AJHG, Volume *84*

Supplemental Data

Mutations in *STIL*, Encoding a Pericentriolar and Centrosomal Protein, Cause Primary Microcephaly

Arun Kumar, Satish C. Girimaji, Mahesh R. Duvvari, and Susan H. Blanton

Figure S1. Haplotype analysis of families IIS-17 and IIS-28. The regions of homozygosity in affected individuals are boxed. The disease haplotype is boxed in normal individuals. D1S2134, which showed homozygosity in all affected individuals (V-1, V-2 and V-3) and heterozygosity in both parents (III-1 and IV-1) from family IIS-17 during a genome-wide screen, is marked in bold.

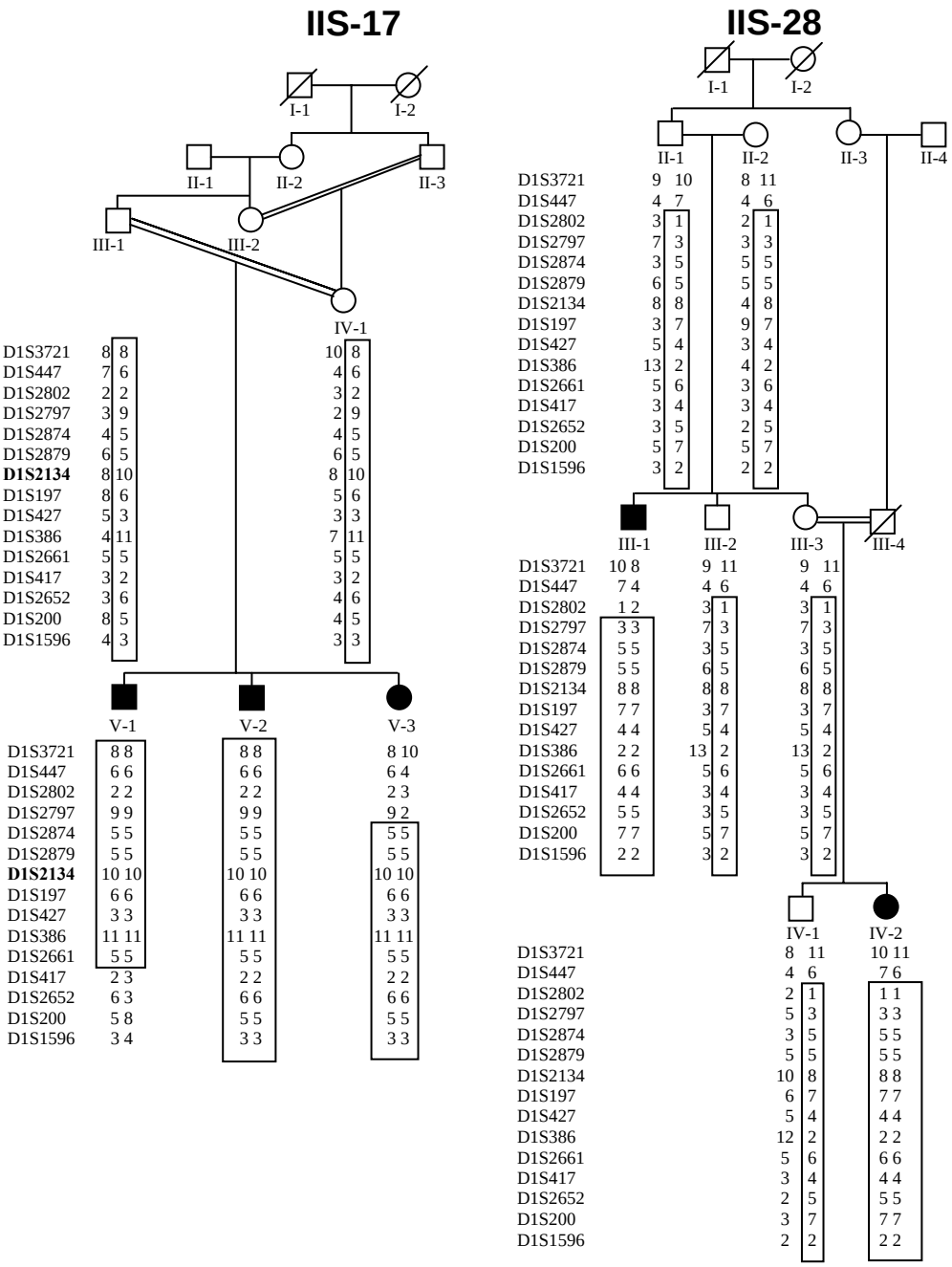

Figure S2. Haplotype analysis of families IIS-3, IIS-35 and IIS-40. The regions of homozygosity in affected individuals are boxed. The disease haplotype is boxed in normal individuals.

