Mosaic *PPM1D* mutations are associated with predisposition to breast and ovarian cancer

Ruark et al., Nature, 2013

Journal Club

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Introduction

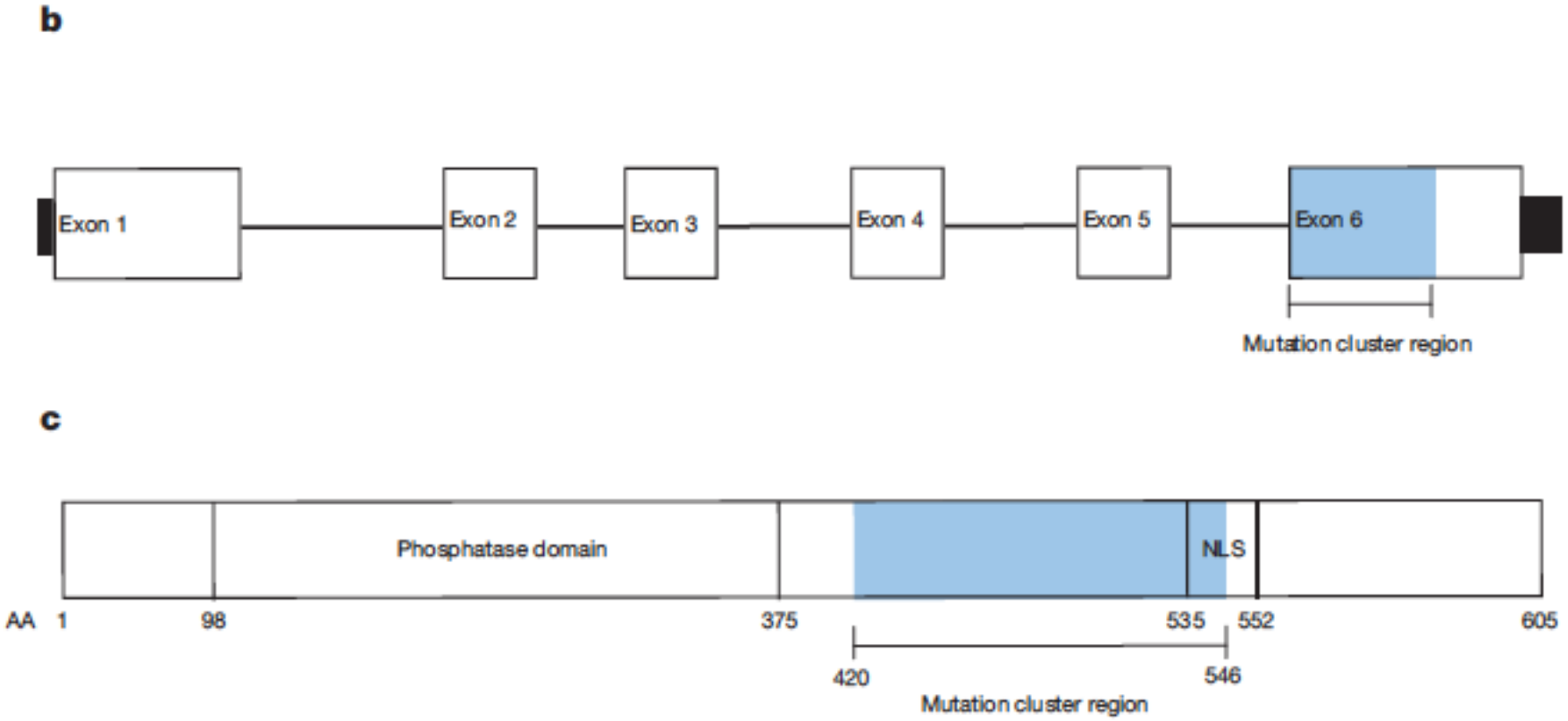
- They sequenced 507 genes involved in DNA repair in 1,150 samples
- All samples had breast cancer, 69 also had ovarian cancer
- Found that rare protein-truncating variants (PTVs) in *PPM1D* are associated with predisposition to breast cancer and ovarian cancer

