The emerging role of genomics in the diagnosis and workup of congenital urinary tract defects: a novel deletion syndrome on chromosome 3q13.31-22.1

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Abstract

Background Copy number variants (CNVs) are increasingly recognized as an important cause of congenital malformations and likely explain over 16 % of cases of congenital anomalies of the kidney and urinary tract (CAKUT). Here, we illustrate how a molecular diagnosis of CNV can be beneficial to the clinical management of a pediatric patient presenting with CAKUT and other organ defects.

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Methods We describe a 14-year-old girl with a large de novo deletion of chromosome 3q13.31-22.1 that disrupts 101 known genes. The patient presented with CAKUT, neurodevelopmental delay, agenesis of corpus callosum (ACC), cardiac malformations, electrolyte and endocrine disorders, skeletal abnormalities and dysmorphic features. We performed extensive annotation of the deleted region to prioritize genes for specific phenotypes and to predict future disease risk.

Results Our case defined new minimal chromosomal candidate regions for both CAKUT and ACC. The presence of the *CASR* gene in the deleted interval predicted a diagnosis of hypocalciuric hypercalcemia, which was confirmed by the serum and urine chemistries. Our gene annotation explained clinical hypothyroidism and predicted that the index case is at increased risk of thoracic aortic aneurysm, renal cell carcinoma and myeloproliferative disorder.

Conclusions Extended annotation of CNV regions refines the diagnosis and uncovers previously unrecognized phenotypic features. This approach enables personalized treatment and prevention strategies in patients harboring genomic deletions.

Keywords Copy number variants · Chromosome deletion · CAKUT · Neurodevelopmental delay · Agenesis of corpus callosum · Genetics · Clinical management

Introduction

Structural genomic defects have been increasingly recognized as important causes of syndromic and isolated congenital malformations. However, because of limited resolution of standard karyotyping, large chromosomal aberrations are identified only in a small fraction of cases using current cytogenetic tools. Submicroscopic genomic structural defects

that escape standard cytogenetic screening are likely to explain a substantial portion of congenital malformations [1, 2]. Such defects can be either inherited (in familial disease) or occur de novo (in sporadic disease).

In recent years, substantial technological progress has allowed for improved detection of small genomic rearrangements. Two commonly used methods include array-based comparative genomic hybridization (aCGH) and high-density single nucleotide polymorphism (SNP) arrays. Recent studies have demonstrated the ability of these methods to detect pathogenic copy number defects in a large percentage of patients with neurodevelopmental phenotypes [2, 3], schizophrenia [4, 5], autism [6], cardiac malformations [7, 8] and short stature [9, 10]. In one of the largest studies to date, diverse pathogenic copy number variants (CNVs) accounted for 14.2 % of cases among 15,767 children with intellectual disability and variable congenital defects [3]. A number of criteria are commonly used to establish the pathogenicity of CNV events, including (1) large CNV size, (2) disruption of coding gene sequence, (3) inheritance pattern (e.g. CNVs co-segregating with a phenotype in familial cases or occurring de novo in sporadic cases) and (4) absence or extremely low frequency in the general population [11]. Despite these criteria, however, some pathogenic CNVs may have incomplete penetrance and, consequently, their clinical interpretation has to be carefully considered on an individual basis.

We recently studied a large cohort of patients with congenital renal defects (renal hypodysplasia and agenesis) and found that over 16.6 % of such defects could be explained by pathogenic submicroscopic deletions or duplications [1]. The majority of these genomic variants were previously associated with variably penetrant neurodevelopmental disorders, extra-renal malformations or systemic disorders. These data indicated that disruption of genes within the CNV intervals could also explain extra-renal manifestations and that early molecular diagnosis could positively impact the clinical care of patients. For example, congenital anomalies of the kidney and urinary tract (CAKUT) patients with cystic renal dysplasia who carry a molecular diagnosis of deletions at the *HNF1B* locus are also at risk for developing diabetes mellitus, hyperuricemia, genital defects and infertility [12, 13]. Thus, early detection of this molecular defect is highly relevant to an individual's reproductive health and may also alert clinicians to frequent screening for glucose intolerance, thus facilitating early detection, treatment and prevention of long-term diabetic complications.

Here, we provide detailed data on the clinical and molecular characterization of a sporadic case carrying a new de novo 15-Mb deletion of chromosome 3q13.31-22.1 that was initially missed by standard cytogenetic tools. The patient presented for re-evaluation at the age of 14 years with dysmorphic features and multiple malformations of internal organs, including right renal agenesis and grade IV left vesicoureteral reflux (VUR), at which time the CNV analysis was performed. With the results in hand, we re-evaluated the patient in the context of her genetic diagnosis.

