SUPPLEMENTARY MATERIALS AND METHODS

Variant confirmation

RNA was extracted from lymphoblastoid cell lines using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and cDNA was transcribed using the TaqMan Reverse Transcription Reagent kit (Applied Biosystems, Foster City, CA) for all samples. *JAG1* gene expression for the promoter variant (Proband 11) and the inversion (Proband 12 and the affected father, Proband 12-F) was performed via droplet digital PCR (ddPCR). Two FAM-labelled TaqMan primer and probes sets (ThermoFisher Scientific, Waltham, MA) were designed to map within exon 1 (Hs05024303_s1) or spanning exons 25-26 (Hs01070036_m1) of *JAG1*. A third VIC-labelled TaqMan primer and probe set was used for the reference gene, *TBP* (Hs00427620_m1). Expression of each *JAG1* probe was normalized to *TBP* for each sample, and two replicates were run for each sample. Four individuals without *JAG1* pathogenic variants were used as negative controls and an individual with a known pathogenic *JAG1* frameshift variant (c.2122_2125del) was used as a positive control. The four negative controls were averaged together and plotted as a single value with error bars showing standard deviation. Values for all samples were normalized to the negative control value.

NOTCH2 copy number assays were performed for Proband 10 and both unaffected parents via ddPCR. A TaqMan copy number probe (ThermoFisher Scientific, Waltham, MA) was designed within the deletion using the coordinates: chr1:120,457,929-120,462,236.

All samples for both gene expression and copy number analysis were run on a BioRad QX100 ddPCR system (Hercules, CA), and data analysis was performed using QuantaSoft (BioRad, Hercules, CA).

SUPPLEMENTARY FIGURES

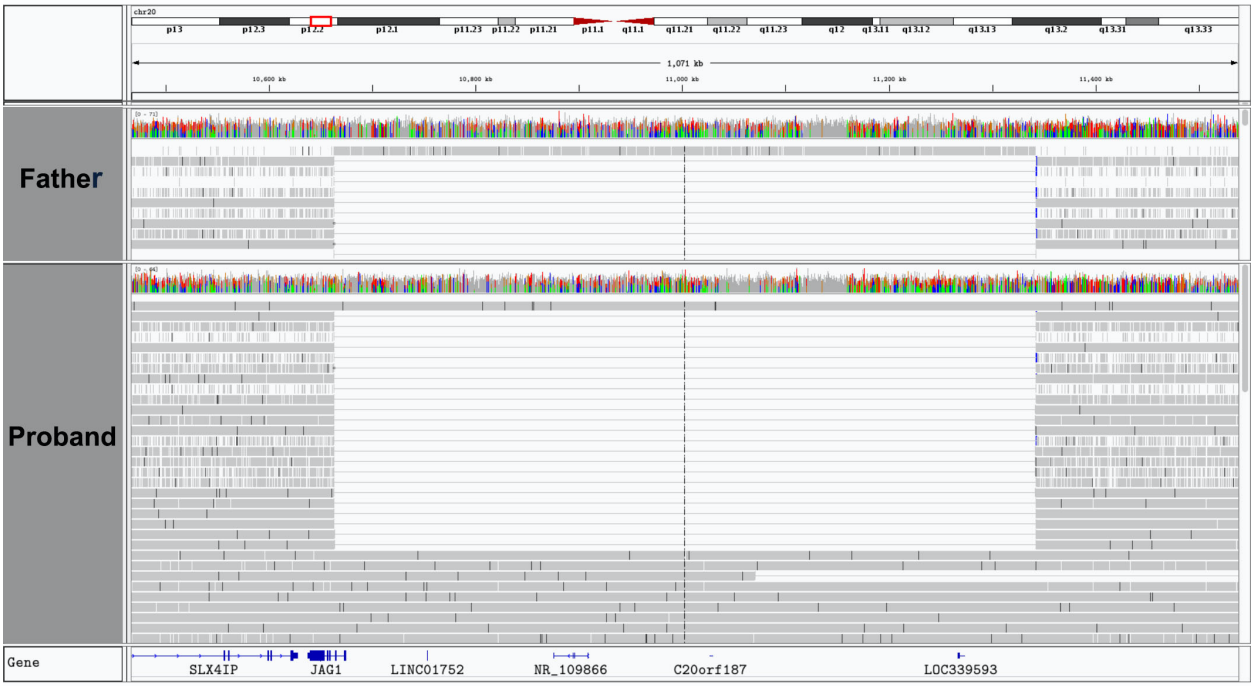

Figure S1. IGV screenshot of the *JAG1* inversion in Proband 12 and the father of Proband 12. Visualization of the region involving the inversion shows several read-pairs with an abnormal insert size equivalent to the size of the inversion and no copy-number change.

