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Clinical Report

Clinical Report

Maternal Inheritance of *BDNF* Deletion, with Phenotype of Obesity and Developmental Delay in Mother and Child[1].

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Abstract

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ABSTRACT

Childhood obesity is a significant world health problem. Understanding the genetic and environmental factors contributing to the development of obesity in childhood is important for the rational design of strategies for obesity prevention and treatment. Brain-derived neurotrophic factor (BDNF) plays an important role in the growth and development of the central nervous system; there is also evidence that BDNF plays a role in regulation of appetite. Disruption of the expression of this gene in a child has been previously reported to result in a phenotype of severe obesity, hyperphagia, impaired cognitive function and hyperactivity. We report a mother and child, both with micro-deletions encompassing the BDNF gene locus, who both have obesity and developmental delay, although without hyperactivity. This report highlights the maternal inheritance of a rare genetic cause of childhood obesity.

INTRODUCTION

In the USA, approximately 17% of children aged 2-19 are obese, as defined by a body mass index (BMI) at or above the 95th centile [Ogden et al., 2014]. A multitude of variables underlie the development of obesity in childhood: lifestyle, diet, activity levels, hormones and socioeconomic status [Juonala et al., 2011; Waters et al., 2011]. The list of genes implicated in both monogenic and polygenic obesity is ever-growing with overlap between the two. Some of these identified genes include *SIM1* (encoding single-minded homologue 1), *BDNF* (brain-derived neurotrophic factor) and its receptor tropomyosin-related kinase B (TrkB), which is encoded by *NTRK2* gene [Walley et al., 2009; Choquet and Meyre 2011; Sabin et al., 2011; Mou et al., 2015].

BDNF is a neurotrophin localized within neuronal cell bodies and axon terminals throughout the CNS. It is postulated that via TrkB, BDNF has enduring effects on the potentiation of synaptic transmission in the brain [Conner et al., 1997; Kokaia et al., 1998], and therefore may be important for cognitive function, memory, behaviour and regulation of appetite [Urabe et al., 2013]. Mice that have homozygous *Bdnf* null mutations have marked reduction in neurons in peripheral sensory ganglia, are ataxic and smaller than control littermates [Ernfors et al., 1995]. Heterozygous *Bdnf* mice exhibit increased aggression, hyperphagia, and higher bodyweights compared to their control littermates [Lyons et al., 1999]. Conditional mutants with a postnatal brain specific deletion of *Bdnf* demonstrate abnormal responses to stressors, marked increases in bodyweight and elevated metabolic hormones including, leptin, insulin and glucose [Rios et al., 2001]. Therefore, central control of energy balance is mediated in part by BDNF expression in the ventro-medial hypothalamus [Cordeira et al., 2014], and metabolic dysfunction can be ameliorated via delivery of BDNF to this region [Rios et al., 2001; Toriya et al., 2010].

In genome-wide association studies, *BDNF* mutations are associated with increased body mass, behavioral disorders and eating disorders [Thorleifsson et al., 2009; Walley et al., 2009]. However, the frequency of *BDNF* mutations in the general population is unknown. We hypothesize that with increased

proclivity to access genetic screening in childhood and align phenotypic presentation with a genetic cause, patients with combination; BDNF mutation, obesity, and developmental delay will become more prevalent. To date only one report, with albeit tenuous associations, has been presented in the literature. Friedel et al. [2005] reported an extremely obese male with a non-conservative amino acid substitution (p.T2I) in *BDNF*, which was inherited from his overweight mother. The patient also had an overweight sibling who had inherited the wild type maternal allele. Studies confirming the loss of function of BDNF peptide were not done.

Herein, we report a 3-year-old female with significant developmental delay and obesity who was found to have a 2.0 Mb heterozygous deletion at 11p14.1 encompassing *BDNF*. We identified that this deletion was of maternal origin, and that her mother demonstrated a similar, though less pronounced phenotype. This is the first report of an inherited isolated deletion in this region of chromosome 11 resulting in an obesity phenotype.

CLINICAL REPORT

The patient was the firstborn of a 28-year-old mother and 37-year-old father. Following a natural conception and an uncomplicated pregnancy without gestational diabetes, the patient was delivered naturally at 36 weeks gestation, and weighed 2.58 kg (50^{th} centile). When the child was initially seen at The Royal Children's Hospital in Melbourne, Australia at 3 years of age, she was noted to have persistent developmental delay. The child began walking at approximately 2.5 years and had a very limited vocabulary; communicating mainly through gestures and grunts. Language comprehension appeared to be less delayed than expressive language as the child was able to follow simple verbal commands. Nociception was not assessed at that time.

