CURRENT TOPIC

Frasier and Denys-Drash syndromes: different disorders or part of a spectrum?

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Frasier and Denys-Drash syndromes are both characterised by renal disease, intersex, and a predisposition to develop tumours. The association of mutations within the Wilms's tumour suppressor gene (WT1) and the Denys-Drash syndrome is now well described. More recently, mutations of the WT1 gene have also been found to cause Frasier syndrome. The clinical and genetic overlap between these two syndromes has opened up the debate as to whether these two conditions are part of the same spectrum or are different disorders caused by different mutations within the same gene. Molecular studies of these rare syndromes are providing valuable insights into the role of the WT1 gene in genitourinary development and underline the increasing importance of genetic analysis for diagnostic, prognostic, and therapeutic purposes.

Clinical aspects

The Denys-Drash syndrome[1 2] consists of a triad of intersex, Wilms's tumour, and nephrotic syndrome. In Denys-Drash syndrome, the characteristic glomerular damage causing the nephrotic syndrome manifests as diffuse mesangial sclerosis and this may be the presenting feature, either at birth or in the 1st few years of life. The presence of nephropathy is the key defining feature of the syndrome, which can exist in either a complete form, consisting of all three components of the triad,[3] or an incomplete form, in which the nephropathy is present in association with either one of the other features—either Wilms's tumour or intersex.[4–6] Progression of the nephropathy into renal failure is inevitable. A wide variety of genital abnormalities is seen: most patients with 46 XX appear normal, but might have streak gonads, whereas most patients with 46 XY exhibit ambiguous genitalia or male pseudohermaphroditism. Most, but not all, patients with Denys-Drash syndrome develop Wilms's tumour, the median age at presentation is 18 months, with 20% of cases being bilateral[5 6]—classic features of a Wilms's tumour predisposition syndrome.[7] In contrast, the median age of sporadic Wilms's tumour is 44 months and the incidence of bilateral tumours is 8%.[8–10] The histological features of Wilms's tumour in Denys-Drash syndrome are no different from the sporadic form, but intralobar nephrogenic rests are found in the kidneys of most patients with Denys-Drash syndrome compared with only 15% of patients with sporadic Wilms's tumour.[11] Intralobar nephrogenic rests are thought to be caused by the disruption of early events in nephrogenesis and to be precursor lesions of Wilms' tumour.[12]

Frasier syndrome[13] presents in a similar fashion but with some important clinical differences. The nephropathy tends to present later in life and is caused by focal segmental glomerulosclerosis rather than diffuse mesangial sclerosis. Progression into renal failure is more gradual. There is no known predisposition to Wilms's tumour, but gonadoblastoma is far more common in Frasier syndrome than in Denys-Drash syndrome, and may be the presenting feature. Gonadoblastomas arise in dysgenetic testes and are predominantly benign tumours, the rare malignant cases are usually germinomas. Furthermore, individuals with Frasier syndrome who have a 46 XY karyotype present with a pure intersex state, whereas those with a 46 XX karyotype have normal gonadal development. Table 1 compares the clinical features of the Denys-Drash and Frasier syndromes.

Genetic aspects: the WT1 gene

WT1 was originally isolated from the short arm of chromosome 11, as the result of a positional cloning effort aimed at identifying a gene for Wilms' tumour.[14 15] The region was of particular interest because cytogenetically visible deletions of 11p of varying size, but always involving band p13, had been reported in both sporadic Wilms's tumour and Wilms's tumour in association with the complex phenotype known as the WAGR syndrome (Wilms's tumour, aniridia, genitourinary abnormalities, and mental retardation).[16] It was presumed that a tumour suppressor gene lay in this region and that inactivation of both copies would lead to tumorigenesis.[7] However, WT1 now appears to have a number of different roles in health and disease and its function is far more complex

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Table 1 Clinical features of Denys-Drash and Frasier syndromes

Denys-Drash syndrome	*Frasier syndrome*
Intersex	Intersex
Nephropathy: diffuse mesangial sclerosis	Nephropathy: focal segmental glomerulosclerosis
Early presentation: 0–3 years	Later presentation: 10–20 years
Gonadoblastoma	Gonadoblastoma
High risk of Wilms's tumour	?No risk of Wilms's tumour

