Generation of the Sotos syndrome deletion in mice

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Abstract Haploinsufficiency of the human 5q35 region spanning the NSD1 gene results in a rare genomic disorder known as Sotos syndrome (Sotos), with patients displaying a variety of clinical features, including pre- and postnatal overgrowth, intellectual disability, and urinary/renal abnormalities. We used chromosome engineering to generate a segmental monosomy, i.e., mice carrying a heterozygous 1.5-Mb deletion of 36 genes on mouse chromosome 13 (4732471D19Rik-B4galt7), syntenic with 5q35.2–q35.3 in humans (Df(13)Ms2Dja+/− mice). Surprisingly, Df(13)Ms2Dja+/− mice were significantly smaller for their gestational age and also showed decreased postnatal growth, in contrast to Sotos patients. Df(13)Ms2Dja+/− mice did, however, display deficits in long-term memory retention and dilation of the pelvicalyceal system, which in part may model the learning difficulties and renal abnormalities observed in Sotos patients. Thus, haploinsufficiency of genes within the mouse 4732471D19Rik–B4galt7 deletion interval play important roles in growth, memory retention, and the development of the renal pelvicalyceal system.

Introduction

Sotos syndrome (Sotos; MIM# 117550) is an autosomal dominant, multiple-anomaly syndrome characterized by overgrowth, a distinctive craniofacial appearance, advanced bone age, and variable learning disabilities. However, there is significant clinical heterogeneity in Sotos syndrome, with some affected individuals also showing frequent ear and chest infections, cardiac and urinary/renal defects, seizures, scoliosis, and behavioural problems (Tatton-Brown et al. 1993). The diagnosis of Sotos syndrome relied solely on clinical criteria until haploinsufficiency of the NSD1 gene (encoding a histone methyltransferase implicated in chromatin regulation) was identified as causative (Kurotaki et al. 2002). Subsequent analysis of patients clinically diagnosed with Sotos showed that haploinsufficiency of NSD1 due to intragenic NSD1 mutations, partial NSD1 deletions, or chromosomal microdeletions spanning the 5q35 region encompassing the entire NSD1 gene accounted for more than 90 % of cases (with the prevalence of intragenic NSD1 mutations and 5q35 microdeletions encompassing the NSD1 gene depending greatly on ethnic origin) (Baujat et al. 2004;
Tatton-Brown and Rahman 2007; Tatton-Brown et al. 2005a, b). It has also been proposed that GPC3 mutations or 11p15 abnormalities or both may be responsible for some Sotos cases without NSD1 abnormalities (Baujat et al. 2004; Li et al. 2001; Tatton-Brown and Rahman 2007).

The major clinical features of Sotos, including overgrowth, facial abnormalities, and intellectual disabilities, are diagnosed in Sotos patients with intragenic NSD1 mutations and in Sotos individuals carrying 5q35 microdeletions (Tatton-Brown et al. 2005a, b). However, in contrast to Sotos individuals with NSD1 mutations, Sotos patients with 5q35 microdeletions tend to show less pronounced overgrowth but more profound intellectual disability (Kurotaki et al. 2003; Nagai et al. 2003; Saugier-Veber et al. 2007; Tatton-Brown et al. 2005a), and several studies have reported an increased frequency of cardiovascular and urinary/renal abnormalities in 5q35 microdeletion Sotos patients (Kurotaki et al. 2003; Nagai et al. 2003; Saugier-Veber et al. 2007). Thus, it is possible that genes other than the NSD1 gene could be dosage-sensitive and therefore responsible for the extended variability and degree of severity of phenotypes observed in Sotos patients who carry 5q35 microdeletions. Furthermore, selected patients with Sotos can have low laboratory values of the factor XII blood-clotting protein, a phenotype thought to result from the hemizygous deletion of 5q35, unmasking a functional single nucleotide variant (SNV) on the remaining nondeleted allele of the FXII gene within the common Sotos deletion interval (Kurotaki et al. 2005). The latter genetic mechanism has recently also been shown to be responsible for TAR (thrombocytopenia absent radius) syndrome where 1q21.1 deletions appear to unmask functional variants associated with the RBM8a gene (Albers et al. 2012).

