Supplementary Material

Detailed description of variant frequencies in each cohort

TMEM216

TMEM216 c.218G>T is a well-known founder variant in the Ashkenazi Jewish population.[6,7] Two unrelated Italian JS probands were homozygous for this variant (allelic frequency - AF 0.36%), while only one Italian control was heterozygous. This variant was much more common among US JS patients, with 11 homozygous and four heterozygous carriers (AF 2.16%). Global gnomAD AF is 0.016%, but this is mostly driven by Ashkenazi Jewish controls (0.34%). When removing this sub-cohort, gnomAD AF drops to 0.0037%.

TMEM237

TMEM237 c.52C>T was reported as a founder variant in the Hutterite population.[8] We found this variant in heterozygosity in two unrelated Italian JS probands (AF 0.18%) and in one Italian non-JS control, while it was not present in the US JS cohort. In gnomAD, the variant is present in 16 heterozygous individuals (global AF 0.006%), of whom 15 are European non-Finnish (AF in this subgroup 0.012%).

MKS1

MKS1 c.1408-34_1408-6del variant was reported as a founder variant explaining up to 70% cases of Meckel syndrome in the Finnish population, with a disease prevalence of 1 in 9000 live births.[10,11] We found this variant in heterozygosity in three European and two US JS patients (AF 0.27% and 0.16% respectively), as well as in seven Italian controls. gnomAD has 308 heterozygous carriers (AF 0.11%), of whom more than half are Finnish (AF in this subgroup 0.70%).

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Another recurrent variant in *MKS1* (c.1476T>G) was present in eight unrelated JS probands from the vast Mediterranean area (AF 0.73%), and in one patient of Greek origin from the US cohort (AF 0.08%). Of note, this variant had been previously reported in a Turkish patient with Bardet-Biedl syndrome.[12] The variant was also detected in 11 Italian controls (AF 0.03%), and in 14 individuals from gnomAD (global AF 0.006%), all non-Finnish Europeans (AF in this subgroup 0.01%). Of note, the healthy mother of one JS patient was homozygous for the variant.

KIAA0586

The most common *KIAA0586* variant, c.428delG, was reported in several studies on JS patients worldwide.[14-17,20] This variant was detected in 22 unrelated European probands, including 3 homozygotes (AF 2.27%), and in 34 unrelated US probands, of whom 5 homozygotes (AF 3.26%). Among Italian controls, allele frequency was 0.52%. The gnomAD database lists a total of 781 alleles (global AF 0.31%), including two homozygotes, with highest frequency in Ashkenazi Jewish (0.83%), intermediate in non-Finnish Europeans and Latin Americans (0.48% and 0.24%), and lowest in African-Americans, European Finnish and Asians (0.10% to 0.006%). A homozygous clinically unaffected parent of a JS patient was reported by Pauli et al.[18]

In addition to this variant, we detected three other recurrent *KIAA0586* variants in our JS cohorts. The first was a deletion of exons 8-10 detected in heterozygosity in four European patients (AF 0.36%) and one US patient (AF 0.10%). Comparison with non-JS cohorts was not possible, since genomic and population databases do not report large deletions such as this one. *KIAA0586* c.863_864delAA was found in three European (AF 0.27%) and three US (AF 0.25%) JS patients; it was absent from Italian non-JS controls, and it was detected in heterozygosity in eight subjects of various ethnicity in gnomAD (AF 0.003%). Finally, *KIAA0586* c.1006C>T occurred only in two JS patients from Sardinia (AF 0.18%).

Analysis of Italian controls identified three heterozygotes, while in gnomAD, this variant is present in two non-Finnish Europeans (AF 0.0008%).

RPGRIP1L

RPGRIP1L c.1843A>C was detected in four European JS probands, two homozygous and two heterozygous (AF 0.54%), and in four US patients (AF 0.32%) all heterozygous. The variant, always in heterozygosity, was reported in one Italian control and in 17 individuals from gnomAD (AF 0.006%), all but one of non-Finnish European origin.

CC2D2A

CC2D2A c.4667A>T was found in ten European JS patients (AF 0.91%) and 16 US JS patients (AF 1.32%), always in compound heterozygosity with other pathogenic variants. The variant was also detected in the heterozygous state in 17 Italian controls and in 51 subjects from gnomAD (AF 0.02%), of whom 45 non-Finnish Europeans.

TMEM67

TMEM67 c.755T>C was found in three European and two US JS patients (AF 0.27% and 0.16%, respectively). gnomAD lists 23 heterozygous carriers (AF 0.008%), of whom 19 non-Finnish European, three Latin-Admixed Americans and one from the “other” group. This variant was also present in three Italian controls.

Supplementary Table 1 - Microsatellite markers genotyped across the three gene loci