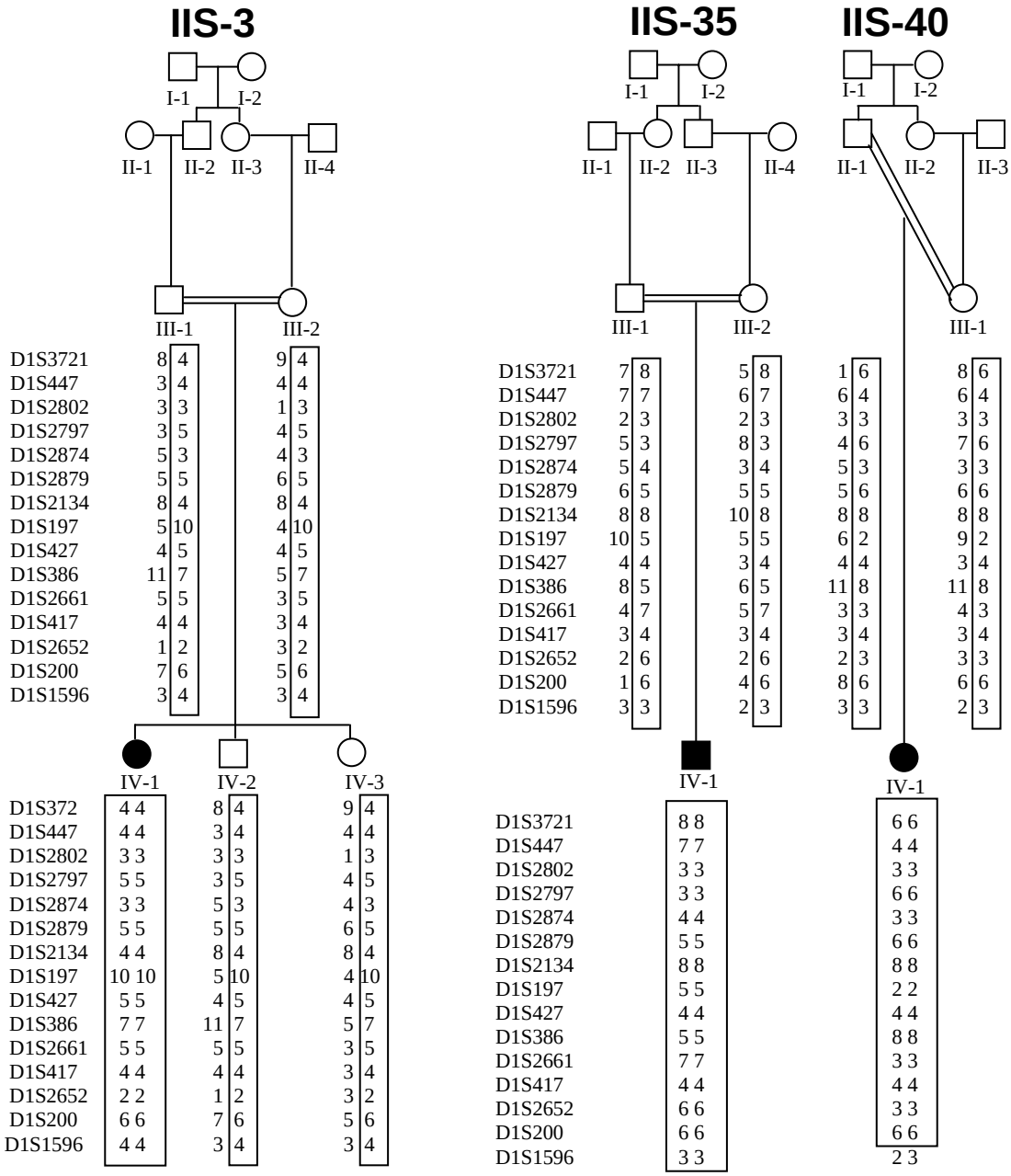

Figure S3. Combined multi-point analysis of five MCPH7 families. A maximum combined multi-point LOD score of 6.97 is obtained for the region flanked by D1S2797 and D1S417.

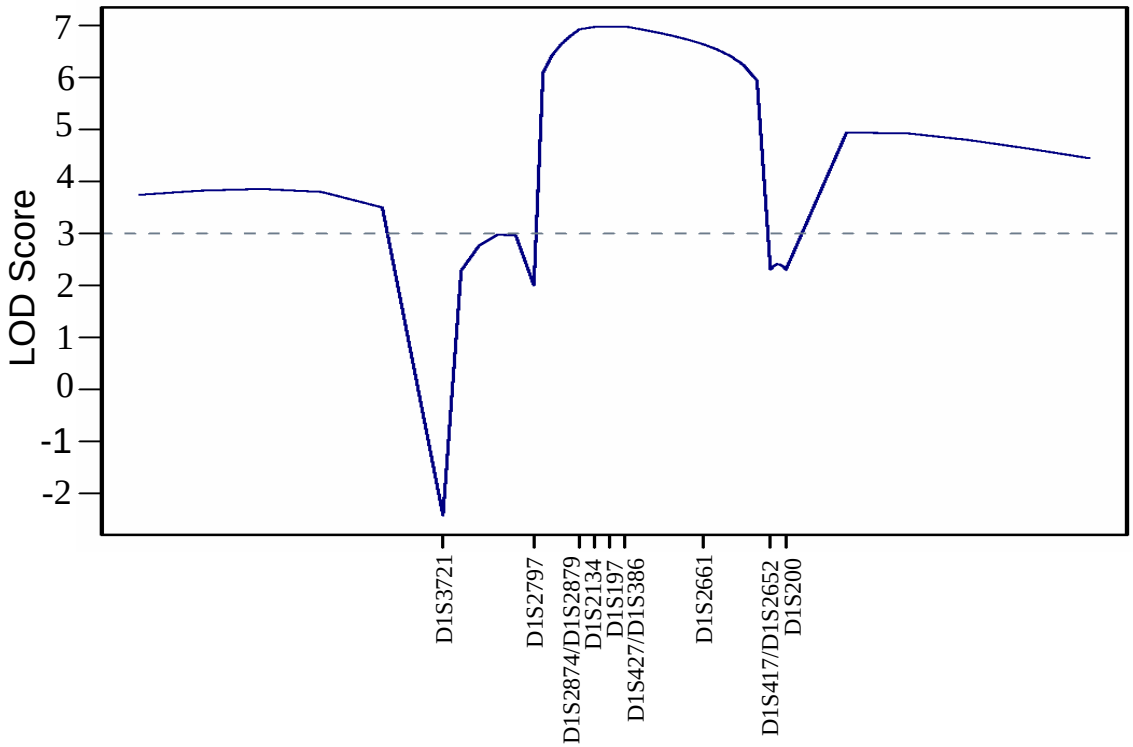

Figure S4. Sequence conservation of donor splice site residues gt in intron 16 across species and the effect of the splice mutation in family IIS-3. A) Note conservation of donor splice site dinucleotide residues gt in different species (boxed). The changed nucleotide residue g in family IIS-3 is marked by an arrow. B) Note that the G>A change results in the introduction of a premature stop codon tag in intron 16. The exonic and intronic sequences are in capital and small letters, respectively. Codon numbers are shown.

