BRIEF REPORT

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Paternal or Maternal Uniparental Disomy of Chromosome 16 Resulting in Homozygosity of a Mutant Allele Causes Fanconi Anemia

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ABSTRACT: Fanconi anemia (FA) is a rare inherited disorder caused by pathogenic variants in one of 19 FANC genes. FA patients display congenital abnormalities, and develop bone marrow failure, and cancer susceptibility. We identified homozygous mutations in four FA patients and, in each case, only one parent carried the obligate mutant allele. *FANCA* and *FANCP/SLX4* genes, both located on chromosome 16, were the affected recessive FA genes in three and one family respectively. Genotyping with short tandem repeat markers and SNP arrays revealed uniparental disomy (UPD) of the entire mutation-carrying chromosome 16 in all four patients. One FANCA patient had paternal UPD, whereas FA in the other three patients resulted from maternal UPD. These are the first reported cases of UPD as a cause of FA. UPD indicates a reduced risk of having another child with FA in the family and has implications in prenatal diagnosis.

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KEY WORDS: Uniparental disomy; UPD16; Fanconi anemia; Recurrence risk; FANCA; FANCP

Fanconi anemia (FA) is characterized by debilitating developmental abnormalities, progressive bone marrow failure, increased incidence of myelodysplastic syndrome, acute myeloid leukemia, and solid tumors, especially of the head and neck and genitourinary system [Kottemann and Smogorzewska, 2013]. This rare genetic disorder, caused by mutations in any of at least 19 different genes, is both clinically and genetically heterogeneous. The genotype–phenotype relationship in FA remains largely unexplained. The Next-Gen sequencing (NGS) and arrayCGH technologies have been adopted to identify the gene and causal variants in FA families [Chandrasekharappa, et al., 2013; Flynn, et al., 2014]. The inheritance of two mutant alleles resulting in FA can be from one of 17 autosomal recessive genes (*FANCA, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4/XPF, FANCS/BRCA1, FANCT/UBE2T*). Alternatively, acquiring only one X-linked mutant *FANCB* allele, or one autosomal dominant *RAD51/FANCR* mutant allele [Hira, et al., 2015; Rickman, et al., 2015; Wang, et al., 2015; Wang and Smogorzewska, 2015] can also cause FA. With the exception of *FANCB* and *FANCR* mutation carriers, affected individuals carry two mutations in the same FANC gene, generally compound heterozygous mutations. Though primarily a recessive disease, uniparental disomy (UPD) as a cause of disease has not been reported for FA patients. Search for parental inheritance of mutations is a critical component of a comprehensive molecular diagnosis in an FA family. Such an analysis, particularly, for FA patients with homozygous causal variants may reveal instances where only one of the parents is a heterozygous carrier of the obligatory mutation while the other is not, and thus may lead to discovery of UPD as a cause of FA.

A search for biallelic pathogenic variants was performed in patients diagnosed with FA who were enrolled in the International Fanconi Anemia Registry (IFAR) (http://lab.rockefeller.edu/smogorzewska/ifar/). NGS and arrayCGH technologies were employed for mutation search in DNA from FA patients (see Supp. Methods). For those FA patients whose parental DNA were available, we also examined the parental inheritance of the observed pathogenic variants. Among the many families whose molecular diagnosis and the inheritance patterns of pathogenic variants were uncovered, there were four patients with unusual inheritance pattern. These include three patients with homozygous mutations in FANCA:FA1, FA2, and FA3, and one patient with a homozygous mutation in FANCP/SLX4: FA4 (Table 1). All four variants were independently confirmed by Sanger sequencing (Fig. 1A). The c.2546delC variant in patient FA1, if expressed, is predicted to generate a non-functional truncation. It appears 22 times in the Fanconi Anemia Mutation Database (FAMutDB) (http://www.rockefeller.edu/fanconi/) and is confined to FA patients of Japanese and

Additional Supporting Information may be found in the online version of this article.

