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De novo 3q22.1 q24 deletion associated with multiple congenital anomalies, growth retardation and intellectual disability

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ABSTRACT

We describe a boy with a *de novo* deletion of 15.67 Mb spanning 3q22.1q24. He has bilateral micropthalmia, ptosis, cleft palate, global developmental delay and brain, skeletal and cardiac abnormalities. In addition, he has bilateral inguinal hernia and his right kidney is absent. We compare his phenotype with seven other patients with overlapping and molecularly defined interstitial 3q deletions. This patient has some phenotypic features that are not shared by the other patients. More cases with smaller deletions defined by high resolution aCGH will enable better genotype–phenotype correlations and prioritizing of candidate genes for the identification of pathways and disease mechanisms.

1. Introduction

Deletions on 3q in the published literature can be subdivided into pericentromeric-proximal (involving 3q11.1–q13.33 only), proximal (involving 3q21–q28) and subtelomeric/terminal (involving 3q29 only). Subtelomeric/terminal deletions cause 3q29 microdeletion syndrome. Deletions which include the more proximal region (3q21–q25) have various clinical presentations depending on the size of the deletion and the genes involved. Patients with interstitial deletions can present with manifestations of (i) blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES: OMIM#110100), (ii) Dandy–Walker syndrome (DWS: OMIM%220200) including Dandy–Walker malformation (DWM), (iii) Wisconsin syndrome (Cohen, 1986), (iv) Pierre–Robin sequence (OMIM%602196) and (v) Seckel syndrome-1 (SCKL1: OMIM#210600).

The most frequent region involved in interstitial 3q deletions is 3q22–23, accounting for approximately half of the documented cases. These patients have features of BPES which can also be caused by point mutations in the gene. In addition to dysplasia of the eyelids, other common features include microcephaly, skeletal anomalies, congenital heart defects, cranial anomalies, intellectual disability and developmental delay (de Ru et al., 2005; Rea et al., 2010; Willemsen et al., 2010). Unfortunately, the majority of these previously published cases were identified by conventional cytogenetic studies and this has hampered genotype–phenotype correlations and identification of candidate genes for each specific feature. Exceptions are forkhead box L2 (*FOXL2*) gene mutations/deletions in patients with BPES (Beysen et al., 2009; Crisponi et al., 2001), mutations in the gene encoding ataxia-telangiectasia and RAD3-related protein (*ATR*) in Seckel syndrome patients (O'Driscoll et al., 2003), and evidence associating the ZIC family member (*ZIC*) genes, *ZIC1* and *ZIC4* with Dandy–Walker malformation (Grinberg et al., 2004; Lim et al., 2011; Tohyama et al., 2010).

We describe a boy with a *de novo* deletion of 15.6 Mb spanning 3q22.1q24. He has developmental delay and some features of BPES and

Abbreviations: *A4GNT*, alpha-1,4-N-acetylglucosaminyltransferase; *AGTR1*, angiotensin II receptor, type 1; *ATR*, ataxia telangiectasia and Rad3 related; BAC, bacterial artificial chromosome; *BFSP2*, beaded filament structural protein 2; BPES, blepharophimosis, ptosis, and epicanthus inversus syndrome; CT, computer tomography; CCD, charge-coupled device; CGH, comparative genomic hybridization; *CLDN18*, claudin 18; DWM, Dandy–Walker malformation; *DZIP1L*, DAZ interacting protein 1-like; *FOXL2*, forkhead box L2; GRCH36/hg18, Genome Reference Consortium Human Reference Build 36; ID, intellectual disability; kg, kilogram; Mb, million bases; *MRPS22*, mitochondrial ribosomal protein S22; OFC, occipito-frontal circumference; OMIM, Online Mendelian inheritance in man; *PCCB*, propionyl CoA carboxylase, beta polypeptide; *PCOLCE2*, procollagen C-endopeptidase enhancer 2; *PLOD2*, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2; PDA, patent ductus arteriosus; PCR, polymerase chain reaction; qRT-PCR, quantitative real time-polymerase chain reaction; RQ, relative quantitation; SCKL1, Seckel syndrome-1; *SLC9A9*, solute carrier family 9, subfamily A (NHE9, cation proton antiporter 9), member 9; SNP, single nucleotide polymorphism; *SOX14*, SRY (sex determining region Y)-box 14; *TF*, transferrin; VSD, ventricular septal defect; *ZIC*, Zinc finger protein of the cerebellum.

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Seckel syndrome-1. We compare his phenotype with seven other patients with overlapping and molecularly defined interstitial 3q deletions.

2. Clinical description

The patient was the second child of non-consanguineous Chinese parents. Antenatal history was unremarkable and his older brother was well. He was born at term by normal delivery with a birth weight of 2.5 kg, length of 45 cm and occipito-frontal circumference (OFC) of 30 cm. He was noted to be dysmorphic and had a cardiac murmur soon after birth. The child was referred to the Genetics Department at KK Women's & Children's Hospital at 3 weeks of age.

On examination, he was noted to be small with microcephaly. At six weeks of age his OFC was 31.9 cm, 2 cm less than the 3rd centile. Dysmorphic features included bilateral micropthalmia, bilateral ptosis and blepharophimosis, micrognathia, midline cleft palate, camptodactyly and prominent ear lobules. He also had rocker bottom feet and bilateral inguinal hernia. Ultrasound examination showed the absence of the right kidney and 2D Echocardiogram showed a moderate sized perimembranous ventricular septal defect (VSD) and a small patent ductus arteriosus (PDA). Cranial CT scan revealed turricephaly and fusion of bilateral lambdoid and coronal sutures.