Sequencing details

- Illumina HiSeq2000 sequencer that generated a minimum coverage per pool of 480X for at least 90% of the target region
- Sequence variants called using Syzygy (SNVs and indels)
- 34,564 variants identified
- Focused on 1,044 PTVs
- Stratify genes according to the number of different, rare truncating mutations present within the samples
- *PPM1D* showed the strongest signal
- Confirmed by sanger sequencing of 5 individuals carrying different *PPM1D* mutations – 2 of these also had ovarian cancer

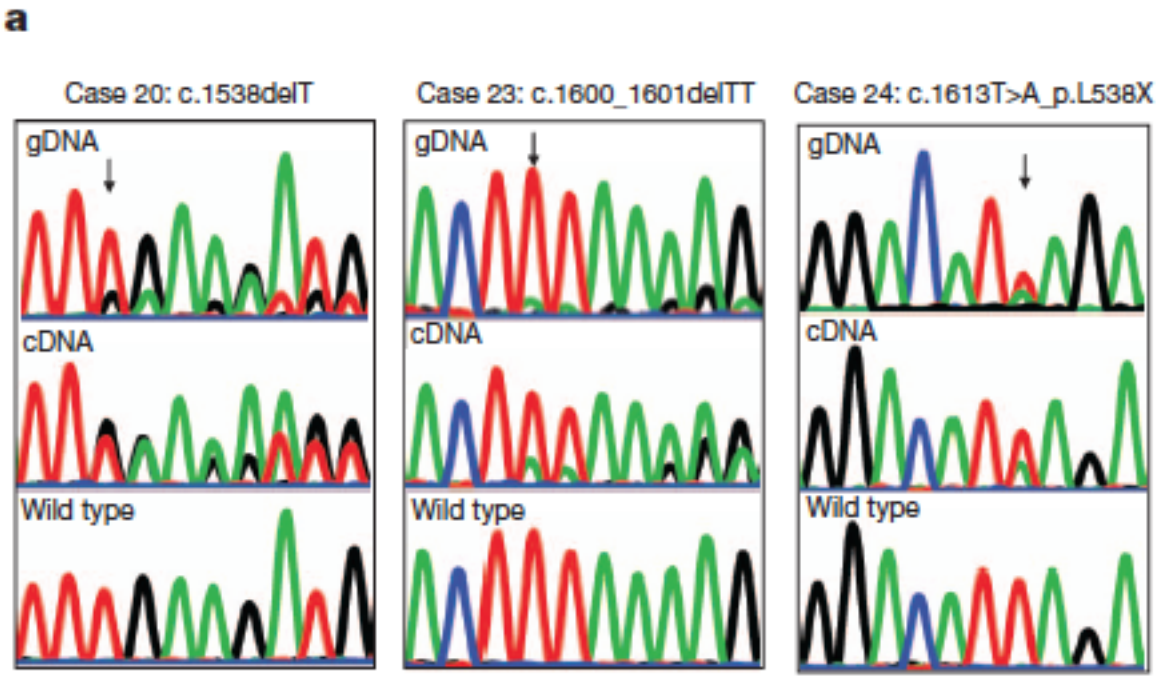
Sequencing details (contd.)

- To explore the role of *PPM1D* further, case control Sanger sequencing in 13,642 individuals – 7,781 with breast and/or ovarian cancer and 5,961 controls
- All 25 PTV mutations occurred in last exon of the gene (Figure 1)