The mouse orthologue of the NSD1 gene (Nsd1) is highly conserved (83% homology at amino acid level) (Kurotaki et al. 2001); however, heterozygous Nsd1 mice do not display any gross phenotypic abnormalities (Rayasam et al. 2003). Similarly, mice carrying knockout mutations of other genes within the most frequently detected human Sotos 5q35 microdeletion interval have failed to display dominant phenotypes, suggesting that Sncb, Unc5a, Fgfr4, Mxd3, Rgs14, Slc34a1, F12, Grk6, Dbn1, and Dok3 are not dosage-sensitive and, therefore, at least individually are not likely to be responsible for the extended variability and degree of severity of clinical features observed in 5q35 microdeletion Sotos patients (Mouse Genome Informatics; http://www.informatics.jax.org). In order to allow investigation of the contribution of the 36 genes at the distal end of human chromosome 5 to the development of clinical features commonly diagnosed in Sotos patients, we used chromosome engineering to generate a new genetic mouse model of Sotos syndrome, Df(13)Ms2Dja+, carrying a deletion syntenic to the human chromosome 5q35.2–q35.3 region.

Materials and methods

Gene targeting in ES cells and generation of deletion mice

The 5′ Hprt MICER targeting vector MHPP55m07 (Adams et al. 2004) was linearized with KpnI and electroporated into E14Tg2a embryonic stem (ES) cells (129P201a), which were selected in G418 as described previously (Ramirez-Solis et al. 1995). Southern blotting was performed on BstEI-digested ES cell genomic DNA (gDNA) using a probe amplified from E14Tg2a gDNA (5′-GTC TGT TGT TAA AAG CTA AAA CCT TAG A-3′ and 5′-TGA GCT ACA GTA GTG TGG TGC ATG TTA GTC CAT-3′) to identify correctly targeted clones. The 5′ Hprt MICER targeting vector MHPP265c24 (Adams et al. 2004) was linearized with NcoI and electroporated into MHPP55m07-targeted E14Tg2a ES cells, which were then selected in puromycin. Southern blotting was performed on SpeI-digested gDNA using a probe amplified from E14Tg2a gDNA (5′-CAG TAA TAT AGT AGA AGC ATG GTC CAT-3′ and 5′-ATG ATA CTG AAC ACA GAC AGA GGC TGC T-3′). Double-targeted ES cell clones were electroporated with a Cre-expression vector and selected in hypoxanthine, aminopterin, and thymidine (HAT) medium, as described previously (Ramirez-Solis et al. 1995), to identify whether the 5′ Hprt and 3′ Hprt vectors were targeted in cis or in trans. Clones of ES cells that had undergone cis recombination were identified and confirmed by PCR (5′-AGG GTT GTT TAC CCA TGG ACT AAT TAT G-3′ and 5′-CCT TCA TCA CAT CTC GAG CAA GAC GTT CAG-3′; presence of a 1.7-kb band confirmed the cis orientation). The deletion allele was designated Df(13)Ms2Dja+. ES cell clones carrying the conditional deletion (prior to electroporation with Cre) were injected into C57BL/6-J for three to four generations and maintained on a mixed C57BL/6J background. All mice were housed and experimental procedures were carried out in accordance with UK Home Office guidelines.

Comparative genomic hybridization (CGH)

CGH analysis was performed on the Agilent 244A array using tail DNA. The data were processed and analysed in R (R Foundation for Statistical Computing, Vienna, Austria).

Fluorescent in situ hybridization (FISH)

Chromosome spreads of activated splenocytes and ES cells were generated and labelled as described previously.
Generation of Df(13)Ms2Dja+/- mice carrying a 1.5-Mb deletion of the 4732471D19Rik–B4galt7 region