Both parents were markedly overweight, with paternal weight 130.0 kg and maternal weight approximately 120.0 kg. There was no reported family history of diabetes or cardiovascular disease. The mother self-reported delayed achievement of cognitive milestones in childhood, delayed intellectual development and being overweight throughout her own childhood.

On examination the child was found to be well, with a rounded face, similar to her father's, with large eyebrows and peg-like teeth (Fig 1A). No facial dysmorphism was noted in the child or mother (Fig 1A and 1B). Hands were normal to inspection. Cardiorespiratory, neurological and abdominal examinations revealed no gross abnormalities. One small birthmark on the right lower abdomen was noted. Serum analysis at the initial appointment revealed hypercholesterolemia, hypernatremia, hyperinsulinemia, normal glucose, normal vitamin D, elevated CRP and ALT, and normal thyroid function (Table 1).

The mother and child both appeared to be significantly overweight (Fig 1C and 1D). The child had a weight at 3 years, 7 months of 40.2 kg (more than 20 kg above the 97th centile for age (Fig 2A)), with height 110.6 cm (3 cm above 97th centile for age, (Fig 2B)) and head circumference in the normal range (49 cm, 50th centile (Fig 2C)). Growth charts showed that the child's weight trajectory suddenly increased at approximately 2 years of age, though appeared to have slowed in the eighteen months since her initial visit. Her BMI z-score at the age of 5 years remains at the 99.93rd percentile (Fig 2D).

The child's parents were asked to complete a 6-day food record for the patient after her initial medical assessment. Food intake was again documented after one year of diet and lifestyle counselling. The food record was analysed using FoodWorks 2009 Professional Edition (Version: 7.0.3016, Xyris, Australia). Total mean energy intake was significantly reduced after one year of nutritional education (Table 2). Mean total energy consumption remains less than the estimated basal metabolic rate (BMR) energy requirement suggested for girls aged three (812 cal/day) [NHMRC and MOH 2006]. Total consumption of protein and fat were essentially unchanged between the two food diaries, however, a decrease in consumption of simple refined

carbohydrate food types significantly reduced the percentage contribution of carbohydrates to the total energy intake (Table 2). Qualitative analysis of the food records showed the child had increased consumption of meat and vegetables, reduced consumption of fruits and grains, and had switched refined carbohydrate grain based foods to lower glycemic index alternatives. This resulted in increased fiber intake and the child was now meeting the recommended daily intake (RDI) for more micronutrients despite consuming fewer calories. While the child's parents did not initially report food seeking behaviors , they did report that they had locks on the pantry and fridge to prevent self-serving when reviewed in September 2015. It was reported that the child has a history of being sedentary for extended periods and watches 4-5 hours of television per day.

CYTOGENIC ANALYSIS

Chromosomal microarray analysis was performed using the Illumina HumanCytoSNP - 300k v2.1 array platform. Raw data were uploaded in KaryoStudio software (Illumina) and log2 ratios (LogR) and B allele frequency (BAF) was calculated by normalisation to an internal reference 'cluster' of approximately 300 clinical samples. Automated detection of copy number changes was carried out using the cnvPartition algorithm (versions 1.2.1 to 3.1.6) in KaryoStudio software. All identified abnormalities were further characterised by visual inspection of the LogR and BAF chromosomal plots. Quality assessment of the array data utilizes BAF (ie, BAFdev) and LogR (ie, LogRdev) with samples only accepted for analysis where BAFdev <0.03 and LogRdev <0.16. The laboratory uses a conservative threshold of 20 consecutive SNP markers for CNV calling as well as the logR and BAF variance, which over the genome on average gives a reporting threshold down to 200kb.

The microarray performed on the child revealed an approximate 2.0 Mb heterozygous deletion of chromosome band 11p14.1 which involves 102 SNP markers (genomic coordinate's chr11:27,593,590-29,594,921 UCSC Genome Browser March 2006 (NCBI36/hg18) (Fig 3). An additional CNV was detected in

this patient which is a well-established benign variant. Parental SNP microarray analysis showed the mother to carry the same 2.0Mb deletion, whilst the father's microarray showed a normal LogR of BAF within 11p14.1. The mother also had four other CNVs, two of which did not contain genes and two which were common well-established benign variants.