microsatellite marker	repeat	primer forward	primer reverse
CC2D2A			
chr4_13377406	(AC)13.5	CTTTTTCTGTGTCTGGGAGCTA	GCTTCAGAAGTGGTTTGGTTAG
chr4_13950888	(TG)15.5	TGAGAGTTTTGGGAGGCTTACG	CATGGATGGAGGGAGGGATTC
chr4_14449343	(GT)18	TTTTCGTAAGCAGCCCTCTG	TTGAGCCCAAGTCCAAACTT
chr4_14730789	(TG)12.5	CCTGCCTTAGACAAGCTACTTCA	CAAACCCCAAACTTCTGCTTACA
chr4_16131330	(CA)14	GTCCTAGGAGAGGCAGCCTTATAG	TAGTGGCCTTCTCAGCATCTTTTC
chr4_16594109	(AT)20.5	TGTGCTTGTTAAGTGGGAGTGT	ATGCACCCACCAGTTTTCCTAT
chr4_17143227	(AC)23	CCTGGGAGGGCACTATTTGATT	GGATACAGCCCAACCCTTTCA
chr4_17606757	(CA)15.5	CCATGCCCTTCACAGCCA	TAACAGTGGTGTGGACATTCGA
KIAA0586			
chr14_57281491	(GT)16	GATGTGGTGCTGTGAGACATTT	CTACCACCCTTCATCAATCCAT
chr14_57704958	(AC)16	AAGGCCATATTAATCTGTGTGAA	CAAGGCTTGGTGAAGAGG
chr14_58333698	(AAT)15	TCTTCTGCTTTAGCCAGAGACC	ACCTCCACCTTTTCATTTCTGA
chr14_59195234	(GT)13	GCTCTAGAGAATGGCCTAGTGG	CCCCAGTAATTCTCAAGTTTGG
chr14_59298006	(AC)24.5	AGGAGGTGTTAGGTTCTTTGTAGG	AGCACATAACCTCTTTCTCAGC
chr14_59729395	(GT)23	AGCATTTTCAACATTTTCCCATA	AAGCCAGGACTTGAGCTAGATG
chr14_60259097	(CA)19	AGGACTGTACTCCCAGAAGCAG	ATGTGGCCAGTTTTCTATTTGG
chr14_60798045	(CT)13	CAAGTCTTTGTGACAACCAAC	AAGCCGAACACAATACCAGTTT
RPGRIP1L			
chr16_52016326	(AC)16.5	TAGCTGCTGCACAAATTAGGTC	TGGCAGGGTTCTGTTTTGTT
chr16_52478429	(CA)17.5	TAGCTGCTGCACAAATTAGGTC	GGTGGCAGGGTTCTGTTTT
chr16_52822271	(AC)22.5	AAAGGAGGGAGGGGATTTG	GTGTGAAAAGTTGGGCTCTACC
chr16_53204512	(GT)21	TGACCTTGTAAAATGCATCGTG	ACACAAATCCCTTCGCTCAATA
chr16_53637604	(TG)17.5	CACACCTGGCTTAGTTTCCAA	TGCACCTAACTCTTTGTGTATCAAG
chr16_53951639	(GT)17	CCTATTTGAGAGCCATACCACA	GCAGAGTTGCTGTACTTGTCCTT
chr16_54311628	(AC)22.5	ATCCAGGCAGGAGGAGTCT	GCAAGAGTTTCCAGAGGTAATG
chr16_54545329	(GA)15	CTGGGAGGCAGTTTTCATTC	GGTCTTCCCATTGTCTCGAA
chr16_55014711	(GT)22.5	GCCCAGCCTCAAGTGAGTATAA	GCAGATACTGCCTGCTTTGACT
MKS1			
chr17_54402639	(TG)15	TGCCTAGCAGAAGAGTCCAA	GGCTCCTTTCACAGCATTGT
chr17_55427602	(GT)15	GAGGTTGTTAATGCTCCAAAGC	AATCCCTACAACGGCTATTCC
chr17_55618526	(AG)13.5	GCCTGACTTGGTTCACACCT	TATGGTTTGTGTCGCTGGAG
chr17_56256012	(AC)19.5	AAACCCAATTACAAACCTGGACAC	CAATCTGGAGGCCAAAGTACCA
chr17_56700558	(AC)15.5	GTCTGTGCCCACAGGAACTT	GTTTCTGGGCAGCCAATATC
chr17_57224756	(AC)17.5	GGCCAGGGCTGGTAACTAA	AGGCATGAACTCAAGGACATC
chr17_57365673	(AC)15.5	AGAGATCCTGTCCCCAAAAGA	CTTGAATCCAGAAAGCAGAGGT
chr17_57720512	(GT)18	GACAGTGGTGGTGTTTGTGG	TATCCCCCTTTCCTCCAAAG
chr17_58239929	(AC)21	GGGATGGTGGCTCTGAGTT	TCAGAGAAGTCAGGGTGCAG
chr17_54402639	(TG)15	TGCCTAGCAGAAGAGTCCAA	GGCTCCTTTCACAGCATTGT

The name of each microsatellite marker refers to its genomic position (reference assembly: hg19).

Supplementary Table 2 – Functional assessment of primary cilia from fibroblasts

Cell lines and phenotypes	Genotype	ciliated cells (%) (mean±SD)	p	ciliary length (µm) (mean±SD)	p
Four controls (averaged)	wild type	79.58±3.44	-	5.63±0.40	-
MKS1 affected compound het (son)	*MKS1* c.1476T>G + c.1024+1G>A	66.16±5.07	p<0.0001	3.41±0.36	p<0.0001
MKS1 healthy hom (mother)	*MKS1* c.1476T>G hom	69.47±2.71	p<0.001	4.90±0.08	p<0.01
KIAA0586 affected compound het	*KIAA0586* c.1476T>G + c.863_864delAA	57.26±2.50	p<0.0001	4.53±0.29	p<0.001
KIAA0586 affected hom	*KIAA0586* c.428delG hom	49.67±4.05	p<0.0001	4.01±0.20	p<0.0001
KIAA0586 healthy hom	*KIAA0586* c.428delG hom	80.73±3.55	n.s.	5.85±0.34	n.s.

The percentage of ciliated cells was calculated by dividing the number of cells showing a primary cilium for the total number of cells for each field (up to 15 fields per experiment, in triplicate). The two *MKS1* carriers are mother and son; the three *KIAA0586* carriers are unrelated (affected son of the healthy homozygous individual was not available for skin biopsy). Ciliary length was measured from the centrosome (γ-tubulin) to the tip of the cilium (acetylated-α-tubulin) (up to 100 primary cilia per sample for each experiment, in triplicate). p is given for comparisons vs healthy controls. n.s.=non-significant