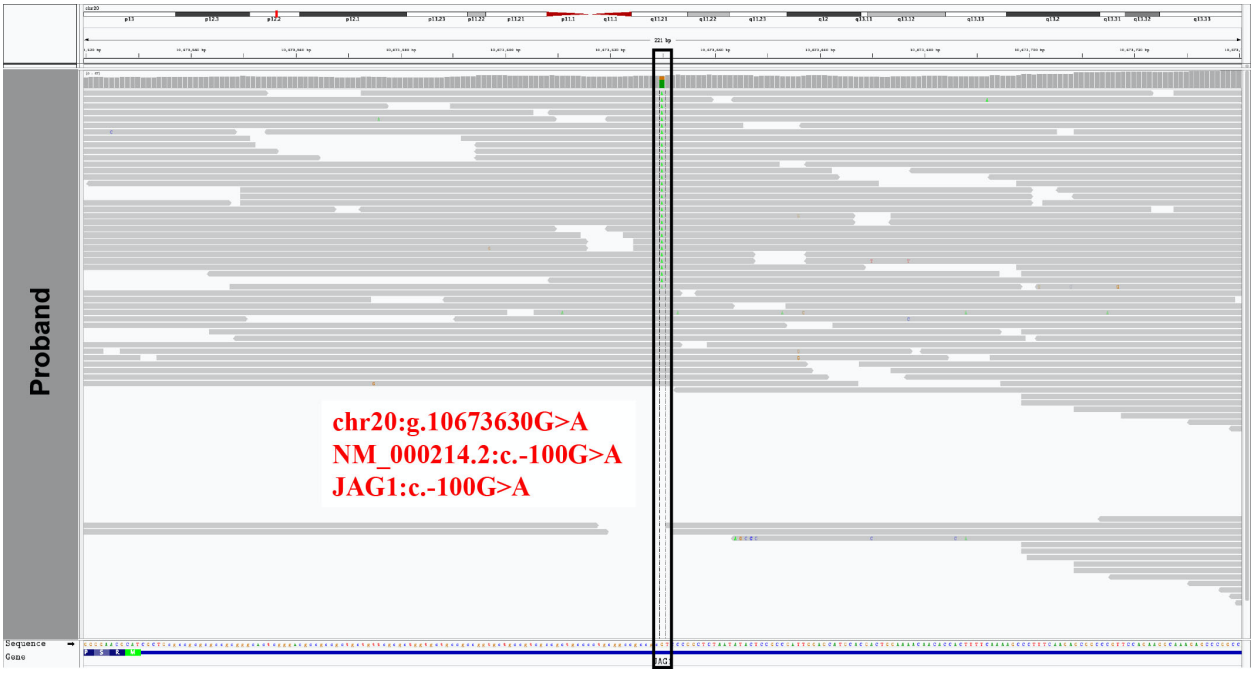

Figure S2. IGV screenshot of the heterozygous variant identified in the *JAG1* promoter region in Proband 11.