Case

A 14-year-old Polish girl with dysmorphic features was referred to us for clinical evaluation. The proband was the first child born to healthy, non-consanguineous parents. At the time of conception, the mother was 22 and the father was 25 years old. The pregnancy was complicated by placental abruption requiring a C-section in the 35th week of pregnancy. Asphyxia was present at birth with the Apgar scores being 0, 0 and 4 at 1, 5, and 10 min after birth, respectively. Regular strong heartbeat was observed at 10 min and regular spontaneous breathing at 50 min after birth. The karyotype was 46XX, and no major chromosomal aberrations were detected by initial cytogenetic evaluation.

At the age of 14 years the proband was seen in the clinical genetics clinic at the Center for Medical Genetics (GENESIS, Poznan, Poland). At this time, multiple dysmorphic features were clearly noticeable (Fig. 1). Skeletal abnormalities included short stature (height 133 cm, weight 23 kg), scoliosis, contraction of the left forearm and left hand, phalangeal hypoplasia, hypoplastic thumbs, absent radial bones, left clubfoot varus and syndactyly (2nd and 3rd toes). Facial dysmorphic features included macrostomia (repaired surgically at the age of 15 months), narrow palpebral fissures and right auricular tags. Neurological evaluation revealed severe neurodevelopmental delay. A generalized hypotonia was present since birth. The patient had a reported history of epilepsy characterized by involuntary ocular movements for which she was maintained on antiepileptic medications. Brain imaging documented cerebral atrophy with agenesis of corpus callosum (ACC) (Fig. 2). In addition, the patient had a history of mild myopia, but fundoscopic exam revealed no major retinal abnormalities. Her hearing was intact.

The patient also had several malformations of internal organs. Early echocardiographic evaluation revealed patent ductus arteriosus with aortic coarctation (repaired surgically at the age of 7 weeks), a small ventricular septal defect, a patent foramen ovale and an asymmetric bicuspid aortic valve. The urogenital abnormalities included complete right renal agenesis, grade IV left VUR, left-sided hydronephrosis and a prominent clitoris. A successful surgical anti-reflux procedure was performed at the age of 2 years, with follow-up voiding cystourethrogram showing only mild (grade I) residual left-sided VUR. The parameters of renal function (blood urea nitrogen and creatinine) remained within normal limits for her age, and there was no evidence of proteinuria on serial urinalyses after the procedure. Additionally the patient had left thyroid lobe hypoplasia and associated hypothyroidism. This
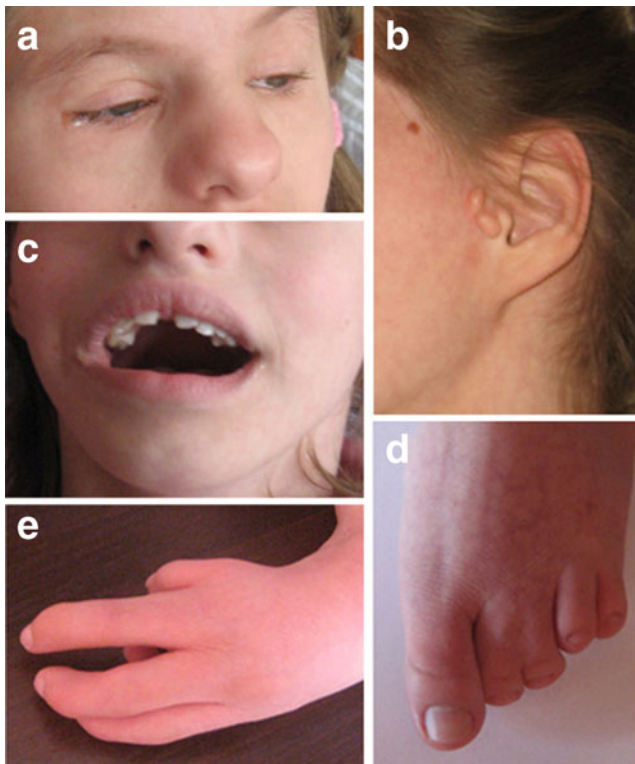
Fig. 1 Dysmorphic features of the proband. **a** Blepharophimosis, **b** preauricular tags, **c** macrostomia, **d** left foot syndactyly involving second and third toe, **e** left upper extremity contracture with underdeveloped phalanges and vestigial left thumb

diagnosis was established at the age of 16 months: thyroid-stimulating hormone (TSH) level was 4.2 mU/l (normal 0.4–4.0 mU/l), FT4 level was 8.1 pmol/l (normal 8.5–19.0 pmol/l), and thyroid ultrasound revealed a small hypoechoic thyroid gland with severe left lobe hypoplasia. At this time, the patient was initiated on thyroid hormone replacement therapy.

