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Letter

Biallelic alterations in *PLXND1* cause common arterial trunk and other cardiac malformations in humans

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Zhou *et al.* (1) recently reported in this journal an association between heterozygous variants in *PLXND1* and a subtype of anomalous pulmonary venous return (APVR), in a case–control study of individuals recruited in China. From two cohorts of APVR patients and controls (144 patients and 1636 controls in the discovery cohort and 82 patients and 82 controls in a replication study), they identified eight unrelated patients harboring heterozygous truncating, essential splice site or predicted deleterious missense variants in *PLXND1* (all of which were absent from gnomAD), representing a statistically significant enrichment of such variants compared to control groups. Parental segregation was not reported for six of the variants, while one was *de novo* and one inherited from a healthy father. The lack of cardiac evaluation in controls was noted as a limitation to their study. In contrast to the presumed dominant effect of the heterozygous *PLXND1* variants identified by Zhou *et al.*, an earlier publication had reported a consanguineous family with recurrence in four siblings of common arterial trunk (CAT, also known as truncus arteriosus, a conotruncal malformation characterized by a single vessel exiting both ventricles) (2) without APVR, with a homozygous missense variant in *PLXND1* (p.Arg1299Cys; Fig. 1) identified in the only affected sib that could be studied (3). In addition, three *de novo* heterozygous variants in *PLXND1* have been reported in three patients with Moebius syndrome (a disorder involving abnormal development of cranial nuclei VI and VII) without associated cardiac anomalies (4) (Fig. 1). Two of the Moebius-associated variants were missense and one was synonymous. The latter (p.Leu1006Leu) has since been reported in gnomAD (v2.1.1) with a frequency of 11/272 916 alleles. Moebius-related signs were not reported by Zhou *et al.* in their cohort of patients with *PLXND1* variants. It is unclear whether these different phenotypes and modes of inheritance associated with *PLXND1* variants are due to complex genetics (i.e., the influence of modifying alleles) and/or to different forms of Mendelian disease.

PLXND1 encodes a type I transmembrane protein composed of an extracellular region that interacts with semaphorin ligands and an intracellular signal-transducing GTPase activating protein domain. A number of pathways downstream of PLXND1 signaling have been identified, including those with effects on cell guidance via cytoskeletal regulation, especially in

