RESEARCH NOTE

Novel compound heterozygous frameshift mutations of *C2orf37* in a familial Indian case of Woodhouse–Sakati syndrome

MANSOOR C. ABDULLA[1]*, ANAS M. ALAZAMI[2], JEMSHAD ALUNGAL[1], JASSIM M. KOYA[3] and MOHTHASH MUSAMBIL[4]

[1]*Department of Internal Medicine,* [3]*Department of Radiodiagnosis, and* [4]*Medical Biotechnology Central Research Lab, MES Medical College, Perinthalmanna 679 338, India*

[2]*Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh MBC-03 PO BOX 3354, Saudi Arabia*

[Abdulla M. C., Alazami A. M., Alungal J., Koya J. M. and Musambil M. 2015 Novel compound heterozygous frameshift mutations of *C2orf37* in a familial Indian case of Woodhouse–Sakati syndrome. *J. Genet.* **94**, 489–492]

Introduction

Woodhouse–Sakati syndrome (WSS, MIM: 241080), a rare autosomal recessive disorder characterized by hypogonadism, alopecia, diabetes mellitus, mental retardation and extrapyramidal manifestations, was first described in a few consanguineous Saudi families (Woodhouse and Sakati 1983) and is now recognized in other ethnicities as well (Koshy *et al.* 2008; Gul *et al.* 2000; Steindl *et al.* 2010; Habib *et al.* 2011). The other manifestations of this syndrome are sensorineural deafness, decreased signal intensity in the basal ganglia, T-wave abnormalities and depressed insulin-like growth factor 1 levels. A mutation in the *C2orf37* gene was described as the cause of WSS in 2008 in the Saudi families including the ones originally described by Woodhouse and Sakati (Alazami *et al.* 2008). Additional mutations in *C2orf37* were also described by them in patients from other ethnicities. Here, we report two novel frameshift mutations in *C2orf37* present in the compound heterozygous state in an Indian family with WSS, and discuss the interfamilial and intrafamilial phenotypic heterogeneity. The patients showed all the clinical features previously described in WSS, except for the extrapyramidal features. The knowledge of phenotypic variability of WSS in patients from different ethnicities help in facilitating the diagnosis of this rare syndrome.

Patients and methods

Patients

The WSS family included in this study is from Kerala state, south India. The proband was a 40 year-old widow, the second child of a nonconsanguineous couple. She was born by vaginal delivery at term without complications. Two other affected members of the family (the proband's younger brother and sister) and two unaffected members (father and elder sister) were included in the study. The age at onset of the proband and sister was six years, and 10 years for the proband's brother. Informed consent was obtained from all members of the family before testing. The study was approved by the Ethical Committee of the MES Academy of Medical Sciences. Detailed clinical examination, blood investigations, electrocardiogram and pure-tone audiogram were carried out for all affected members. Magnetic resonance imaging (MRI) of the brain and pelvis was done for two affected female patients.

Molecular testing

Peripheral blood samples were collected from all individuals for genomic DNA extraction and molecular diagnosis. Primers were designed to flank the coding regions and exon/intron boundaries of *C2orf37*, as identified on the UCSC website and were directly sequenced with the dideoxy chain-termination method (Amersham ET Dye Termination Sequencing, GE Healthcare, Pittsburgh, USA). The resultant chromatograms were assessed using the SeqMan Pro suite (Dnastar, Madison, USA). Mutations described in the text are based on accession NM_025000.3, with A of the ATG initiation codon numbered as +1. All primer sequences and PCR conditions are available from the authors upon request.

Results

Clinical finding

The proband (patient 1) had primary amenorrhoea, alopecia from the age of six years and sensorineural hearing loss from the age of eight. The proband was diagnosed with diabetes

*For correspondence. E-mail: drcamans@gmail.com.

Keywords. Woodhouse–Sakati syndrome; Indian family; *C2orf37* gene.

at the age of 30 years. She had alopecia totalis, madarosis, a triangular and progeric face with prominent ears (figure 1c). She had underdeveloped secondary sex characters and mild cognitive impairment. Electrocardiogram showed symmetrical T-wave inversion in leads V1, V2, V3, V4, lead III and avF. MRI of pelvis showed a rudimentary uterus with no identifiable ovarian tissue. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin were normal. Brain MRI showed small discrete and confluent T2 weighted and fluid attenuated inversion recovery (FLAIR) hyperintensities in the periventricular white matter and centrum semiovale bilaterally (figure 1, a&b).