Notably, the family history was negative for neurodevelopmental disorders, hypothyroidism, heart disease or kidney
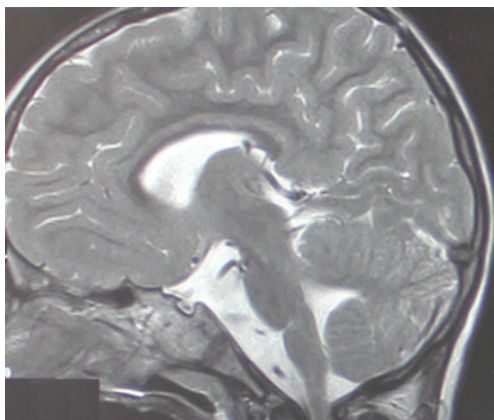

Fig. 2 Magnetic resonance image of the head (*sagittal view*) demonstrates generalized cortical atrophy and agenesis of corpus callosum

failure. There was no maternal history of pre-term deliveries, miscarriages or abortions.

Results

Detection of the novel 3q13.31-22.1 deletion

Taking the extensive multi-organ involvement into consideration, we hypothesized that a submicroscopic CNV disrupting multiple genes may be responsible for the severe phenotypic features observed in this case. As outlined in our recent paper [1], we have developed a stringent analytic pipeline for identification of pathogenic CNVs in individuals with severe congenital malformations. Briefly, the first step of the protocol is a genome-wide analysis of CNVs using the 610-Quad SNP array (Illumina, San Diego, CA). The CNVs are identified using generalized genotyping methods, as implemented in the PennCNV software [14] and then mapped to the human reference genome hg18. In the case of our 14-year-old patient, we identified a large deletion of 14,960,554 bp involving chromosome 3q13.31-22.1 (Fig. 3). The breakpoints of the deletion were from rs11921574 (chr3:118,151,853 bp) to rs957919 (chr3:133,112,406 bp) and spanned over 3,088 probes on the Illumina chip. This deletion was completely novel and was absent in public databases and in our panel of 13,839 population controls genotyped with SNP chips of comparable density (Illumina's 550 K or higher).

Deletion confirmation and annotation of the region

The deletion was confirmed by quantitative real time-PCR and SNP genotyping via traditional Sanger sequencing in the index case and the unaffected parents. The CNV was absent in both parents, indicating a de novo event. Additional genotyping of highly informative SNPs (heterozygosity approx. 0.5) in the region confirmed that the index case carried a maternal haplotype in homozygosity, thereby also confirming that the deletion occurred de novo in the paternal chromosome. We next performed comprehensive annotation of this region using public databases, including the National Center for Biotechnolgy Information (NCBI) Genome Browser (URL: http://www.ncbi.nlm.nih.gov/), Genome Reviews (URL: http://www.ebi.ac.uk/GenomeReviews/), Online Mendelian Inheritance in Man (OMIM; URL: http://www.omim.org/) and PubMed (www.ncbi.nlm.nih.gov/pubmed/). According to the NCBI Genome Browser (build 37.2), the region of the 3q13.31-22.1 deletion contains 101 known genes (Fig. 3). We cross-referenced these transcripts against the OMIM database and identified 23 annotated genes previously associated with a disease phenotype in humans (Table 1). Using the probabilistic model that integrates genomic, evolutionary, functional

Fig. 3 Detailed view of the 3q13.31-22.1 deletion region: *top panel* absence of heterozygous calls within the 3q13.31-22.1 locus, *middle panel* decreased probe intensity (Log R Ratio) in the same region, *bottom panel* known annotated genes within the deleted interval (NCBI v.37.2)
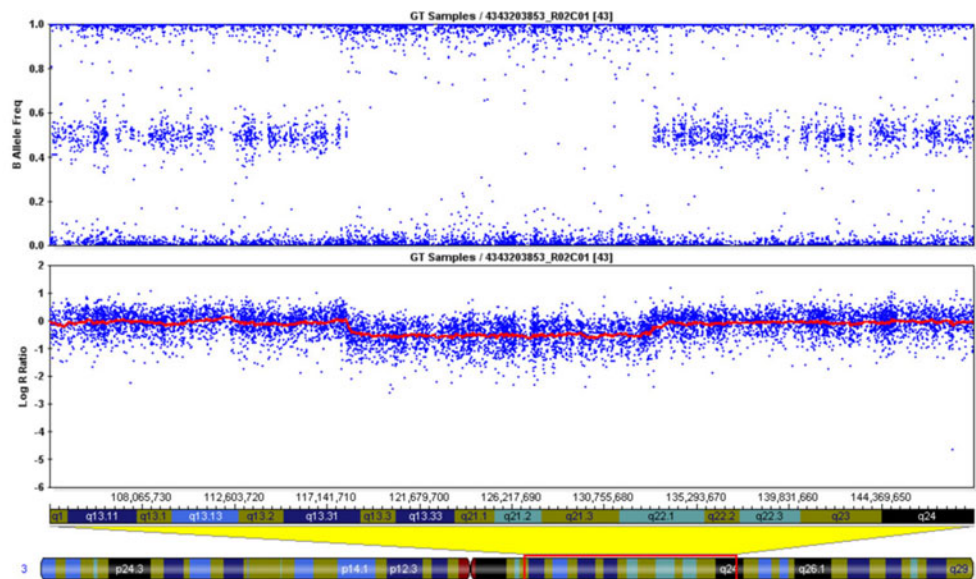
101 known genes encompassed by the 3q13.31-22.1 deletion (NCBI Genome Browser):
IGSF11 UPK1B B4GALT4 CDGAP TMEM39A KTELC1 CD80 ADPRH PLA1A POPDC2 COX17 NR1I2 GSK3B GPR156 FSTL1 NDUFB4 HGD RABL3 GTF2E1 STXBP5L POLQ ARGFX FBXO40 HCLS1 GOLGB1 IQCB1 EAF2 SLC15A2 ILDR1 CD86 CASR CSTA CCDC58 FAM162A WDR5B KPNA1 PARP9 DTX3L PARP15 PARP14 HSPBAP1 DIRC2 SEMA5B PDIA5 SEC22A ADCY5 MYLK ROPN1 KALRN UMPS ITGB5 MUC13 SLC12A8 ZNF148 SNX4 OSBPL11 ALG1L ROPN1B SLC41A3 ALDH1L1 KLF15 ZXDC UROC1 CHST13 TXNRD3IT1 TXNRD3 CHCHD6 PLXNA1 TPRA1 MCM2 PODXL2 ABTB1 MGLL KBTBD12 SEC61A1 RUVBL1 EEFSEC DNAJB8 GATA2 RPN1 RAB7A ACAD9 GP9 RAB43 ISY1 CNBP COPG H1FX MBD4 IFT122 RHO H1FOO PLXND1 TRH COL29A1 PIK3R4 ATP2C1 ASTE1 NEK11 MRPL3 CPNE4