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Korean ancestry [Yagasaki, et al., 2004; Park, et al., 2013]. The c.3520_3522delTGG variant in patient FA2 appears 31 times in the FAMutDB, and its pathogenicity has been clearly demonstrated by complementation studies [Adachi, et al., 2002]. The c.523_792del variant in patient FA3 is the result of a 9,290 bp genomic deletion (g;chr16:89867339-89876628), caused by non-allelic homologous recombination of *AluY* and *AluSx1* repeats residing in IVS5 and IVS8, respectively (Supp. Fig. S1), eliminating exons 6–8 (aa 175–264) of *FANCA*. Patient FA4 harbors a novel homozygous *FANCP/SLX4* variant, c.1366G>A. Contrary to a predicted protein with a missense amino acid, this mutation, altering the last nucleotide of exon 6, results in aberrant splicing by retaining IVS6 in cDNA, creating an unstable message. A patient fibroblast cell line carrying this homozygous variant expressed no SLX4, and the

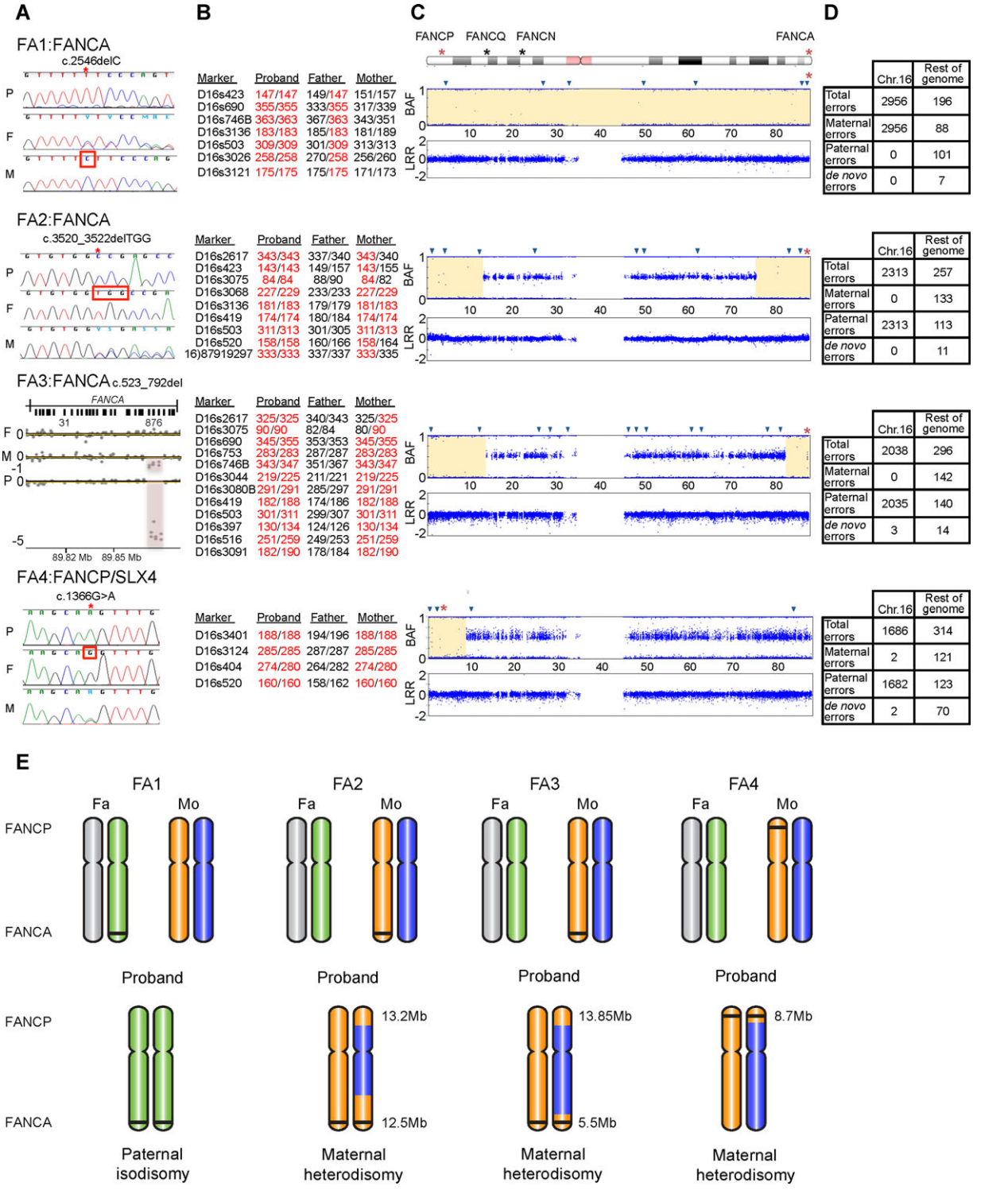

Figure 1. See figure legend on next page.

defect of interstrand crosslinking and Camptothecin sensitivity was rescued by expression of the WT SLX4 cDNA (Supp. Fig. S2). Thus, homozygous pathogenic variants caused FA in all four patients.

Sequencing of parental DNA for each patient showed that the *FANCA* variant in FA1, c.2546delC, was present only in the paternal DNA, and not the patient's mother. For patients FA2, FA3, and FA4, the mother was a heterozygous carrier (Fig. 1A). ArrayCGH or multiplex ligation-dependent probe amplification was performed to confirm that the nature of the homozygous variants in *FANCA* (patients FA1, FA2) and *FANCP/SLX4* (patient FA4) was not the result of an overlapping large heterozygous deletion (data not shown). This prompted the exploration of UPD as the cause of homozygous variants in the patients.

Each family was genotyped using STRP markers. Both parental alleles are accounted for in the proband (Supp. Table S1), except for the markers on chromosome 16 (Supp. Table S2) which showed inheritance from only one parent (Fig. 1B). Similarly, SNP arrays demonstrate a contribution from both parental alleles for the entire genome of the probands (Supp. Fig. S3), except for chromosome 16 (Fig. 1C). Homozygosity was observed for the entire chromosome 16 in FA1, and large regions near the termini harboring the affected FA genes, *FANCA* (qter) in