The proband's affected younger sister (patient 2) had alopecia from the age of six, primary amenorrhoea, bilateral moderate sensorineural hearing loss from the age of eight and had diabetes from the age of 34 years. She had madarosis, a triangular and progeric face with prominent ears (figure 1f), anodontia and underdeveloped secondary sex characters. Electrocardiogram showed symmetrical T-wave inversion in leads V1, V2 and V3. MRI of the pelvis showed rudimentary uterus with two thin-walled cysts in the right half of the pelvis with no identifiable ovarian tissue. Brain MRI showed abnormalities similar to the proband (figure 1, d&e).

The proband's affected younger brother (patient 3) had alopecia from the age of 10, bilateral severe sensorineural hearing loss from the age of 12 and diabetes from the age of 32 years. He had madarosis, mild cognitive impairment and small external genitalia and testes. His electrocardiogram was normal. He refused to take photographs and MRI of brain. Other members of the family including an elder sister and father were normal.

Mutation screening

To assess the underlying genetic cause of WSS in this family, DNA extracted from whole blood was assayed using the polymerase chain reaction (PCR). Bidirectional sequencing of *C2orf37* revealed compound heterozygosity of two novel mutations in all patients (figure 2). The first of these, c.459-7_499del, involves a deletion that begins 7-bp upstream of exon 5 (NP_079276) and ends at cDNA position 499, thereby removing the splice acceptor site of exon 5 along with half its coding region. The second mutation, c.1238delA, produces a frameshift in exon 12. Hence, both mutations are predicted to result in premature protein truncation. The father was wild type for the first allele and heterozygous for the second. The mother's DNA was not available for screening.

Discussion

Woodhouse and Sakati (1983) described a rare autosomal recessive disorder in seven individuals from two highly

