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An Ashkenazi founder mutation in the *PKHD1* gene

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ABSTRACT

Autosomal recessive polycystic kidney disease (ARPKD) is usually detected late in pregnancies in embryos with large echogenic kidneys accompanied by oligohydramnios. Hundreds of private pathogenic variants have been identified in the large *PKHD1* gene in various populations. Yet, because of the large size of the gene, segregation analysis of microsatellite polymorphic markers residing in the *PKDH1* locus has commonly been utilized for prenatal diagnosis. Keeping in mind the limitations of this strategy, we utilized it for testing 7 families with affected fetuses or newborns, of which in 5 at least one parent was Ashkenazi, and identified that the same haplotype was shared by the majority of the Ashkenazi parents (7/9). This led us to suspect that they carry the same founder mutation. Whole Exome analysis of DNA from a fetus of one of the families detected an already known pathogenic variant c.3761_3762delCCinsG, an indel variant resulting in frameshift (p.Ala1254GlyfsX49). This variant was detected in 9 parents (5 families), of them 7 individuals were Ashkenazi and one Moroccan Jew who shared the same haplotype, and one Ashkenazi, who carried the same variant on a recombinant haplotype. Screening for this variant in 364 Ashkenazi individuals detected 2 carriers. These findings suggest that although c.3761_3762delCCinsG is considered one of the frequent variants detected in unrelated individuals, and was thought to have occurred independently on various haplotypes, it is in fact a founder mutation in the Ashkenazi population.

1. Introduction

Autosomal recessive polycystic kidney disease (ARPKD, MIM# 263200) is a hereditary fibrocystic disease that involves mainly the kidneys and the hepatobiliary tract with an estimated incidence of 1:10,000 to 1:40,000 live births (Dell, 2011). The majority of cases are detected either late in pregnancy with large echogenic kidneys accompanied by oligohydramnios or in the neonatal period (Halvorson et al., 2010). About 30–50% of the affected newborns die shortly after birth from respiratory insufficiency due to pulmonary hypoplasia (Zerres et al., 1998).

The locus for ARPKD, was mapped to chromosome 6p (Zerres et al., 1994) and the gene *PKHD1* (polycystic kidney and hepatic disease 1, MIM# 606702) was identified to be responsible for the disease (Ward et al., 2002; Onuchic et al., 2002). The large gene (67 exons) encodes for the 447 kDa multi-domain fibrocystin protein, which is localized in the primary cilium and basal body of the renal and bile duct epithelium (Wang et al., 2004). Fibrocystin has numerous protein–protein interactions and is likely required in signal transduction pathways which are important for normal kidney epithelial cell divisions, polarity and differentiation (Fischer et al., 2006; Fischer and Pontoglio, 2009).

Over 300 pathogenic variants, distributed throughout the *PKHD1* gene, have been reported; most of them are rare private variants and only 10%–20% of ARPKD cases are associated with commonly occurring *PKHD1* variants. The most common variant reported in central Europe, c.107C > T; p.T36M, represents a founder mutation but there is also evidence for a mutational "hotspot" at this site (Bergmann et al., 2004; Sharp et al., 2005). Additional variants, like c.9689delA, c.3761_3762delCCinsG and c.5895dupA, which were detected in several unrelated individuals, support the concept of mutational hotspots (Sharp et al., 2005).

Correlations between *PKHD1* genotypes and the phenotypes are limited, yet studies support the view that genotypes consisting of two truncating variants are severe and tend to be lethal, while

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those with at least one missense variant that might retain partially-functional protein tend to be compatible with life (Furu et al., 2003).

Here we describe the molecular study of families referred to our center in which newborns or fetuses have been diagnosed with infantile severe polycystic kidneys and the identification of a common founder Ashkenazi mutation.

2. Methods

DNA extraction from blood and chorionic villi was performed with FlexiGene DNA kit and QIAamp DNA mini kit (QIAGEN).

Haplotype analysis was performed by using polymorphic SNPs and microsatellites located within and near the *PKHD1* gene using the USCS browser (http://genome.ucsc.edu/). PCR was performed in standard conditions using primers flanking the SNPs or repeats (Primer sequences available upon request). PCR products were separated using the Applied Biosystems® 3130 Genetic Analyzer and analyzed with the GeneMapper® Software v4.0 (Applied Biosystems). Restriction based analysis was performed for rs2076308, rs2766124, rs9382035, rs473642 and rs2235723.

2.1. Whole-Exome sequencing

DNA sample that was extracted from fetal tissue of family 6 was enriched for exonic sequences, with the SureSelect Human All Exon v.2 Kit that targeted 50 Mb (Agilent Technologies, Santa Clara, California, USA). Sequencing was carried out on a HiSeq2000 (Illumina, San Diego, California, USA) and 90-bp were read paired-end. Reads alignment and variant calling were performed with DNAnexus software (Palo Alto, California, USA) using the default parameters with the human genome assembly hg19 (GRCh37) as a reference.