A

B

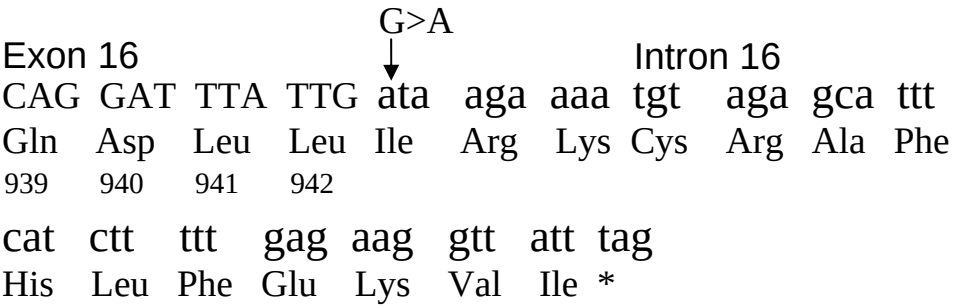

Figure S5. Genotyping of families IIS-28 and IIS-45 with markers flanking the *STIL* gene. The disease haplotypes (boxed) are different in both families, suggesting that the mutation c.3655delG has independent origins in these families.

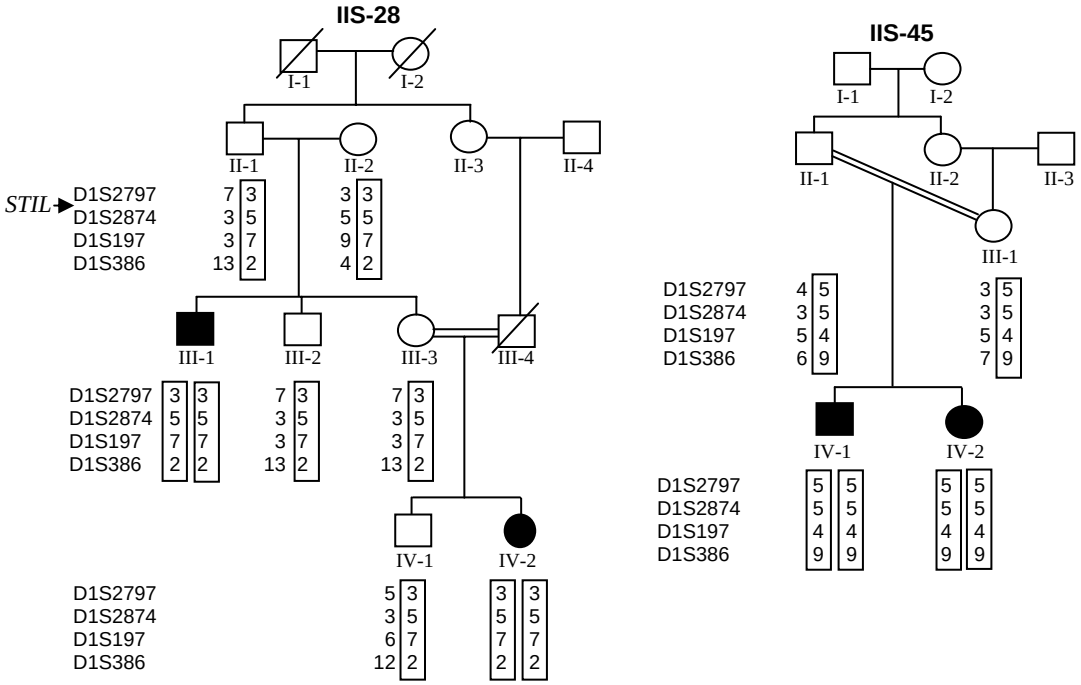

Figure S6. RT-PCR analysis of the *STIL* gene expression in different human fetal tissues. The *STIL* gene was amplified by the following primers: forward, 5'-cttaagcaactaagaagccttggag-3', and reverse, 5'-gcctccatgctcaaatccacacca-3'. *GAPDH* expression was used as a control. RNA from tissues was isolated using the Tri REAGENT™ (Sigma-Aldrich, USA). The 1st-strand cDNA template from RNA samples was synthesized using the Revertaid™ H Minus First Strand cDNA Synthesis kit (Fermentas, USA). Fetal tissue samples were collected at the Kempegowda Institute of Medical Sciences, Bangalore following approval from the Institute's ethical committee and with the consent of the mother. *STIL* is clearly ubiquitously expressed.

