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SHORT REPORT

No evidence for pathogenic variants or maternal effect of *ZFP57* as the cause of Beckwith–Wiedemann Syndrome

Susanne E Boonen*[,1,2], Johanne MD Hahnemann[1], Deborah Mackay[3,4], Niels Tommerup[2],
Karen Brøndum-Nielsen[1,5], Zeynep Tümer[1,5] and Karen Grønskov[1,5]

Beckwith–Wiedemann syndrome (BWS) is an overgrowth syndrome, which, in 50–60% of sporadic cases, is caused by hypomethylation of *KCNQ1OT1* differentially methylated region (DMR) at chromosome 11p15.5. The underlying defect of this hypomethylation is largely unknown. Recently, recessive mutations of the *ZFP57* gene were reported in patients with transient neonatal diabetes mellitus type 1, showing hypomethylation at multiple imprinted loci, including *KCNQ1OT1* DMR in some. The aim of our study was to determine whether *ZFP57* alterations were a genetic cause of the hypomethylation at *KCNQ1OT1* DMR in patients with BWS. We sequenced *ZFP57* in 27 BWS probands and in 23 available mothers to test for a maternal effect. We identified three novel, presumably benign sequence variants in *ZFP57*; thus, we found no evidence for *ZFP57* alterations as a major cause in sporadic BWS cases.

INTRODUCTION

Beckwith–Wiedemann syndrome (BWS, OMIM 130650) is an imprinting syndrome with an incidence of 1:13 700.[1] The most prominent clinical features are pre- and postnatal overgrowth (>97th centile), macroglossia and abdominal wall defects.[1–3] The underlying pathology of BWS includes genetic and epigenetic alterations on chromosome 11p15.5, resulting in dysregulation of growth regulatory genes.[1–3] Hypomethylation of the differentially methylated region (DMR) of imprinting control region 2/*KCNQ1OT1* DMR on the maternal allele occurs in 50–60% of BWS cases and is mostly due to an apparently pure epigenetic modification.[1,2]

Recently, mechanisms influencing imprinting disorders in trans have been identified. Hypomethylation of multiple imprinted loci (HIL) is described in different imprinting syndromes including transient neonatal diabetes mellitus type 1 (TNDM1; MIM 601410),[4] BWS,[5–7] Silver–Russell syndrome (SRS; MIM 180860)/growth restriction,[8,9] and in a single patient with the clinical phenotype of BWS and Prader–Willi syndrome.[10] Recessive *ZFP57* mutations were identified in more than half of the TNDM1 cases displaying HIL.[11] *ZFP57* is localized at chromosome 6p22.1 and encodes the KRAB zinc finger protein ZFP57, containing seven zinc finger domains (ZF1–ZF7).[11] In mice, when the zygotic function of *Zfp57* is lost (*Zfp57^{−/−}*), offsprings show partial hypomethylation at multiple maternally and paternally imprinted loci, but when both maternal and zygotic zfp57 function are lost (*Zfp57^{−/−}* offspring of *Zfp57^{−/−}* mothers), the offsprings show complete loss of methylation. This indicates that *Zfp57* is a maternal-zygotic effect gene required for maintenance of DNA methylation imprints.[12,13] The maternal effect of a gene is the genetic phenomenon in which a phenotype in the progeny is caused by a genetic alteration in the maternal genome rather than an alteration of its own; this has been observed both in humans[7,14] and in mice.[12,13] Mutations in *NLRP7* were described in females with familial recurrent biparental complete hydatidiform moles.[14] These females had normal methylation, but failed to establish methylation imprints in their oocytes. More recently, a homozygous *NLRP2* mutation was found in a female; both her sons had BWS with hypomethylation of the *KCNQ1OT1* DMR, and furthermore, one of them also had hypomethylation of *PEG1* DMR.[7] In this study, we present the mutation analysis of *ZFP57* in sporadic BWS patients and their mothers to test two hypotheses: (1) mutations in *ZFP57* lead to isolated hypomethylation of *KCNQ1OT1* DMR and thereby lead to BWS; (2) mutations in *ZFP57* in a mother may lead to BWS in her children.

MATERIAL AND METHODS

Study population

Twenty-seven sporadic BWS probands (14 females and 13 males), 23 from Scandinavia, three from other parts of Èurope and one of unknown origin, were referred for diagnostic molecular genetic testing for BWS. Patients with hypomethylation at *KCNQ1OT1* DMR were selected for the study. This was tested either by Southern blot analysis using a *KCNQ1OT1* probe and a methylation sensitive restriction enzyme, or by methylation-specific MLPA (MS-MLPA) analysis using kit ME030 (MRC-Holland, Amsterdam, the Netherlands). The mean methylation indices were for Southern blot analysis 0.12 (based on nine patients; minimum 0.05 and maximum 0.24; median 0.07) and for MS-MLPA 0.09 (based on four patients; minimum 0.01

[1]Center for Applied Human Molecular Genetics, The Kennedy Center, Glostrup, Denmark; [2]Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, Panum Institute, University of Copenhagen, Copenhagen N, Denmark; [3]Wessex Genetics Service, Southampton University Hospital Trusts, Southampton SO16 5Y, UK; [4]Salisbury Hospital NHS Foundation Trust, Salisbury, UK; [5]Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
*Correspondence: Dr SE Boonen, Center for Applied Human Molecular Genetics, The Kennedy Center, Gl. Landevej 7, 2600 Glostrup, Denmark. Tel: +45 43 26 01 68; Fax: + 45 43 43 11 30; E-mail: seb@kennedy.dk

and maximum 0.14; median 0.10). Ten patients were further tested for HIL by MS-PCR as described previously.[4] Three of the 27 probands were from discordant twin pairs (one from a pair of female monozygotic (MZ) twins, one from a pair of female twins with unknown zygosity, and one from a pair of male twins of unknown zygosity). Twenty-three mothers of BWS probands were available for investigation, and all had normal methylation at *KCNQ1OT1* DMR tested as described above. To our knowledge, none of the probands were from consanguineous families. We have no information about whether the probands were conceived by assisted reproductive technology. The control group consisted of 50 normal Danish individuals.

