* and *Prkra*) (online suppl. table 1).

The other gene for which deletions in mice produce a growth plate or other skeletal phenotype, *Tnfrsf11b*, located in the deleted region found in case II.3, encodes osteoprotegerin (OPG), which negatively regulates osteoclast formation, and null mutations in mice cause severe

Table 3. Auxologic and biochemical features of patients with type II CNVs (see table 2 for abbreviations)

ID, M/F	Age, years	BW SDS	BL SDS	BHC SDS	Height SDS	BMI SDS	HC SDS	Pat height SDS	Mat height SDS	IGF-I SDS	IGFBP-3 SDS	BA/CA	Clinical details
II.1, F	12.19	-2.2	-0.8	-2.7	-3.0	-2.1	-1.5	0.9	0.4	-1.4	-0.1	8.5/12.2	No dysmorphic features
II.2, M	11.97	-1.3	-2.6	-0.7	-2.5	-0.2	-1.0	-1.5	-2.7	0.1	0.1	10.5/11.4	No dysmorphic features
II.3, F	9.14	-2.1	-2.4	-1.6	-2.8	-0.5	-1.2	-1.0	0.4	-0.4	0.4	7.0/9.1	Stout trunk, inverse neck hair
II.4, M	2.83	-1.9	-2.2	-2.9	-2.5	-1.3	1.0	-0.4	1.1	-0.6	-1.7	2.0/2.8	Triangular face, GH treatment
II.5, M	4.53	-2.1	-2.6	-1.4	-3.1	-0.8	-2.2	-1.5	-0.1	-1.7	-1.7	2.0/4.5	No dysmorphic features
II.6, F	10.12	-4.1	-5.0	n.a.	-2.8	-3.4	-1.0	1.2	0.6	-0.4	0.6	10/10.1	Triangular face, SRS
II.7, M	7.14	-2.2	-2.4	-1.7	-3.2	-0.2	-2.4	-0.8	-0.8	-1.7	-1.4	5.0/7.1	No dysmorphic features
II.8, F	2.43	-3.5	-2.4	-2.1	-4.6	-3.4	-1.5	-0.1	-0.8	-0.8	2.2		Triangular face, body asymmetry, SRS

Table 4. Type II CNVs: de novo or segregating with short stature (height SDS in carrier ≤ -1.0)

ID M/F	Karyotype (ISCN 2009)	Size, kb	Protein-coding genes ^a and miRNAs	Arguments pro pathogenicity	Arguments contra pathogenicity
II.1 F	arr 2q31.1q31.2 (177,004,116–179,748,446)×1 dn	2,744.3	18 protein-coding genes; from <i>HOXD3</i> to <i>CCDC141</i> , including <i>HOXD4</i> , <i>AGPS</i> , <i>PDE11A</i> , <i>OSBPL6</i> , <i>PRKRA</i> , <i>PLEKHA3</i> ; MIR10B, MIR4444-1, MIR3128	<i>Hoxd4</i> knockout mice show skeletal abnormalities; <i>AGPS</i> associated with rhizomelic chondrodysplasia; <i>PDE11A</i> listed as candidate gene in GWAS Differential expression of <i>Osbpl6</i> , <i>Prkra</i> , and <i>Plekha3</i> in rodent growth plate; de novo	
II.2 M	arr 7p21.2 (14,876,918–15,081,347)×1 pat	204.4	Part of diacylglycerol kinase, beta 90 kDa (<i>DGKB</i>)	Key role in cellular processes by regulating the second messenger diacylglycerol (DAG); overlap with various small losses in DGV, not overlapping <i>DGKB</i> ; father carrying the CNV is rather short (–1.5 SDS)	Mother not carrying the CNV is short (–2.7 SDS)
II.3 F	arr 8q24.12 (119,272,494–119,937,740)×3 pat	665.2	Part of <i>SAMD12</i> , part of <i>TNFRSF11B</i>	Father carrying the CNV is rather short (–1.0 SDS) <i>Tnfrsf11b</i> strongly expressed in the murine growth plate, knockout mice showed osteoporosis and destruction of growth plate	Father is less short than his child

dn = De novo; pat = paternally inherited.