The 4732471D19Rik and B4galt7 genes are located at the proximal and distal ends of a 1.5-Mb region in the B1 band of mouse chromosome 13 (MMU13), which is syntenic to the human cytogenetic region 5q35.2–q35.3 and the genomic interval Chr 5: 175–177 Mb (Fig. 1a). This region on human chromosome 5 (HSA5) contains 36 genes (NCBI build GRCh37), all of which have orthologous counterparts found on the syntenic region of MMU13 (NCBI build m37) (Supplementary Table 1). We used chromosomal engineering to generate the deletion (Zheng et al. 1999), whereby E14Tg2a ES cells were sequentially electroporated with targeting vectors containing a portion of the Hprt selection cassette (5' or 3' Hprt), a loxP site, and a coat-colour marker (Agouti or Tyrosinase). The targeting vector containing the 5' Hprt cassette (MICER clone: MHPN55m07) (Adams et al. 2004) was inserted proximal to 4732471D19Rik and the targeting vector containing the 3' Hprt cassette (MICER clone: MHPP265c24) (Adams et al. 2004) was inserted distal to B4galt7 (Fig. 1b). The correct insertion of both targeting vectors was confirmed by Southern blot analysis on BstEII- or SpeI-digested genomic DNA extracted from ES clones using external probes. Double-targeted ES cell clones were electroporated with a Cre-expression vector and selected in medium containing hypoxanthine, aminopterin, and thymidine (HAT) to identify lines carrying chromosomal deletions generated via loxP-mediated recombination. Clones in which the vectors were targeted in cis generated hundreds of colonies, while those targeted in trans generated virtually no colonies. The deletion allele was designated Df(13)Ms2Dja, and deletion of the interval between the targeting vectors was confirmed by fluorescence in situ hybridisation (FISH) on Hprt-resistant ES clones (Fig. 1d). Cis-targeted conditional ES cell clones were injected and the double-targeted un-recombined (floxed) chromosome was transmitted through the germline. The resulting F1 mice were bred with a Cre deleter strain (CMV-Cre mice) to generate Df(13)Ms2Dja+/− mice. Df(13)Ms2Dja+/- mice were backcrossed to C57BL/6J for three to four generations and maintained on a mixed C57BL/6J-129P2/Ola background. FISH analysis was performed on splenocytes, as described above, to revalidate the allele before experiments were performed using Df(13)Ms2Dja+/- mice. CGH analysis of tail DNA also confirmed the establishment of the Df(13)Ms2Dja deletion in mice (Fig. 1c).

Df(13)Ms2Dja+/- embryos are small for their gestational age

To determine whether clinical features of Sotos patients could be observed in Df(13)Ms2Dja+/- mice, we initiated our studies during embryonic development. At embryonic day 15.5 (E15.5), Df(13)Ms2Dja+/- embryos showed significantly reduced growth (as measured by reduced body
weight) for their gestational age when compared with wild-type littermate embryos (Fig. 2a). It is unlikely that this difference is due to the genetic background of these mice (mixed C57–129), as the same phenotype was observed after a one-generation backcross to different strains (specifically, BALB/cJ, CBA/J, and 129/SvEv; Supplementary Fig. 1). Reduced growth of Df(13)Ms2Dja−/− animals was also evident upon examination of 1-day-old pups compared with wild-type littermates (Fig. 2b). These observations are contrary to human Sotos foetuses and newborns that typically show an overt overgrowth phenotype and suggest differences in the role that NSD1 and Nsd1 play in embryonic and postnatal development in human and mouse, respectively.

Adult Df(13)Ms2Dja+/− mice are smaller and lighter than their littermates. Df(13)Ms2Dja+/− mice fed on a normal-fat diet were lighter and shorter than their wild-type littermates at 28 weeks (males, Fig. 2c, d; females, data not shown). Detailed analysis of the body composition of these mice revealed a statistically significant decrease in the lean mass, but not fat mass, of Df(13)Ms2Dja+/− mice (males, Fig. 2e, f; females, data not shown).

In addition, to determine whether clinical features of Sotos patients, including macrocephaly, facial abnormalities, hypotonia, seizures, and/or scoliosis, could be observed in adult Df(13)Ms2Dja+/− mice, they were subjected to...
could be observed in adult Df(13)Ms2Dja
To identify whether the learning disability of Sotos patients
mice
Behavioural phenotyping of adult
Df(13)Ms2Dja
or craniofacial phenotypes (data not shown).
However, Df(13)Ms2Dja+/− mice were viable, fertile, and did not show any overt skeletal or craniofacial phenotypes (data not shown).

Fig. 2 Phenotypic analysis of Df(13)Ms2Dja+/− mice at various time points. a Body weight measurements in control (+/+, n = 12) and deficiency (Df(13)Ms2Dja+/−, n = 12) littermates at embryonic day 15.5. b Body weight measurements in control (+/+, n = 25) and deficiency (Df(13)Ms2Dja+/−, n = 21) littermates at 1 day of age. c, d Body weight and length measurements in control (+/+, n = 12) and deficiency (Df(13)Ms2Dja+/−, n = 11) littermates at 28 weeks of age. e, f DEXA analysis of lean mass and fat mass in control (+/+, n = 12) and deficiency (Df(13)Ms2Dja+/−, n = 11) littermates at 28 weeks of age. All data were statistically analysed using the two-tailed Student’s t-test, and the asterisk indicates statistical significance. The error bars represent the standard deviation of the measurements.