DNA studies

Mutation analysis. DNA was extracted from peripheral blood by standard procedures. The five coding exons of *ZFP57* and 20 bp of the flanking intronic sequences were amplified in eight fragments by PCR as previously described.[11] PCR primer sequenced are listed in Supplementary Table 1 and PCR conditions are available upon request. All *ZFP57* alterations are described according to den Dunnen and Antonarakis,[15] with GenBank accession number: NM_001109809.1.

In silico analysis

The possible impact of the novel amino acid substitutions was evaluated using the SIFT database, (http://sift.jcvi.org/) and the PolyPhen-2 database, (http://genetics.bwh.harvard.edu/pph2/).

RESULTS

By sequencing *ZFP57* in 27 sporadic BWS patients with hypomethylation at *KCNQ1OT1* DMR and in 23 mothers with normal methylation, we identified three novel sequence variations. All the variations were located in exon 6. Ten out of the 27 patients were investigated for HIL, but no signs of HIL were observed (data not shown).

In the affected twin from the MZ female twins (from Scandinavia), we detected a heterozygous sequence variation c.503C>T (p.Ser168Phe) affecting a non-conserved amino acid in ZF1. The variation was also found in the non-affected twin and in the father, but not in the mother. The SIFT database predicts the variation to be tolerated with a score of 0.30 (normal >0.05), but the PolyPhen-2 database predicts it to be probably damaging with a score of 0.971 (sensitivity: 0.56; specificity: 0.91). The variant was not detected in 50 controls or present in the 1000 genomes database (http://browser.1000genomes.org/index.html).

In a proband from Europe, we found two silent sequence variations, c.723C>T (p.=) and c.1026T>C (p.=). The c.723C>T is located in ZF4 and the c.1026T>C is located between ZF4 and ZF5. Both variations were inherited from the unaffected father and thus were in *cis*. Both variations were absent in the mother. The variants were not detected in 50 controls or in the 1000 genome database.

In a proband from Scandinavia, we identified a sequence variation c.499C>T (p.Arg167Cys) affecting a non-conserved amino acid in ZF1. The variation was inherited from the mother. This variation was present in seven out of 50 controls and was reported in the SNP database (http://ncbi.nlm.nih.gov/snp) (rs61730330). The SIFT database predicts the variation to affect protein function with a score of 0.02 (normal > 0.05) and the PolyPhen-2 database predicts it to be probably damaging with a score of 0.914 (sensitivity: 0.67; specificity: 0.86).

DISCUSSION

The etiology of the hypomethylation of *KCNQ1OT1* DMR observed in BWS patients is unknown. Recently, mutations in *ZFP57* were found to cause HIL in some TNDM1 patients.[11] As *KCNQ1OT1* DMR is among the loci observed to be hypomethylated in TNDM HIL patients, and further, as some BWS patients were also shown to have HIL, we investigated whether sporadic BWS could be caused by mutations in *ZFP57*.

We identified three novel sequence variations in *ZFP57*. Two of the variations are silent mutations, and furthermore, not located at the splice sites. The causative effect of the third variation is questionable as suggested by *in silico* analysis. We also identified a known SNP. None of the probands or mothers was homozygous or compound heterozygous for variations in *ZFP57*.

It could be argued that *ZFP57* mutations would only be found in patients with HIL, but notably the methylation profile for the different imprinted loci varies considerably in patients with *ZFP57* mutations.[11] Other studies reported 11–25% of BWS patients[5,6] and 14% of SRS patients[8,9] to have HIL. In our BWS cohort, none of the 10 investigated patients had HIL, but the cohort is too small to deduce conclusions. In SRS patients, mutational analysis of *ZFP57* similarly has shown no pathogenic alterations.[16] In contrast to BWS, however, the hypomethylation in SRS affects a paternally imprinted locus (*H19* DMR), albeit cases of both SRS and BWS have been reported with hypomethylation at both maternally as well as paternally methylated DMRs.[8,9,17,18]

Other trans-acting factors and mechanisms involved in the establishment and maintenance of methylation in DNA imprints could be responsible for the methylation and demethylation at *KCNQ1OT1*, maybe in cooperation with *ZFP57*, including DNA methyltransferases, methyl-binding proteins, imprinting genes network and/or RNA interference. Furthermore, alterations in *cis* are known to cause aberrant methylation, for example, in SRS and growth retardation where deletions and translocations of the *H19/IGF2* enhancer region can cause hypomethylation of the IGF2P0 promoter element.[19]

Mice studies have shown that *Zfp57* is a maternal effect gene, and it has been hypothesized that a gradual loss of DNA methylation takes place in the *Zfp57*−/− progeny of *Zfp57*−/− mothers and account for the spatial and temporal discrepancy of the molecular defect and the phenotypic effect;[13] however, we do not find *ZFP57* to be a major maternal effect gene in sporadic BWS, neither do our data support the hypothesis that alterations in *ZFP57* are a major cause of *KCNQ1OT1* DMR hypomethylation in sporadic BWS cases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)