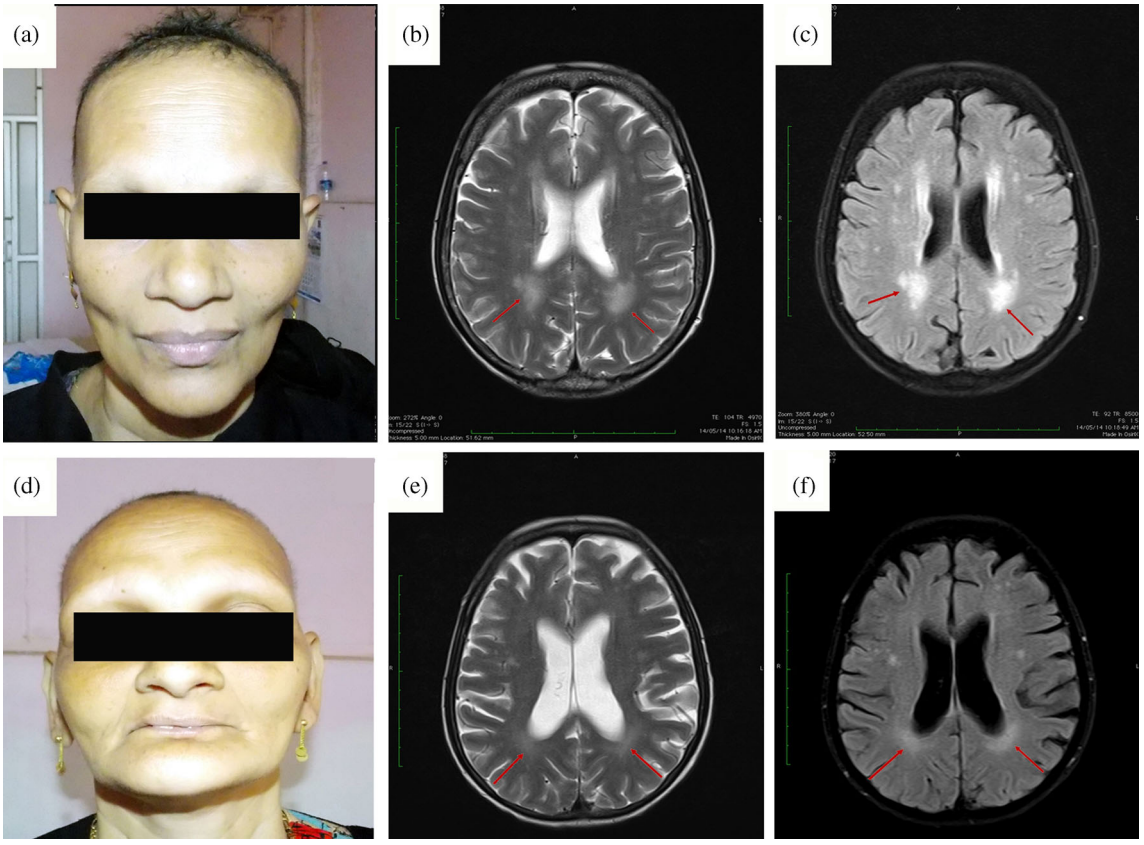

Figure 1. Clinical photographs of (a) proband and (d) affected younger sister showing alopecia totalis, madarosis and a triangular and progeric face. (b&c) MRI brain of proband and (e&f) affected younger sister showing T_2 weighted and FLAIR hyperintensities (indicated by red arrows) in the periventricular white matter and centrum semiovale bilaterally.

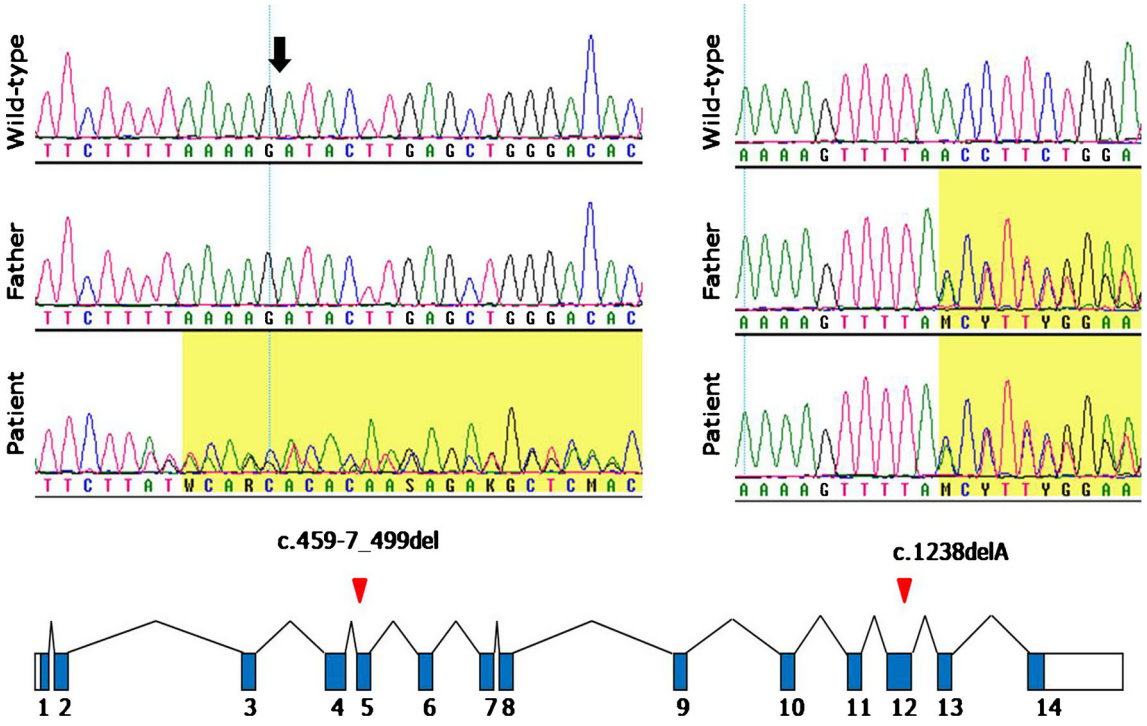

Figure 2. DNA sequence chromatograms of the two novel mutations for a patient, the father and one normal control. Sequence that is highlighted in yellow is indicative of a heterozygous frameshift. The black arrow indicates the beginning of exon 5. Red arrowheads denote the location of that particular mutation along the genomic stretch of *C2orf37* (coding regions are filled boxes, untranslated regions are empty boxes and introns are represented by lines).

consanguineous Saudi families, which became known as WSS. This syndrome was initially thought to be confined to Saudi families but was described later in patients from other ethnicities as well. In 2008, Alazami *et al.* (2008) defined a mutation in the *C2orf37* gene as the cause of WSS. This gene encodes a previously uncharacterized nucleolar protein whose precise function is poorly known.

Nine different mutations leading to protein truncations including three deletions, three nonsense and three splicing errors have been described in *C2orf37* (Alazami *et al.* 2010; Habib *et al.* 2011). Our Indian family is the first case of compound heterozygosity described in WSS. Our findings increase the total number of published mutations to 11 and reinforce that, the missense variants do not appear to be a genetic feature of this disorder.

