Supporting Information

Detailed description of patients in Figure 1

The mother of **family 1** (Fig. 1A, Fig. 3 A II-1) came to genetic counselling with her youngest daughter (Fig 1 B-E, Fig. 3 A 111-2), aged 12 years after the clinical diagnosis of OFD had been suggested. The mother had had two miscarriages in the 10th and 19th weeks of pregnancy, respectively, the second pregnancy having been a twin pregnancy, before the birth of her two affected daughters. Chromosome analysis in herself and her husband revealed normal karyotypes. Oral frenulae and a "very small chin" had been surgically corrected soon after birth, and an extra set of teeth in both her upper and lower jaws had been removed. She had unilateral kidney failure after the birth of her second daughter, but she was otherwise healthy. Her first daughter (Fig. 3A III-1) had delayed motor development after birth and left-sided hemiplegia, which responded to intensive physiotherapy. She had cutaneous syndactyly of the 4th and 5th fingers of the left hand and an extra oral frenulum. A cranial MRI at age 18 years (Fig. 2A and B) showed asymmetrically widened posterior lateral ventricles and corpus callosum agenesis. The second daughter (Fig. 1B to E, Fig. 3A III-2) presented with an abnormal head shape (caput quadratum), mid-face hypoplasia (Fig. 1B), suspected cleft lip and palate, a bifid tongue (Fig. 1C), tongue frenulae, a high-arched palate, a split alveolar ridge (Fig. 1D), short neck, syndactyly of the 4th and 5th fingers of the right hand, and polysyndactyly of the first digit of the left foot (Fig. 1E). She has global developmental delay, she walked at age 2 years, talked at age 3,5 years and attends a school for children with special learning needs. Ultrasound of the head revealed corpus callosum agenesis, but no other abnormalities. As a child she had unexplained recurrent fevers which were diagnosed as Kawasaki syndrome. Mutation analysis in the family revealed the mutation c.13-10T>A in intron 1 which affects the splicing of exon 2. Haplotype analysis in the family revealed that the mutation had arisen on the mother's paternal allele.

The daughter in **Family 6** (Fig. 1G-I, K-L, Fig. 3BII-1) was diagnosed clinically with OFDS at birth, however genetic testing was first performed at age 28 years. In addition to common facial, oral and skeletal features of OFD1, she had cystic kidney disease diagnosed in adulthood and onset of epilepsy at age 27 years. Brain MRI had been performed and was reported as normal,

the patient was intelligent and had achieved a university degree. Mutation analysis revealed the mutation c.148insG (p.His50Alafs*2) in exon 3. Her mother (Fig. 1F and J, Fig. 3B I-1), who also carried the mutation, was relatively mildly affected with slight facial dysmorphism, syndactyly and brachydactyly (Fig. 1J), other than having cystic kidneys. Interestingly, her father (maternal grandfather of the index patient) was also reported to have cystic kidneys, suggesting possible mosaicism for the family's mutation. He was not available for testing.