and network properties of haploinsufficient genes [15], we predicted that the deletion is highly deleterious with a haploinsufficiency LOD score of 49.2. We next compared the deleted region against the ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosomal Aberrations; URL: http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp) and DECIPHER (URL: http://decipher.sanger.ac.uk/) databases of rare cytogenetic abnormalities. All previously reported deletions overlapping with the 3q13.31-22.1 region are summarized in Table 2. For each of the observed phenotypes in our case, we performed detailed analysis of candidate genes. Moreover, considering the frequent occurrence of CAKUT and ACC among these cases, we performed detailed mapping of partially overlapping deletions in patients with these phenotypes (Fig. 4). The analysis was performed under the assumption of incomplete penetrance, thus only the cases with reported CAKUT or ACC phenotypes were included in the mapping.

Neurodevelopmental delay and ACC

Intellectual disability and neurodevelopmental delay are frequently observed in individuals harboring a variety of large genomic rearrangements, suggesting that the appropriate dosage of many different genes is critical for proper development of the central nervous system. Accordingly, neurodevelopmental delay was present in virtually all of previously reported patients carrying 3q13.31-22.1 deletions (Table 1). However, six patients (including the present case) had a specific rare finding of ACC on brain imaging (Fig. 2) [16–23]. Detailed analysis of deletion breakpoints revealed that our case effectively narrows down the minimal region of overlap for ACC to bands 3q13.32-13.33 (chr3:118,152–123,400 kb; Fig. 4). This chromosomal interval contains a total of only 34 NCBI reference genes and has a predicted haploinsufficiency LOD of 12.6. Ranking these genes based on their individual haploinsufficiency prediction scores defined *GSK3B* (OMIM 605004) as the top candidate gene, with 97.5 % estimated probability of having serious phenotypic manifestations [15]. The protein encoded by this gene is a serine-threonine kinase involved in neuronal cell development [24] and body pattern formation [25]. Notably, a recent aCGH study found two independent overlapping duplications of 3q13.33 as a cause of holoprosencephaly, one of the most common forms of forebrain malformations in humans [26]. While the first duplication spanned 3.8 Mb (3q13.32q21.1) and was associated with a severe alobar holoprosencephaly, the second variant was smaller (267 kb, 3q13.33) and disrupted only one gene—this gene encompassed ten of 12 exons of *GSK3B* and manifested with microform holoprosencephaly, including partial ACC.