2.2. Mutation testing

The variant c.3761_3762delCCinsG (NM_138694.3; NP_619639.3) was tested by restriction enzyme analysis with *Scrf*I using the primers PKHD1EX32F- GGACTTCCACAGGTGCTATG and PKHD1EX32frag1R- AGGCTCAGGCTGCTATTTGT. The PCR product of 398 bp was cut into 2 fragments, 206 bp and 192 bp, for the mutant allele while the normal allele remained uncut (398 bp).

Detection of g.32796_36455del [NG_008753.1] (deletion of exons 20–21) was performed using the primers PKHD1ex19F-TAGTTGGGCTGCTGTGGGA and PKHD1int21R- ATAAA-GAGTCCTCTTGCC ACCCATC which resulted in 350 bp fragment specific to the mutant allele.

2.3. Carrier frequency estimation

Anonymous DNA samples of 236 Ashkenazi individuals were tested for the c.3761_3762delCCinsG variant by allele specific PCR using the primers PKHD-3761M- GCATCTGGTGTGAAACCCTGC-CACG and PKHD1-3761C- ACTGAGTTGGAGAGG TTACTTCCTCCCAC. PCR product of 247 bp specific for the variant was detected on 4% Nusieve agarose gel and visualized with UV illumination. The c.3761_3762delCCinsG variant was also looked up in the recently reported whole-genome sequencing of 128 Ashkenazi Jews (Carmi et al., 2014).

2.4. Mutation age estimation

To estimate the age of the c.3761delCCinsG allele, we used a simple composite-likelihood based estimator, similarly to Genin et al. (Genin et al., 2004) (see also (Slatkin and Rannala, 2000)). The likelihood of observing each haplotype on each side of the mutation was assumed to be independent, and computed according to the HapMap2 recombination map (Frazer et al, 2007). We also assumed that the ancestral haplotype is known (according to the majority genotype at each position), and neglected the possibility of returning to the ancestral haplotype following recombination. We also ignored mutations at SNPs.

Denote the genetic distance from the focal mutation to marker k as x_k, the age of the mutation (time to coalescence of all carriers) as g generations, and consider a (one-sided) haplotype extending to marker k, but not to marker k+1. The likelihood of the haplotype is $(1-x_k)^g \cdot [1-(1-(x_{k+1}-x_k))^g]$. The second term is omitted if the haplotype is unbroken until the last genotyped marker. The likelihood is then multiplied by $(1-\mu)^g$ for each microsatellite marker between 1 and k, where μ is the microsatellite mutation rate, taken as $2.5 \cdot 10^{-4}$ per locus per generation (Sun et al., 2012 Oct). We multiplied the likelihood over both sides of all nine haplotypes carrying the c.3761delCCinsG allele (eight Ashkenazi + one Moroccan self-reported ancestry), and iterated over all values of g between 1 and 100 to obtain the maximum likelihood estimate. This likelihood is "composite", and becomes exact (under the assumptions described above) under a "star" genealogy, which is a reasonable approximation for a population that expanded as rapidly as Ashkenazi Jews (Carmi et al., 2014). Nevertheless, to guarantee that our confidence intervals are not too optimistically narrow, we used 10 k bootstrapping iterations over the 18 one-sided haplotypes, and reported the 2.5%–97.5% quantile.

3. Results

In total, 7 families were referred for molecular analysis after having either a fetus or a newborn affected with polycystic kidneys, suspected to have autosomal recessive polycystic kidney disease (ARPKD) (Table 1). The first 5 families (1–5, Table 1), were studied by linkage analysis in order to enable prenatal diagnoses in future pregnancies.

Segregation analysis of microsatellite polymorphic markers residing in and near the *PKDH1* gene was performed for each family. Using this strategy, we were able to perform 11 prenatal diagnoses, in which 3 fetuses were carriers for the disease haplotype, 4 were homozygous for the disease haplotype, 3 were homozygous of the normal haplotypes and one fetus was found to be a triploid. The results of the linkage analysis also led us to suspect that a common variant might underlie the disease in 3 families as out of 6 parents 5 (4 Ashkenazi and 1 Jewish Moroccan) shared the same haplotype [C-189-140-129-A-230-C-C-A or C-189-140-129-A-230-C-C-G] and the mother in family 4 that seemed to have another haplotype in fact shares the same haplotype to the right of the mutation (families 2, 4,5 in Table 1). Another haplotype, [189-154-129-A-222-C-253], was found in the Palestinian family and yet another one, [197-144/146-132-G-224-C-249], in a consanguineous couple of Jewish Algerian origin (Table 1, families 1 and 3, respectively).

When the sixth family, also of Ashkenazi origin, was referred, we opted for whole Exome sequencing as a more efficient method due to the large size of the *PKHD1* gene. Through this strategy 184,157 variants were called and 632 remained after removing variants of high MAF. Of these 632 variants, only one heterozygous variant was relevant for ARPKD and it was found in the *PKHD1* gene. This was an indel variant c.3761_3762delCCinsG resulting in frameshift (p.Ala1254GlyfsX49) (NM_138694.3; NP_619639.3). Comparing the coverage of each *PKHD1* exon to the same run samples, revealed half coverage of exons 20–21, suggesting a heterozygous genomic deletion encompassing the two exons and was confirmed by PCR crossing the junction point – g.32796_36455del [