mutations is more heterogeneous than was previously thought. The rapid progress of nephropathy in these cases might be the consequence, in part, of a possible influence of additional genes related to development of the kidneys.

In conclusion, this review of the clinical features of nephropathy in patients with *WT1* gene mutations indicates that differences exist in the progression of nephropathy, the degree of genital abnormalities, and the incidence of Wilms tumours between two types of mutations, that is, exonic and intronic mutations, although in a few cases (patients 8 and 9) features occasionally overlap. Thus, detecting and clarifying types of *WT1* mutations is considered to be useful for prognostic estimation of the clinical course in children with progressive nephropathy.

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Interstitial deletion of chromosome 17 (del(17)(q22q23.3)) confirms a link with oesophageal atresia

EDITOR—There have been five previously described cases with a de novo interstitial deletion within the distal long arm of chromosome 17.[1–5] We describe a sixth case, 46,XY,del(17)(q22q23.3)de novo. Three patients, including the proband in this report, presented with tracheo-oesophageal fistula (TOF)/oesophageal atresia, highlighting a potential genetic locus for this significant congenital anomaly.

The patient was born at 32 weeks' gestation by Caesarean section for fetal distress and intrauterine growth retardation. The liquor volume was low. He was the first child of a 20 year old mother and 22 year old father who were non-consanguineous and healthy. The birth weight was 1420 g (9th centile) and head circumference was 27 cm (2nd centile). Apgar scores were 7 at one minute and 9 at five minutes. The patient was noted to be dysmorphic at birth and oesophageal atresia was diagnosed at 2 days of age (fig 1). The patient had a small fontanelle, a sloping forehead with wrinkled skin, a round facial appearance, hypertelorism, small eyes with upward slanting palpebral fissures, a broad nasal tip with a short philtrum, a downturned mouth, and thin lips. His palate and ears were normal. He had small nails, proximally placed thumbs, and a deep crease between his first and second toes. He had knee contractures and a fixed flexion deformity of his hips. There was no palpable hip instability and ultrasound confirmed this.

Echocardiography showed a ventricular septal defect with an overriding aorta, pulmonary valve stenosis, and a left to right shunt. Cranial ultrasound was normal, as was renal ultrasound. At surgery oesophageal atresia was

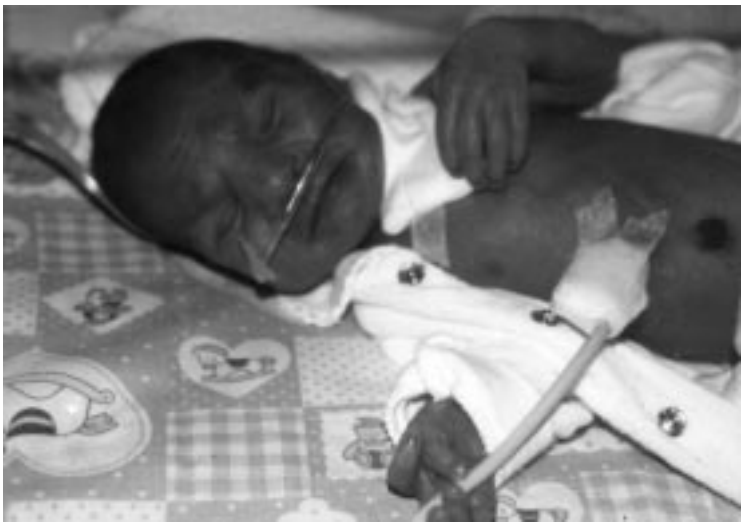

Figure 1 The proband aged 3 days. Note the wrinkled forehead, hypertelorism, small, upward slanting palpebral fissures, broad nasal tip, and downturned mouth. Proximal placement of the thumb is shown on the right.

confirmed with tracheo-oesophageal fistulae communicating with both the upper and lower oesophageal pouches. A successful oesophagostomy and gastrostomy were performed. Anal stenosis was noted and required repeated dilatations and eventually an anoplasty was performed.

The baby's recovery was complicated by several episodes of sepsis but he was discharged at 2 months of age. He made little developmental progress. Readmission was required for cyanotic episodes related to a deterioration in his cardiac condition. He developed bronchiolitis and died at 3½ months.