Table 1 Known disease-associated genes within the 3q13.31-22.1 deletion region

| Genomic location (hg19)	Band	Gene symbols	Gene name	OMIM number	Phenotype association (inheritance pattern and details if known)
3:118,892,424	3q13.3	UPK1B	Uroplakin 1B	602380	Candidate gene for vesicoureteral reflux disease (unknown)
3:119,013,219	3q13.3	ARHGAP31	Rho GTPase-activating protein 31	610911	Adams-Oliver syndrome (AD, scalp and distal limb reduction defects)
3:120,347,014	3q13.3	HGD	Homogentisate 1,2-dioxygenase	607474	Alkaptonuria (AR)
3:121,488,609	3q13.3	IQCB1	Nephrocystin 5	609237	Senior-Loken syndrome (AR, nephronophthisis and retinitis pigmentosa)
3:121,706,169	3q13.3	ILDR1	Ig-like domain-containing receptor 1	609739	Deafness (AR)
3:121,902,529	3q13.3	CASR	Calcium-sensing receptor	601199	Familial hypocalciuric hypercalcemia (AD), hypocalcemia (AD)
3:122,044,010	3q21	CSTA	Cystatin A (stefin A)	184600	Exfoliative ichthyosis, ichthyosis bullosa of Siemens-like (AR)
3:122,513,900	3q21	DIRC2	Disrupted in renal carcinoma 2	602773	Renal cell carcinoma (AD)
3:123,001,142	3q21	ADCY5	Adenylate cyclase-5	600293	Familial dyskinesia with facial myokymia (AD)
3:123,331,142	3q21	MYLK	Myosin-light-polypeptide kinase	600922	Familial thoracic aortic aneurysm (AD)
3:124,449,212	3q21	UMPS	Uridine monophosphate synthetase	613891	Orotic aciduria (AR, megaloblastic anemia and orotic acid crystalluria)
3:126,200,007	3q21	UROC1	Urocanase domain-containing protein 1	613012	Urocanase deficiency (AR, behavioral and neurological symptoms)
3:128,198,264	3q21	GATA2	GATA-binding protein-2	137295	Dendritic cell, monocyte, B cell and NK lymphocyte deficiency; Emberger syndrome
3:128,444,978	3q21	RAB7	Ras-associated protein RAB7	602298	Charcot-Marie-Tooth disease, type 2B (AD)
3:128,598,332	3q21	ACAD9	Acyl-CoA dehydrogenase family member 9	611103	ACAD9 deficiency (AR)
3:128,779,644	3q21	GP9	Glycoprotein IX, platelet	173515	Bernard-Soulier syndrome, type C (AR)
3:128,886,657	3q21	ZNF9	Zinc finger protein-9	116955	Myotonic dystrophy 2 (AD, microsatellite expansion)
3:129,158,967	3q21	IFT122	Intraflagellar transport 122, homolog of	606045	Cranioectodermal dysplasia 1 (AR, ciliopathy with systemic manifestations)
3:129,247,481	3q22	RHO	Rhodopsin (opsin 2)	180380	Congenital night blindness (AD and AR, retinitis pigmentosa)
3:129,693,113	3q22	TRH	Thyrotropin-releasing hormone	613879	Thyrotropin-releasing hormone deficiency (unknown)
3:130,569,368	3q22	ATP2C1	ATPase, Ca(2+)-sequestering	604384	Hailey–Hailey disease (AD, blistering skin disorder)
3:131,181,044	3q22	MRPL3	Mitochondrial ribosomal protein L3	607118	Combined oxidative phosphorylation deficiency (AR)

AR, Autosomal recessive; AD, autosomal dominant

Table 2 Reported chromosomal aberrations overlapping with the 3q13.31-22.1 deletion region

Karyotype	Age at last exam (years)	Short stature	Ear abnormality	Blepharophimosis	Epicanthic folds	Ptosis of eyelids	Other	Oral abnormality	Genital abnormalities	CAKUT[a]	Hand abnormality	Foot abnormality	Joints contractures	Agenesis of corpus callosum	Hypotonia	Developmental delay and/or delayed speech[b]
		Spectrum of malformations		Ocula:										Neurologica:		
46,XX,del(3)(q31.1q13.3)dn [22]	4	+					+							+	+	+
46,XY,del(3)(q13.11q13.33)dn [47]	3				+		+	+	+							+
46,XX,del(3)(q12.3-q13.33)dn [23]	17				+		+	+			+	+			+	+
46,XX,del(3)(q13.11-q13.33)dn [23]	10						+								+	+
46,XX,del(3)(q13.13-q13.33)dn [23]	5						+							+		+
46,XY,del(3)(q13.2-13.32)dn [23]	18		+				+	+			+				+	+
46,XY,del(3)(q13.12q21.3)dn [19]	5		+		+		+	+	+	+	+	+		+	+	+
46,XY,del(3)(q13.11q21.3)dn [23]	1.5		+		+		+		+	+	+				+	+
46,XY,del(3)(q13.32q21.2)dn [23]	8		+		+	+	+				+					+
46,XX,del(3)(q12q21)dn [16]	4						+	+			+				+	+
46,XY,del(3)(q12q21)dn [16]	1.5		+		+	+	+				+	+		+	+	+
46,XX,del(3)(q11q21)dn [18]	7				+	+	+								+	+
46,XX,del(3)(q12q21)dn [17]	8	+			+			+		+		+	+		+	+
46,XX,del(3)(q12-q21)dn [20]	0								+	+		+	+	+	+	NA
46,XX,del(3)(q21q23)dn [48]	5		+	+	+			+								+
46,XY,del(3)(q21q23)dn [49]	0			+	+	+	+									NA
46,XY,del(3)(q21.3q22.3)dn [50]	1	+		+	+	+	+	+	+		+				+	+
46,XY,del(3)(q12q23)dn [21]	6		+	+	+	+	+	+	+		+	+			+	+
46,XY,del(3)(q13.31q22.1)dn (present report)	14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