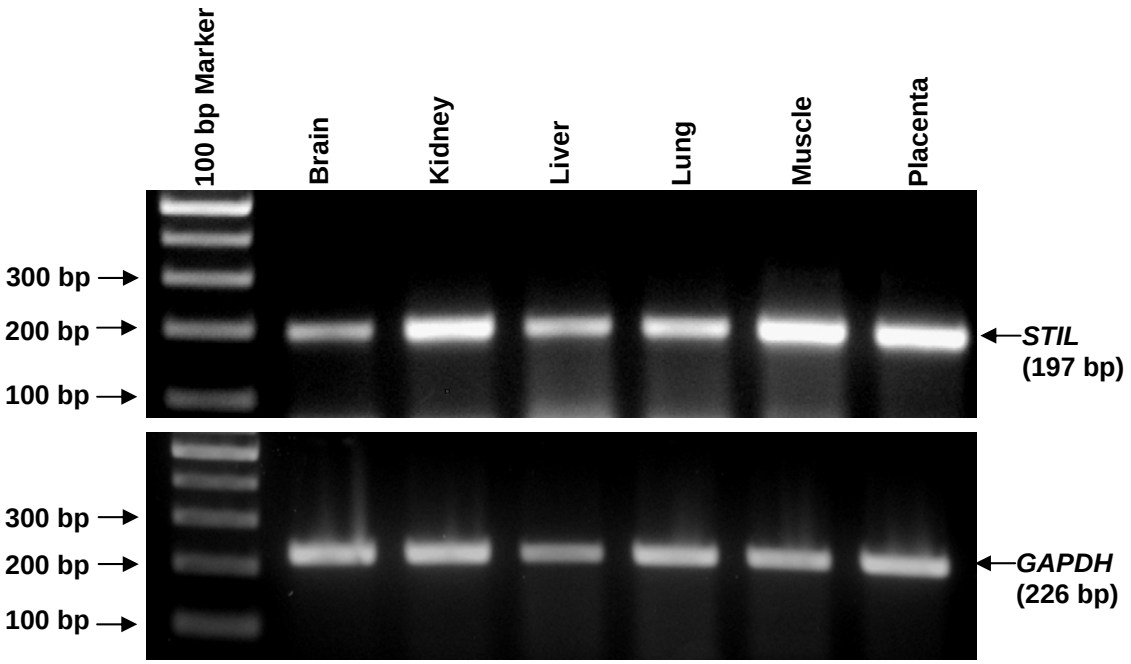

Table S1. Microsatellite markers from the MCPH7 candidate region.

Sl.#	Marker[a]	Genetic distance (cM)[b]
1	D1S3721	72.59
2	D1S447	73.81
3	D1S2802	75.66
4	D1S2797	75.66
5	D1S2874	75.66
6	D1S2879	75.66
7	D1S2134	75.66
8	D1S197	76.27
9	D1S427	76.27
10	D1S386	77.18
11	D1S2661	78.25
12	D1S417	79.80
13	D1S2652	80.77
14	D1S200	82.41
15	D1S1596	89.49

[a] The order of markers is according to the UCSC Genome Bioinformatics site. Markers were either purchased from Research Genetics (USA) or synthesized by Sigma-Aldrich (India). Genotyping was carried out as described in Kumar et al.[3]. Total genomic DNA from blood samples was isolated using a Wizard™ Genomic DNA Purification kit (Promega, USA).

[b]Genetic distances were obtained from the Marshfield Medical Research Foundation web site for linkage maps.

Table S2. PCR primers used for mutation analysis of the *STIL** gene.