FA2 and FA3, or *FANCP/SLX4* (pter) in FA4 (Fig. 1C; Supp. Table S3). Mendelian error (ME) calculations revealed several chromosome 16 errors, ranging from 1,682 to 2,956 genotypes, originating exclusively from one parent. ME for the rest of the genome, ranging from 196 to 314 genotypes, were derived evenly from both parents (Fig. 1D). The UPD16 extended throughout the entire chromosome 16 in all four families, with contribution from only one parent.

UPD describes the inheritance of a pair of chromosomes from only one parent, either both homologues (heterodisomy), or two copies of one homologue (isodisomy) [Shaffer, et al., 2001]. In patient FA1, the paternal chromosome 16 bearing the *FANCA* variant c.2546delC was duplicated resulting in complete paternal isodisomy (Fig. 1E). FANCA-deficient patients, FA2 and FA3, and FANCP/SLX4-deficient patient FA4 showed maternal heterodisomy with regions of isodisomy at the qter or pter harboring the maternal *FANCA* or *FANCP/SLX4* mutant alleles. A mechanism involving monosomy rescue may explain the isodisomy in FA1, while trisomy rescue may have caused maternal heterodisomy in FA2, FA3, and FA4 (Fig. 1E, see legend). We observe both paternal and maternal UPD16 in FA and, consequently, conclude no bias for parental origin of UPD.

UPD is often associated with defects in imprinting disorders resulting in lack of expression of a functional allele [Ishida and Moore, 2013]. Potential consequences of imprinted genes in the UPD region may have additional clinical implications for patients [Engel, 2006]. UPD of the entire chromosome 16, inherited paternally (FA1) and maternally (FA2–FA4), should expose phenotypes potentially associated with imprinting. However, typical clinical presentation of the four FA patients presented here (Table 1) suggests a lack of imprinted genes on chr16. Previous reports of UPD16 in five different recessive disorders involving genes *MLYCD* (Malonic aciduria), *HBA1* (HB Bart hydrops fetalis), *ABCA3* (Inherited surfactant deficiency), *APRT* (Adenine phosphoribosyltransferase deficiency), and *GALNS* (Morquio A syndrome) [Catarzi, et al., 2012] also showed no indication of imprinted genes causing a major phenotypic defect. The severe phenotype of patient FA4 could be due to the affected gene, *FANCP/SLX4*, or the 8.7 Mb pter homozygous region may harbor another unknown defective recessive gene.