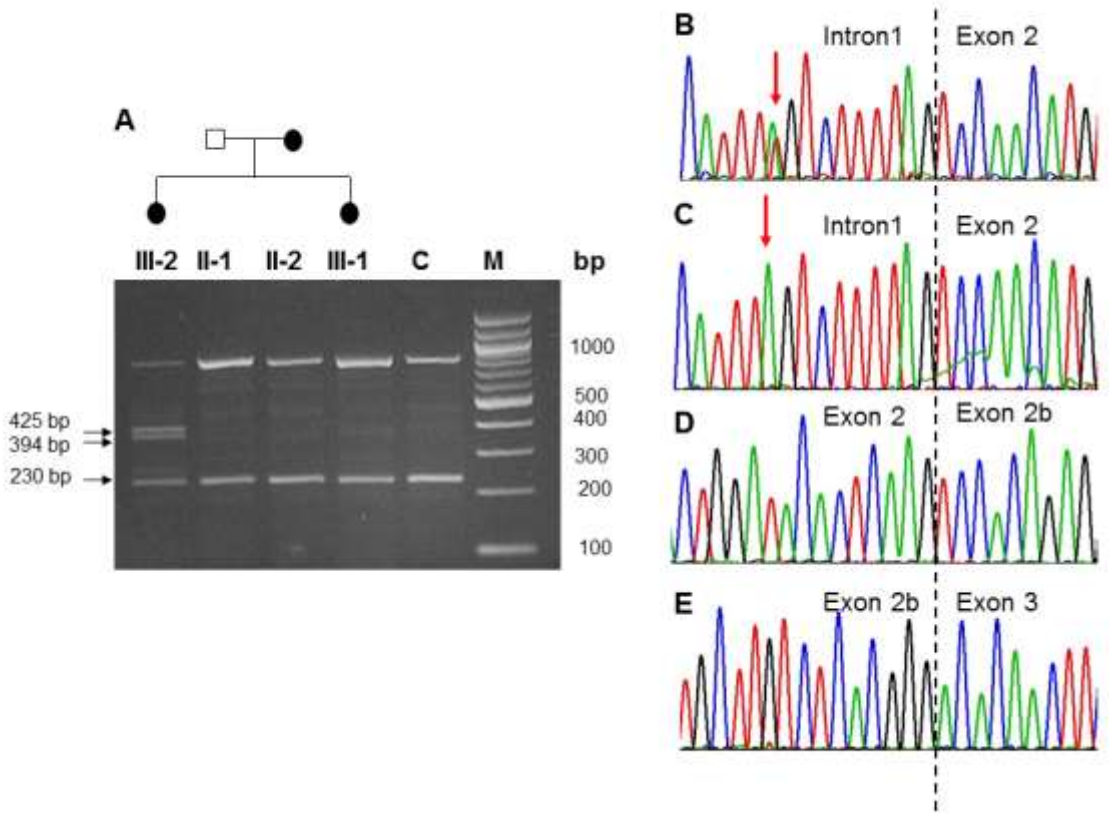
In **Family 9** both mother and daughter are very mildly affected with OFD1. The mother (Fig. 1M) was diagnosed during childhood based on the presence of tongue hamartomas, a short lingual frenulum, a cleft palate, extra teeth, small milia on the ears and sparse hair. She had no learning disabilities or seizures and brain MRI was not performed. Ultrasound of the kidneys was performed at age 29 years and was normal. The daughter showed no abnormalities at age one year (Fig. 1M and N) other than a somewhat lobulated tongue. Both mother and daughter carry the only missense mutation that we found: c.422T>G, p.Met141Arg.

In **Family 10** the mother (Fig. 1O and Q, Fig. 3C I-1) had had six miscarriages before the birth of two daughters. Cystic kidneys were detected incidentally at age of 20 years. She had very mild facial features of OFD, her jaw had been operated on as a child because of a very small chin, she had extra teeth and subtle brachydactyly (Fig. 1Q). Her mother (maternal grandmother of the index patient) had no features suggestive of OFD, however a maternal aunt had a cleft palate and had intellectual disability. Her first daughter is not affected with OFD and does not carry the mutation. The second daughter (Fig. 1 P to S) had clear signs of OFD1 at birth, with a typical facies, medial cleft lip and palate, tongue hamartomas, brachydactyly, proximal syndactyly and corpus callosum agenesis which was observed on ultrasound. Mutation analysis of *OFD1* was performed after the birth of this child and the frameshift mutation c.506_507delGA (p.D170Ffs*3) was detected in her and her mother.