DISCUSSION

This report presents a case of a well characterized inherited deletion of 11p14.1 in both mother and child resulting in a phenotype characterized by developmental delay and childhood obesity, likely the result of *BDNF* gene deletion. There are no comparable deletions in the Children's Hospital of Philadelphia (CHOP), ISCA or DECIPHER copy number variant (CNV) databases nor in VCGS' internal database representing CNVs detected in over 50,000 individuals from the paediatric referral population. However, Ernst et al. [2012] reported a mother and child with intellectual difficulties and obesity who had a similar BDNF containing deletion (, chr11:23, 484,198-27,857,928).. These findings suggest that the combination of obesity and developmental delay in a child and mother should raise suspicions of an underlying genetic cause. Furthermore, this case demonstrates that despite a genetic predisposition for obesity, the condition can be managed in a specialist multidisciplinary pediatric clinic.

This novel 2.0 Mb deletion detected in the child and mother is relatively gene poor, containing only three genes; *BDNF, KIF18A* and *METT5D1*. Neither *KIF18A*, a member of the mitotonic kinesin family which plays a role in controlling microtubule length; nor *METT5D1*, a member of the methyltransferase superfamily, are reported in the literature to have a role in neurological development or obesity.

The prevalence of *BDNF* deletion in the population is currently unknown, although there are at least 26 individuals reported in the literature [Gray et al., 2006; Ernst et al., 2012], including patients with WAGR syndrome and *BDNF* haploinsufficiency [Han et al., 2008]. In a comparative analysis these patients

with combined WAGR and *BDNF* haploinsufficiency were compared to 14 patients with WAGR syndrome without *BDNF* deletions, and were found to have reduced BDNF expression, reduced nociception and significantly increased rates of obesity (100% of patients with *BDNF* haploinsufficiency *vs.* 20% without *BDNF* deletion, at 10 years of age). This group also later described that patients with WAGR syndrome and *BDNF* deletion had reduced cognitive functioning and greater social impairment [Han et al., 2013].

Grey et al. [2006] reported on an 8year-old child with a chromosomal inversion involving the 11p14 region including the *BDNF* locus. BDNF expression was shown to be decreased and her phenotype included obesity and developmental delay, nociception and hyperactivity.

Obesity is associated with a negative stigma among both the general and medical communities. Identifying genetic causes of obesity, such as the *BDNF* deletion described here, may help to relieve patients of the guilt often associated with obesity. Therefore, there may be a role for *BDNF* gene screening of patients presenting in this manner. Although there are certain genes and hormonal conditions that predispose an individual to the development of obesity, the presence of an underlying genetic cause does not reduce the benefits of a program of dietary and exercise interventions. Evidence supports that patients with an underlying genetic cause of obesity, in particular *MC4R* and *FTO*, who implement lifestyle modifications can achieve significant weight reduction [Zlatohlavek et al., 2013]. Indeed, our results indicate that early intervention and implementation of a healthy-eating regime is beneficial for weight management and improvement of health parameters.

Despite having an estimated energy intake below the predicted BMR for a 3-year-old child, prior to intervention this child was still gaining weight excessively. Estimated energy intake for 3-4 year olds was previously been reported to be 1,500cal/day which is nearly three times that consumed by this child [Reilly et al., 2001]. These results are in contrast to other reports which find that deletion of *BDNF* generally leads to a phenotype of hyperphagia

[Ramachandrappa et al., 2013], which was not observed in this case, although the child's parents did report severe food-seeking behavior, and therefore underreporting of intake may be a factor.

There has been much discussion about the role of BDNF in the development of obesity and in the regulation of appetite [Waterhouse and Xu 2013]. Indeed, it has been shown that a central injection of BDNF into the brains of rats resulted in significant loss of appetite together with severe weight loss [Pelleymounter et al., 1995]. Another clinical report described an 8-year-old with *NTRK2* mutation, the gene encoding BDNF receptor, which resulted in a similar phenotype [Yeo et al., 2004]. Future development of a pharmacological means of delivery of BDNF, or target of its receptor may lead to the development of a potential treatment for the obesity and food-seeking/hyperphagia phenotype described in these patients. Indeed, BDNF has been studied in pre-clinical trials in both Parkinson disease and Huntington disease for its role as a neuroprotective agent [Perez-Navarro et al., 2000; Fumagalli et al., 2006; Allen et al., 2013].