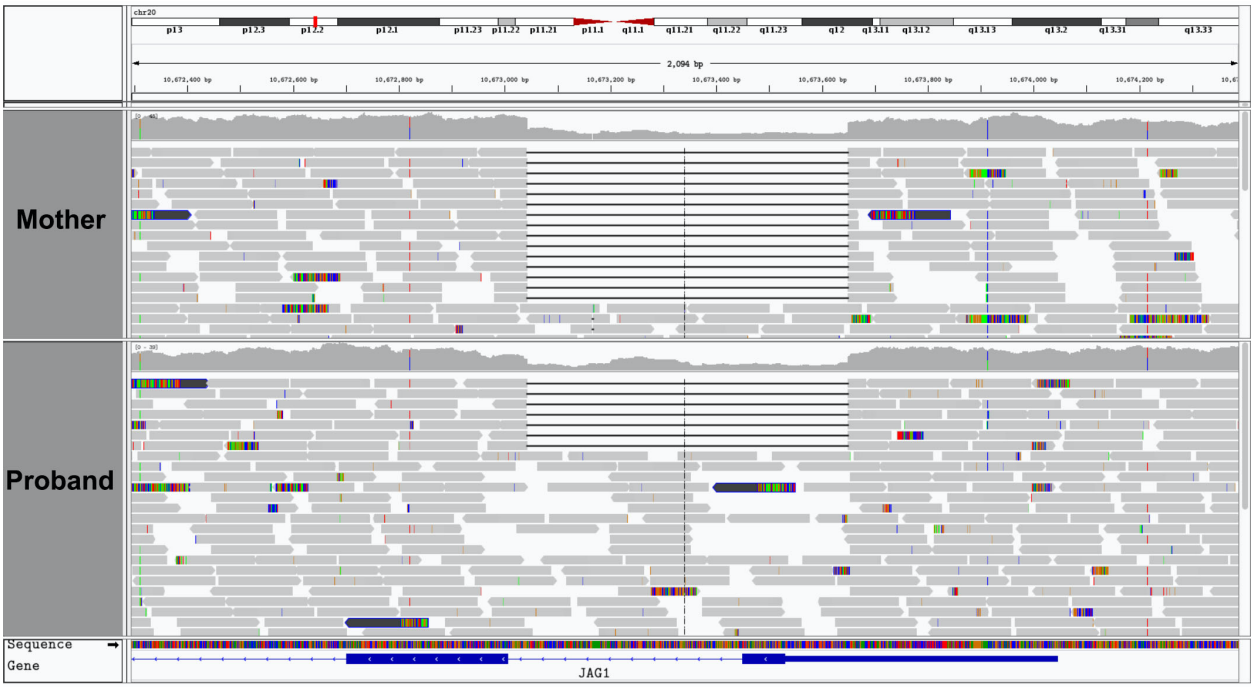

Figure S3. IGV screenshot of the *JAG1* exon 1 deletion in Proband 15. Visualization of the region overlapping the deletion shows read alignments with gaps equivalent to the size of the deletion and a reduction in the read depth across the deleted region. Exact breakpoints of the deletion were mapped by blatting the reads with gapped alignment.