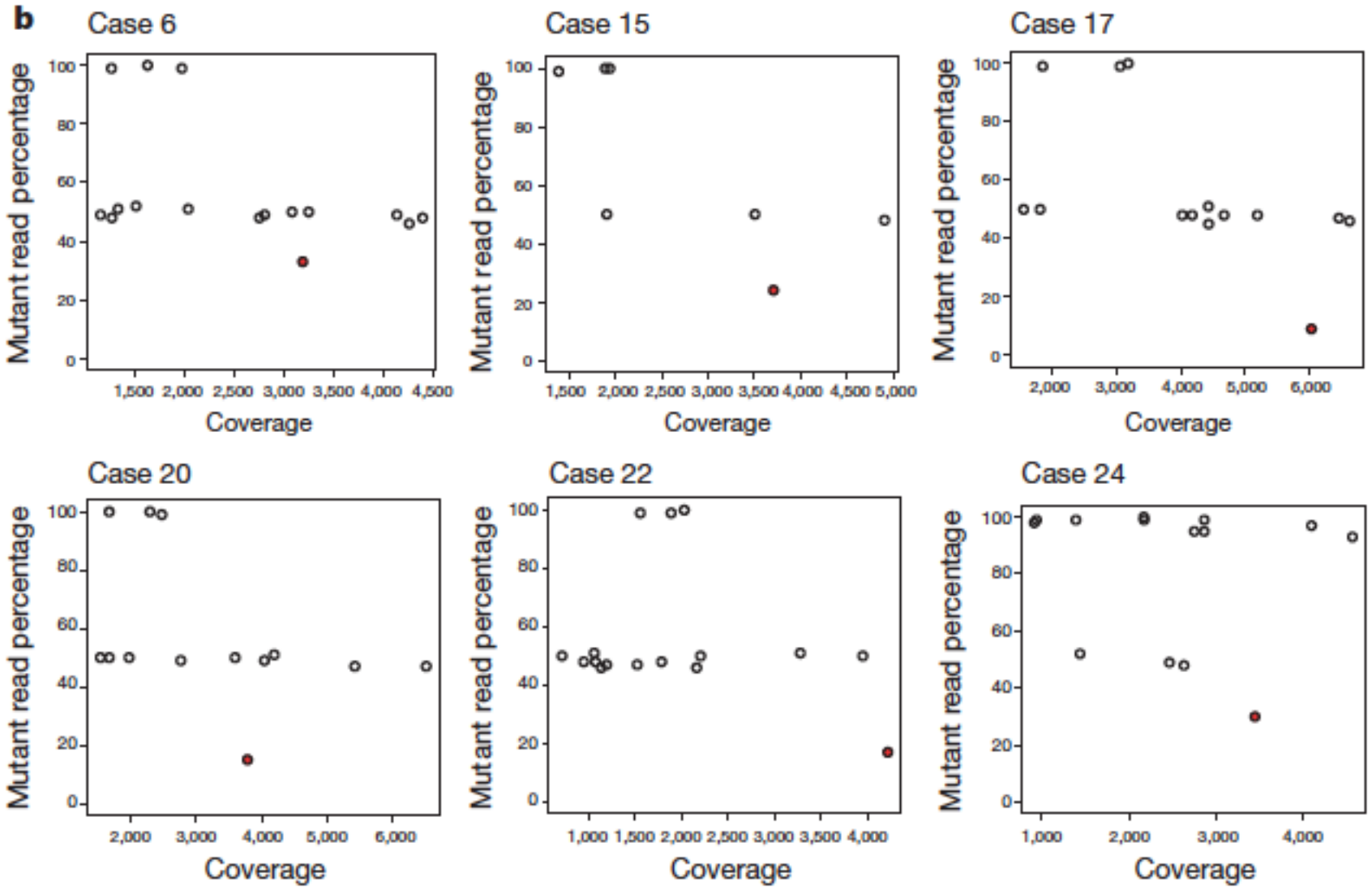

b
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
Mutation cluster region
c
Phosphatase domain
NLS
AA 1
98
375
535
552
605
420
546
Mutation cluster region

Unusual allele frequencies (mosaic mutations)