This clinical report of a mother and child with the same 11p14.1 deletion and phenotype of obesity and developmental delay serves to suggest the importance of BDNF in regulating appetite and in normal neuronal development. This patient also highlights a potential candidate gene for investigation in patients presenting with a phenotype of obesity and developmental delay. We hypothesize that in the future there may be a role for *BDNF* gene screening of patients presenting in this manner.

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TABLES

Table 1: Biochemical analysis of serum obtained from the child at the initial clinic visit.

Biochemistry	**Value**
Glucose (mmol/L)	5.0
Insulin (mU/L)	**44.6**

Cholesterol (mmol/L)	**6.2**
HDL Cholesterol (mmol/L)	1.1
LDL Cholesterol (mmol/L)	**3.7**
Triglycerides (mmol/L)	**3.1**
Sodium (mmol/L)	**148.0**
Potassium (mmol/L)	**5.2**
Urea (mmol/L)	3.9
Creatinine (mmol/L)	27.0
Albumin (g/L)	**48.0**
Total Protein (g/L)	**78.0**
CRP (mg/L)	**33.0**
ALT (IU/L)	**67.0**
ALP (IU/L)	290.0
GGT (IU/L)	16.0
TSH (mIU/L)	1.7

Bold indicates that value is elevated beyond normal range
TSH: thyroid stimulating hormone

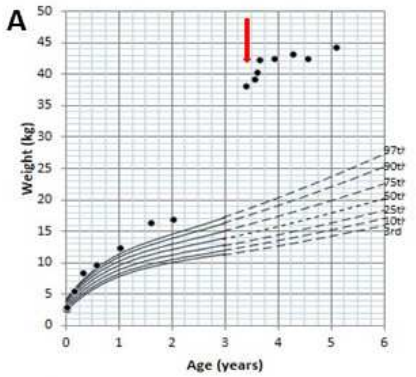
Table 2: Comparison of the child's average daily intake for energy and macronutrients.

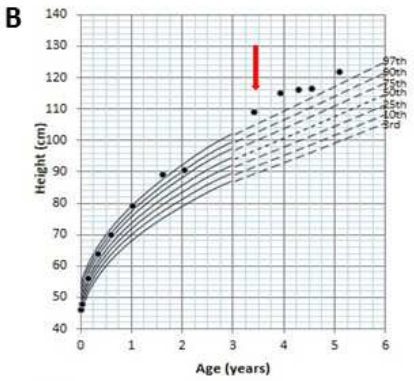
Average daily intake	Oct 2014 Mean ±SD	Sept 2015 Mean ±SD	*p* value
Total energy (kJ)	2868 (±396)	2317 (±509)	**0.050**
Protein (g)	31.4 (±5.8)	30.3 (±4.1)	0.772
Fat (g)	21.1 (±5.8)	20.7 (±8.5)	0.864
Carbohydrate (g)	92.7 (±20.7)	61.7 (±14.7)	**0.001**
% Energy from			
Protein	18.3 (±4.7)	22.3 (±3.7)	0.198
Fat	26.5 (±7.4)	31.2 (±7.2)	0.365
Carbohydrate	51.5 (±5.8)	42.2 (±5.6)	**0.008**

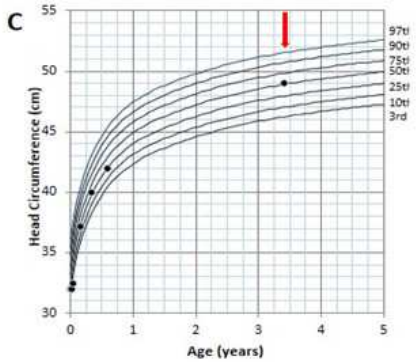
p-value determined via two-tailed students t-test, values <0.05 are statistically significant, SD; standard deviation

FIGURES

Figure 1: Phenotype of BDNF deletion in mother and child. (A) Patient's dentition, and facial structure, (B) Mothers facial structure, (C) Body habitus of patient and (D) Mother







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Age (years)	BMI	BMI z-score
2	20.63	2.39
3.36	31.98	4.36
3.87	32.14	3.89
4.22	31.92	3.63
4.49	31.31	3.46
5.03	29.81	3.18

Figure 2: Growth pattern of child with BDNF deletion. (A) Weight for age (kg). (B) Height for age. Recumbent length from 0-3 years, standing height from 3-6 years (cm). (C) Head circumference (cm). (D) BMI-for-age and BMI z-score. Percentile and BMI z-score data taken from CDC Growth Charts [Kuczmarski et al., 2002]. Arrows indicate the time point where the patient was first seen at the Royal Children's Hospital Melbourne.