Short term peripheral blood cultures were initiated and harvested by standard protocols. G banded analysis was carried out using trypsin digestion followed by Leishman staining. All metaphases examined showed a male karyotype with an apparent interstitial deletion of the region 17q22→q23.3 (fig 2). Parental karyotypes were normal. The patient's karyotype was therefore 46,XY,del(17)(q22q23.3)de novo.

Five cases of interstitial deletions within the distal long arm of chromosome 17 have been described with a similar phenotype, including microcephaly, facial dysmorphism, proximal placement of the thumbs, and developmental delay.[1–5] The features are shown in table 1. Congenital heart disease has been reported in all the cases that have died prematurely including the present case and those of Levin *et al*[4] and Dallapiccola *et al*.[2] The cardiac malformations described in these latter two cases differ from the present case and include bicuspid aortic valve, atrial septal defect, patent ductus arteriosus, and dilated left atrium and ventricle. Both of the other patients have a more distal breakpoint, involving 17q24. The patient in this report has similar breakpoints to that of Mickelson *et al*,[5] who reported a female with no cardiac defects. Our finding is

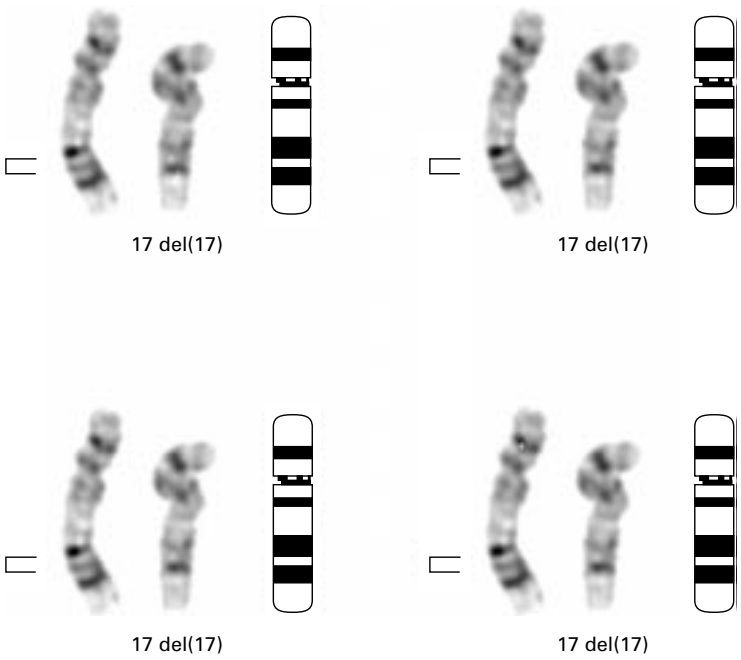

Figure 2 A partial G banded karyotype indicating the normal chromosome 17 (on the left) and the deleted chromosome 17 (in the centre). A chromosome 17 ideogram is also shown (on the right). The bracket indicates the deleted region.

Table 1 Clinical features of the six cases with deletions of chromosome 17(q21-24)

	Park et al[1]	*Dallapiccola et al*[2]	*Khalifa et al*[3]	*Levin et al*[4]	*Mickelson et al*[5]	*Present case*
Birth details						
Gestation	41.5 wk	Term	41.5 wk	35 wk, 3 d	39 wk	32 wk
Delivery	Caesarean	SVD	SVD	Caesarean	SVD	Caesarean
Birth weight (g)	2370	2780	3425	1160	2590	1430
Birth OFC (cm)	30.5	33	32	27	–	27
Birth length (cm)	47	47	52	36	49	–
Sex	F	M	M	F	F	M
Craniofacial						
Microbrachycephaly	+	+	+	–	+	–
Round face	+	?	+	–	+	+
Hypertelorism	+	+	+	+	–	+
Upward slanting palpebral fissures	+	+	+	–	+	+
Posteriorly rotated and low set ears	+	–	+	+	–	–
Long philtrum	+	+	–	–	–	–
Downturned mouth	+	–	–	–	+	+
Abnormal palate	–	+	–	+	+	–
Abnormal uvula	+	–	–	+	–	–
Micrognathia	+	+/–	+	–	Mild	–
CNS						
Radiological cerebral abnormalities	+	+	–	–	–	–
Developmental delay	Mild to mod	?	Mod to severe	?	Mod to severe	?
Skeletal						
Proximal placement of the thumbs	+	+	+	+	+	+
Symphalangism	?	+	+	–	+	–
Other						
TOF	+	+	–	–	–	+
Oesophageal atresia	–	+	–	–	–	+
Congenital heart defect	–	+	–	+	–	+
Death	–	4 mth	–	17 d	–	3.5 mth
Cytogenetics						
Karyotype	46,XX,del(17)(q21.3q23)	46,XY,del(17)(q21.3q24.2)	46,XY,del(17)(q21.3q23)	46,XX,del(17)(q23.2q24.3)	46,XX,del(17)(q23.1q23.3)	46,XY,del(17)(q22q23.3)
Parental karyotype	Normal	Normal	Normal	Normal	Normal	Normal