^a For CNVs containing ≤ 5 protein-coding genes, all protein-coding genes are depicted. For CNVs containing ≥ 6 protein-coding genes, the number, and the first and last protein-coding gene in the CNV are given.

osteoporosis, growth retardation, and destruction of the growth plate [33, 34]. Also *DGKB*, part of which is duplicated in case II.2, can be considered as a potential candidate gene for short stature.

Five cases had inherited the CNV from a parent with a height within the normal range (online suppl. table 2). For 4 cases this makes it a priori unlikely that these CNVs are causally related to short stature, although several genes in the CNVs are preferentially expressed in the murine growth plate, or spatially or temporally expressed in the rat

(online suppl. table 1). Two cases were later found to show features of Silver-Russell syndrome (II.6 and II.8), which was genetically confirmed (online suppl. table 2), and compatible with the phenotype. For the duplication located in the X chromosome in case II.4, the normal maternal height does not decrease the likelihood of an association with short stature, because an X-linked inheritance pattern would be expected. In fact, this duplication contains two interesting candidate genes (*SERPINA7* and *NRK*).

Discussion

Whole-genome SNP array analysis in 49 patients born SGA with persistent short stature led to the detection of 2 maternal UPDs, 3 CNVs that are known to cause short stature, 1 de novo deletion and 2 CNVs segregating with short stature. The de novo 2q31.1 deletion contained several potential candidate genes (*HOXD4*, *AGPS*, *PDE11A*, *OSBPL6*, *PRKRA*, and *PLEKHA3*), and the two CNVs segregating with short stature contained genes that also could be candidates for further study (*DGKB* and *TNFRSF11B*). In addition, two genes in the duplication in Xq22.3 (*SERPINA7* and *NRK*) may be associated with an X-linked syndrome of growth failure based on previous similar observations.

With regard to the cases where the SNP array analysis led to the diagnosis of a syndrome known to be associated with short stature, it should be emphasized that at the time of the analysis these syndromes were not suspected. However, in retrospect, the clinical features of the children with UPD 7 and UPD14 were consistent with these diagnoses. The 22q11.21 deletion (1.5) (velocardiofacial or DiGeorge syndrome) is known to be associated with short stature and several other clinical and biochemical features [35–37].

The cases with the *SHOX* duplication and *IGF1R* deletion showed only few of the known clinical features of these syndromes, but with the clinical scores that we developed previously, there was sufficient basis for genetic testing [13]. The phenotype of case I.3, with a duplication of the *SHOX* downstream enhancer region, was reported in more detail in a previous paper from our group [13]. It illustrates that the clinical picture of *SHOX* haploinsufficiency is not only observed in cases with a *SHOX* mutation or deletion but also in cases with a duplication of *SHOX* [38, 39], or deletions or duplications of the downstream enhancer region (PAR probes 3–12) or upstream enhancer region [13, 40–46]. Case I.4 carries a large terminal deletion of 15q containing *IGF1R*, as recently reported [13], which is now a well-established cause of persistent short stature of SGA born children [13, 47–49].

We reasoned that some of the identified CNVs might cause short stature because they contain genes that are expressed and function in the growth plate. We therefore used existing expression microarray data to identify genes that show greater expression in mouse growth plate than in soft tissues, temporal regulation in rat growth plate, or spatial regulation in rat growth plate. For the three type II CNVs which were either de novo or segregated with short stature, this approach implicated four genes

(*Osbp16*, *Plekha3*, *Prkra* and *Tnfrsf11b*) (online suppl. table 1). In addition, we performed a bioinformatic analysis to establish which deletions in mice produce a growth plate or other skeletal phenotype. This information, in combination with clinical data and data retrieved from online biological knowledge bases, was used to formulate the arguments pro and contra an association of these genes with short stature (summarized in table 4).