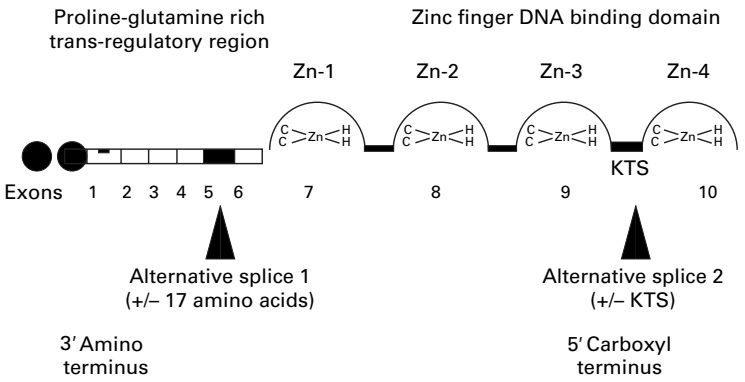

Figure 1 A diagrammatic representation of the Wilms's tumour suppressor (WT1) gene and protein.

than simply "tumour suppression". In situ hybridisation studies have shown that the WT1 gene is expressed in a very specific set of tissues during embryogenesis, namely the glomerular precursor cells of the fetal kidney, stromal cells of the gonads and spleen, and the mesothelium lining the heart, pleural, and peritoneal cavities.[17 18] In the developing kidney, the highest level of WT1 gene expression is in the condensing mesenchyme, whereas in the mature nephron, expression is confined to the podocytes, whose role is to maintain the integrity of the glomerular filtration barrier. The crucial role of WT1 function in urogenital development is emphasised by the WT1 knockout mouse, where homozygous inactivation of WT1 causes absent kidneys and malformation of the gonads.[19] To date, WT1 remains the principal gene to be associated with disorders of renal disease and intersex. WT1 contains 10 exons, covering ~ 50 kb, and encodes a nuclear protein with four zinc fingers that binds DNA and is thought to function as a transcription regulator (fig 1).

Numerous genes have been proposed as targets for WT1, but the physiological relevance of these observations remains unclear.[20] WT-1 may also bind RNA and has a putative role in mRNA processing because it can colocalise with elements of the nuclear splicing machinery.[21] Alternative splicing of the gene at two sites results in four different zinc finger protein isoforms with molecular weights of between 52 and 54 kDa.[22 23] Splicing at the first site results in either inclusion or exclusion of exon 5 (encoding 17 amino acids) and, at the second, the inclusion or exclusion of three amino acids—lysine, threonine, and serine (KTS), resulting in either KTS positive or negative isoforms. This is highly conserved throughout evolution and is thought to have great biological importance.[22] In the normal situation, approximately two to four times more KTS positive than negative isoforms are produced. The possibility of RNA editing and the use of alternative initiation codons increases the number of potential isoforms to 16.[24] The precise ratio of the WT1 isoforms appears to be crucial for normal gene function. Accumulating evidence supports a regulatory role for WT1 in genitourinary development, although little is known about which cascades may be affected by its abnormal function.

Molecular pathology of Denys-Drash and Frasier syndromes

The Denys-Drash and Frasier syndromes now have a defined genetic basis because > 96% of patients with Denys-Drash syndrome and all patients with Frasier syndrome characterised at the molecular level carry constitutional heterozygous mutations of the WT1 gene.

DENYS-DRASH SYNDROME

A wide variety of constitutional WT1 gene mutations are seen in Denys-Drash syndrome and genotype–phenotype correlations are difficult, even with the aid of computer programs.[25] A unifying feature is that the WT1 mutations result in the disruption or inactivation of DNA binding by the zinc fingers of the WT1 protein.[26] Nearly half the patients have missense mutations affecting a crucial arginine residue in zinc finger 3 (Arg394) and, collectively, missense mutations in zinc fingers 2 and 3 account for 80% of Denys-Drash syndrome mutations.[5] It is now recognised that these mutations affect amino acids crucial for the stability of DNA binding of the zinc fingers.[27 28] Because these mutations were only detected in one allele of the WT1 gene it was initially thought that Denys-Drash syndrome mutations were acting in a dominant manner. However, in a few patients with Denys-Drash syndrome, WT1 mutations either within or upstream of the zinc finger region produced a truncated non-functional protein, suggesting that the mutant protein that results may behave in a dominant-negative fashion, somehow interfering with the function of the normal protein produced from the remaining allele[26] (for a review of WT1 mutations, see Little and Wells[29]). Effective WT1 concentrations in cells are probably reduced to below 50% because the WT1 protein can dimerise, resulting in abundant non-functional homodimers and heterodimers of mutant WT1 protein.[30] In summary, the general effect of Denys-Drash syndrome mutations is to produce an abnormal protein with abnormal functions, resulting in severely impaired kidney function, varying degrees of gonadal abnormality, and loss of tumour suppressor capabilities. How WT1 mutations result in the nephropathy characteristically found in Denys-Drash syndrome is not known. Such effects might be mediated via aberrant DNA, RNA, or protein interactions perhaps during the progression of nephrogenesis or alternatively in the terminally differentiated podocytes.