Exon	Primer sequence (5'→3')	T_m	Amplicon size (bp)
3	F:ttggcagaatgactctaacgtatga R:taccttcccaagttatgtgaaaagc	58	329
4	F:cctaccaaatgtctaccaagtttgc R:aaattttgcctgaggggagattcta	65	311
5	F:ccctgcctttgaccctgggatttc R:ggattacaggcgtgagcactggtc	65	304
6	F:gctataatgtagtcttgcttggttga R:ctaaacatagtttgattcttgtgttga	58	324
7	F:actttggcgatatgggcacatttca R:ggacaattctttcacattacatggac	65	390
8	F:gtgtgcaagacaacgttcatttgaa R:cctgacaaccacttatctttgtgg	58	338
9	F:gtatggctgataaaagccatacgtc R:taaactatgaatgggggaaaggatc	58	253
10	F:catgaagttatttgagtgtcatcatg R:gttgggtttaaactgccaaagtgca	65	289
11	F:ccatcatctagtgtttcagagctac R:cttcctagtgaatatgaaggaatgca	58	370
12	F:gggtagtttattaaggctggctgg R:gtgttttaaccatgaagagacttagg	58	385
13	aF:ggtctgaggaaagtaaagtatacag aR:gagatggcccattatgagaagatg	58	418
13	bF:actgcaaagtaagacagccacctgc bR:ttcagactggacatcttgaatggatc	58	401
13	cF:catagtcatattcaatatagtccgcta cR:tgaggagaggtgccagtttttaaga	58	465
14	F:gttttgttgattacttccctgtcag R:ctactgcaccatgcaccaatttgaa	65	317
15	F:attcttaacaaaggtttggcagagta R:ctgtaaacatgcatctaaacattgtg	58	424
16	F:atacaaccaggtgctgcttgagtc R:cttaaggtctcaatcagtttaaaccta	58	420
17	F:gaaccaccacacccagccctaac R:ttctgatgagattgccacaatacatc	58	443
18	aF:ggaacacattgagaactatgtattac aR:attcactcttgctatctgcattgtc	58	443
18	bF:tatggactcctacaaagcagtgaca bR:taagtatcttcaggagacaccctg	58	545

Abbreviations: F, forward primer; R, reverse primer; bp, base pairs; and T_m, annealing temperature.

*For mutation analysis of this gene (GenBank accession no. NM_003035), entire coding exons and intron-exon junctions were amplified and sequenced on an ABIprism A370-autimated sequencer.

Table S3. PCR primers used for mutation analysis in normal controls by single-stranded conformation polymorphism analysis (SSCP).

Mutation	**Primer sequence (5'→3')**	**T_m (^oC)**	**Amplicon size (bp)**
c.3715C>T and c.3655delG	F:tgcagacaaaagcaaaacagcagttg R:tgctttagcgtttcagaaggttgca	61	184
c.IVS16+1G>A	F:atcagaggaaccaaaaattgagcatg R:ctgtaaacatgcatctaaacattgtg	58	184

Table S4. Clinical details of patients with mutations in the *STIL* gene.

Family/patient	Mutation	Age (years)	HC (SD)[a]	Development milestone	Degree of mental retardation[b]	Motor and neurological symptoms
IIS-3/ IV-1	c.IVS16+1G>A	19	47.0 (5)	All development milestones delayed	MILMR	None
IIS-17/ V-1	c.3715C>T	11.5	43.0 (7)	Independent walking at 2 years, meaningful speech at 5 years	MODMR	None
IIS-17/ V-2	c.3715C>T	5	39.3 (8)	Independent walking at 1.5 years, meaningful speech at 2 years	MILMR	None
IIS-17/ V-3	c.3715C>T	9	41.5 (8)	Independent walking at 1.5 years, meaningful speech at 4 years	MILMR	None
IIS-28/ III-1	c.3655delG	20	44 (7)	All development milestones delayed	MILMR	None
IIS-28/ IV-2	c.3655delG	8	38.5 (10)	Independent walking at 1 year, meaningful speech at 4 years	SMR	None
IIS-45/ IV-1	c.3655delG	23	49.0 (4)	Independent walking at 3 years, meaningful speech at 4 years	SMR	None
IIS-45/ IV-2	c.3655delG	14	42.5 (8)	Independent walking at 3 years,	SMR	None

				meaningful speech at 4 years		

[a] Head circumference in cm with SD below mean for age and sex in parentheses.

[b] MODMR, moderate mental retardation; MILMR, mild mental retardation; and, SMR, severe mental retardation.