Bioinformatic analysis of case II.1 with de novo type II CNV led to several potentially interesting candidate genes (table 4). The first is *HOXD4*, because heterozygous and homozygous null mutations of *Hoxd4* in mice exhibit homeotic transformation of the second cervical vertebrae and malformed neural arches of C1–C3 and of the basioccipital bone [50]. The second is *PDE11A*, because a SNP located within this gene is associated with adult height variation in GWAS [1]. However, the clinical syndrome of individuals with a heterozygous mutation in this gene (primary pigmented nodular adrenocortical disease, PPNAD2; MIM 610475) [51] is not consistent with the characteristics of our patient. Homozygous or compound heterozygous mutations in *AGPS*, the gene encoding alkyl-dihydroxyacetonephosphate synthase (alkyl-DHAP synthase) (MIM 603051), cause rhizomelic chondrodysplasia punctata type 3 (RCDP3) [52]. There are no published data on heights of heterozygous carriers, but they appear of normal stature [Dr. Nancy Braverman, pers. commun.]. Homozygous or heterozygous mutations in the *PRKRA* gene are associated with generalized dystonia (DYT16, MIM 612067) [53, 54]. Interestingly, *Prkra* is preferentially expressed in the murine growth plate and its expression is higher in the hypertrophic zone than the proliferative zone in the rat. Two other genes are also differentially expressed in rodent growth plates (*Osbp16* and *Plekha3*). We speculate that in this case either haploinsufficiency of one of the genes located in this region may have an impact on growth, or there may be a combined effect of haploinsufficiency of several genes in this locus.

In case II.2, the deletion of part of *DGKB* segregates with moderate short stature of the father (–1.5 SDS). *DGKB* plays a key role in cellular processes by regulating the second messenger diacylglycerol (DAG). However, no human disorders have been associated with *DGKB* variants. Since the mother is as short as her child (–2.7 and –2.5 SDS, respectively), it appears more likely that a maternal gene variant plays a role.

One of the genes in the small duplication observed in case II.3 (*TNFRSF11B*, encoding OPG), may be an interesting candidate gene, since *Tnfrsf11b* is very strongly expressed in the murine growth plate and temporally regu-

lated in the rat growth plate, and null mutations in mice cause severe osteoporosis, growth retardation, and destruction of the growth plate [33, 34]. However, homozygous mutations in this gene cause juvenile Paget disease [55] and are not associated with abnormal body growth. The duplication also contains part of the *SAMD12* gene, for which no function is known.

Out of the 5 cases in whom the CNV was transmitted from a parent with normal height, case II.4 is noteworthy. This refers to the maternal Xq22.3 duplication containing *SERPINA7*, encoding thyroxine-binding globulin (TBG). In three papers, TBG excess by gene duplication was reported to be associated with short stature [56–58], which responded to T₃ treatment in terms of catch-up growth [56]. In one of these cases, GH deficiency was observed [57]. However, in two other reports [59, 60] no mention was made about short stature. Our patient had indeed an elevated serum TBG, so that this may be a further example of the possible role of TBG excess on growth. Another patient with an identical CNV (DECIPHER, id 249139) and short stature (height –2.1 SDS) was reported by us in a previous paper (case II.23, online suppl. tables [7]). Interestingly, another gene in this region is strongly expressed in the murine growth plate (*Nrk*), so it is also possible that growth failure is associated with overexpression of *NRK*.

In conclusion, whole-genome SNP array analysis in this exploratory study on 49 patients born SGA with persistent short stature identified 13 CNVs (27%), including

2 maternal UPDs (UPD 7 and UPD14) and 3 CNVs for which the association with short stature is virtually certain (*SHOX* duplication, *IGF1R* deletion and 22.q11 deletion). This percentage is in the same range as observed in previous studies [7–9]. Several of the deleted genes in the de novo CNV may be considered potential candidate genes, but confirmation is needed by future studies. From the two type II CNVs segregating with short stature, *DGKB* and *TNFRSF11B* deserve further study, and we speculate that a *SERPINA7* or *NRK* duplication may be associated with X-linked short stature.

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Disclosure Statement

J.M.W. has served on advisory boards for Tercica, Ipsen, Pfizer, Prolog, Teva and Biopartners and has received speaker honorariums from Pfizer, Lilly, Ipsen and Ferring. The remaining authors have no conflicts of interest to disclose.

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