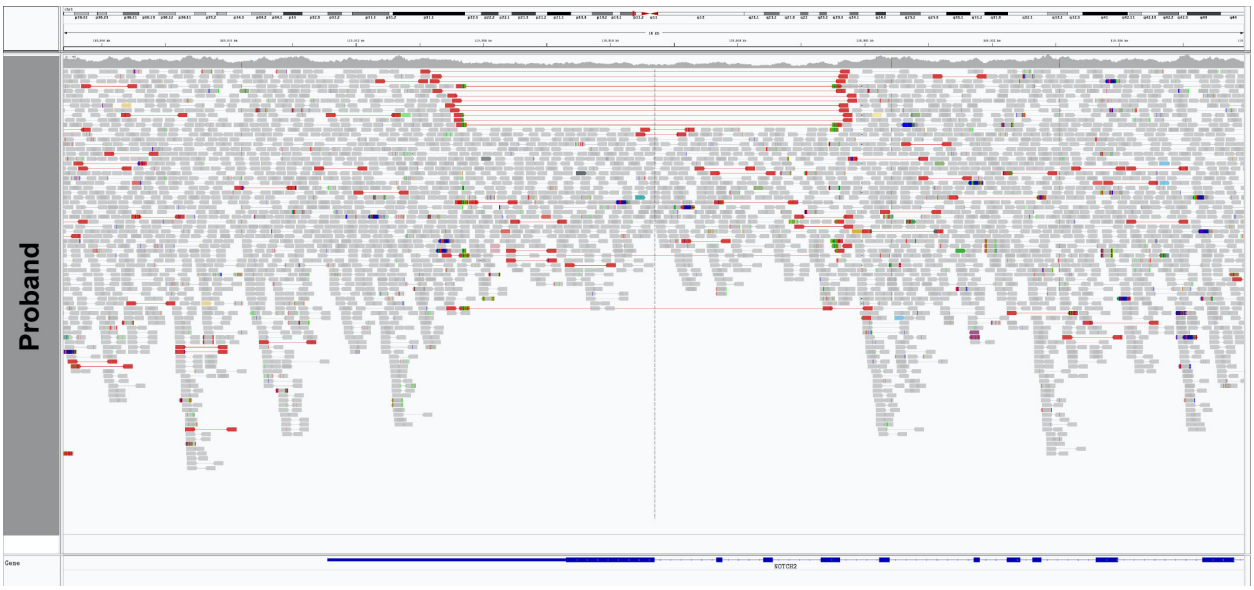

Figure S4. IGV screenshot of the *NOTCH2* deletion in Proband 10. Visualization of the region shows paired-end reads with abnormal insert sizes and a reduction of read-depth across the deleted region. Breakpoints of the deletion were mapped by directly analyzing breakpoint-spanning reads with soft-clipping.

