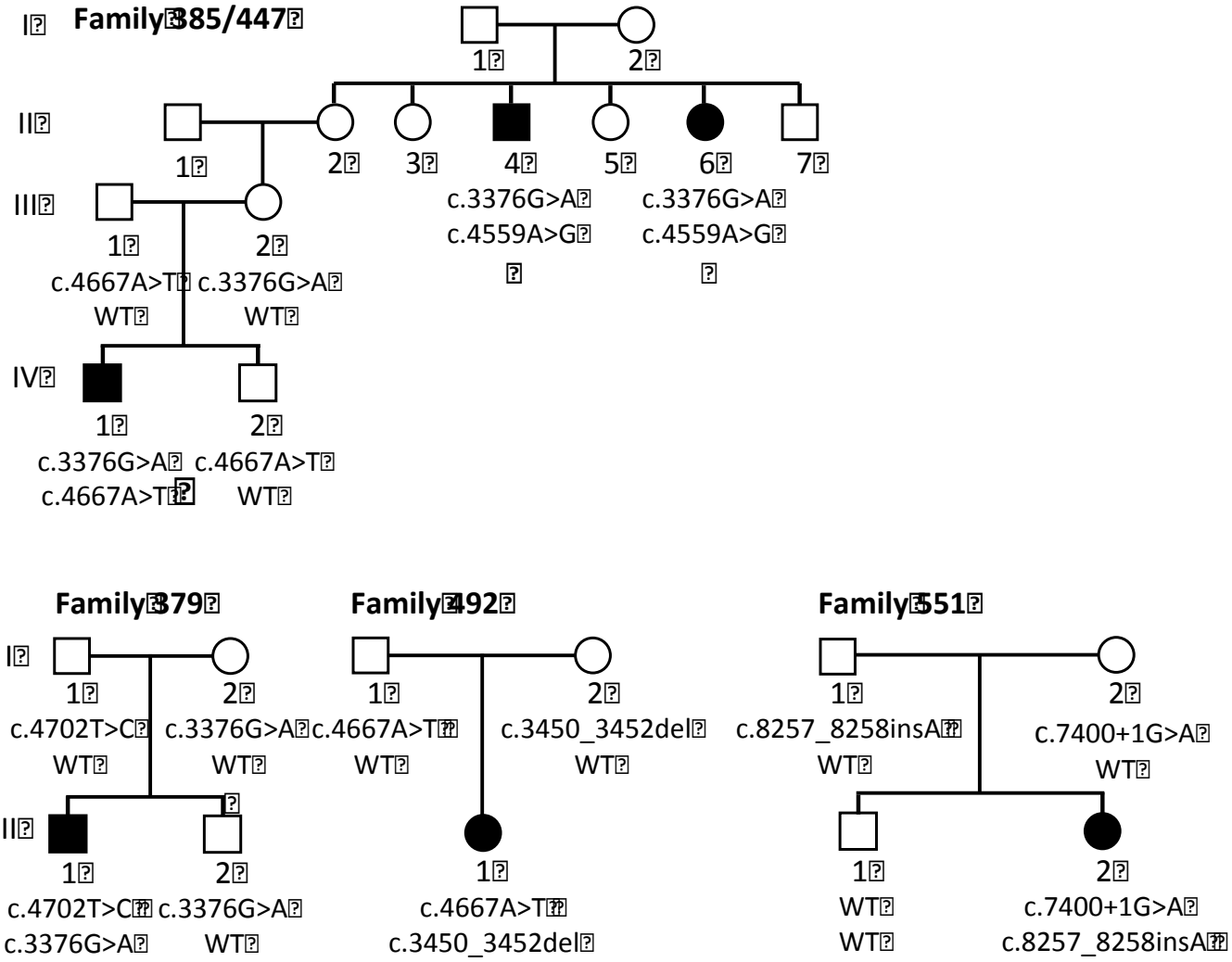
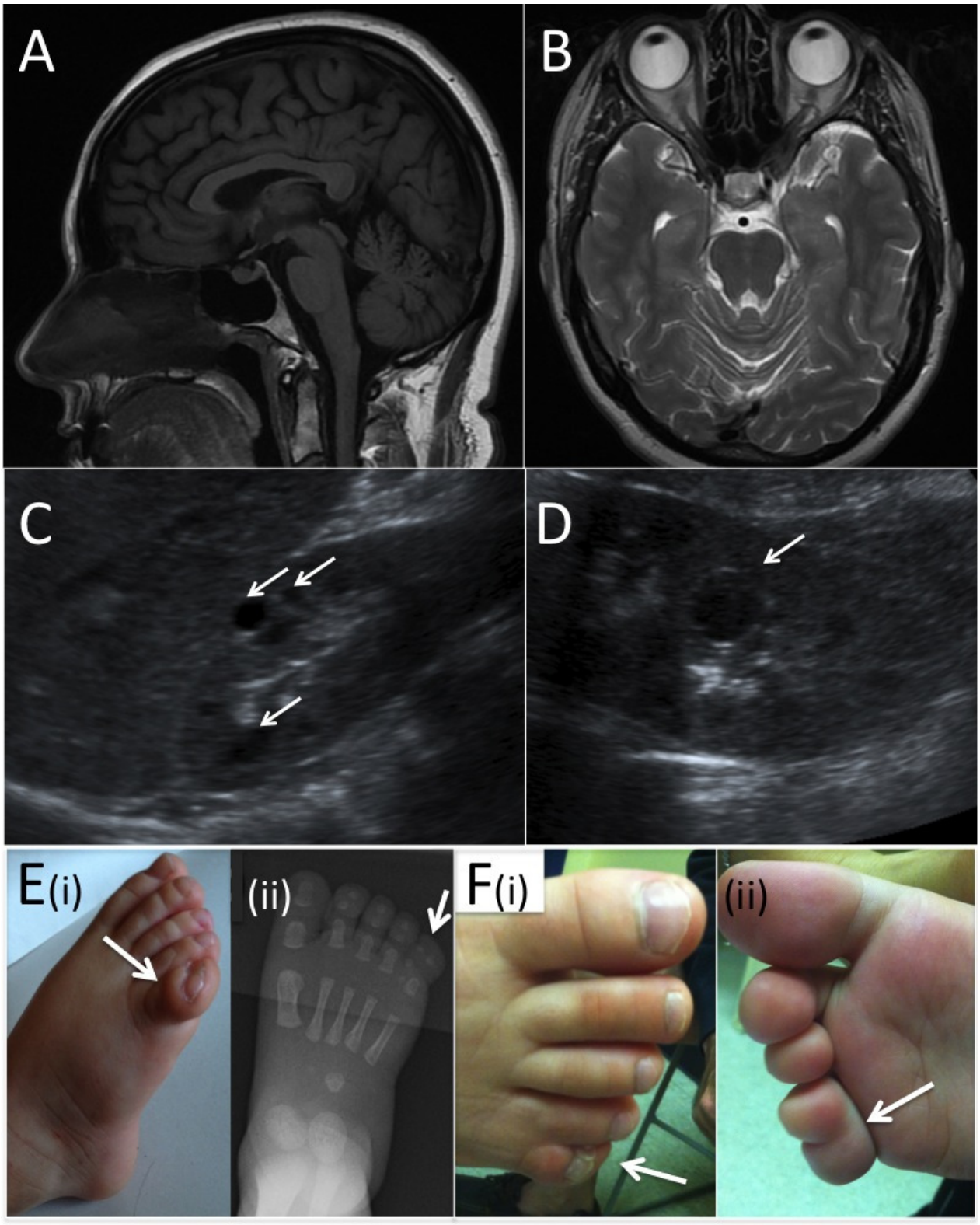
Supplemental Figure 1: Segregation studies of mutations in *CC2D2A* (NM_001080522.2) in families 385/447, 379 and 492, and of mutations in *C5ORF42* (NM_023073.3) in family 551.