Before age two, the child had fronto-orbital advancement operation for craniosynostosis, cleft palate repair, brow suspension surgery for ptosis, and bilateral herniotomy. Coil-occlusion of the PDA was carried out when he was 4 1/2 years of age. At age 10, he is asymptomatic for his small VSD and closed PDA and no further interventions are planned. He has impaired visual acuity and continues to have problems with his vision. He also shows severe growth retardation with height, weight and OFC below the 3rd centile. He has global developmental delay — he sat unsupported at 10 months, walked at 2 3/4 years and started speaking single words at 3 years. Currently aged 11, he has a learning disability and attends Special School but is able to speak in sentences.

3. Materials and methods

3.1. Molecular karyotyping

DNA from the peripheral blood of the patient was used on an Affymetrix SNP 6 array according to the manufacturer's instructions (Affymetrix Inc., USA). Data were processed using the Affymetrix Genotyping Console and further analyzed by the Chromosome Analysis Suite software (Version 1.0.1392(r2426)). Copy number changes were calculated based on hybridization signal intensity data from calculated intensity distributions derived from a reference set from Affymetrix with the setting for marker count at 50, size at 100 kb and confidence at 85.

3.2. Fluorescence-in situ-hybridization (FISH)

Peripheral blood lymphocytes from the child's phenotypically normal parents were cultured using phytohemagglutinin (PHA) stimulation and methotrexate (MTX) synchronization/thymidine release methods. Molecular cytogenetic analysis was performed using probes obtained from The Hospital for Sick Children (Toronto, Canada). Slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in Vectashield mounting medium (Vector Laboratories, Inc., USA) and analyzed by fluorescence microscope Olympus BX51 equipped with a CCD Progressive Scan Video Camera (JAI, Japan). Image analysis was carried out with Cytovision software (version 3.93.2) (Applied Imaging Corp, USA).

3.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Gene copy number was also investigated by relative quantitative real-time-PCR with SYBR Green dye and *SLC9A9* (solute carrier family 9 (sodium/hydrogen exchanger), member gene) as the target gene for quantifying gene copy number. Primers were designed using Primer Express (Version 3.0), and the experiment was carried out in triplicate. The patient's and parents' DNA samples were amplified in the same experiment with HBB (hemoglobin beta gene) as the internal reference. Amplification was done using Applied Biosystems StepOnePlus real time PCR system (Applied Biosystems, USA). Results were analyzed using Applied Biosystems StepOne software (version 2.1).

3.4. Network/pathway analysis

The coordinates of the minimum deleted region were searched against the Human reference genome (hg18) for known genes in the region. The resulting list of genes was imported into Ingenuity Pathway Analysis (IPA) software using the Entrez ID mapped to the Ingenuity Pathway Knowledge Base identifier. The reference set used was Ingenuity Knowledge Base (Genes only); relationship to include was both direct and indirect. The analysis included endogeneous chemicals and the filter summary was set to consider only relationships where confidence = experimentally observed. The statistical significance for the enrichment of genes of interest in each pathway was evaluated by a Fisher Exact test under the Core Analysis function of IPA.

4. Results

4.1. Molecular karyotyping

Genome-wide analysis of the child from the SNP array showed a minimum loss of 15.67 Mb at 3q22.1q24. The first probe with copy number loss was at 134,366,905 and the last probe with copy number lost was at 150,039,405 [hg18]. The last probe with normal copy number was at 134,362,222 and the first probe with normal copy number is at 150,044,473 (Fig. 1). The maximum size of the deletion is 15.68 Mb.

4.2. Fluorescence-in situ-hybridization (FISH)

Parental chromosomes tested using BAC probes RP11-1023P20 (chr3: 134,159,391–134,326,599 [hg18]), RP11-883M5 (chr3: 134,732,494–134,899,911 [hg18]), RP11-505J9 (chr3: 149,845,223–150,049,901 [hg18]) and RP11-80I8 (chr3: 153,554,710–153,735,639 [hg18]) showed normal hybridization pattern at band 3q22.1 to q25.2 on the two homologues of chromosome 3 for both parents.

4.3. Quantitative real-time polymerase chain reaction

Quantitative real-time PCR confirmed the copy number loss in the child with the RQ (relative quantitation) at 0.500. The respective RQ values for the father and mother are 1.143 and 1.149 (Fig. 2).

4.4. Network/pathway analysis

The top scoring networks from the IPA Core Analysis function are displayed in Table 1. For each biological function identified, the range for level of significance of the genes involved in the pathway is displayed in Table 2.

5. Discussion

In addition to the 22 cases of interstitial 3q deletions that Ramieri et al. (2011) cited, there are at least four more cases with deletions limited to 3q13.1–q13.33 (Lawson-Yuen et al., 2006; Shimojima et al., 2009; Simovich et al., 2008), and 19 more cases involving at least part of 3q21–q23 (with some extending to 3q13) (Almenrader et al., 2008; Arai et al., 1982; Brueton et al., 1989; Callier et al., 2009; Croft and