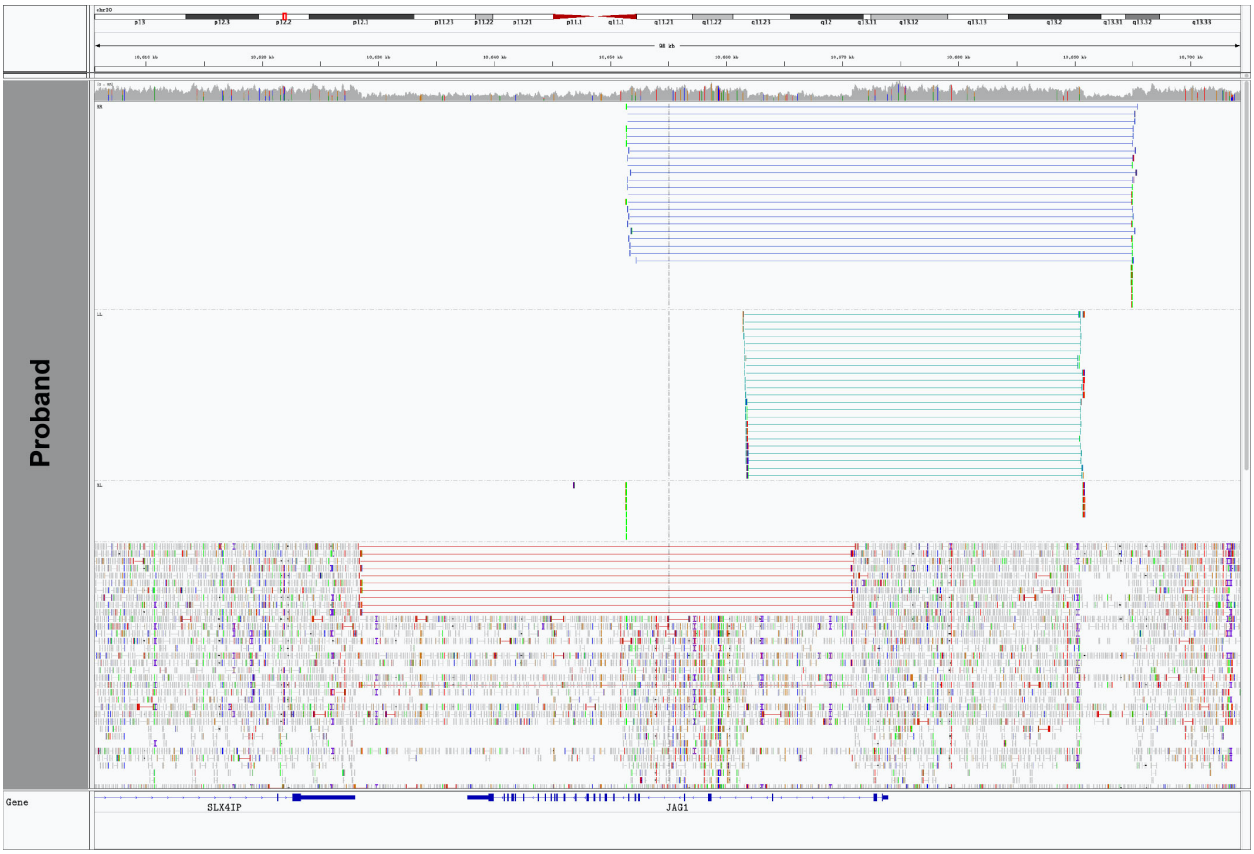

Figure S5. IGV screenshot of the disjointed *JAG1* deletions in Proband 8. Visualization of the region involving the complex variant in the IGV revealed clustering of read-pairs with different insert sizes, read-pair orientation, and read-depth across the deleted regions. Combined analysis of the read-depth, read-pair orientation, and soft-clipped reads spanning the breakpoints revealed the exact rearrangement present in the proband.

Figure S6. IGV screenshot of the disjointed *JAG1* deletions in Proband 14. Visualization of the region involving the complex variant in the IGV revealed two deletions with a very small region of normal copy-number (246bp) in between. Combined analysis of the read-depth, read-pair orientation, and soft-clipped reads spanning the breakpoints revealed the exact rearrangement present in the proband.

SUPPLEMENTARY TABLES

Table S1. Demographic information and standard of care serial testing strategy of probands included in this study

Patient ID	Sex	Age at Enrollment (years)	Geographic Location	*JAG1* Sequencing Test Method	*JAG1* Del/Dup Test Method	*NOTCH2* Sequencing Test Method	Other Screening Tests
Proband 1	M	3	Virginia	SSCP, CSGE, Sanger sequencing (genomic and cDNA)	MLPA	Sanger sequencing	N
Proband 2	M	0.25	Massachusetts	SSCP, CSGE, Sanger sequencing	FISH, MLPA	Sanger sequencing	N
Proband 3	M	0.33	Tennessee	SSCP, CSGE, Sanger sequencing (genomic and cDNA)	MLPA	Sanger sequencing	N
Proband 4	M	8	Denmark	CSGE, Sanger sequencing	FISH, MLPA	Sanger sequencing	N
Proband 5	F	14	New York	SSCP, CSGE, Sanger sequencing	FISH, MLPA	Sanger sequencing	N
Proband 6	M	2	Tennessee	SSCP, Sanger sequencing	MLPA	Sanger sequencing	N
Proband 7	M	8	Brazil	SSCP, Sanger sequencing	MLPA	Sanger sequencing	N
Proband 8	F	1	Washington	CSGE	MLPA[a]	Sanger sequencing[b]	N
Proband 9	F	10	CHOP	Sanger sequencing	FISH, MLPA	Sanger sequencing	ES
Proband 10	F	9	New York	Sanger sequencing	MLPA	Sanger sequencing	N
Proband 11	F	0.58	Vietnam	Sanger sequencing	MLPA	Sanger sequencing	N

Proband 12	M	16	CHOP	Sanger sequencing	MLPA	Sanger sequencing	ES
Proband 13	M	0.83	North Carolina	Sanger sequencing	MLPA	Sanger sequencing	N
Proband 14	F	26	CHOP	Not performed[b]	MLPA[a]	Not performed[b]	N
Proband 15	F	0.92	England	Sanger sequencing	MLPA	Sanger sequencing	N
Proband 16	M	1.5	India	Sanger sequencing	MLPA	Sanger sequencing	N
Proband 17	F	0.92	Turkey	Sanger sequencing[c]	MLPA	Sanger sequencing	N
Proband 18	M	0.42	Vietnam	Sanger sequencing[c]	MLPA	Sanger sequencing	N

Abbreviations: F–female; M–male; CHOP–Children's Hospital of Philadelphia; SSCP–single strand conformation polymorphism; CSGE–conformation sensitive gel electrophoresis; FISH–fluorescence *in situ* hybridization; MLPA–multiplex ligation-dependent probe amplification; N–No; ES–exome sequencing

[a]Test provided evidence of a structural rearrangement

[b]Test was not completed due to evidence of a pathogenic variant via another testing strategy

[c]Testing strategy identified a pathogenic variant that was missed by manual inspection

