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LETTER TO THE EDITOR

XRCC2 mutation causes premature ovarian insufficiency as well as non-obstructive azoospermia in humans

To the Editor

Meiotic homologous recombination, which is mediated by the ubiquitous *RAD51* and meiosis-specific *DMC1* recombinase systems, is a vital process, for both oogenesis and spermatogenesis. A c.41T>C (p.Leu14Pro) substitution in *XRCC2*, a paralog of *RAD51*, was recently reported to be associated with non-obstructive azoospermia (NOA) in humans.[1] Here, we found that this gene could also cause premature ovarian insufficiency (POI). POI is defined as the depletion or loss of normal ovarian function in women aged below 40 years and is an important cause of female infertility.[2] NOA causes spermatogenesis failure within the testis and affects approximately 10% of infertile men.[3]

In this study, we described two infertile patients from a four-generation consanguineous Chinese family (brother with NOA and sister with POI; Figure 1A). The proband (IV-2), a 29-year-old woman, was diagnosed with POI at age 16. She attained menarche with

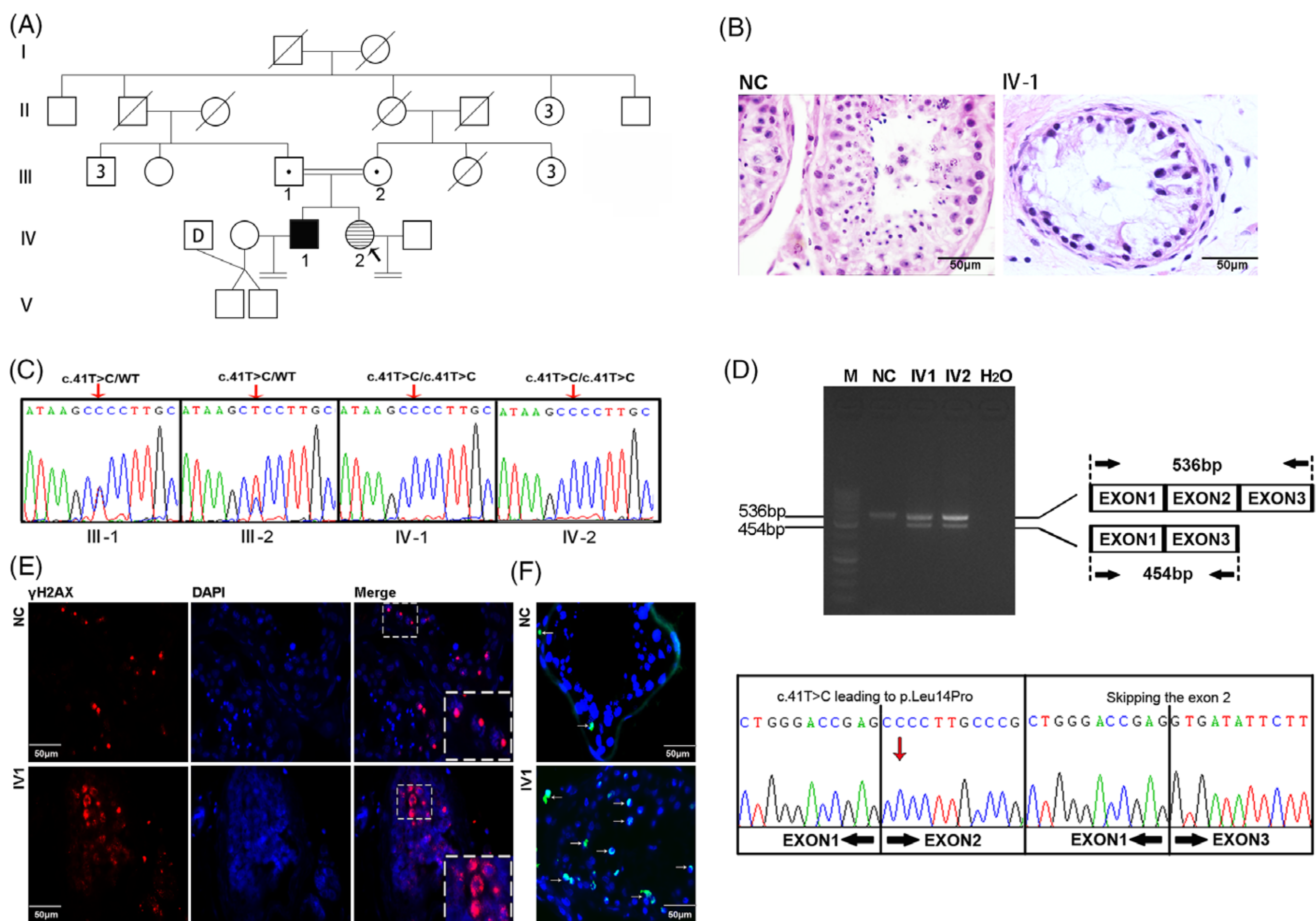

FIGURE 1 Pedigree and impact of *XRCC2* mutation in function. A, pedigree of the family. B, H&E staining of testicular biopsy samples from a normal control (NC) and IV-1. C, Sanger sequencing chromatograms of the subjects in the family. D, RT-PCR products of *XRCC2*; Sanger sequencing revealed exon 2 was skipped in the smaller fragment. E, immunofluorescence staining with γH2AX. F, TUNEL staining of apoptotic cells in testis sections [Colour figure can be viewed at wileyonlinelibrary.com]

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hypomenorrhea at age 15 and subsequently experienced amenorrhea in the next year. Transvaginal ultrasound examination revealed that her uterus was relatively small (40 × 23 × 37 mm), as were both ovaries (left ovary: 17 × 10 × 14 mm; right ovary: 19 × 11 × 13 mm). She had low estradiol level (15 pg/mL; normal range 21-251 pg/mL), high levels of follicle stimulating hormone (FSH) (39.67 mIU/mL; normal range 3.03-8.08 mIU/mL) and luteinizing hormone (LH) (12.07 mIU/ mL; normal range 1.80-11.78 mIU/mL), and normal levels of prolactin and thyroid-stimulating hormone. In addition, her anti-mullerian hormone (AMH) and inhibin B levels were too low to be detected. She had a normal karyotype and normal range of *FMR1* CGG trinucleotide repeats. The brother of the proband (IV-1), a 31-year-old man, suffered from infertility for 5 years after marriage due to complete azoospermia (only primary spermatocytes found in his testicular biopsy, Figure 1B). He presented normal bilateral testicular size and hormone levels, a normal karyotype, and no Y-chromosome microdeletion. Other causes of infertility in both patients were excluded.