The putative molecular mechanisms underlying the gonadal abnormalities are better understood since the recent work published by Nachtigal *et al*.[31] In boys/men, steroidogenic factor 1 (SF1) participates in sexual development by regulating expression of the polypeptide hormone Mullerian inhibiting substance (MIS). Nachtigal *et al* were able to show that in the normal situation, WT1 isoforms lacking a KTS insert associate and synergise with SF1 to promote expression of the gene encoding MIS.

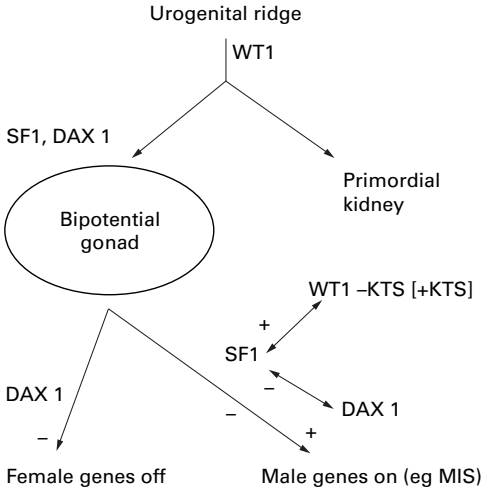

Figure 2 The interplay of the Wilms's tumour suppressor (WT1) protein isoforms (KTS negative (−KTS) and positive (+KTS)), steroidogenic factor 1 (SF1), Mullerian inhibiting substance (MIS), and DAX1 in boys/men with an XY karyotype.

They also showed that DAX1, a gene thought to direct ovarian development, antagonises this synergy (fig 2). In contrast, if WT1 mutations characteristically seen in Denys-Drash syndrome are introduced, the resultant WT1 protein and SF1 fail to associate and synergise, functional opposition of the DAX1 gene no longer occurs, and testis development fails. Thus, the severity of the genital phenotype is dependent on the degree to which WT1/SF1 binding is disrupted. Because different mutations might be expected to have different effects on the synergistic action of the protein cascade, this might explain why such a wide variety of genital phenotypes is seen in patients with Denys-Drash syndrome and a 46 XY karyotype. Interestingly, because an intact WT1 gene is not an absolute requisite for female gonadal development, individuals with Denys-Drash syndrome and a 46 XX karyotype usually have no gonadal abnormality.

Molecular analysis of Wilms's tumours arising in patients with Denys-Drash syndrome has revealed that most, but not all, tumours have lost the remaining normal WT1 allele, consistent with the "two hit hypothesis".[7] Furthermore, the same WT1 mutation results in the formation of WT in some patients with Denys-Drash syndrome but not others, suggesting that the "window of opportunity" for the "second hit" is temporally limited. Although homozygous inactivation of WT1 appears to be important in the formation of WT in patients with Denys-Drash syndrome, it is now clear that WT1 mutations play a limited role in the formation of sporadic Wilms's tumours. Exhaustive molecular analysis has revealed that only 10–15% of patients with sporadic Wilms's tumours harbour WT1 mutations, this suggests that genes other than WT1 are likely to be important in the genesis of most cases of Wilms's tumour in children.[32–34]

FRASIER SYNDROME

In contrast to Denys-Drash syndrome, Frasier syndrome is caused by very specific WT1 mutations that disrupt splicing at the second alternative splice donor site.[35–37] Mutations are present in intron 9 of the WT1 gene and result in deficiency of the usually more abundant KTS positive isoforms and reversal of the normal KTS positive/negative ratio from 2:1 to 1:2.[37] Because the WT1 protein produced in Frasier syndrome is normal, with normal binding abilities, the effect of these mutations highlights the importance of precisely balanced expression of WT1 isoforms for normal function. This has a great impact on tumour risk, because patients with Frasier syndrome have one normal copy of WT1 and one that can only produce the KTS negative isoform. Allele loss leads to cells that cannot produce the KTS positive isoform of WT1, but still have large amounts of the KTS negative isoform. It is interesting that in the experimental situation, the tumorigenicity of the G401 Wilms's tumour cell line in nude mice can be suppressed by both KTS positive and negative isoforms to the same extent.[38] This might explain why patients with Frasier syndrome do not develop Wilms's tumour. It is of note that the three patients reported with diffuse mesangial sclerosis and WT1 intron 9 mutations, associated more commonly with Frasier syndrome, did not develop Wilms's tumour. The high frequency of gonadoblastoma in Frasier syndrome might be the result of the obligatory presence of dysgenetic gonads, which carry a higher risk of tumorigenesis. However, there may also be differences in the stage at which gonadal development is halted in Frasier syndrome and Denys-Drash syndrome, which might potentially increase the tumour risk.