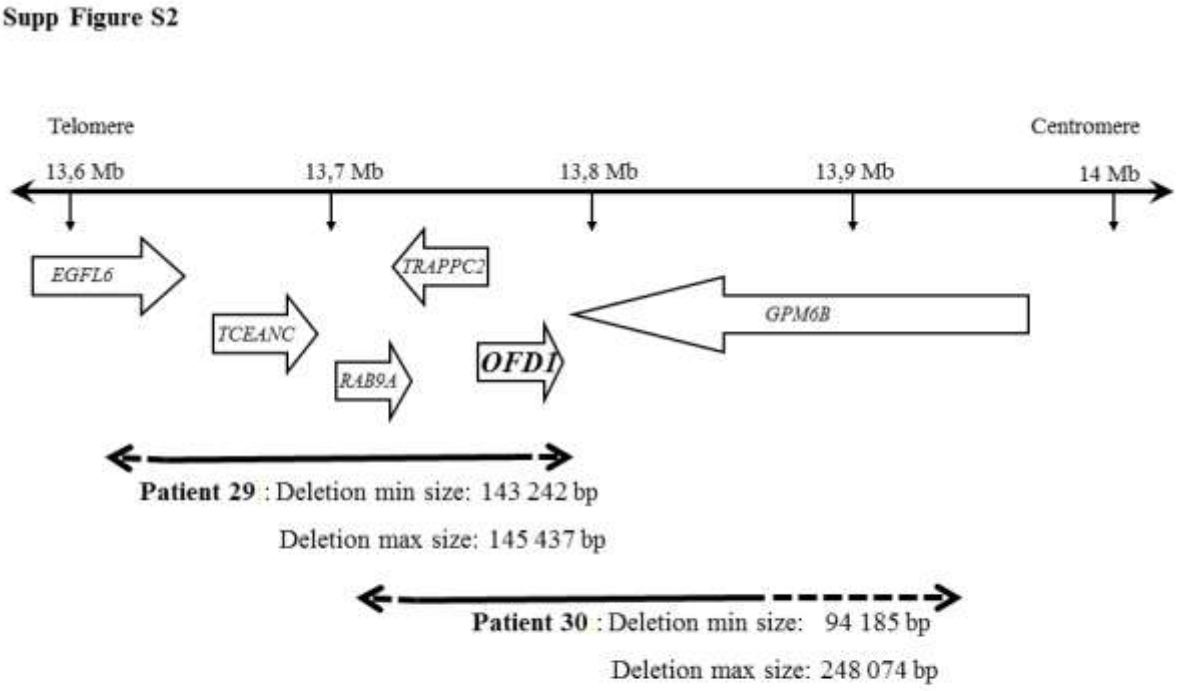
Patient 29 (Fig. 1T to Z) was 30 years old at the time of molecular genetic diagnosis. She was the younger child of young (21 and 23 years old at the time of her birth) healthy, unrelated parents and had a healthy older sister . She was born by spontaneous vaginal delivery at 39 weeks with a birth weight of 4200 g, length 52 cm and occipitofrontal head circumference (OFC) of 35 cm. She had a cleft palate and was noted to be dysmorphic. The patient was re-evaluated at age 26 months because of hydrocephalus (OFC was 52 cm, 1,2 cm > 97th percentile) and significantly delayed psychomotor development (developmental age equivalent to 16 months) and the clinical diagnosis of OFD1 was made based on the additional findings of facial dysmorphism (prominent forehead, thin hair, epicanthus, broad nasal ridge, hypertelorism, palpebral fissures, Fig. 1T), a high cleft palate (Fig. 1W), oral frenulae, multilobulated tongue with hamartomas (Fig. 1X), malpositioned teeth, bilateral skin syndactyly of fingers II-III (Fig. 1Y) and bilateral dysplastic metacarpals I-III and metatarsals II-III (Fig. 1Z). Cytogenetic karyotype was normal. She has severe intellectual disability and since puberty showed an autoaggressive behavioural disturbance which manifested as biting her left index finger and hand. At age 21 years a diagnosis of chronic kidney failure due to polycystic kidneys was made and she received a kidney transplant at age 25 years.

Patient 30 was diagnosed newborn with OFD based on the findings of midface hypoplasia, hypertelorism, micrognathia, a cleft palate, bilateral broad thumbs, syndactyly of the 4th and 5th fingers of the left hand and short metacarpals. Her birth weight, birth length and head circumference (36 cm) were all within normal range. Ultrasound of the heart revealed myxomatose changes of the mitral and tricuspid valves. Cranial ultrasound revealed abnormal cortical gyration, mild dilatation of the lateral ventricles and agenesis of the corpus callosum. Molecular genetic testing confirmed the clinical diagnosis of OFD1 at 5 months with the finding of the large deletion.

Supp. Figure S1



Supp. Figure S1. cDNA transcripts in family 1 (A). Genomic sequence from the index patient (daughter II-2) showing the c.13-10T>A mutation in intron 1(B), sequence of a cDNA clone from the same patient, showing the retention of intron 1 in the cDNA (C); sequence of a transcript from her healthy father showing the retention of an additional exon 2b between exons 2 and 3 (D and E).



Supp. Figure S2. Schematic representation of the two deletions found to span *OFD1* and flanking genes on Xp21 in patients 29 and 30). The deletion in Patient 29 is between 143,2 and 145,4 kb large and completely encompasses the *TCEANC*, *RAB9A* and *TRAPPC2* genes and part of *EGFL6*. The deletion in patient 30 is between 94,2 and 248 kb large and spans *RAB9A* and *TRAPPC2* and ends in the 152,7 kb large first intron of *GPM6B*.