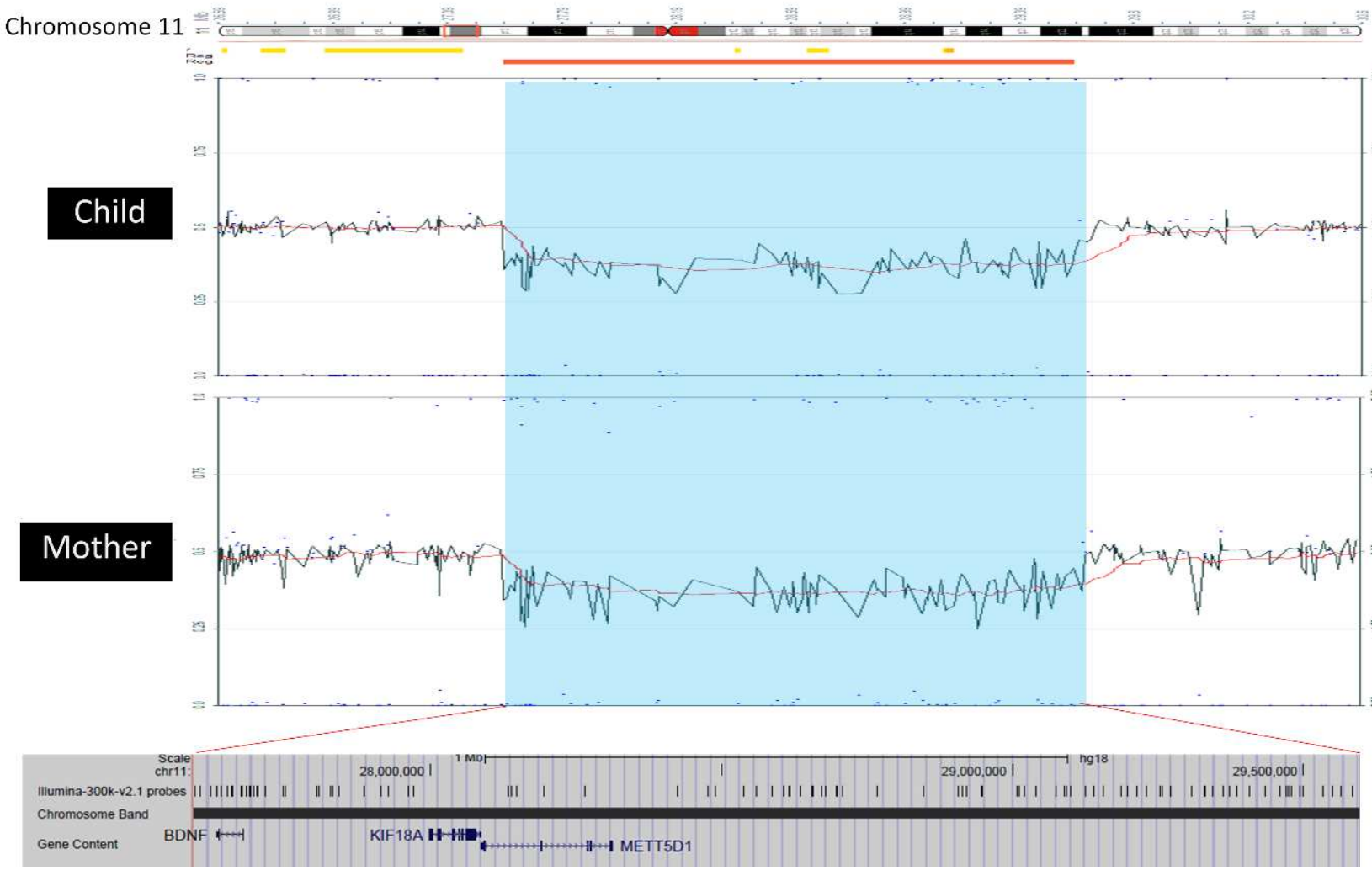

Figure 3: Schematic illustrating the heterozygous microdeletions in chromosome 11p14.1 in the child's and maternal genome. Highlighted in blue is the region deleted (chr11:27,593,590-29,594,921) in both individuals with expansion out to the gene content output from the UCSC Genome Browser March 2006 (NCBI36/hg18) assembly. The father microarray showed normal LogRdev and BAF for this region (not shown).