UPD as a cause of FA is a novel mechanism that has not been previously reported. Four UPD cases in our mutation analysis of ~400 FA families suggests we may discover other FA cases with UPD by screening more patients using these methodologies. Exploring the inheritance pattern of a larger number of patients with homozygous pathogenic variants may define the precise frequency of UPD causing FA. Trisomy16 is the most common trisomy observed in human conceptions [Griffin, 1996], and thus as a consequence of trisomy rescue, children with UPD16 may be relatively more common. In addition to *FANCA* and *FANCP/SLX4*, *FANCQ/ERCC4/XPF* (chr16:g.14014014-14046205) and *FANCN/PALB2* (chr16:g.23614483-23652678) also reside on chromosome 16, which suggests the possibility of unknown UPD16 in patients from other FA groups. The diagnosis of UPD as a cause of recessive disease drastically reduces the recurrence risk in a family from 25% to nearly zero, potentially making invasive prenatal

Figure 1. Homozygous mutations resulting from UPD of chromosome 16 in four FA patients. **A**: Homozygous mutation in each proband and the heterozygous carrier status of each parent. For each family, data from the proband (P), father (F), and mother (M) are shown. Families FA1, FA2, and FA3 carry mutations in *FANCA*, and family FA4 carries a mutation in *FANCP/SLX4.* Sequence traces from Sanger sequencing are shown for families FA1, FA2, and FA4. The red star indicates the mutation site, homozygous in the proband and heterozygous in the carrier parent, and the red square indicates the presence of the WT sequence in the non-carrier parent. ArrayCGH intensity plot is shown for FA3 family. The red-shaded region indicates the site of deletion, and the length of the shaded region shows the state of zygosity: a heterozygous loss in the parental carrier and a homozygous loss in the proband. **B**: Genotypes of proband/parents trio using chromosome 16 STRP markers. The markers and their two alleles in each member of the family are shown. The alleles of the proband are marked in red to emphasize the parent from whom they were inherited. Only the informative markers from STRP analysis are shown. The full list of tested markers is provided in Supp. Table S2. The positions of the informative markers on chromosome 16 are represented by blue triangles in Figure 1C. **C**: Probands' chromosome 16 SNP genotype data. An ideogram of chromosome 16 indicating the location of the four FA genes. The two affected genes in these four families, *FANCA* and *FANCP/SLX4*, are marked with a red asterisk. The top plot, for each proband, is the B Allele Frequency (BAF) showing the state of zygosity for each SNP. A BAF of 1 or 0 indicate a homozygous genotype, whereas a BAF of 0.5 indicates a heterozygous genotype. The bottom plot is the Log R Ratio (LRR) which shows the SNP intensity and represents the allelic copy number. A LRR of 0 indicates a copy number of two. The regions of isodisomy are highlighted in yellow. **D**: Mendelian errors in the probands' SNP genotypes. A mendelian error is identified as the presence of a genotype in the proband that cannot be explained by a classical inheritance pattern from both parents. The table shows the number of errors present in chromosome 16 in comparison to the number of errors present throughout the rest of the genome. Comparing the number of errors contributed from each parent elucidates whose chromosome 16 is absent from the proband. **E**: Graphical representation of the likely mechanism leading to UPD in each family. The two chromosome 16 homologues of each parent and their presence in, or absence from, the proband are shown. The location of *FANCA* and *FANCP/SLX4* are indicated, and the mutation is indicated with a black line. The sizes of the isodisomy regions in probands FA2-FA4 are presented in megabases. The complete paternal isodisomy in FA1 may have been generated by a mechanism involving monosomy rescue: maternal nondisjunction results in no chromosome 16 (nullisomy), leading to monosomy of paternal chromosome 16, which would be lethal and would be rescued by duplicating the paternal chromosome 16 [Engel, 2006]. The maternal heterodisomy in FA2, FA3, and FA4 may be explained by a mechanism involving trisomy rescue: where a supernumerary chromosome from a trisomic conceptus is lost, leaving behind two homologues from the same gamete [Engel, 2006]. The homozygous regions distal to the crossover events during meiotic recombination appear as isodisomic regions in the pter of FA4 (a single crossover), and the pter and the qter of FA2 and FA3 (two crossover events). This reduction to homozygosity, of the regions harboring the *FANCA* (FA2 and FA3) and *FANCP/SLX4* mutations (FA4), leads to FA.