a CAKUT (congenital anomalies of the kidney and urinary tract) phenotypes include: duplicated collecting system (Okada et al. [17]), unilateral renal hypoplasia/agenesis (Molin et al. [23]), hydroureters and stenosis of urethral valves (Genuardi et al. [19]), hydronephrosis (McMorrow et al. [20]), and vesicoureteral reflux and agenesis (present case)

b Information on developmental delay not available (NA) for infants

Fig. 4 The reported deletions in all cases with agenesis of corpus callosum (*ACC*, *middle panel*) and congenital anomalies of the kidney and urinary tract (*CAKUT*, *bottom panel*) and their relationship to the reference map of the chromosomal region 3q12.1-22.3 (*top panel*). Our case defines new minimal candidate regions for ACC (chr3q13.31-13.33: 118,152–123,400 kb) and CAKUT (chr3q13.31-21.3: 118,152–128,178 kb). A complete list of candidate genes in these minimal candidate regions is provided as Electronic Supplementary Material Dataset 1
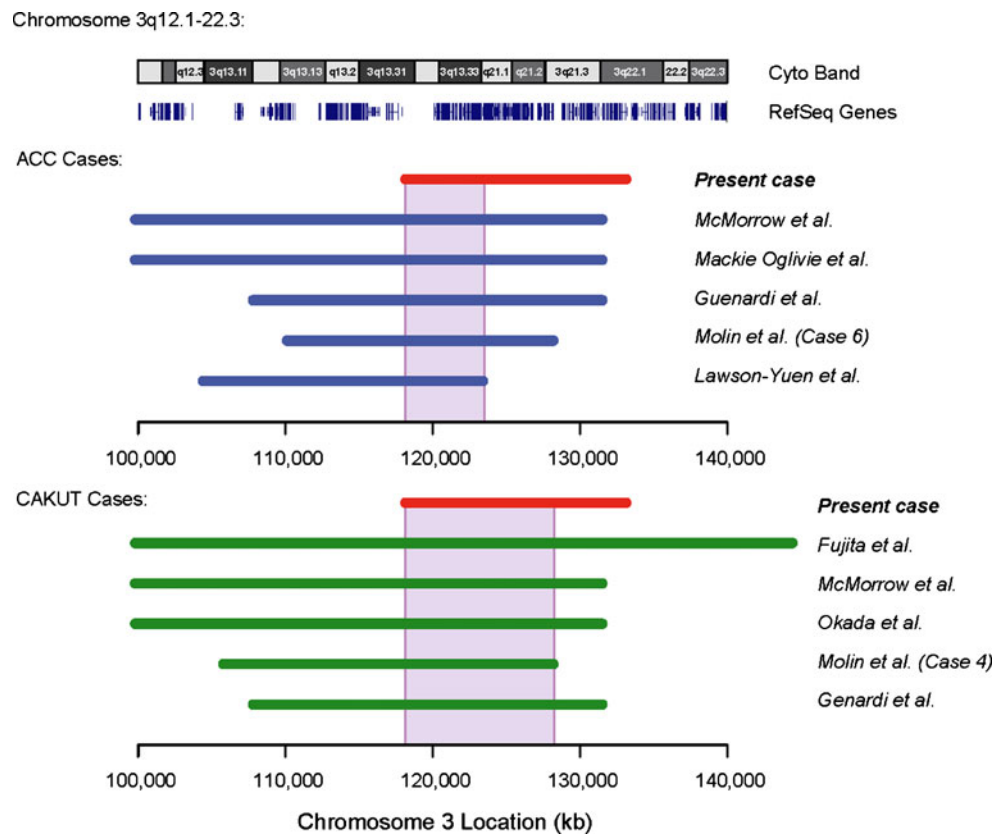
Therefore, our results provide further support for *GSK3B* involvement in the development of the central nervous system and suggest that biallelic dosage of this specific gene is critical for proper formation of the corpus callosum.

Kidney and urinary tract malformations

Among all of the deletions of 3q13.31-22.1 reported to date, six cases had a diagnosis of CAKUT. Among these cases, kidney phenotypes were highly variable, ranging from unilateral duplicated collecting system [17], unilateral hydronephrosis [20] and bilateral hydroureters with stenosis of the urethral valve [19], to grade IV left VUR with contralateral agenesis (present case). In addition to phenotypic heterogeneity, not all patients carrying the deletion of 3q13.31-21.3 had CAKUT, implying incomplete penetrance, although it is not clear if adequate screening was performed in all of the cases reported. These observations, however, are consistent with the fact that pleiotropy and incomplete penetrance are common features of the known CAKUT mutations. For example, mutations in *PAX2* cause the renal-coloboma syndrome, but kidney defects vary significantly between affected individuals, ranging from relatively mild (e.g. VUR or secondary obstruction) to extremely severe (e.g. bilateral renal agenesis) [27].