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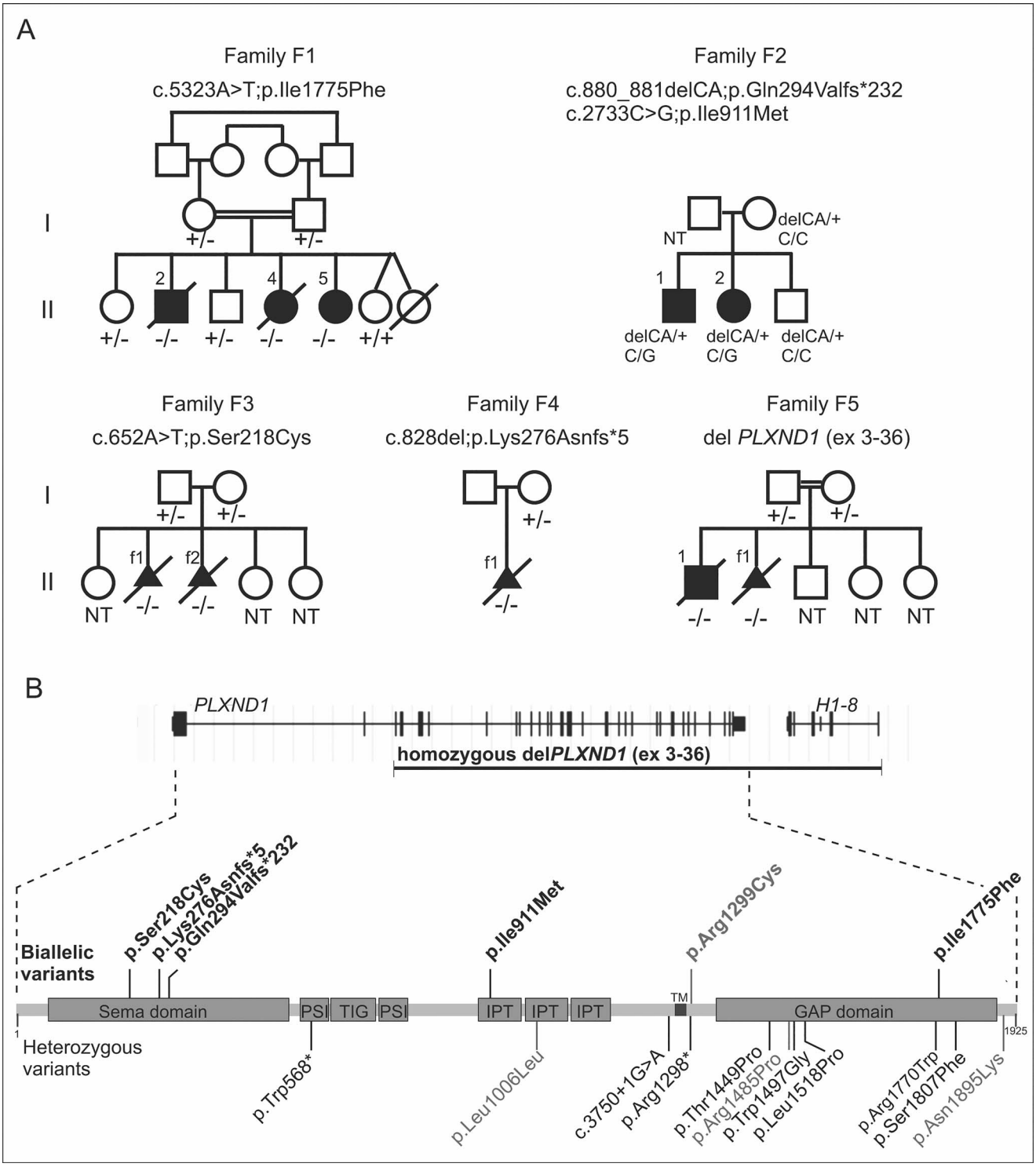

Figure 1. Biallelic variants in *PLXND1* cause cardiac malformations. (**A**) Pedigrees of five affected families with biallelic *PLXND1* alterations and cardiovascular defects. *PLXND1* variants refer to transcript NM_015103.2. Filled symbols represent affected individuals and triangles represent fetal cases; +: wild-type allele, −: altered allele. (**B**) Representations of the *PLXND1* gene and PLXND1 protein domain structure (as annotated by NCBI for reference sequence NP_055918.3). Biallelic variants and a homozygous deletion identified in patients with CAT or other cardiac defects are in black bold text for families reported here and in gray bold for the family described in Ta-Shma *et al.* Heterozygous variants are indicated below the protein and are in black for variants identified by Zhou *et al.* and in gray for variants identified by Tomas-Roca *et al.* PSI, Plexin-Semaphorin-Integrin repeat; TIG, Transcription factor ImmunoGlobin domain; IPT, immunoglobulin-like fold, plexins, transcription factors domain; TM, transmembrane domain; GAP, GTPase activating protein domain.

the context of blood vessel development and axonal growth (5,6). During mouse embryonic development, *Plxnd1* is highly expressed in vascular and cardiac endothelial cells, and *Plxnd1*-null mice display neonatal lethality with a high penetrance of CAT and peripheral vascular patterning defects, including anomalies of the aortic arch arteries (7). Conditional inactivation of *Plxnd1* in endothelial cells (by *Tie2-cre*-mediated deletion)

recapitulates CAT (8), and also results in ventricular hypertrabeculation and noncompaction defects (9), suggesting a role in cardiac chamber development. Zhou *et al*. generated a new *Plxnd1* knockout mouse model, which displayed cardiovascular phenotypes similar to those previously reported, including CAT. They observed defects in connection of the coronary sinus and in pulmonary vascular development, but they did not observe APVR in null embryos and they suggested that this may be due to the known differences between mice and humans in the structure and development of pulmonary venous return. However, an APVR phenotype has been demonstrated in *Sema3d* mutant mice (10).