Peripheral blood samples were taken from the patients and their parents, after obtaining informed consent, following the guidelines approved by the ethics committee of Reproductive and Genetic Hospital of CITIC-Xiangya. Whole-exome sequencing was performed on the two affected siblings. We filtered the variations with minor allele frequencies <0.05 in any of the following databases: dbSNP, 1000 Genomes Project and ExAC. We then focused on the variants that were homozygous, and relevancy for phenotype using Gene Ontology terms (biological process associated with gametogenesis) and model organism data. According to this filtering strategy, only the *XRCC2* variant (c.41T>C, p.Leu14Pro), previously described by our group, was identified, confirmed by Sanger sequencing. Both their parents were heterozygotes (Figure 1C).

We used functional analysis to explore the effects of this mutation further. Firstly, total RNA was extracted from patients' blood, and reverse transcription PCR (RT-PCR) showed this variant caused partially aberrant splicing (Figure 1D), consistent with the earlier report.[1] In addition, immunofluorescence revealed that γH2AX staining appeared prominently in the spermatocytes undergoing preleptotene to zygotene in the seminiferous tubules of the male patient, and no sex body was observed (Figure 1E). These results suggested that spermatogenesis was blocked in the zygotene stage in our male patient. Furthermore, terminal dexynucleotidyl transferase(TdT)-mediated dUTP nick end labeling (TUNEL) assay showed a significant increase in apoptotic germ cells in his seminiferous tubules, similar to *Xrcc2* knock-in mice (Figure 1F).[1]

Based on the phenotype of the patients and the effects of this mutation, we suggested that the family mutation is responsible for POI in the sister and NOA in the brother. Our earlier report showed that *DMC1* is responsible for NOA and POI in humans.[4] This is our second report that a meiosis-related gene is associated with infertility in both genders and the first report that XRCC2 mutation can cause POI as well as NOA in humans.

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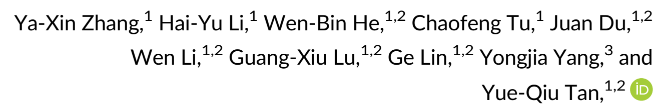

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