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Mutations in *CEP57* cause mosaic variegated aneuploidy syndrome

Katie Snape^{1,+}, Sandra Hanks^{1,+}, Elise Ruarik¹, Patricio Barros-Núñez², Anna Elliott¹, Anne Murray¹, Andrew H Lane³, Nora Shannon⁴, Patrick Callier⁵, David Chitayat⁶, Jill Clayton-Smith⁷, David FitzPatrick⁸, David Gisselsson⁹, Sebastien Jacquemont¹⁰, Keiko Asakura-Hay¹¹, Mark Micale¹², John Tolmie¹³, Peter Turnpenny¹⁴, Michael Wright¹⁵, Jenny Douglas¹, and Nazneen Rahman^{*,1}

¹Section of Cancer Genetics, Institute of Cancer Research, Sutton, UK ²División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México ³Division of Pediatric Endocrinology, State University of New York at Stony Brook, NY, USA ⁴Clinical Genetics Service, City Hospital, Nottingham, UK ⁵Service de Cytogénétique, CHU de Dijon, Dijon, France ⁶Division of Clinical & Metabolic Genetics, Department of Pediatrics, Hospital for Sick Children, Toronto, Ontario, Canada ⁷Department of Clinical Genetics, St. Mary's Hospital, Hathersage Road, Manchester, UK ⁸MRC Human Genetics Unit, Institute of Genetic and Molecular Medicine, Western General Hospital, Edinburgh, UK ⁹Department of Clinical Genetics, Lund University, University and Regional Laboratories, Skåne University Hospital, Lund, Sweden ¹⁰Division of Medical Genetics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland ¹¹Ninewells Hospital and Medical School, Tayside University Hospitals NHS Trust, Dundee ¹²Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak & Oakland University, William Beaumont School of Medicine, Rochester, MI, USA ¹³Department of Clinical Genetics, Ferguson Smith Centre, Royal Hospital for Sick Children, Glasgow, UK ¹⁴Peninsula Clinical Genetics Service and Peninsula Medical School, Royal Devon & Exeter Healthcare NHS Trust, Exeter, UK ¹⁵Northern Genetics Service, Newcastle upon Tyne Hospitals Foundation Trust, Newcastle upon Tyne, UK

Abstract

Using exome sequencing and a variant prioritisation strategy that focuses on loss-of-function variants, we identified biallelic, loss-of-function *CEP57* mutations as a cause of constitutional mosaic aneuploidies. *CEP57* is a centrosomal protein and is involved in nucleating and stabilizing microtubules. Our data indicate that these and/or additional functions of *CEP57* are crucial in maintaining correct chromosomal number during cell division.

*To whom correspondence should be addressed .

⁺These authors contributed equally

Accession codes GenBank: human *CEP57*, NM_014679; UniprotKB: human CEP57, Q86XR8.

URLs

<http://genome.ucsc.edu/>

<http://www.ensembl.org/index.html>

<http://www.ncbi.nlm.nih.gov/projects/SNP/>

<http://www.1000genomes.org/>

<http://www.genenames.org/>

Author contributions S.H., J.D., A.M. undertook exome sequencing. K.S., E.R., A.E., and N.R. performed data management and analysis. S.H. and J.D. undertook *CEP57* mutation and dosage analysis. P. B-N., A.L., N.S., P.C., D.C., J. C-S., D.F., D.G., S.J., K.A-H., M.M., J.T., P.T. and M.W. provided clinical material. N.R. wrote the manuscript with substantial input from K.S., S.H., E.R. and J.D. N.R. designed and oversaw all aspects of the study.

Cell division is a highly complex process that involves chromosomal duplication and separation into two daughter cells. Errors in this process result in gains or losses of chromosomes, which is known as aneuploidy. Aneuploidy is a significant cause of human disease, causally implicated in miscarriage, developmental disorders and cancer¹⁻². Multiple biological processes, including mitotic checkpoints, chromatid attachment mechanisms and centrosome, kinetochore and microtubule functions are required to prevent aneuploidy¹⁻².

Mosaic variegated aneuploidy syndrome (MVA; MIM 257300) is a rare, autosomal recessive disorder characterised by mosaic aneuploidies, diverse phenotypic abnormalities and predisposition to cancer³⁻⁴. Study of this rare condition can inform on the basic processes controlling aneuploidy in humans. For example, we previously demonstrated that biallelic *BUB1B* mutations cause MVA, linking mitotic spindle defects to human disease and providing some of the strongest evidence that aneuploidy can be a cause, as well as a consequence, of cancer⁴.

