SUPPLEMENTARY METHODS

Children with DVD.

All probands fulfilled the following selection criteria, originally chosen to most closely match the phenotype observed in the KE family; difficulty in speech articulation as diagnosed by a qualified clinician (e.g. paediatrician or neurologist), no evidence of mental retardation or other congenital abnormalities, normal hearing, no other diagnosed medical/genetic deficits, and a normal karyotype[1]. This panel comprised 40 singleton probands, 8 probands with 1 affected sibling, and 1 proband with 2 affected siblings, yielding a total of 59 individuals in the screening panel. All cases were Caucasian, residing in Europe, Australia or the USA. Human Random Control (HRC) DNA panels were obtained from European Collection of Cell Cultures (ECACC). Genomic DNA was isolated from blood lymphocytes using standard procedures.

Amplification and screening of FOXP1 exons.

PCR assays amplified at least 50 bp of DNA flanking each intron/exon boundary to ensure screening of the complete exon and splice-sites during subsequent SHPLC or sequencing analysis. Primers had a GC-content of 40-60%, a T_M (Melting Temperature) of 57-63ºC, and were between 18 and 25 bases in length (Table S1).

A touchdown PCR protocol was used to amplify exons in a 50 μl total volume with the following components; 30 ng template DNA, 200 nM of forward/reverse

primer, 200 μM dNTP's, 2.5 mM $MgCl_2$, with 0.9 units Amplitaq gold polymerase (Applied Biosystems) and 0.1 U of Pfu Turbo, in 1X reaction buffer. PCR Cycling conditions were as follows; 95ºC for 18 minutes, followed by 14 cycles of (95ºC for 30 seconds, 60ºC for 30 seconds with -0.5ºC per cycle, 72ºC for 45 seconds), followed by 25 cycles of (95ºC for 30 seconds, 60ºC for 30 seconds, 72ºC for 45 seconds), then at 72ºC for 7 minutes. PCR products were denatured and re-hybridized immediately prior to DHPLC analysis, under the following conditions; 95ºC for 4 minutes, followed by 42 cycles of 95ºC for 1 minute reducing the temperature by 1.6ºC per cycle.

Fragments were analyzed using the Transgenomics WAVE® DHPLC system utilizing the DNASep cartridge, at optimal temperatures for mutation detection, as predicted by the WAVEMAKER™ software package (Transgenomics, UK) (Table S1). Any fragments showing aberrant elution patterns during DHPLC were sequenced directly to confirm and identify variants via BigDye chemistry on the ABI3700 automated capillary sequencer, using the PCR primers (Table S1). Exon 1 was analyzed for the presence of mutations by direct sequencing instead of DHPLC, due to the high GC content of the sequence.

SUPPLEMENTARY REFERENCES

1 MacDermot KD, Bonora E, Sykes N *et al*: Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. *Am J Hum Genet* 2005; **76**: 1074-1080.

Supplementary Table 1 - *FOXP1* DHPLC screening primers.

Exon	Forward/Reverse Primers (5'→3')	Product Size (bp)	DHPLC Temperatures
1	CTTGGAAATCCTTGTATCAGGT/ GCGAGATCGCGATTAAGTGT	293	Sequenced directly
2	CGCACTTCCTGGAATCCTTT/ TGCTCAACACAATCCACTCC	267	56ºC, 58 ºC, 60.5 ºC
3	CTGCGTGCTTCTGATTTCCT/ CTGGGTTCTGGGGGAGAC	236	60ºC, 62 ºC,
4	TTGTGTATGGCACCAAAAGG/ TGAAAGCTGAGAACCGATAGAG	290	57ºC, 57.5 ºC, 59 ºC
5	TGGTGAGTTTTGAAGTGTCCA/ TCCATCATTATCCCACTCCA	380	55ºC, 58 ºC, 61 ºC
6	CGTAGTTGGGAGGGGAAAA/ TGCACATTCAAGTCACATGG	467	56.5ºC, 59.8 ºC
7	GGCTCCTCCTGCCTTTTT/ GGGTGAGGTGAAACTCTCCAT	297	56.5ºC, 58.5 ºC, 60ºC
8	CTAGACCCGCTGCCTAGTTT/ GGTTTTGGACCTTCCATTCA	300	49.5ºC, 54.5 ºC, 55.5 ºC, 57.5ºC
9	TGGTGCCATAGCGTAATTTG/ AGTAGGCTGGTCCTCCTTCC	276	55ºC, 58.5ºC, 61.5ºC
10-11	CCACGCATCCTCTGTGTTAC/ CAGCATGCTTGCATACTAAACG	585	53.5ºC, 55 ºC, 58ºC, 60ºC, 62.5ºC
12	CGAGAAACTGTGGAATGACG/ GCTTCCTTATAGCACAACTGCAT	261	54ºC, 55.5 ºC, 57.5ºC
13	TGCTTTTGGAAAAGACATCC/ AAACAGGAGGGATGAAATGC	445	57ºC, 58ºC
14	GCACCAGCAAGCTTAAAACAAAA/ GGGAGTATGATGCTTTGTGC	300	53.5ºC, 57 ºC, 60.5 ºC
15	TGCCAGCCATGCTACAATTA/ AGCCCAGAGAAAGGGCTGT	367	56ºC, 57.7 ºC, 61 ºC
16	TCTGTTGCCCCAAACTTTTC/ AAACGTAGTGAAAATCCTCCAGAC	352	56ºC, 60.5 ºC