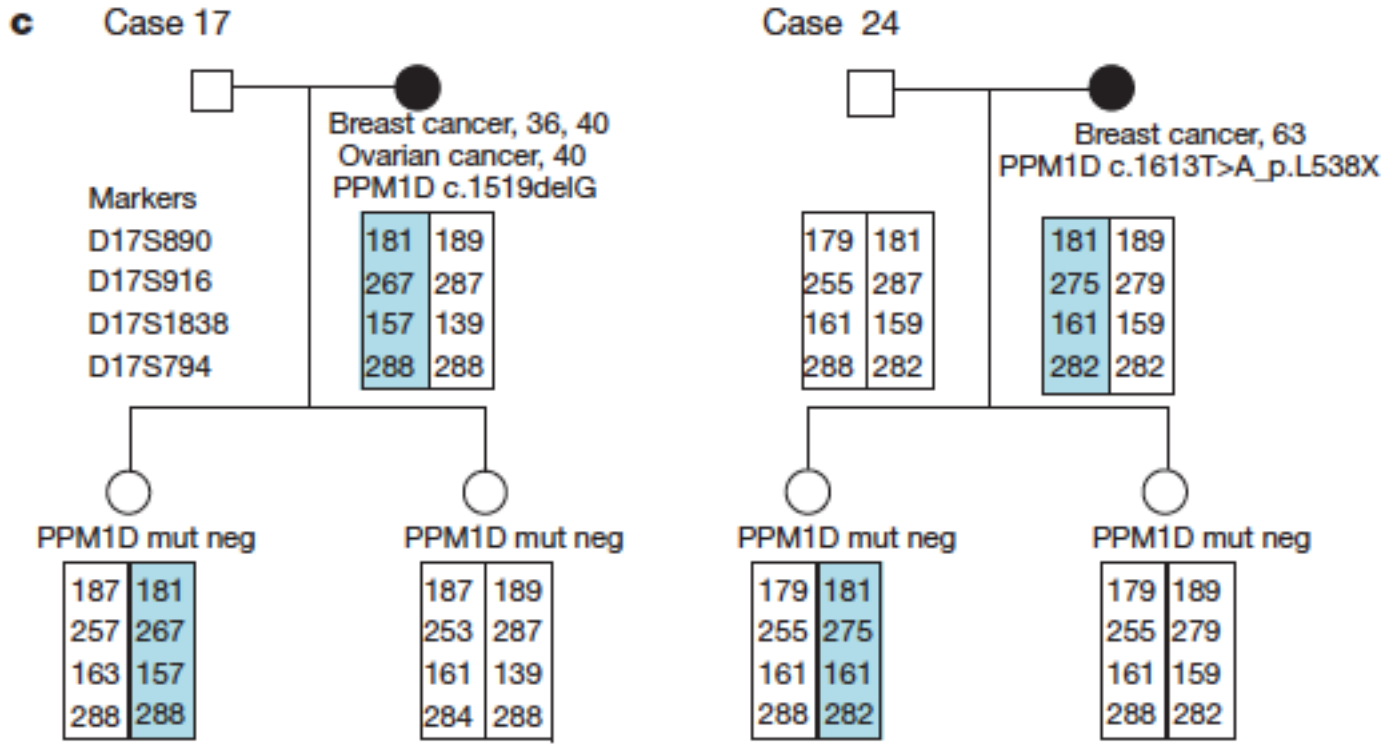
- Mutant allele considerably and consistently lower than wild type allele in lymphocyte DNA
- Chromatograms inconsistent with heterozygous (or homozygous mutations) (Fig. 2a)
- Saliva available for 2 individuals and results consistent
- Deep PCR amplicon sequencing confirmed this (Fig. 2b)
- For 2 samples, the offspring did not carry the *PPM1D* mutation at the inherited *PPM1D* maternal haplotype (Fig. 2c)



Sanger sequencing traces showing mutation is lower in genomic DNA (gDNA) than is typical for heterozygous mutations



Deep PCR amplicon sequencing showing BRCA1/2 variants at 50% (open dots) and PPM1D mutation at lower precentage (red dots)



Haplotype analysis in two families. The offspring of PPM1D-mutation carriers have different maternal haplotypes spanning PPM1D, but neither of them carries the mutation.