BUB1B mutations underlie only a proportion of MVA and we adopted an exome sequencing strategy to identify additional causes. We undertook exome sequencing in two siblings with MVA, (ID 633_1 and 633_2), in whom we previously excluded *BUB1B* mutations³. We used exome capture followed by paired-end sequencing on an Illumina GAIIX Solexa sequencer (Supplementary Methods). 90% of target bases in the resulting sequence had 10x coverage. We used NextGENe software to detect sequence variants and applied a series of filters to identify the variants that were most likely to be causative of MVA. (Supplementary Methods, Supplementary Table 1). We removed variants with read coverage <10 and/or with a wildtype / mutant ratio of <30% as these are more likely to be false. We also removed variants in known pseudogenes. As MVA is a very rare condition we removed all variants present in 50 exomes we have performed in individuals with other conditions and all variants recorded in dbSNP and/or 1000 Genomes. We also removed all synonymous and intronic variants outside splice junctions, as these are unlikely to be causative. After these filters there were 439 variants in sibling 1 and 439 in sibling 2. We used this final dataset in the analyses outlined below.

We first applied a loss-of-function (LOF) script that identifies nonsense mutations, coding insertions or deletions that result in translational frameshifts, and insertions, deletions or base substitutions at consensus splice residues (Supplementary Methods). We removed LOF variants in genes in which we had identified >1 LOF variant in the 50 in-house exomes on the assumption that LOF variants detected in such genes are too common to cause MVA and/or are more likely to be artefactual. After this, there remained 20 LOF variants in sibling 1 and 24 in sibling 2 (Supplementary Table 2). We focussed our analyses on LOF variants in the first instance because of the strong prior evidence of association of this class of mutation with human disease.

There was no history of consanguinity in family 633 and therefore our expectation was for the siblings to be compound heterozygotes for two different pathogenic mutations in the same gene. We identified no genes with two different LOF mutations and 12 genes with one LOF mutation shared by the two siblings (Supplementary Table 2). We next applied a conventional recessive script to identify all genes with two variants present in both siblings. There were no genes with one LOF mutation together with a second variant. There were four genes with two non-LOF variants that were both present in each sibling, but none were likely to be causative (Supplementary Table 3).

Of the twelve genes with one shared LOF mutation in the siblings, *CEP57* was notable because it localises to the centrosome⁵⁻⁶. Using Sanger sequencing we confirmed the *CEP57* 520_521delGA deletion detected through the exome sequencing and identified a

second LOF mutation, an 11bp insertion, c.915_925dup11, which was also present in both siblings (Supplementary Methods, Supplementary Figures 1 and 2, Supplementary Table 4). This insertion is a duplication of the preceding 11 bases (Supplementary Figure 2). Analysis of parental DNA demonstrated that the deletion was present in the father and the duplication in the mother, consistent with autosomal recessive inheritance. On review of the exome data the 11bp insertion was clearly present in 30-40% of reads in each sibling. However, it had not been called in many reads, particularly those in which the insertion was towards the end of the read, presumably because the reduced flanking sequence impaired alignment. As a result, the mutant / wildtype read ratio was below the NextGENe calling threshold in both siblings, and the insertion was disregarded (Supplementary Table 1, Supplementary Figure 3).

We used Sanger sequencing to analyse *CEP57* in 18 cases from 13 additional *BUB1B*-negative families with MVA and identified two further individuals with biallelic *CEP57* mutations, confirming the causative role of *CEP57* in aneuploidy predisposition (Table 1, Supplementary Figure 2). Child 638 was homozygous for a nonsense mutation, c.241C>T; p.R81X, and each of his parents, who are first cousins, are heterozygous for the mutation. Child 657 is homozygous for c.915_925dup11 and using dosage analysis we confirmed that the duplication was present on both of his alleles (Supplementary Methods, Supplementary Figure 4, Supplementary Table 5). This child was fostered and no parental samples or information regarding a possible relationship between his parents is obtainable⁷.

There were some shared clinical features in the *CEP57* cases (Table 1 and Supplementary Methods). All the affected individuals had random gains and losses of chromosomes in ~25-50% of examined cells (Figure 1a). They all had growth retardation at the milder end of the spectrum but with relative sparing of the head. There was no gross dysmorphology and development was normal or mildly delayed. Of note, two of the cases had hypothyroidism and two had rhizomelic shortening of the upper limbs which may be specific, associated features of *CEP57* mutations.

CEP57 encodes a 500 amino acid protein⁵. Secondary structure prediction suggests it is composed of two α -helical coiled-coil domains connected by a flexible linker region (Figure 1b). The N-terminal coiled-coil domain is within a region required for localisation of *CEP57* to the centrosome and for multimerization of the protein. The C-terminal half of *CEP57*, including the second coiled-coil domain, is required for nucleating, bundling and anchoring microtubules to the centrosomes within basket-like structures⁶. In addition *CEP57* is involved in intracellular bidirectional trafficking of factors, such as FGF2, along microtubules⁸.