Supplemental Figure 2: Sagittal T1(A) and axial T2 (B) brain MRI of individual II:6 from family 385/447 who has compound heterozygous mutations in *CC2D2A*, and ocumolotor apraxia as the sole manifestation. Right (C) and left (D) renal ultrasounds from individual II:2 from family 387 with compound heterozygous mutations in *TMEM231* showing macroscopic cysts (arrows). Post-axial polysyndactyly in individual II:1 from family 483 (Ei and ii) and II:2 from family 387 (F i and ii).



Supplemental Table 1. Analysis of combined exome sequences from the 6 individuals (from 4 families) with unexplained JBTS

Variant prioritization steps in the combined exomes dataset	
Filters applied (sequentially)	***Number of variants retained***
Non-synonymous/splicing/coding indel variants	19884
After excluding variants present in > 2 in-house exomes	1035
After excluding variants reported in 1000 genomes and EVS datasets (frequency > 0.5%)	987

*Total number of variants identified in the combined 6 exomes; redundant variants were counted only once.

Supplemental Table 2. Genes with rare homozygous or multiple heterozygous variants in the 6 individuals (from 4 families) with unexplained JBTS

Number of families with mutations in the same gene	Number of genes	Genes
1 family	19	*C14orf135,C9orf174,CLCN1, ENTPD3,FBXL22,FCGR3B,FLG,LRRK2,MUC12,PDE8A,PPL,PUS10, RASIP1,RCC2,SHROOM4,TACC3,* ***TMEM231****, TRAF5,TTN*
2 families	1	***TMEM231***
> 2 families	0	-

Supplemental Table 3: This table shows the called genotypes for SNVs upstream and downstream of the two TMEM231 mutations reported. SNVs are included until 4 discordant SNVs are seen.Only positions where all three of the samples had at least 10 reads of coverage are included. Regions with lower than average mapping quality (below 50) have genotypes shown as "?", since the lower quality of mapping in these regions indicates that genotype calls may not be accurate.Positions with discordant genotypes are shown in red text. The two TMEM231 mutations are highlighted in yellow.