All the affected members had the classical features of WSS including intellectual disability, deafness, hypogonadism, alopecia and diabetes mellitus. Extrapyramidal defects were not evident in any of the family members. Movement disorders described in WSS include dystonia and chorea of the limbs with onset in adolescence (Schneider and Bhatia 2008). The members of the first family with WSS reported from India also had no extrapyramidal manifestations (Koshy *et al*. 2008).

Brain MRI showed white matter changes as previously reported in both affected females. Widespread, confluent periventricular T2 white matter hyperintensities are characteristic of WSS (Kruer *et al.* 2012). WSS is included in a group of inherited neurologic disorders called 'neurodegeneration with brain iron accumulation' (Gregory and Hayflick 2011). Decreased signal in the globus pallidus, substantia nigra and other regions of the basal ganglia on T_2-weighted images are consistent with iron accumulation.

Anodontia was found only in one among the three patients and ECG changes in two. Anodontia was described in all the three members of the Italian family with WSS (Steindl *et al.* 2010). Interfamilial and intrafamilial phenotypic heterogeneity in WSS was reported previously (Ben-Omran *et al.* 2011) and is evident from the aforementioned family.

In this study, we have identified the first compound heterozygous mutation in the *C2orf37* gene resulting in WSS. Reporting of phenotypic and genotypic variations in WSS will help explicate the pathophysiological aspects of this rare nucleolopathy, as well as open the scope for more study and research on the key roles played by the nucleolus in humans.

Acknowledgements

We thank the family members for their kind participation, and Fowzan Alkuraya for invaluable assistance.

References

Alazami A. M., Al-Saif A., Al-Semari A., Bohlega S., Zlitni S., Alzahrani F. *et al.* 2008 Mutations in *C2orf37*, encoding a nucleolar protein, cause hypogonadism, alopecia, diabetes mellitus,

mental retardation, and extrapyramidal syndrome. *Am. J. Hum. Genet.* **83**, 684–691.

Alazami A. M., Schneider S. A., Bonneau D., Pasquier L., Carecchio M., Kojovic M. *et al.* 2010 *C2orf37* mutational spectrum in Woodhouse–Sakati syndrome patients. *Clin. Genet.* **78**, 585–590.

Ben-Omran T., Ali R., Almureikhi M., Alameer S., Al-Saffar M., Walsh C. A. *et al.* 2011 Phenotypic heterogeneity in Woodhouse–Sakati syndrome: two new families with a mutation in the *C2orf37* gene. *Am. J. Med. Genet. Part A* **155**, 2647–2653.

Gregory A. and Hayflick S. J. 2011 Genetics of neurodegeneration with brain iron accumulation. *Curr. Neurol. Neurosci. Rep.* **11**, 254–261.

Gul D., Ozata M., Mergen H., Odabaşi Z. and Mergen M. 2000 Woodhouse and Sakati syndrome (MIM 241080): report of a new patient. *Clin. Dysmorphol.* **9**, 123–125.

Habib R., Basit S., Khan S., Khan M. N. and Ahmad W. 2011 A novel splice site mutation in gene *C2orf37* underlying Woodhouse–Sakati syndrome (WSS) in a consanguineous family of Pakistani origin. *Gene* **490**, 26–31.

Koshy G., Danda S., Thomas N., Mathews V. and Viswanathan V. 2008 Three siblings with Woodhouse-Sakati syndrome in an Indian family. *Clin. Dysmorphol.* **17**, 57–60.

Kruer M. C., Boddaert N., Schneider S. A., Houlden H., Bhatia K. P., Gregory A. *et al.* 2012 Neuroimaging features of neurodegeneration with brain iron accumulation. *Am. J. Neuroradiol.* **33**, 407–414.

Schneider S. A. and Bhatia K. P. 2008 Dystonia in the Woodhouse Sakati syndrome: a new family and literature review. *Mov. Disord.* **23**, 592–596.

Steindl K., Alazami A. M., Bhatia K. P., Wuerfel J. T., Petersen D., Cartolari R. *et al.* 2010 A novel *C2orf37* mutation causes the first Italian cases of Woodhouse Sakati syndrome. *Clin. Genet.* **78**, 594–597.

Woodhouse N. J. and Sakati N. A. 1983 A syndrome of hypogonadism, alopecia, diabetes mellitus, mental retardation, deafness, and ECG abnormalities. *J. Med. Genet.* **20**, 216–219.

Received 23 November 2014, in final revised form 11 February 2015; accepted 4 March 2015
Unedited version published online: 9 March 2015
Final version published online: 14 August 2015