SVD - spontaneous vaginal delivery
OFC - occipitofrontal circumference

thus consistent with a candidate region for cardiac malformations being more proximal to 17q24 and is against the hypothesis put forward by Mickelson *et al*,[5] who suggested a more distal locus for cardiac abnormalities.

TOF/oesophageal atresia is rarely associated with a consistent chromosome abnormality. Brunner and Winter[6] commented that an abnormal karyotype can be detected in 6% of patients with oesophageal atresia and associated anomalies. Schinzel[7] noted that oesophageal atresia and TOF occur uncommonly in at least 10 different chromosomal abnormalities. These have been isolated cases in all instances except for two, where two out of 15 patients with dup(3)(pter-p21) had a TOF as did two out of three cases with del(6)(q13-q15); there were no cases associated with chromosome 2 abnormalities. The discovery therefore of three patients with an interstitial 17q deletion and TOF is significant and unlikely to be coincidental. The breakpoints seen in our patient (q22-23.3) fall within the same region as reported in the two other patients[1 2] and define a possible critical region. This is emphasised by two of the three cases without TOF having more distal breakpoints.[4 5] The one case with no TOF and breakpoints in our critical region may confound this hypothesis, but is more likely to be because of variable expression and should not deter further research into this region. There are many other examples of deletion syndromes with a different clinical outcome in the presence of haploinsufficiency to support this.[12]

It is of note that the clinical findings have some overlap with Feingold syndrome,[8] where oesophageal/duodenal atresia is commonly reported along with microcephaly and mesobrachyphalangy. However, the hand and foot findings seen in the 17q deletion cases such as symphalangism and thumb anomalies are not specific for Feingold syndrome and none of the patients have 4,5 toe syndactyly which is characteristic of this inherited condition. A recent report from Celli *et al*[10] showing linkage to 2p22.3 would confirm these differences.

Human *Nog* has been shown by Gong *et al*[11] to map to 17q22. Noggin encodes a bone morphogenetic protein (BMP) antagonist which is expressed in the node, notochord, and dorsal somite. In mice lacking Noggin, cartilage condensations initiated normally but developed hyperplasia. In humans, heterozygous *Nog* mutations have been shown in multiple synostoses syndrome (SYNS1, OMIM 186500) and proximal symphalangism (SYM1, OMIM 185800). Our patient did not have similar joint abnormalities, nor is TOF a feature of either of these syndromes. Cartilage is, however, present in the oesophagus which could represent a rather tenuous link between these findings.

We believe that this case provides further evidence of a distinct phenotype associated with deletions of 17q22-q23 and confirms an association between haploinsufficiency of this region and oesophageal atresia. This is of particular interest as familial oesophageal atresia is rare[9] and there are no other clues to genomic locations of importance for this malformation.

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Mutational analysis of Sanfilippo syndrome type A (MPS IIIA): identification of 13 novel mutations

EDITOR—Sanfilippo syndrome or mucopolysaccharidosis type III (MPS III) encompasses a group of four lysosomal storage disorders resulting from a failure to break down the glycosaminoglycan heparan sulphate. Each of the four subtypes, A, B, C, and D, is caused by the deficiency of a different enzyme in the degradative pathway of heparan sulphate: heparan-N-sulphatase (EC 3.10.1.1), α-N-acetylglucosaminidase (EC 3.2.1.50), acetyl-CoA N-acetyl transferase (EC 2.3.1.3), and N-acetylglucosamine-6-sulphatase (EC 3.1.6.14), respectively.[1] Clinical symptoms usually occur after two years of apparently normal development and include hyperactivity, aggressive behaviour, delayed development (particularly in speech), sleep disturbances, coarse hair, hirsutism, and diarrhoea. There are only relatively mild somatic manifestations. There then follows a period of progressive mental retardation with death usually between the second and third decade of life. In a small number of patients with Sanfilippo syndrome type B, there is a more slowly progressive form of the disease with later onset known as the attenuated phenotype.[2–4] A late onset phenotype has also been described for Sanfilippo syndrome type A.[5]