As evident in Fig. 4, our case effectively defined a new minimal candidate gene region for CAKUT (3q13.31-21.3: 118,152–128,178 kb). This region contains 78 annotated

transcripts and has a predicted haploinsufficiency LOD score of 25.7 (Electronic Supplementary Material Data 1). Two OMIM-annotated genes within this interval have previously been associated with a developmental renal phenotype: *UPK1B* (3q13.32) and *IQCB1* (3q13.33). The *UPK1B* gene encodes uroplakin 1B, a protein strongly expressed in normal uroepithelium. UPK1B is a binding partner for UPK3, forming the UPK1B–UPK3 complex that is inserted into the apical membrane of urothelial cells and necessary for their proper differentiation [28]. Upk3-deficient mice develop VUR with evidence of overexpression and abnormal targeting of Upk1b [29]. Moreover, de novo heterozygous and potentially deleterious single nucleotide variants in *UPK3A* have been described in four cases of VUR and renal dysplasia [30]. However, no deleterious mutations in human *UPK1B* have been reported to date. The second candidate gene, *IQCB1* (*NPHP5*), encodes a ciliary protein. Homozygous or compound heterozygous mutations in *IQCB1* are responsible for Senior–Loken syndrome, a rare recessive disorder characterized by kidney disease (nephronophthisis) and retinitis pigmentosa [31]. It is not clear if haploinsufficiency for *IQCB1* contributes to the observed renal defects, or if the deletion unmasks the effect of a deleterious allele in trans, or modifies the effect of other risk alleles carried by the patient. Notably, our case is also haploinsufficient for *IFT122*, another ciliary protein residing on 3q21. Recessive mutations in *IFT122* cause cranioectodermal dysplasia-1 (Sensenbrenner syndrome) which usually involves renal failure

in addition to short stature, limb defects and multiple craniofacial abnormalities [32]. Although this gene resides outside of the minimal candidate region for CAKUT, combined haploinsufficiency of both ciliary genes might modify the severity of the observed kidney defects in our case.

Skeletal and cardiac defects

Similar to CAKUT discussed above, *IFT122* haploinsufficiency may act as a modifier of some of the skeletal phenotypes, such as the short stature or distal limb malformations observed in our patient. Moreover, our patient is haploinsufficient for *ARHGAP31*, another gene implicated in skeletal development. *ARGHAP31* encodes a GTPase-activating protein that regulates two GTPases involved in protein trafficking and cell growth, Cdc42 and Rac1. It has been shown that rare gain-of-function mutations in *ARHGAP31* cause an autosomal dominant form of Adams–Oliver syndrome, a disorder characterized by skin aplasia and terminal transverse limb defects (short or absent distal phalanges with or without syndactyly) [33]. Interestingly, in mice, the expression of *ARHGAP31* is strongest in the developing heart, and while there have been reports of cardiac defects in patients with other forms of Adams–Oliver syndrome, individuals harboring the gain-of-function mutations in *ARHGAP31* had no cardiac involvement. However, there are no human loss-of-function mutations in this gene reported to date. Therefore, it is still possible that lower dosage of this gene contributes to the cardiac and/or skeletal defects observed in our case. Based on a literature search, we identified no other obvious known candidates for cardiac or skeletal defects in the region.

Thyroid hypoplasia and clinical hypothyroidism

Based on our gene annotations, we speculate that thyroid hypoplasia with hypothyroidism is likely due to haploinsufficiency of *TRH* (gene for thyrotropin-releasing hormone). The *TRH* gene is located in the distal portion of the 3q13.31-22.1, specifically within the band 3q22.1. Unfortunately, thyroid tests are not consistently reported for all deletion cases summarized in Table 2. Okada et al. reported a deletion of chromosome 3q12-3q21 in a patient with clinical features similar to those of our patient, including severe psychomotor retardation, progressive scoliosis, multiple joint contractures and CAKUT [17]. However, the thyroid function tests were normal in the patient described by these authors [17], suggesting that the locus responsible for hypothyroidism resides distal to 3q21.

TRH is a hypothalamic peptide responsible for the regulation and release of TSH, a critical regulator of thyroid gland function. Congenital deficiency of TRH and loss-of-function mutations in the TRH receptor gene have previously been described as a very rare cause of central hypothyroidism [34, 35]. In central hypothyroidism, the serum free T4 level is low, but serum TSH level may vary. Normal or high serum TSH concentrations in some cases of central hypothyroidism are due, in part, to secretion of TSH that has reduced biologic activity but normal immunoreactivity [36]. Reduced bioactivity is due to abnormalities in glycosylation of the TSH subunits, which is under the control of TRH [37, 38]. A prolonged period of decreased activation of thyroid cells by TSH may result in a hypoplastic thyroid and clinical hypothyroidism, as observed in our patient.