The molecular mechanisms underlying the intersex state in patients with Frasier syndrome and 46 XY is more difficult to explain than in Denys-Drash syndrome because KTS positive isoforms only have a limited ability to activate SF1.[31] WT1 isoforms may be able to take over each other's functions in certain situations,[21] so a deficiency of the usually more abundant KTS positive isoform may in turn causes a relative deficiency of the KTS negative isoform, while it takes over essential KTS positive functions. Alternatively, the KTS positive isoform might participate in another pathway required for normal male urogenital development (fig 2). Individuals with a 46 XX karyotype and Frasier syndrome nephropathy have normal gonadal development,[36] affirming the less crucial role of WT1 in normal female gonadal development. Again, the cause of the nephropathy at a molecular level in Frasier syndrome is poorly understood. Figure 3 summarises the interplay of the various WT1 isoforms, SF1, MIS, and DAX1 in the Denys-Drash and Frasier syndromes.

Relevance to clinical practice and conclusions

In view of the clinical and genetic overlap between Frasier syndrome and Denys-Drash syndrome, it appears that they form part of a

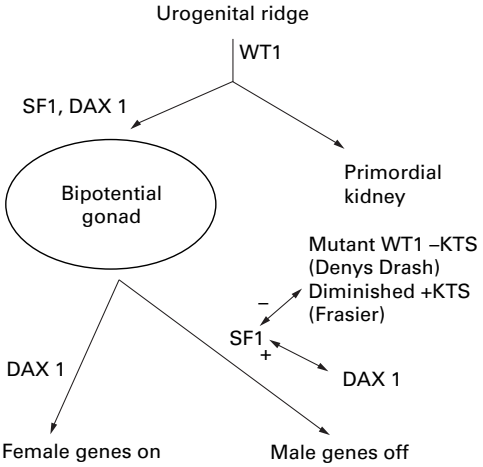

Figure 3 The interplay of the Wilms's tumour suppressor (WT1) protein isoforms (KTS negative (−KTS) and positive (+KTS)), steroidogenic factor 1 (SF1), Mullerian inhibiting substance (MIS), and DAX1 in Denys-Drash and Frasier syndromes.

spectrum of disorders. Despite the very different effect on the type of protein produced by Denys-Drash syndrome and Frasier syndrome WT1 mutations, it has been shown recently that Frasier syndrome mutations can also be associated with Denys-Drash syndrome nephropathy[39] and, conversely, that Denys-Drash syndrome type WT1 mutations can produce Frasier syndrome glomerulosclerosis.[40] Therefore, two quite different renal histologies can be caused by the same type of WT1 mutation. However, there are some important differences in the molecular mechanisms underlying the intersex state, the tumour type, and the subsequent risk of malignancy in Denys-Drash syndrome and Frasier syndrome. This suggests that Frasier syndrome and Denys-Drash syndrome might be different disorders caused by mutations within the same gene, as is seen—for example, with the haemoglobin gene.

Molecular analysis of patients' with renal disease and intersex for mutations of the WT1 gene can provide a method of identifying patients with Denys-Drash syndrome and Frasier syndrome. In Frasier syndrome, the small number of 46 XX karyotypes reported may be the result of underdiagnosis because these patients suffer primarily from nephrotic syndrome caused by Frasier syndrome glomerulosclerosis and renal failure. However, analysis may be of benefit for both prognostic and therapeutic reasons. Anecdotally, patients with Frasier syndrome glomerulosclerosis and WT1 mutations are less likely to respond to immunosuppressive treatment, although the risk of relapse after renal transplant is low. These observations hold, regardless of whether a 46 XX or 46 XY karyotype is present. In addition, transmission to offspring has been reported—this may manifest either as Frasier syndrome or Denys-Drash syndrome.[41] The type of WT1 mutation present can also provide useful information about the patient's risk of developing Wilms's tumour. A key investigation in this group of disorders remains karyotype analysis, in particular, in phenotypically normal girls with Frasier syndrome glomerulosclerosis, where an intersex state may be missed until the patient presents with a gonadoblastoma in adolescence.

Molecular analysis is now providing the necessary tools to define diseases by their genetic mechanisms. The Frasier and Denys-Drash syndromes are important human disease models of the effects of WT1 gene mutations on genitourinary development and tumorigenesis. Moreover, these syndromes provide an excellent model for the relevance of molecular genetics to clinical practice, particularly as we move towards a taxonomy of disease based on molecular abnormality rather than on phenotype.

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