ORIGINAL RESEARCH

Optimised molecular genetic diagnostics of Fanconi anaemia by whole exome sequencing and functional studies

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ABSTRACT

Purpose Patients with Fanconi anaemia (FA), a rare DNA repair genetic disease, exhibit chromosome fragility, bone marrow failure, malformations and cancer susceptibility. FA molecular diagnosis is challenging since FA is caused by point mutations and large deletions in 22 genes following three heritability patterns. To optimise FA patients' characterisation, we developed a simplified but effective methodology based on whole exome sequencing (WES) and functional studies.

Methods 68 patients with FA were analysed by commercial WES services. Copy number variations were evaluated by sequencing data analysis with RStudio. To test *FANCA* missense variants, wt FANCA cDNA was cloned and variants were introduced by site-directed mutagenesis. Vectors were then tested for their ability to complement DNA repair defects of a FANCA-KO human cell line generated by TALEN technologies.

Results We identified 93.3% of mutated alleles including large deletions. We determined the pathogenicity of three FANCA missense variants and demonstrated that two *FANCA* variants reported in mutations databases as 'affecting functions' are SNPs. Deep analysis of sequencing data revealed patients' true mutations, highlighting the importance of functional analysis. In one patient, no pathogenic variant could be identified in any of the 22 known FA genes, and in seven patients, only one deleterious variant could be identified (three patients each with FANCA and FANCD2 and one patient with FANCE mutations)

Conclusion WES and proper bioinformatics analysis are sufficient to effectively characterise patients with FA regardless of the rarity of their complementation group, type of mutations, mosaic condition and DNA source.

INTRODUCTION

Fanconi anaemia (FA) is a rare genetic DNA repair disease characterised by bone marrow failure (BMF), acute myeloid leukaemia and/or solid tumours especially head and neck squamous cells carcinoma.[1] FA affects about 1 in 360 000 births, and half of the patients die within 20 years.[2] Twenty-two FA-related genes have been identified: *FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG/XRCC9, FANCI, FANCJ/BRIP1, FANCL/PHF9 FANCM, FANCN/PALB2 FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4 FANCS/BRCA1, FANCT/UBE2T, FANCR/RAD51A, FANCU/XRCC2, FANCV/REV7* and *FANCW/RFWD3*.[3]

In the past, after clinical diagnosis and a positive chromosome fragility test (CFT), FA patients' molecular characterisation was achieved by Sanger sequencing of candidate genes often supported by