Previously unrecognized phenotypes and disease predictions

Strikingly, the 3q13.31-22.1 deletion also encompasses the *CASR* gene, encoding a calcium-sensing receptor. This receptor is expressed in the chief cells of the parathyroid gland and is critical for sensing changes in serum calcium concentration and initiation of signaling pathways that modify PTH secretion. Additionally, this receptor is also expressed in regions of the kidney involved in Ca^{2+}-regulated Ca^{2+} and Mg^{2+} reabsorption. Gain-of-function *CASR* mutations cause familial isolated hypoparathyroidism with hypocalcemia [39], while loss-of-function mutations cause familial hypocalciuric hypercalcemia (FHH) [40]. Importantly, the dosage of the *CASR* gene correlates with phenotype severity. Individuals with a single defective allele have relatively mild symptoms of FHH, while individuals carrying two deleterious alleles develop severe neonatal hyperparathyroidism [41]. Consequently, we predicted that the index case carried the diagnosis of hypocalciuric hypercalcemia. Upon subsequent review of the medical records, we noted that indeed our patient had hypercalcemia and hypermagnesemia of unclear etiology on several occasions, including serum Ca^{2+} levels of 12.6, 10.6, and 10.8 mg/dl (normal 8.5–10.5 mg/dl) with corresponding Mg^{2+} levels of 2.3, 2.6, and 2.2 mg/dl (normal 1.3–2.2 mg/dl) at the ages of 1, 8 and 12 years, respectively. As often observed in familial hypocalciuric hypercalcemia, the parathyroid hormone levels were within normal limits. Similarly, vitamin D levels were normal. Taking these findings into consideration, we performed a 24-h collection of urine and confirmed a low daily calcium clearance of 119.3 mg/24 h (normal <200 mg/24 h). Since the normal clearance threshold of 200 mg per day was established based on studies of adult subjects, we also estimated weight-normalized calcium clearance at 3.7 mg/kg/day, which was inappropriately low for a hypercalcemic patient (serum Ca^{2+} was 10.9 mg/dl at the time of urine collection). Finally, the calcium fractional excretion was calculated at 0.009, further supporting the diagnosis of hypocalciuric hypercalcemia.

We also noted that our patient is haploinsufficient for *ADCY5*, a gene encoding a membrane-bound adenylyl cyclase enzyme that mediates G protein-coupled receptor

signaling. It has recently been shown that heterozygous loss-of-function mutations in this gene cause autosomal dominant familial dyskinesia with facial myokymia [42]. Individuals affected by this disorder exhibit adventitious movements associated with perioral and periorbital myokymia (brief episodes of repeated facial muscle contractions that can sometimes also involve ocular muscles). In our patient, there is a possibility that ocular myokymia was misdiagnosed as focal epilepsy early in childhood, thus the patient would benefit from additional neurological assessment and reevaluation of her antiepileptic regimen.

Finally, by identifying literature reports of disease-causing heterozygous loss-of-function mutations in the genes contained within the 3q13.31-22.1 interval, we predict that our patient may be at an increased risk of developing a thoracic aortic aneurysm and dissection (haploinsufficiency of *MYLK* [43]), renal cell carcinoma (*DIRC2* [44]) and myelodysplasia or acute myeloid leukemia later in life (*GATA2* [45, 46]). Notably, *MYLK* is listed as one of the 37 genes recently recommended by the American College of Medical Genetics (ACMG) for reporting to patients when confirmed to harbor pathogenic mutations detected as incidental findings in genetic studies (URL: http://www.acmg. net). Personalized screening protocols for aneurysms and malignancy should therefore be considered in this highly unusual case.

Conclusion

We report a novel 15-Mb deletion of chromosome 3q13.31-22.1 that disrupts over 101 known genes and gives rise to a variety of phenotypic features, including developmental delay, ACC, dysmorphic facial features, limb abnormalities, hypothyroidism and malformations of several internal organs, including heart and kidneys. There are several possible mechanisms through which a deletion can manifest phenotypically, including a reduced gene dosage effect of a single gene or a contiguous set of genes (a haploinsufficiency effect), unmasking of a deleterious allele(s) on the non-deleted chromosome or epistasis with deleterious allele(s) outside of the deleted locus. Some of the phenotypic features observed in our patient can be clearly explained by the gene dosage effect of individual genes within this interval, while others likely involve more complex mechanisms.

We demonstrate that genotype-driven phenotyping combined with an exhaustive analysis of positional candidate genes within the regions of structural abnormality may provide novel insights into gene function and disease pathogenesis. Our case illustrates the great utility of publicly available genomic databases in facilitating diagnoses and in establishing connections between disrupted genes and specific phenotypic features. Notably, the rate of discoveries in the field of Mendelian genetics has been greatly accelerated by

the use of whole exome and genome sequencing in gene mapping studies. This technology continues to uncover new genotype–phenotype connections and, as the databases of loss-of-function phenotypes become more complete across the genome, our ability to interpret the consequences of large genomic deletions will improve. Importantly, through this case we demonstrate that detailed functional annotation of the deleted genes can uncover previously unrecognized or missed phenotypes, guide medical therapies and help to design personalized preventive approaches.

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