Results (I) - Molecular characterization

- 123 different mutations were identified: 79 missense, 36 non-missense mutations (12 splicing, 13 frameshift, 7 stop codons, and 4 large deletions), 5 inframe indels, and 3 promoter variants (Figure 2). Most pts had at least one missense mutation (85%).
- Fifty mutations, affecting PK structural domains, have not been reported previously (31 missense, 3 stop codon, 7 frameshift, 3 inframe indel, 5 splicing mutation, 1 promoter mutation); 22% of the patients (56/255) carried at least one new molecular variant (Table 1).
- The new missense mutations affected conserved residues in multiple domains of the PKLR gene, were not detected in 1000 Genomes and HGMD databases, and were considered pathogenic by different mutation algorithms.

Results (II) - Genotype-phenotype correlation

- **Age at Diagnosis.** There is a trend for pts with more severe mutations to be diagnosed at a younger age (p=0.049).
- **Splenectomy.** The NM/NM group had the highest rate of splenectomy (72%) vs. 50% in the M/NM and 44% in the M/M group (p=0.024). In pts splenectomized and not on regular transfusions, the Hb levels were significantly different between the groups (p=0.003).
- **Transfusion status.** There were significant differences in the transfusion status; 86% of the NM/NM group were previously or currently regularly transfused, versus 50% of the M/NM and 42% of the M/M group (p=0.001). There were also significant differences in the total number of transfusions (p=0.0013); 96% in the NM/NM group received at least one lifetime transfusion compared to 81% and 75% of the M/NM and M/M groups, respectively.
- **Iron status.** The NM/NM group had the highest iron overload (p=0.0001) and was more likely to have iron overload, defined as ferritin >1000 mg/ml or having received chelation in the year prior to enrollment (p=0.0013). However, iron overload was not uncommon in the M/M group (43%).
- **Enzyme activity.** There was no association of PK enzyme activity with the genotype.

Conclusions

- Genotype-phenotype associations were observed in a large international cohort of pts with PKD.
- Pursuing molecular testing may be useful to discuss prognosis and to establish a monitoring plan in pts based on genotyping results.
- Fifty new mutations were identified, thus confirming the wide heterogeneity of the molecular genotype and diagnostic complexities in PKD.