

1 **Supplementary Files**

2 **Methods**

3 *Laboratory examination*

4 Serum tests for pituitary-related hormones, thyroid and parathyroid hormones, blood
5 glucose, insulin, C-peptide, and bone metabolism-related tests were accomplished in
6 the proband, as well as the related gland function evaluation test. These examinations
7 were performed by the professional Laboratory Medicine Staff in the Medical
8 Laboratory Center, the First Hospital of China Medical University. Pituitary-related
9 hormones levels were measured using IMMULITE2000XPi (SIEMENS AG FWB :
10 SIE, NYSE : SI), thyroid related hormones levels were measured using Cobas 601
11 analyzer (Roche Diagnostic, Switzerland). Blood glucose, insulin, C-peptide were
12 measured using maglumi4000 (Shenzhen New Industries Biomedical Engineering Co.,
13 Ltd, China). Bone metabolism related hormones levels were measured using
14 cobas®8000 e 801 (Roche Diagnostic, Switzerland).

15 *Imaging examination*

16 For a diagnosis of MEN1, the proband completed several imaging examinations,
17 including an ultrasound, CT (computed tomography), MRI (magnetic resonance
18 imaging), endoscopic ultrasound (EUS), and parathyroid ECT (emission computed
19 tomography).

20 *Sanger sequencing*

21 A 5-ml sample of peripheral blood was collected from the proband and each of her
22 six relatives mentioned, anticoagulated with ethylenediaminetetraacetic acid (EDTA),

23 and stored at -80°C . Later, whole-genome DNA was extracted. Sanger sequencing was
24 performed on the peripheral blood samples of the six family members using an ABI
25 3730 sequencer to verify the co-segregation of genotype and phenotype. The DNA
26 sequence of *MEN1* (NM_130802) was queried in the Ensembl Genome Browser, and
27 Primer Premier 6.0 was used to design primers for verification, the gene sequence
28 corresponding to the primer are as follows (Supplementary Fig. 2):

29 1st times detection: GGGTGG AACCTTAGCGG, AGATGAAATTGGGCTGCA.

30 2nd times detection: GGGTGG AACCTTAGCGG, CCTCTTTGCAGTTGGGAAAC.

31 3rd times detection: GACCTGGTGCTCCTTTCCTT, TGTCTATCATCGCCGCCC.

32 ***Multiplex ligation-dependent probe amplification (MLPA)***

33 DNA (100 ng) was denatured by heating to 98°C in a thermocycler. The multiplex
34 ligation-dependent probe amplification (MLPA) probes (probeset name: P017-D1
35 MEN1, version number: Lot D1-0716, MRC Holland) and buffer were added, and then
36 the samples were left overnight at 60°C for hybridization. The ligase and ligase buffer
37 were added, and the samples were ligated at 54°C for 15 min. The ligase was
38 inactivated by heating to 98°C . PCR primers, dNTPs, and polymerase were added, and
39 the PCR was started. The products were analyzed by capillary electrophoresis.

40 Coffalyser.Net Server (versions: v.140721.1958) was used for the analysis of
41 sequencing and MLPA reads.

42

43 **Table 1. The laboratory examination results of hypoglycemia occurred.**

Items	Results	Normal range
Hypoglycemia occurred for the first time		
Intravenous glucose (mmol/l)	2.63	3.89-6.38
Synchronous insulin (mIU/L)	25.86	4.03-23.46
Synchronous c-peptide (pmol/L)	2052.1	99.9-1242.09
Synchronous proinsulin (pg/mL)	253.10	30-180
Insulin release index	0.55	<0.3
Hypoglycemia occurred for the second time		
Intravenous glucose (mmol/l)	1.72	3.89-6.38
Synchronous insulin (mIU/L)	16.21	4.03-23.46
Synchronous c-peptide (pmol/L)	1994	99.9-1242.09
Synchronous proinsulin (pg/mL)	264.1	30-180
Insulin release index	0.52	<0.3

44 Table 1. The proband's serum glucose, insulin (INI), C-peptide (CPS), and pro-insulin
 45 (pro-INS) levels were tested when hypoglycemia occurred. The insulin release indexes
 46 were 0.55 and 0.52 (normal range <0.3) during the twice hypoglycemia, respectively.
 47 Glucose was decreased while the insulin level was either in the normal reference range
 48 or increased at the time of the hypoglycemia.