PPM1D role

- *PPM1D* down regulates several targets, particularly proteins associated with DNA damage response pathway, including some tumor suppressors such as *p53*, *ATM* and *CHK2*.

PPM1D PTVs are gain-of-function

- Clustering of mutations downstream of the phosphatase catalytic domain (Fig. 1)
- Up regulated *PPM1D* would enhance *p53* suppression
- Confirmed experimentally (Fig. 3) that *PPM1D* PTVs encode hyperactive isoforms

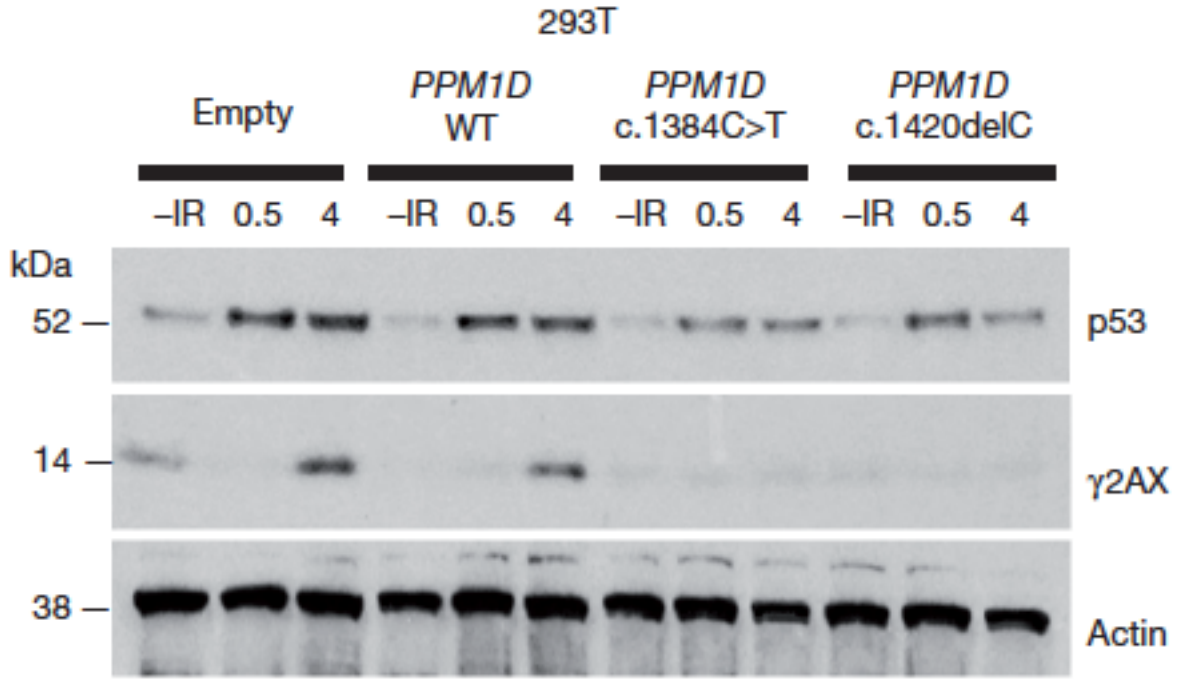


Figure 3 | The effect of mutant *PPM1D* isoforms on p53 activation. p53

Mystery continued (mutations absent in tumor)

- Analyzed 8 tumors from 5 individuals – mutations not present in tumor (confirmed present in blood of all 5 individuals)
- Possibilities
  - Mutations needed for oncogenesis but not required or perhaps even detrimental for cancer progression
  - Or may be oncogenesis is driven by mutations in circulating blood cells
  - Or these mutations not directly involved in causing cancer but manifestation of another lesion that causes them and cancer in other cells

Summary

- All results confirm that individuals with these mutations are at increased risk of cancer
- A new class of genetic defect that lies somewhere in between inherited and somatic cancer variants
- Huge clinical implications for biomarker detectable in blood (but not inherited)