Supp. Table S1. Primers used for real-time quantitative PCR (qPCR) of *OFD1* and flanking genes

Amplicon	**Location of forward primer (F)**	**Primers**	**Product size (bp)**	**Distance to next amplicon (bp)**
EGFL6 Ex 6	chrX:13624461	F: TTGCTATCACTGACACCTTCTGG R: CTATACAGTCATATCGTCCACTGAT	172	1 774
EGFL6 Ex7	chrX:13626406	F: GTACCACAGAGTCGTTCACTG R: CACCATGACCAAATGCTACTTAC	183	9 268
EGFL6 Ex8	chrX:13635856	F: TGAAAATTCTGTGAAGGAAGTCCTC R: GGGCTGCAAGTTCACCTTAG	160	1 280
EGFL6 Ex9	chrX:13637295	F: TGAAGCAGGTGAATTCGGCC R: GTACCAGTCACACAAGGACC	249	5 108
EGFL6 In10 (1)	chrX:13642651	F :CCCATTGCCTACTTAAACACTATTG R: GAAAGATCTTTTAACTGAGGGCTGAG	214	1 529
EGFL6 In10 (2)	chrX:13644393	F: GAAGACGACCCATTCCCCAATG R: GTGTAGTGGACTTTTGCTCATATC	152	614
EGFL6 Ex11	chrX:13645158	F: CTTGGCAGGTCACAAGAAAGAC R: CAACTGAATTTTCCCTGTCTTCCAC	211	5 757
EGFL6 Ex12	chrX:13651125	F: GGCAAGGGCAAAACCGGC R: ATGCCAGAGGTCCTATGATGC	167	31 947
TCEANC Ex2	chrX:13683238	F: CTCTCCGTGTCTTCCCAGG R: ACACCGACTTGAACTAATCAAATTTGG	188	23 465
RAB9A Ex5´	chrX:13706890	F: CTTGTTTTTCCACAGTATCACAACTG R: GTTTACGTAAAGTGGAAATAAACAGACC	145	14 846
RAB9A Ex2	chrX:13721880	F: GAACCTGTCTGTAAAACTGCCAG R: CTGTCAGAAAAGAAAACTTCTTCCTG	254	4 914
RAB9A Ex3	chrX:13727047	F: GGGACACGGCAGGTCAG R:GCCGTTGTCCCTGCACC	257	25 767
***OFD1* Ex1**	chrX:13753070	F: ACAGAGGCAGGGTTCTGAGG R: CTTTATTTCCGCCCGAGAAG	169	128
OFD1 Ex2	chrX: 13753366	F: GTCCAACATGTTTACCGTGGC R: GTTTCAGGATACAGAGTTATTCGGG	258	1 036
OFD1 Ex3	chrX:13754659	F: GCCTCGGTCCATTTCAGTAG R: TGGACCTAGTCGAGGAGCAC	242	2 129
***OFD1* Ex4/5**	chrX: 13757029	F: CACTGGTAAGATGGCTTAGTTTCTG R: GTTCTCTACAAAGATGACGGCTC	150	5 298
***OFD1* Ex6**	chrX: 13762476	F: CAGTGACTGAGCAGTGGTTGAAAG R: GGCTATAATTACCCAGAGAATCTCTG	175	1 719
OFD1 Ex7	chrX: 13764369	F: CAGAGGTCTGGCGTCTTACG R: CATATACCTATGGTTAAGACTTTAGAAGAC	281	349
***OFD1* Ex8**	chrX: 13764998	F: GCTTGTCAAGCAAAATCTGAAGC R: GATCTAACCCTCTAATCTGCCTGA	364	2 041