Position	rsID	Ref	MAF from 1000genom	EVS MAF	Sample 1329-483	Sample 998_387	Sample 997_387
chr16:72993708	rs62640010	C	0.0298	0.032309	C/T	C/C	C/C
chr16:72993831	rs7193297	A	0.4322	0.471418	A/C	A/A	A/A
chr16:72993860	rs62639999	G	0.0238	0.03756	G/G	G/A	G/A
chr16:73126750	rs72795164	G	0.022		G/G	G/A	G/A
chr16:73126858	rs7189194	T	0.9945	0.011149	G/G	G/G	G/G
chr16:74255440	.	G			G/C	G/C	G/C
chr16:74425548	rs2868591	A		0.362811	G/G	G/G	G/G
chr16:74443433	rs62055232	C		0.138795	?	?	?
chr16:74444361	rs3931746	C			?	?	?
chr16:74444384	rs60469648	C		0.180212	?	?	?
chr16:74444538	.	G		0.149081	?	?	?
chr16:74444838	rs62054261	A	0.4954	0.272234	?	?	?
chr16:74444976	.	C		0.003845	?	?	?
chr16:74445003	.	C			?	?	?
chr16:74445142	rs55906605	C			?	?	?
chr16:74445689	rs62054260	C	0.1145	0.091932	?	?	?
chr16:74445817	rs2650552	C			?	?	?
chr16:74447514	rs2650549	T		0.036624	?	?	?
chr16:74451884	rs78597831	C			?	?	?
chr16:74499668	.	C		9.30E-05	C/T	C/T	C/T
chr16:74501856	rs6564117	C	0.7051		G/G	G/G	G/G
chr16:74503078	rs79193356	T	0.0508		T/C	T/C	T/C
chr16:74504005	rs2303279	T	0.7253	0.215932	T/C	T/C	T/C
chr16:74504097	rs12716764	G	0.9881		C/C	C/C	C/C
chr16:74513985	rs918781	C	0.951		T/T	T/T	T/T
chr16:74517155	rs2303281	A	0.7248		G/G	G/G	G/G
chr16:74519533	rs968649	C	0.9675		A/A	A/A	A/A
chr16:74526752	rs2010910	G	0.9895		A/A	A/A	A/A
chr16:74537591	rs4887772	C	0.7038	0.33984	T/T	T/T	T/T
chr16:74660174	rs12933037	G	0.4103		G/A	G/A	G/A
chr16:74662597	rs11149759	C	0.3214	0.249117	C/T	C/T	C/T
chr16:74664743	rs7193541	T	0.418	0.454545	T/C	T/C	T/C
chr16:74664810	rs7188880	A	0.6305	0.398587	T/T	T/T	T/T
chr16:74664969	rs7193959	T	0.745		A/A	A/A	A/A
chr16:74666634	rs4072450	G	0.7431		C/C	C/C	C/C
chr16:74670458	rs4888262	C	0.6305	0.399052	T/T	T/T	T/T
chr16:74694692	rs56143602	T	0.4066		T/C	T/C	T/C
chr16:74695079	rs8058922	G	0.7605	0.272448	T/T	T/T	T/T
chr16:74706298	rs4888274	A	0.6255		T/T	T/T	T/T
chr16:74709737	rs7184423	C	0.3947		C/A	C/A	C/A
chr16:74712905	rs6564158	A	0.6474	0.387897	A/T	A/T	A/T
chr16:74750396	rs11554621	T	0.1305	0.170385	T/C	T/C	T/C
chr16:74750405	rs2301865	G	0.7546	0.218628	A/A	A/A	A/A
chr16:74752841	rs6564161	C	0.1287	0.169057	C/G	C/G	C/G

Pseudogenes make the read mappings here ambiguous and many of the genotypes may be wrong

