

PM3 For recessive disorders, detected in *trans* with a pathogenic variant

- Fanconi Anemia (FA) of any subtype is generally considered an exceedingly rare, severe, early-onset disease with variable features. In the case of BRCA2, hypomorphic FA patients have been described who are diagnosed at older ages with less severe phenotypes. The criteria set forth in the tables below are designed to accommodate such hypomorphs and are recommended to be applied to all FA-associated genes which may not be as well described due to the extreme infrequency of their identification, and due to ascertainment bias (for severe phenotype) in the literature.
- General Considerations
 - Variant may not exceed general population frequency >0.01%.
 - Consider other gene panel test results as potential explanation for phenotype.
 - Multiple unrelated cases are additive.
- Phenotype consistent:
 - Chromosomal breakage with 1 clinical feature **OR**
 - Ex. Chromosomal breakage testing + microcephaly / triangular face
 - At least 2 of 3 clinical features from separate categories without chromosomal breakage studies
 - Ex. (Without chromosomal breakage): Myelodysplastic Syndrome and microcephaly / triangular face.
- Positive for chromosome breakage test:
 - Increased chromosome breakage and/or radial forms on cytogenetic testing of lymphocytes with diepoxybutane (DEB) or mitomycin C (MMC)
- Clinical features indicative of FA, including
 - Physical features (in ~75% of affected persons), include:
 - prenatal and/or postnatal short stature
 - abnormal skin pigmentation (e.g., café au lait macules, hypo- pigmentation)
 - Skeletal malformations (e.g., hypoplastic thumb, hypoplastic radius)
 - Microcephaly, triangular face
 - Ophthalmic anomalies
 - Genitourinary tract anomalies.
 - See Orphanet for full list of >100 HPO terms (and their reported frequency).
 - Pathology findings and laboratory findings (non-cancer related) include
 - progressive bone marrow failure (unrelated to cancer treatment)
 - aplastic anemia
 - Myelodysplastic syndrome
 - Inordinate toxicities from chemotherapy or radiation
 - macrocytosis
 - cytopenia (especially thrombocytopenia, leukopenia, and neutropenia)
 - increased fetal hemoglobin (often precedes anemia).
 - Cancer diagnosis ≤5yr, particularly

- Blood cancers (AML)
- Brain cancers (medulloblastoma, neuroblastoma)
- Wilms Tumor
- Specifications are adapted from GeneReviews (last revision June 3, 2021)

BS2

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

- Do not use for individuals in population based cohorts, such as gnomAD
- VUA should not be bioinformatically predicted (or experimentally proven) to have a clinically important effect on protein or mRNA splicing. Co-occurent P or LP variant should be assigned classification using VCEP specifications
- Consider multiple instances of co-occurrence with the same variant are more likely to be in cis in unrelated individuals when assessing BS2 application

Fanconi Anemia PM3 | BS2 tables:

Points per unrelated <i>PALB2</i> -related (FANCN) Fanconi Anemia Proband (PM3)		
Phenotype and Classification/ Zygosity of other variant ¹	Confirmed in <i>trans</i> or homozygote ²	Phase unknown
Phenotype <i>consistent</i> with <i>PALB2</i> -related FA	2.0	1.0

Points per proband meeting age/cancer diagnosis criteria (BS2)			
Phenotype and Classification/ Zygosity of other variant	VUA in <i>trans</i> with P or LP variant ³	Homozygote ⁴	Phase unknown, VUA co-occurs with P or LP variant
Patient first cancer onset >50y or cancer-unaffected at age at last follow up of >50y	4.0	2.0	1.0
Patient first cancer onset 40- 50y or unaffected at age of last follow up of 40-50y	2.0	1.0	0.5

¹Variant under assessment (VUA) must be sufficiently rare (not meeting a benign population evidence code). Consider other gene panel test results as potential explanation for phenotype.

²Co-occurent P or LP variant should be assigned classification using VCEP specifications. Stipulation for in *trans* can be met by genotyping of at least one parent or assumed if VUA is seen with at least 2 different P/LP variants. If there are multiple phase unknown co-occurrences with different P/LP variants, at least one should be weighed as phase unknown to allow for the probability of one co-occurrence in *cis* and the remaining can be weighed as in *trans*. Further, co-occurrence in *trans* can be inferred in the case of a homozygote FA-affected patient due to a consanguineous union, or if both maternal and paternal lineages present with family history of cancer consistent with *PALB2* related cancers. Maximum of 2 homozygous individuals.

³VUA should not be bioinformatically predicted (or experimentally proven) to have a clinically important effect on protein or mRNA splicing. Co-occurrent P or LP variant should be assigned classification using VCEP specifications. See footnote 2 for stipulation for in trans.

⁴Apply only for phenotyped individuals from clinical or research cohorts. NOT to be applied for data used to assign frequency-based codes; we have observed that variants observed as ≥ 2 homozygotes in gnomAD data (assumed unaffected adults of unspecified age) are already captured by the BA1 or BS1 frequency codes.

Do not use observations *in cis*

Supporting	Moderate	Strong	Very Strong
PM3_			
1.0	2.0	4.0	8.0
BS2_			
1.0	2.0	≥ 4.0	N/A