Histopathological analysis of Df(13)Ms2Dja+/− mice reveals dilation of the renal pelvicalyceal system

to determine whether histopathological abnormalities of Sotos patients, in particular, advanced bone age, cardiovascular, and/or urinary/renal abnormalities, could be observed in Df(13)Ms2Dja+/− mice, they were subjected to histopathological analysis at 10 weeks of age. Examination of a variety of tissues stained with haematoxylin and eosin (H&E) failed to reveal the presence of anatomical abnormalities of any organs, except the kidneys, where dilation of the pelvicalyceal system was observed in Df(13)Ms2Dja+/− mice with 100% penetrance (Fig. 4a). Kidney and urinary abnormalities are diagnosed in approximately 10% of Sotos patients, suggesting that kidney abnormalities are a highly penetrant trait recapitulated in Df(13)Ms2Dja+/− mice. Interestingly, a range in the severity of the pelvicalyceal system dilatation (mild through moderate to severe) was observed, and in all cases of moderate or severe dilation, only one kidney was affected moderately or severely while the other kidney showed only mild dilation. This dilation did not progress
with age, as the same range of changes observed in 10-week-old mice was also present in 28-week-old (Fig. 4b) and 52-week-old (Fig. 4c) deficiency mice. Blood chemistry analysis revealed no significant change in parameters related to urinary/renal system clearance and metabolic functioning, including creatinine, urea, and electrolytes in 28- and 52-week-old Df(13)Ms2Dja+/- mice when compared to wild-type littermate controls (data not shown). This suggests that dilation of the pelvicalyceal system does not affect overall kidney function. X-ray images of kidneys were not shown). This suggests that dilation of the pelvicalyceal system does not affect overall kidney function. X-ray images of kidneys were not shown).

Discussion

We generated and phenotypically characterized the het-

erozygous deficiency Df(13)Ms2Dja mouse—a genetic

model of Sotos syndrome covering a 1.5-Mb region syn-
tenic to the telomeric part of human chromosome 5. This

region contains 36 genes, which are conserved between

human and mouse (Fig. 1). Df(13)Ms2Dja+/- mice were

viable, fertile, and indistinguishable from wild-type litter-

mate controls in terms of head and facial morphology,

muscle tone, and curvature of their spine. Thus, Df(13)Ms2Dja+/- mice do not recapitulate the macro-

cephaly, facial abnormalities, hypotonia, or scoliosis

observed in some Sotos patients (Tatton-Brown and Rah-
mans 2007). However, it is difficult to reliably model facial abnormalities in mice (Tobin et al. 2008). Sotos individuals

have facial abnormalities that include a high and broad

forehead (the head is said to resemble an inverted pear),

frontotemporal hair sparsity, malar flushing, down-slanting

apalpebral fissures, and a pointed chin, dysmorphology that

is probably hard to observe in mice. 

Deficiency mice fed on a normal-fat diet did, however, show significantly reduced growth at all time points measured (embryonic day 15.5–52 weeks of age; Fig. 2). The presence of reduced postnatal growth in Df(13)Ms2Dja+/- mice contrasts with human data, as Sotos patients with intragenic NSD1 mutations and those with 5q35 microdeletions encompassing the NSD1 gene exhibit overgrowth (Tatton-Brown and Rahman 2007). However, the overgrowth phenotype observed in Sotos patients with 5q35 microdeletions is not very pronounced, even at the early stages of development (Kurotaki et al. 2003; Nagai et al. 2003; Saugier-Veber et al. 2007; Tatton-Brown and Rahman 2007). Collectively, these data suggest divergent roles for Nsd1/NSD1 in regulating growth in mouse and man.