Here, we report a cohort of 10 individuals, including 4 fetal cases, from 5 unrelated families, presenting with cardiac defects and harboring biallelic variants in *PLXND1* (Fig. 1 and Supplementary Material, Table S1). In these individuals, CAT was the most frequent anomaly. In family F1, three sibs from consanguineous parents presented with recurrence of CAT and APVR. These anomalies were associated with interrupted aortic arch in individual II-4 and with right aortic arch and other anomalies of the arterial and venous vasculature in individual II-5. Exome sequencing identified a homozygous missense variant, p.Ile1775Phe, in *PLXND1* in affected individuals of F1. In family F2, two sibs presented with CAT, which was associated with a right aortic arch in individual II-2. Exome sequencing identified compound heterozygous variants in both affected sibs (p.Gln294Valfs*232 and p.Ile911Met). In family F3, fetus II-f1 had ventricular hypoplasia and fetus II-f2 presented with a single ventricle and single outlet artery. A homozygous *PLXND1* missense variant, p.Ser218Cys, was identified in both fetuses. The family F4 fetal case (II-f1) presented with intrauterine growth retardation, cystic hygroma and marked left cardiac axis deviation (which can be suggestive of a heart malformation) followed by fetal demise at 13 weeks of gestation (no autopsy was performed). The fetus was homozygous (by maternal uniparental disomy) for the variant p.Lys276Asnfs*5 in *PLXND1*. In family F5, in which the parents were consanguineous, recurrence of CAT associated with hypoplastic left heart (HLH) was diagnosed in one live-born child (II-1) and in one fetus (II-f1) for which the pregnancy did not go to term. Both were homozygous for a deletion of *PLXND1* exons 3–36. For the families reported here, the patients and their heterozygous parents that were seen in consultation did not display clinical signs of Moebius syndrome. When an echocardiogram was available for the parents, APVR was not reported.

The *PLXND1* variants identified here were absent (or in one case present at very low frequency) in gnomAD v2.1.1, and all missense variants were located in functional domains of the protein and displayed CADD scores above 24 (Fig. 1 and Supplementary Material, Table S1). The recessive inheritance in these cases and in particular the biallelic deletion of *PLXND1* in family F5, in combination with the concordance of CAT between these patients and the *Plxnd1* knockout mice, support biallelic loss of function as the disease-causing mechanism in our cohort. Whether the association of CAT with HLH in family F5 represents the extreme end of the phenotypic spectrum, due to complete loss of *PLXND1*, and whether certain missense alleles in our cohort retain some residual function, leading to CAT without HLH, will require comparison with further patients with biallelic truncating or deletion variants in the future. Our findings are in agreement with the previous case report of a homozygous missense variant in *PLXND1* identified in sibs with CAT (3) and thereby confirm a recessive Mendelian disease associated with this gene. It is not clear how to reconcile our results with the reported associations between heterozygous *PLXND1* variants and Moebius syndrome or APVR, unless if in these two latter cases, the association involves complex genetics and/or alternate effects of the mutations on the protein. Interestingly, APVR was associated with CAT in the three patients bearing a homozygous *PLXND1* missense variant in family F1 reported here. Although it could be suggested that heterozygous *PLXND1* variants may increase risk for APVR when present with other modifying alleles, it is unclear why APVR did not have higher penetrance in our cohort of patients with biallelic variants, and it will be of interest to determine the frequency of APVR in a larger number of patients with recessive inheritance of *PLXND1* variants. Finally, we cannot exclude the possibility that the two missense variants previously reported in association with Moebius syndrome lead to an alteration of protein activity that is not equivalent to loss of function, leading to different signaling outcomes. Experimental investigation will be required in order to address this question.

In conclusion, our findings underscore the major role of *PLXND1* in human cardiovascular development, with Mendelian inheritance of biallelic, predicted loss-of-function alleles of *PLXND1* causing CAT and other cardiac defects.

Supplementary Material

Supplementary Material is available at *HMG* online.

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