Sanfilippo syndrome type A (MPS IIIA) is caused by a deficiency in the enzyme heparan-N-sulphatase (sulphamidase). The disease is autosomal recessive and the gene encoding the enzyme is situated on chromosome 17q25.3, contains eight exons, and encodes a protein of 502 amino acids.[6 7] To date, 46 different mutations have been identified in Sanfilippo A patients,[6 8–13] several of which have been found at high frequencies in particular populations. The R245H, R74C, 1091delC, and S66W were the most frequent mutations in the Dutch (56.7%),[11] Polish (56%),[8] Spanish (45.5%),[13] and Italian (33%)[12] populations, respectively. Several polymorphisms have been identified in the sulphamidase gene including R456H, which has a high frequency of 55% in the normal Australian population.[9] In this study, mutational analysis has been carried out on the sulphamidase gene from 23 patients with Sanfilippo syndrome type A in the UK. Twenty three different mutations have been found, 13 of which have not been reported previously. The novel mutations comprise one insertion (1156ins6), two nonsense mutations (R233X, E369X), and 10 missense mutations (D32G, H84Y, R150W, D235N, D273N, I322S, E355K, Y374H, R433W, V486F).

All except one of the 23 Sanfilippo A patients under investigation had the classical Sanfilippo phenotype. The one milder patient had slight developmental delay but no hyperactivity or sleep problem. Sulphamidase enzyme activity in leucocytes from the patients ranged from 0 to 5 pmol/h/mg protein (reference range 52-458 pmol/h/mg protein).[14] The average age at diagnosis was 5 years 6 months. A modified version of the ammonium acetate salting out method was used to extract genomic DNA from either venous blood or fibroblast cell lines of the patients.[15 16]

Each of the eight exons and intron/exon boundaries of the sulphamidase gene was amplified by PCR using intronic primers (table 1). Exon 8 was amplified as two overlapping fragments (exons 8a and 8b). An M13(-21) forward primer sequence (5'-TGTAAAACGACGGCC AGT-3') and an M13 reverse primer sequence (5'-CAGGAAACAGCTATGACC-3') were tagged at the 5' end of the sense and antisense primers, respectively. These sites were used as universal primer binding sites in the fluorescent DNA sequencing procedure.

A typical PCR reaction using 100 ng of genomic DNA contained 25 pmol of each primer, 1 × NH_4 reaction buffer (Bioline), 4% (v/v) DMSO (dimethylsulphoxide), 0.2 mmol/l dNTPs, and 0.5 µl (2.5 units) BioPro™ DNA polymerase (Bioline) (added after a hot start). Details of annealing temperatures and $MgCl_2$ concentrations for each particular amplification reaction are provided in table 1. Cycling conditions were typically 96°C for 10 minutes, followed by 35 cycles of one minute at 96°C, one minute at 60-64°C, one minute at 72°C, and a final extension at 72°C for 10 minutes.

Following amplification, the PCR products were subjected to SSCP (single strand conformation polymorphism) analysis using MDE™ gel (Mutation Detection Enhancement) (FMC Bioproducts). The nine PCR products were digested with a restriction enzyme before SSCP analysis (table 1). To 5 µl of digestion mix, 2 µl of loading dye (95% (v/v) formamide, 10 mmol/l NaOH, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol) was added. Samples were denatured at 94°C for four minutes before loading on a 0.5 × MDE™ gel. Electrophoresis was carried out in 0.5 × TBE at 15 W overnight at 4°C or at 45 W for four hours at room temperature. Bands were detected by silver staining.[17]

Fragments of interest were concentrated and separated from excess primers and dNTPs by ultrafiltration through Microcon™-100 columns (Millipore) before sequencing. Products were sequenced in both the forward and reverse direction using the appropriate M13 dye labelled primer kits (Perkin Elmer Applied Biosystems). Reactions were performed as instructed and samples were analysed on an ABI Prism™ 377 DNA Sequencer (Perkin Elmer Applied Biosystems).

Sequence changes were confirmed either by digestion with a restriction enzyme or by ACRS (amplification created restriction site) PCR.[18] Primer sequences, annealing