Df(13)Ms2Dja+/- mice showed deficits in long-term memory retention in a socially relevant testing paradigm (Fig. 3). In contrast to other artificial novel object discrimination assays, the mechanism of long-term social recognition in mice is multisensory, requiring the integration of both volatile and nonvolatile olfactory and pheromone cues from conspecifics (Noack et al. 2010). Moreover, the sensitivity of the assay is enhanced by its ethological relevance; it does not rely on prior conditioning and instead exploits an innate olfactory-mediated behav-

ioral response of rodents (Engelmann et al. 2011). Social

\[ N = 8 \]

\[ P < 0.05, \text{two-tailed Student’s } t\text{-test} \]

\[ P < 0.0001, \text{post-hoc analysis after two-way ANOVA} \]

\[ P < 0.0001, \text{post-hoc analysis after two-way ANOVA} \]

\[ P < 0.0001, \text{post-hoc analysis after two-way ANOVA} \]

\[ P < 0.0001, \text{post-hoc analysis after two-way ANOVA} \]

\[ P < 0.0001, \text{post-hoc analysis after two-way ANOVA} \]
recognition is hippocampus-dependent (Kogan et al. 2000) and is well established for learning and memory testing (Engelmann et al. 2011; Richter et al. 2005) and for assessing cognitive impairment in mice (Mitsui et al. 2009). Thus, Df(13)Ms2Dja/?- mice may potentially model the intellectual/learning disability observed in Sotos syndrome patients (Tatton-Brown et al. 2005b). Given that the 4732471D19Rik-B4galnt7 region spans 36 genes, it is possible that other genes within the interval might, in addition to Nsd1, contribute to the cognitive phenotype observed in Df(13)Ms2Dja+/? mice. Histopathological examination of tissues from Df(13)Ms2Dja+/? mice revealed no signs of advanced bone age or cardiovascular anomalies, although these abnormalities

Fig. 4 Dilation of the renal pelvicalyceal system in Df(13)Ms2Dja+/? mice. Haematoxylin and eosin-stained kidney sections from control (+/+) and deficiency (Df(13)Ms2Dja+/?) mice at a 10 weeks, b 28 weeks, and c 52 weeks of age. Kidneys of deficiency (Df(13)Ms2Dja+/?) mice showed mild through moderate to severe dilation of the pelvicalyceal system (left to right) (visible as empty spaces; indicated by asterisks) compared with control littermates (normal structure of the pelvicalyceal system indicated by asterisks). Images are representative and taken at x250 magnification.
are observed only in a subset of Sotos patients carrying 5q35 microdeletions (Nagai et al. 2003; Saugier-Weber et al. 2007; Tatton-Brown and Rahman 2007). However, kidney abnormalities were observed in our deficiency animals, specifically dilation of the pelvicalyceal system (Fig. 4). Anomalies of the urinary/renal system, including vesicoureteric reflux, hydronephrosis, and small kidneys, are diagnosed in some (~ 10 %) Sotos patients carrying 5q35 microdeletions (Kurotaki et al. 2003; Nagai et al. 2003; Saugier-Weber et al. 2007; Tatton-Brown and Rahman 2007). Hydronephrosis is characterized by dilation of the renal pelvis and calyces, so Df(13)Ms2Dja+/- mice seem to recapitulate key features of this anomaly. To date, no mice carrying knockout mutations in genes mapped within the 5q35 interval have been shown to exhibit dilation of the pelvicalyceal system, suggesting that Sench, Unc5a, Nsd1, Fgfr4, Mxd3, Rgs14, Slc34a1, F12, Grik6, Dbn1, and Dok3 are unlikely, at least individually, to contribute to this phenotype in Df(13)Ms2Dja+/- mice (Mouse Genome Informatics, http://www.informatics.jax.org). However, it is possible that haploinsufficiency of other genes in the interval may contribute with Nsd1 to the renal organ system anomalies observed in Sotos patients and Df(13)Ms2Dja+/- mice. Synergy between genes within copy number variable regions has recently been demonstrated for cardiac development anomalies associated with Down syndrome (Grossman et al. 2011) and postulated for other genomic disorders (Lupski et al. 2011).

We show here that haploinsufficiency of gene(s) in the 4732471D19Rik-B4galt7 region of MMU13, syntenic to 35.2–q35.3, results in pre- and postnatal undergrowth, cognitive impairment, and dilation of the renal pelvicalyceal system in Df(13)Ms2Dja+/- mice. Thus, Df(13)Ms2Dja+/- mice have contributed new insights into the role that genes within the Sotos syndrome deletion interval play in Sotos-associated intellectual disability, growth, and development of the urinary/renal system.

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