Table S2. ALGS Phenotypes of Probands and Affected Family Members included in this Study

Patient ID	Hepatic[a]	Skeletal[b]	Cardiac[c]	Facies	PE	Renal[d]	FH[e]	Diagnoses made by GS
Proband 1	Y	Y	N	Y	N	Y	Y	
Proband 2	Y	N	Y	Y	N	Y	N	
Proband 3	Y	N	Y	N	Y	N/A	N	
Proband 4	Y	Y	Y	N/A	Y	N	N/A	
Proband 5	Y	N	Y	N/A	Y	N	Y	
Proband 6	Y	N	Y	Y	Y	N	N/A	
Proband 7	Y	N	Y	Y	N/A	N	N/A	
Proband 8	Y	N/A	Y	Y	Y	N/A	N/A	Resolved complex variant
Proband 9	Y	Y	Y	Y	N	Y	Y	
Proband 10	Y	N/A	Y	Y	Y	N/A	N/A	*NOTCH2* deletion
Proband 11	Y	Y	Y	Y	N/A	Y	N/A	c.-100 promoter variant
Proband 12	Y	N/A	Y	Y	Y	Y	Y	Inversion involving *JAG1*
Proband 12-F	N/A	N/A	Y	Y	N/A	N/A	Y	Inversion involving *JAG1*
Proband 13	Y	N	Y	N/A	Y	N	N/A	
Proband 14	Y	N/A	Y	Y	N	N	Y	Resolved complex variant
Proband 15	Y	Y	Y	Y	N/A	N/A	Y	*JAG1* exon 1 deletion
Proband 15-M	Y	Y	N/A	Y	N/A	N/A	Y	*JAG1* exon 1 deletion
Proband 16	Y	Y	N	Y	N	N/A	N	
Proband 17	Y	N	Y	Y	Y	N	N	*JAG1*: c.1978del; p.E660Rfs*82
Proband 18	Y	N/A	Y	N/A	N/A	Y	N/A	*JAG1*: c.401T>C; p.L134S

Abbreviations: F–father; M–mother; PE–posterior embryotoxon; FH–family history; Y–yes; N–no; N/A–not available

[a]Hepatic phenotypes include the observation of one or more of the following features: bile duct paucity, cholestasis, elevated liver enzymes, and cirrhosis.
[b]Skeletal phenotypes include butterfly vertebrae
[c]Cardiac phenotypes include the observation of one or more of the following features: peripheral pulmonary stenosis, atrial septal defect, ventricular septal defect, tetralogy of Fallot, and other structural congenital heart defects.
[d]Renal phenotypes include the observation of one or more of the following features: bilateral kidney obstruction, hydronephrosis, vesicoureteral reflux, renal duplication, and other structural anomalies.
[e]Family history includes family members with suggestive, clinically-confirmed, and/or molecularly-confirmed ALG

Table S3. Comparison of copy-number/structural variants identified in Probands 8 and 14 using CNVnator, ERDS, and Manta

Patient	Actual Breakpoints	Actual Length	SV Type	CNVnator Breakpoints	ERDS Breakpoints	Manta Breakpoints
Proband 8	chr20:10628528-10651408	22.9 kb	Deletion	chr20:10628201-10651400 (23.2kb)	-	chr20:10628561-10670875 (42314)
	ch20:10661832-10670875	9 kb	Deletion	chr20:10661801-10670900 (9.1kb)	chr20:10661833-10670875 (9kb)	chr20:10690719/chr20:10661832 (Breakend); chr20:10651409/chr20:10694980 (Breakend)
	chr20:10690719-10694980	4.3 kb	Deletion	chr20:10690801-10695000 (4.2kb)	chr20:10690782-10694979 (4.2kb)	
	chr20:10651408-10661832	10.4 kb	Inversion	-	-	
Proband 14	ch20:10648536-10649454	918 bp	Deletion	chr20:10648501-10650800 (2.3kb)	-	chr20:10648532/chr20:10649700 (Breakend); chr20:10649455/ chr20:10650867 (Breakend)
	chr20:10649700-10650866	1.2 kb	Deletion			
	chr20:10649454-10649700	246 bp	Inversion	-		

Abbreviations: SV–structural variant
Reference genome hg38