Supplementary data:

A novel missense mutation in collagenous domain of *EDA* gene in a Chinese family with X-linked hypohidrotic ectodermal dysplasia

Daxu Li, Ran Xu, Fumeng Huang, Biyuan Wang, Yu Tao, Zijian Jiang, Hairui Li, Jianfeng Yao, Peng Xu, Xiaokang Wu, Le Ren, Rui Zhang, John R. Kelsoe and Jie Ma

J. Genet. **94**, 115–119

Table 1. Primers used for amplification and sequencing of *EDA*.

Exon	Physical location[a]	Primer sequence		Annealing temperature (°C)
		Forward	Reverse	
1–1	68,835,911	5′-CGGGACCTCCTCCTTCAT	5′- CGCAACTCTAGGTAGCAGCAC	60
1–2	68,835,911	5′-GCCTCAAGAGAGTGGGTGTC	5′-CCTCTAAATTGGCTCGAGGTT	59
2	69,176,877	5′-TACAGTGGAGGGGAAGATGG	5′- GGCTGGTTTTGAATTCCTCA	59
3	69,243,068	5′-ATGGGGAGGTTTGATAGTTGG	5′-CAAGGAAGAATGAAAGAGGTGAA	59
4	69,247,707	5′- AGATCGTGCCACTGAACTCC	5′- TCTGCATCCCTTATTGCACA	60
5	69,249,354	5′- TGACTCTTCCTCCAGCTCTGA	5′- GGAGGCTCAGGTGAATAGGTC	60
6	69,250,319	5′-CTGAGCAAGCAGCCATTACT	5′- AGAATGCCTGGGCTAGATGAT	61
7	69,253,248	5′- CAGAACAGCTTCTCTGCTTTCA	5′- AGTTAGCCATTGGATGGTCTTG	59
8	69,255,207	5′-AAGAACAATGCCTGTCACCTG	5′- TCCACAGCAGCACTTAGAGGT	60

[a]UCSC browser, Feb. 2009; http://genome.ucsc.edu/cgi-bin/hgGateway.

Table 2. Prediction of the mutation effect.

EDA domain	Exon	Protein	PolyPhen-2		Reference
			Prediction	Score	
19 Gly-X-Y repeats (collagenous domain)	5	p.G189E	Probably damaging	1.000	Schneider *et al.* (2001)
		p.G195E	Probably damaging	1.000	Zhao *et al.* (2008)
		p.G198A	Probably damaging	1.000	Vincent *et al.* (2001)
		p.G207R	Probably damaging	1.000	Schneider *et al.* (2001)
		p.P209L	Probably damaging	1.000	Monreal *et al.* (1998)
		p.G218D	Probably damaging	1.000	Schneider *et al.* (2001)
		p.P220L[a]	Probably damaging	1.000	
		p.G224A	Probably damaging	1.000	Monreal *et al.* (1998)
		p.Q232X[b]			Clauss *et al.* (2010)

[a]Current study.
[b]The mutation creates a stop codon in exon 4 and cannot be analysed by Poly Phen-2 prediction program.

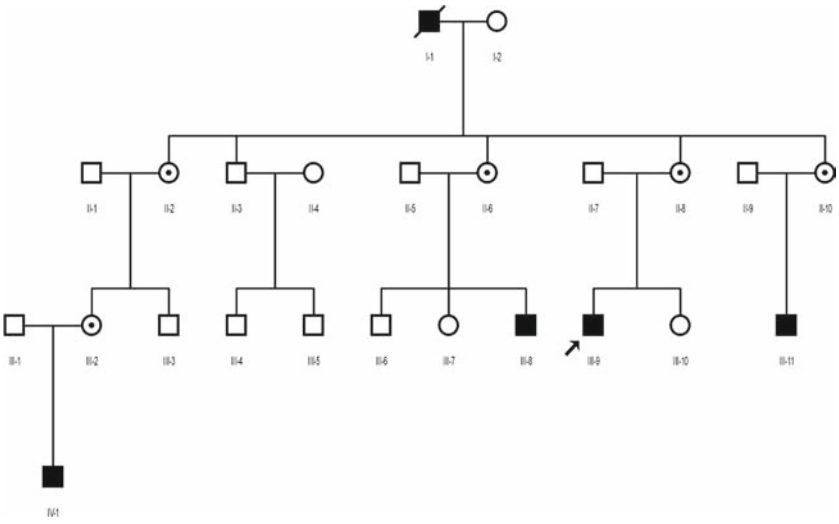

Figure 1. The Chinese family affected by XLHED. Shaded boxes represent affected individuals. Squares and circles symbolize male and female, respectively. Circles with a dark spot symbolize female carriers. The proband is indicated by an arrow (III-9).

Figure 2. The multiple alignments of the amino acid sequences of the human EDA using ClustalW (http://www.ebi.ac.uk/clustalw). The Proline 220 of EDA is highly conserved in 15 vertebrate species at this position. The arrow indicates the Proline residue 220. The p.P220L mutation of EDA responsible for XLHED affects the highly evolution conserved Proline residue 220. The multiple alignments of 19 repeats of Gly-x-y are all highly conserved in 14 species (rabbit, chick, pig, sheep, dog, cow, dolphin, rat, mouse, baboon, macaque, gorilla, chimpanzee and human).