49

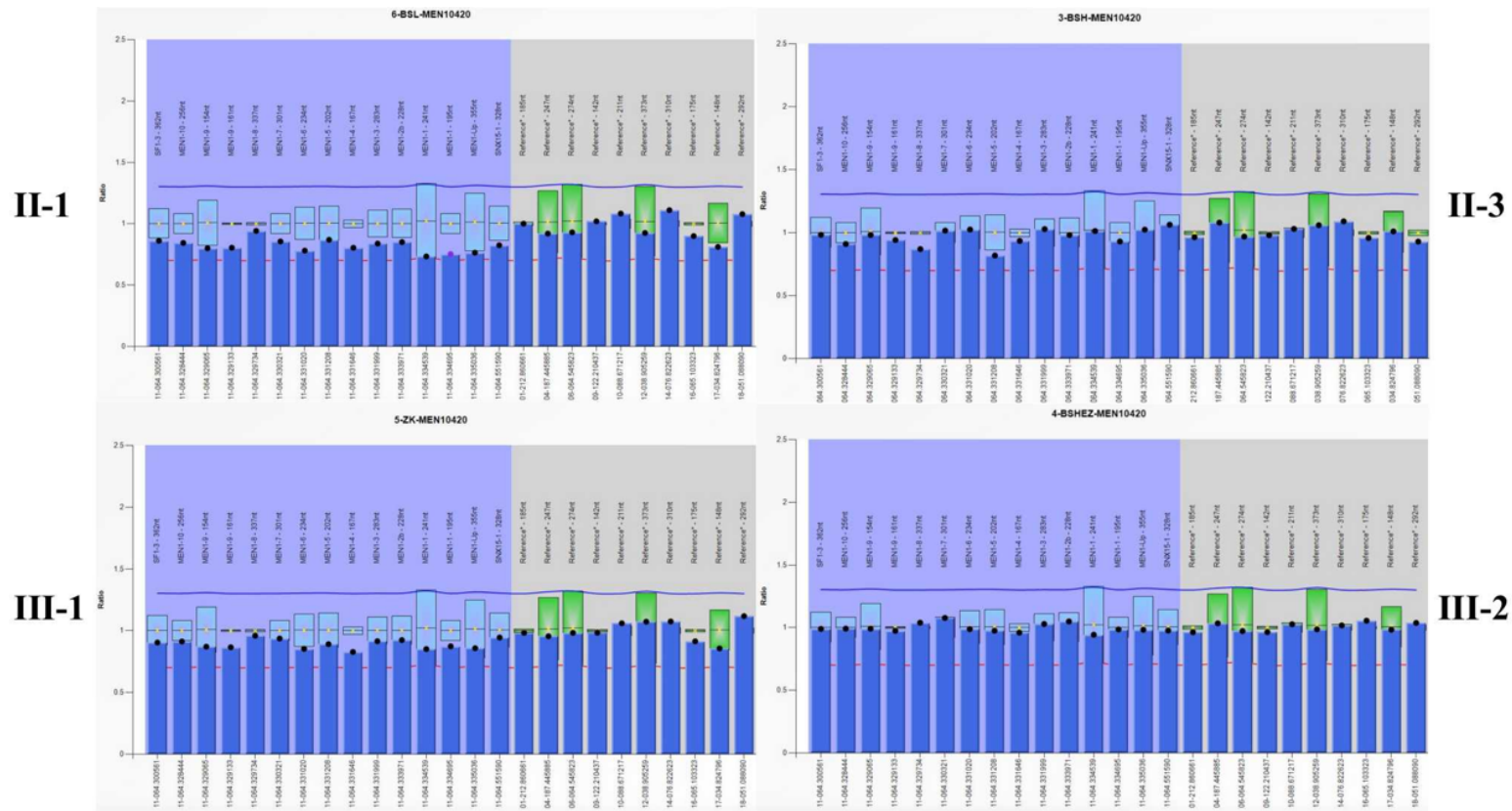
50 **Table 2. The results of prolonged OGTT.**

	Glucose (mmol/l)	Insulin (mIU/L) (4.03-23.46)	C-peptide (pmol/L) (99.9-1242.09)
0 min	5.16	15.45	1480.8
30 min	10.49	231.3	10060.5
60 min	13.11	217.2	10294.6
120 min	6.57	41.31	7194.8
180 min	3.72	21.52	3098.6
240 min	2.01	17.54	2501
300 min	1.95	15.2	1855

51 Table 2. The prolonged oral glucose tolerance test (OGTT) results presented
 52 hypoglycemia at the 240th and 300th minute with glucose levels of 2.01 mmol/L and
 53 1.95 mmol/L, respectively. The corresponding insulin release indexes were 0.48 and
 54 0.43 (normal range <0.3), respectively.

55 **Figure 1. Multiplex ligation-dependent probe amplification (MLPA) results.**

56 MLPA results showed that the *MEN1* gene was not deleted in the proband (II-3), the proband's sister (II-1), the proband's niece (III-1), and the
 57 proband's son (III-2).



59 **Figure 2. Schematic diagram of the gene sequence corresponding to the primers of the three times genetic detection.**