The centrosomal localisation and microtubular stabilisation functions may explicate why *CEP57* loss results in aneuploidy. However, it should be noted that *CEP57* is a relatively under studied protein and it may be that other, currently uncharacterised, functions result in the phenotype we observed in individuals with biallelic inactivating *CEP57* mutations. *BUB1B*, which encodes BUBR1, primarily prevents aneuploidy through a mechanism in which *CEP57* is currently not known to have a role, functioning in the mitotic spindle checkpoint and to maintain stable interactions between microtubules and the kinetochore⁹⁻¹⁰. The phenotypes associated with *BUB1B* and *CEP57* mutations are broadly similar although *BUB1B* mutations are strongly associated with cancer³⁻⁴. Thus far no cancers have been reported in *CEP57* mutation-positive individuals, but the number and ages of individuals is low. The role of *CEP57* as a somatic target in cancer has not been well interrogated¹¹. Given that aneuploidy is present in >90% of solid tumors, our results suggest that further analyses would be of interest.

Exome analysis is proving successful in the identification of many Mendelian disease genes¹². However, the downstream processes of variant calling, filtering, interpretation and prioritisation for follow-up, remain challenging. In particular, many commonly used algorithms find insertion and deletion variants difficult to call with high sensitivity and specificity¹³. As a result, thus far, the majority of new disease genes identified through exome analyses were discovered through the detection of base-substitution mutations¹². Although some LOF mutations, such as nonsense and some splicing mutations result from base substitutions, the majority are due to insertions or deletions. Thus, currently, many exome analyses are not optimally configured for disease gene identification. We have optimised a calling strategy with good sensitivity for small insertions and deletions and this allowed us to detect one of the *CEP57* mutations (a two base-pair deletion). However, large insertions, such as the second *CEP57* mutation, remain challenging to call and particular focus on improving insertion / deletion detection is required for the full potential of exomic analyses to be realised.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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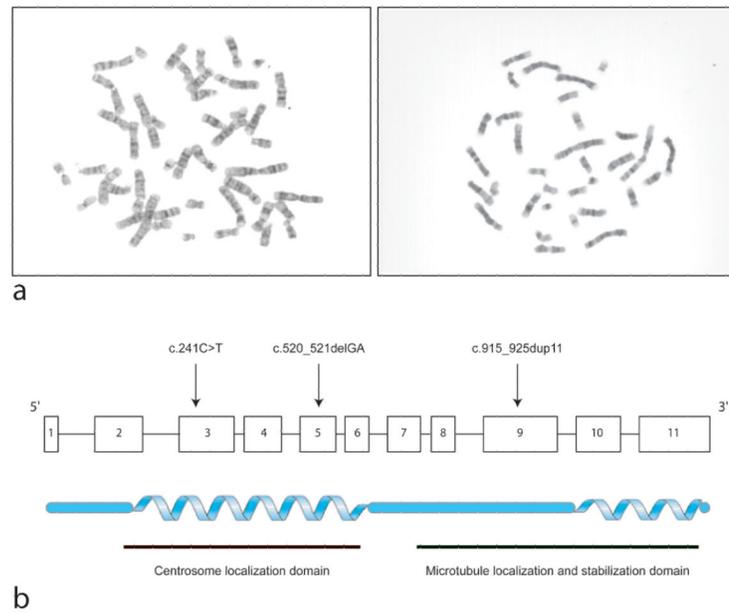


Figure 1. *CEP57* structure, mutations and resulting aneuploidy (a) Aneuploid metaphase karyotypes from family 633, showing gains and losses of whole chromosomes. (b) Schematic representation of the genomic structure of *CEP57* with the positions of mutations indicated with arrows. Below is the protein structure showing the coiled-coil domains, the centrosomal localisation region and microtubular stabilisation region.

Table 1*CEP57* mutations and associated clinical features

| Case ID | 663_1 | 663_2 | 638 | 657 |
|--------------------------|----------------------------------|----------------------------------|--------------------------------------|-----------------------------------|
| Mutations | c.520_521delGA c.915_925dup11 | c.520_521delGA c.915_925dup11 | c.241C>T; p.R81X c.241C>T; p.R81X | c.915_925dup11 c.915_925dup11 |
| Age | 8.5yrs | 4.5yrs | died 3wks | died 15yrs |
| Clinical Features | | | | |
| Mosaic aneuploidies | yes | yes | yes | yes |
| Growth retardation | yes | yes | yes | yes |
| Microcephaly | no | yes | no | yes |
| IUGR | no | yes | yes | no |
| Mental retardation | no | no | n/a | mild |
| Congenital heart disease | no | no | yes | mild |
| Hypothyroidism | yes | no | n/a | yes |
| Rhizomelic shortening | no | no | yes | yes |
| Other features | none | none | duodenal atresia hypotonia | hearing impairment sleep apnea |
| Reference | 3 | 3 | 4 | 7 |

^aMore detailed clinical information is given in the Supplementary Material