Amplicon	**Location of forward primer (F)**	**Primers**	**Product size (bp)**	**Distance to next amplicon (bp)**
***OFD1* Ex9**	chrX: 13767402	F: CCTTGAGTCGGAATCTCTTGAAG R: CATGCATTACTTACAATTCAAAAGCTTC	265	1 523
OFD1 Ex10	chrX:13769189	F: GCTGTAACTAGGTCAGTTCATAAAATAC R: CATTCTTGAGCTTTCGGTCATAGG	289	1 331
***OFD1* Ex11**	chrX: 13770808	F: GTGTCATTGTGACAGCTTCCAC R: CAAATGATAATCAGACGGTCACTTC	328	2 084
OFD1 Ex 12	chrX: 13773219	F: GTATTCACTAGGAAAACATTATGGTGT R: GCAGTCCAAACATCTCAACAGGATG	248	1 139
OFD1 Ex13	chrX: 13774605	F: GTGACACCATGCTTAAAGGAGC R: CATACGGTTTAGGAGACGCAG	287	879
OFD1 Ex14	chrX:13775770	F: CCTTCTTAGGCCTAGCTCAGCCG R: CACTAGTGTGACTTGTGCCAAG	221	340
***OFD1* Ex15**	chrX:13776330	F: CTGTCATAAAGCTTCTTGGAATTGGC R: CATTTTCACAGACACCAGAAGCTTTATAC	302	1 954
OFD1 Ex16	chrX:13778585	F: GCCCACCATCTCTGCACTTG R: TGTCGTGGGCAGGGTGGC	319	295
OFD1 Ex17	chrX:13779198	F: CCTTAGGTCTGGGCAGATCAC R: CAATCAGAACGTTATACTTACCCC	154	883
OFD1 Ex18	chrX:13780234	F: CTGTAGTACCTACTTCACAGCATG R: CCAATGGATACGTTTCACCAAAAC	407	1 194
***OFD1* Ex19**	chrX:13781834	F: GGACTCATGGGACAATTGTGC R: GTATGCGACCCTGCTGACTG	183	3 280
OFD1 Ex20	chrX:13785296	F: GAACAGCAAGTGAAAGAACGAAG R: CAAGGAGAGGACAGGGATGC	252	610
***OFD1* Ex21**	chrX:13786157	F: GGTGCCTGTTTTATAGAAGATGATTG R: CATGGCACTGGCACCTTATCTG	201	501
OFD1 Ex22	chrX:13786858	F: GAAGGCTCCCTAGTGGACAC R: GACTGATTCAATAACTTCTTACAGGC	155	390
***OFD1* Ex23**	chrX:13787402	F: CTAGTCACGTGAAACCTCTTCTC R: GACAAAACAGGAGTGTGCTAGG	233	2 172
GPM6B Ex7	chrX:13789806	F: GACTGTAGGTACTGGGTATTAGTG R: GAAACAGCCAAACCACAAGTTAACT	162	11 875
GPM6B Ex3	chrX: 13801567	F: TTGTATGGGATCATTCTGTTGGCAG R: CTGGTAGCTTTTGCCAAATACAAGG	200	2 584
GPM6B Ex2	chrX: 13804004	F: GAGATGGAAAATTGTAAGCCTTCTC R: GTGGAGAAGTGTTGCTCAAGAATC	255	15 2790
GPM6B Ex1	chrX: 13956587	F: AAGCCCGGTCAGCGCAC R: AGACCGTGGCCGTGGC	157	90 910
GEMIN8	chrX: 14047653	F: CTGCAGTGCTTCCGAATCACT R: CAGATACTGGACTGGATGGGC	205	-
3p1	chr3:1436076	F: GCTGCTGTCGTCCCTGGGAACGACC	186	-

Amplicon	**Location of forward primer (F)**	**Primers**	**Product size (bp)**	**Distance to next amplicon (bp)**
		R: GTGCCAGCCCCACTGCCAGGAACAA		
SALL4 Ex3	chr20:50405598	F: GTTCCTGGCACATTTGTGGGACCC R: GCTGAAAGCCCACACAAACCCACC	245	-

OFD1 primers in bold were used as a screening set for initial duplication/deletion analysis

Supp. Table S2. Criteria used for the assessment of brain malformations in patients with mutations in *OFD1*

Patient	MRI		Brain				
	N	Age	Cortex	IHEM cyst	CC	Brainstem	Cerebellum
12	1	1y0m	1	1	1	1	2
14	1	0y4mo	1	2	2	0	1
26	6	1y4m	2	2	2	0	2
27	1	0y5m	2	2	2	2	2
1	1	18y5m	1	1	1	0	…
28	3	10y10m	0	0	1	0	1
10	1	1y10m	2	2	2	1	2
Abbreviations: Age, age at most recent MRI; CC, corpus callosum; IHEM, interhemispheric							

Key to Supp. Table S2

Cortex			Brainstem	
0	normal		0	normal
1	Unilateral or bilateral single cortical infolds		1	mild hypoplasia or compression
2	Bilateral multiple cortical infolds, some with unconnected PNH		2	thick superior cerebellar peduncle (molar tooth malformation)
Interhemispheric cyst			Cerebellar vermis	
0	none		0	normal
1	small		1	mild vermis hypoplasia
2	large		2	moderate or severe vermis hypoplasia
Corpus callosum				
0	normal			
1	partial agenesis with small or no IHEM cyst			
2	total agenesis with large IHEM cyst			
Abbreviations: IHEM, interhemispheric; PNH, periventricular nodular heterotopia				