chr16:74753079	rs2074629	C	0.2784	0.299684	C/T	C/T	C/T	
chr16:74876541	rs4550476	T	0.9835		C/C	C/C	C/C	
chr16:74876612	rs4328467	G	0.6232		G/A	G/A	G/A	
chr16:74876991	rs7187659	T	0.9382		C/C	C/C	C/C	
chr16:74877062	rs12444292	T	0.1827		T/C	T/C	T/C	
chr16:74877063	rs12446306	G	0.185		G/A	G/A	G/A	
chr16:74877125	rs9930887	G	0.3347		G/A	G/A	G/A	
chr16:74877693	rs79940172	A			A/G	A/G	A/G	
chr16:74877694	rs7187950	C	0.6886		C/T	C/T	C/T	
chr16:74877733	rs12923470	A	0.3063		A/G	A/G	A/G	
chr16:74877754	rs12922918	C	0.3049		C/T	C/T	C/T	
chr16:74877844	rs7186814	G	0.6694		G/A	G/A	G/A	
chr16:74920090	rs9936830	T	0.1799		T/C	T/C	T/C	
chr16:74920191	rs14308	G	0.1113	0.160625	G/A	G/A	G/A	
chr16:74920374	rs4888305	C	0.9487		T/T	T/T	T/T	
chr16:74921661	rs9940014	A	0.168	0.233408	A/T	A/T	A/T	
chr16:74926520	rs11149776	C	0.2601	0.292517	C/T	C/T	C/T	
chr16:74943260	rs2303254	A	0.6662		G/G	G/G	G/G	
chr16:75019070	rs3814875	C	0.331		A/A	A/A	A/A	
chr16:75148647	.	G	0.0023		G/A	G/A	G/A	
chr16:75186727	rs11859007	G	0.7766		A/A	A/A	A/A	
chr16:75200378	rs9929570	T	0.9153		C/C	C/C	C/C	
chr16:75200407	rs11865310	G	0.7761		C/C	C/C	C/C	
chr16:75200416	rs7200789	T	0.9139		C/C	C/C	C/C	
chr16:75201060	rs6564215	T	0.7321		C/C	C/C	C/C	
chr16:75202998	rs12716783	A	0.7798		C/C	C/C	C/C	
chr16:75226297	rs118133067	T	0.0545		T/C	T/C	T/C	
chr16:75238103	rs4737	C	0.8246	0.248094	T/T	T/T	T/T	
chr16:75258617	rs2287990	C	0.1461	0.109221	C/T	C/T	C/T	
chr16:75263974	rs6564241	T	0.9226		C/C	C/C	C/C	
chr16:75269267	rs3169330	A	0.9144	0.154365	G/G	G/G	G/G	
chr16:75269534	rs3743613	C	0.4625	0.305581	C/T	C/T	C/T	
chr16:75270721	rs2278020	C	0.5169	0.359617	C/A	C/A	C/A	
chr16:75276295	rs7195938	A	0.9148		G/G	G/G	G/G	
chr16:75276775	rs1035539	G	0.6016	0.42466	G/A	G/A	G/A	
chr16:75277344	rs4888363	A	0.8471	0.233012	T/T	T/T	T/T	
chr16:75277480	rs4887810	C	0.4922		C/A	C/A	C/A	
chr16:75281964	rs1862737	A	0.5266	0.476976	A/C	A/C	A/C	
chr16:75327916	rs2073619	A	0.7624	0.166853	A/G	A/G	A/G	
chr16:75338855	rs3743607	C	0.3558		C/T	C/T	C/T	
chr16:75339131	rs7192981	T	0.9844	0.025126	G/G	G/G	G/G	
chr16:75445605	rs1109341	G	0.5238		G/A	G/A	G/A	
chr16:75445675	rs1109342	G	0.5234	0.498605	G/A	G/A	G/A	
chr16:75445906	rs247435	C	0.9895		T/T	T/T	T/T	
chr16:75445971	rs247436	T	0.2207		T/G	T/G	T/G	
chr16:75448273	rs8056236	C	0.5252		C/T	C/T	C/T	
chr16:75448659	rs8051407	A	0.5238		A/G	A/G	A/G	
chr16:75563330	rs3826107	G	0.163	0.170106	G/A	G/A	G/A	
chr16:75563746	rs8048818	T	0.2147	0.237821	T/G	T/G	T/G	
chr16:75573884	rs2242407	T	0.2386	0.242384	T/C	T/C	T/C	
chr16:75575410	rs7202717	G	0.1593		G/A	G/A	G/A	
chr16:75576539	.	C		9.90E-05	C/T	C/T	C/T	
chr16:75579111	rs4149500	G	0.1589		?	?	?	

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chr16:75579233	rs2738801	A	0.2505	0.254407	?	?	?	TMEM
chr16:75579470	rs2550894	T			?	?	?	
chr16:75579924	rs8055668	A	0.2491		?	?	?	
chr16:75590096	.	A			A/T	A/T	A/T	
chr16:75634014	rs7206481	A	0.2038		A/G	A/G	A/G	
chr16:75646685	rs3743598	G	0.5261	0.368284	G/T	G/T	G/T	
chr16:75654031	rs11648478	A	0.0911		A/T	A/T	A/T	
chr16:75667954	rs6564270	C	0.2088		C/T	C/T	C/T	
chr16:75670128	rs2289064	C	0.0668		C/A	C/A	C/A	
chr16:76268960	rs11861749	T	0.4913		T/C	T/C	T/C	
chr16:76269501	.	G	0.0082		G/A	G/A	G/A	
chr16:76461273	rs7192076	A	0.7042		A/C	C/C	A/C	
chr16:76461519	rs9927638	C	0.4217	0.39961	C/C	C/A	C/C	
chr16:76461588	rs9938200	A	0.7047		A/G	G/G	A/G	
chr16:76481824	rs35839511	C	0.3292		C/C	C/A	C/C	