Supplementary Material

***Early B-cell factor 3*-related genetic disease can mimic urofacial syndrome**

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Supplementary Methods

The parents of the proband provided consent for familial genetic testing and for identifiable photographs to be used in this report. DNA from index cases with familial primary non-syndromic VUR were sourced from the UK VUR DNA Bank.[S2] DNA from the proband was sent to the Beijing Genomics Institution (BGI) in China where whole exome sequencing (WES) sequencing was performed using a BGI exome kit, version 4 (59M) 6G BGI-Seq500. Exome read data were aligned to the genomic reference sequence (hg19) by our in-house bioinformatics team (Genetic Medicine, St Mary's Hospital, Manchester, UK). Analysis and annotation of genome data was performed in-house using VarSeq™ v2.2 (Golden Helix, Inc., Bozeman, MT). During analysis, variants were filtered as depicted in Figure S1. For Sanger sequencing PCR primers were all designed and optimised in house. Reactions were carried out using the Veriti 96-Well Thermal Cycler (Applied Biosystems) and PCR products were purified and sequenced using an automated Applied Biosystems 3730 XL DNA Analyzer. Results were analysed using the Staden Software package. Sequencing files for patient, parent and control DNA were added to databases created using PreGap4 software, with control DNA processed as the reference sequence. Visualisation of aligned traces allowed for comparative analysis between patient and control or patient and parent sequences. Eight *in silico* prediction tools were used to interrogate variant pathogenicity: Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/); Mutation taster (http://www.mutationtaster.org/); MutationAssessor (http://mutationassessor.org/r3/); SIFT (Sorting Intolerant From Tolerant) (http://sift.bii.a-star.edu.sg/); FATHMM (Functional Analysis Through Hidden Markov Models) (http://fathmm.biocompute.org.uk/inherited.html); FATHMM-MKL (Math Kernel Library) (http://fathmm.biocompute.org.uk/fathmmMKL.htm); CADD (GRCh37-v1.4)[S19]; and REVEL.[S20]

Supplementary Tables

Table S1. *In silico* tools interrogating the possible pathogenicity of the *EBF3* variant c.626G>A found in the index case.

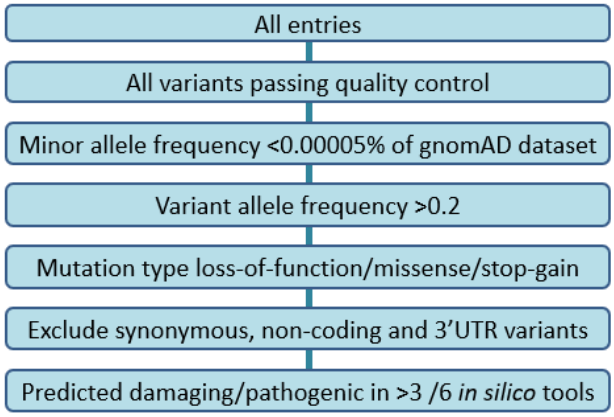
***In silico* tool**	**Prediction**
SIFT	Damaging
PolyPhen2	Probably damaging
Mutation Taster	Disease Causing
Mutation Assessor	Predicted functional
FATHMM	Tolerated
FATHMM-MKL	Damaging
CADD	35
REVEL	0.724

Table S2. Variants detected in *EBF3* sequencing of 80 index cases of the UK VUR cohort. RSID is the reference SNP cluster ID. None of the variants are predicted to be pathogenic using *in silico* tools.

Index cases (n)	**cDNA and *protein* coding variants**	***EBF3* Exon or intron**	**RSID**	**gnomAD allele count/number**	**gnomAD allele Frequency**
5	c.229A>C *p.(Arg77Arg)*	Exon 2	rs75074888	16766/265108	0.0632
1	c.639T>G *p.(Val213Val)*	Exon 8	rs141973685	149/234652	0.000635
1	c.134+40_134+45dup	Intron 1	rs767692640	215/55380	0.003882

Supplementary Figures

Figure S1. Filtering strategy used for whole exome sequencing data analysis. Resulting variants were considered based on their association with clinical data.



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