Table 1. Clinical Presentation of Patients Displaying UPD16

Clinical information	Patient			
	FA1	FA2	FA3	FA4
Gender	F	M	M	F
Gene	*FANCA*	*FANCA*	*FANCA*	*FANCP/SLX4*
Mutation (genomic)[a]	g.89833604delG	g.89811471-89811473delCCA	g.89867339-89876628del	g.3647798C>T
Mutation (cDNA)	c.2546delC	c.3520_3522delTGG	c.523_792del (Δexons 6-8)	c.1366G>A
Mutation (Protein[b])	p.S849FfsX40	p.W1174del	p.S175_Q264del	p.E456SfsX34, not expressed
Mutation (Zygosity)	Homozygous	Homozygous	Homozygous	Homozygous
Mother is carrier	No	Yes	Yes	Yes
Father is carrier	Yes	No	No	No
Ethnicity	Asian (Korean)	Caucasian	Caucasian	Caucasian
Consanguinity	No	No	No	No
Age at diagnosis	9 yrs.	16 yrs.	10 yrs.	2 yrs.
BMF	14 yrs.	16 yrs.	10 yrs.	Birth
BMT	No	26 yrs.	10 yrs.	5 yrs.
Status	Deceased at 27yrs.	Deceased at 26 yrs. Complications of BMT	Alive and well at 22 years	Deceased at 5 yrs. Complications of BMT
Skin	Café-au-lait spots	Normal	Normal	Café-au-lait spots
Musculoskeletal	Hypoplastic thenar eminence Scoliosis Mild pes planus	Abnormal thumb Hypoplastic thenar eminence	Mild thumb anomaly	Bilateral toe syndactyly, microcephaly
Urogenital	Normal	Normal	Normal	Bilateral ectopic kidneys
Ears/hearing	Small canals	Normal	Small canals	Normal
Gastrointestinal	Normal	Normal	Normal	Normal
Cardiopulmonary	Normal	Severe asthma	Normal	L.Superior vena cava, ASD
Eyes/vision	Esotropia	Normal	Normal	Microophthalmia, Esotropia
Development	Normal	Normal	Normal	Severe, Global delay

[a]Coordinates are in accord with UCSC genome build hg19.
[b]Predicted.
BMF, bone marrow failure; BMT, bone marrow transplantation. GenBank reference sequence for *FANCA*: NM_000135.2, and *SLX4*: NM_032444.2

diagnosis in subsequent pregnancies unwarranted. This has important implications for genetic counseling within affected families. Of all possibilities, UPD for only a few chromosomes results in abnormal phenotypes shown to be, or presumed to be, caused by imprinting [Shaffer, et al., 2001]. It is thus important to rule out UPD as a cause of recessive disease in non-consanguineous homozygous patients as UPD cases identified following investigation of single gene often do not manifest additional anomalies beyond those expected for their disease.

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Author Contributions

Study design and manuscript preparation (SCC), patient care, enrollment, and sample collection were done by E.M.S., R.T., J.E.W., M.L.M., E.A.O., A.D.A., and A.S. Experiments and analysis were carried out by F.X.D., D.C.K., Y.K., F.P.L., U.H., M.P.J., A.K., A.S., and S.C.C.

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