SUPPLEMENTAARY DATA

Denys-Drash Syndrome associated WT1 glutamine 369 mutants have altered sequence-preferences and altered responses to epigenetic modifications

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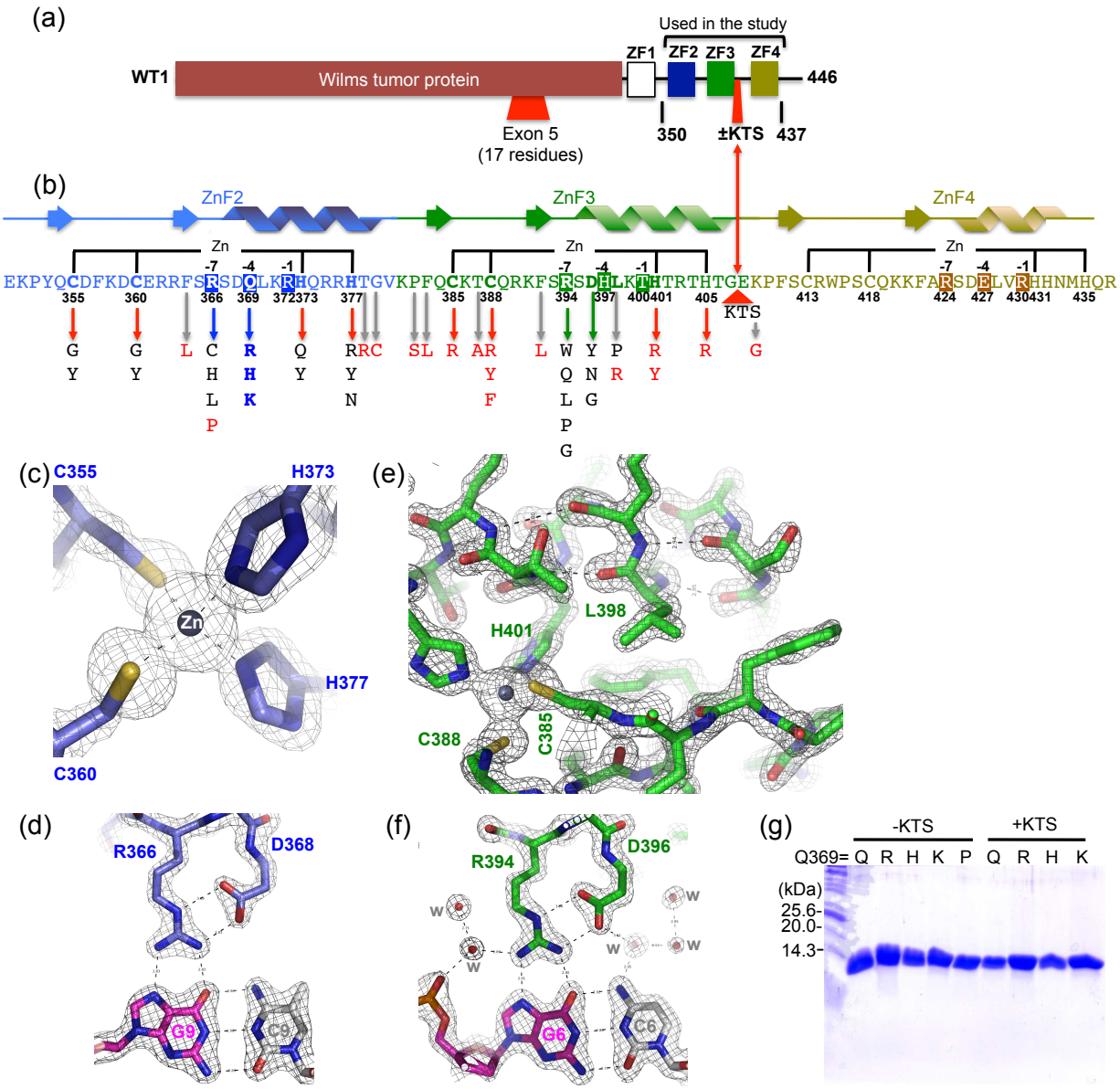
1 Supplementary Figure, Supplementary text and 1 Supplementary Table



Supplementary Figure S1. Denys-Drash Syndrome (DDS) mutations in the WT1, cluster predominantly in ZF2 and 3 at the C-terminus of WT1. (**a**) Schematic representation of human WT1. **(b)** DDS mutations in ZF2 and ZF3 that alter either the Cys2-His2 structural amino acids that coordinate the zinc ions, or the sequence-recognition amino acids at the protein-DNA interface. The information is extracted from the Human Gene Mutation Database (HGMD). (**c**) The four zinc-binding Cys2 and His2 residues of ZF2 all have mutations, including a common mutation to Tyr (see panel b). For comparison, the four zinc-binding residues of ZF3 have a common mutation to Arg. (**d**) R366 recognizes the 3' Gua. The mutation of R366 to C, L, H or P could result in loss of DNA binding affinity, loss of base specificity or altered specificity. (**e**) L398 of ZF3 lies in the hydrophobic core of ZF3, mutation of which to Pro or Arg could affect protein stability. (**f**) D396 of ZF3 stabilizes the conformation of R394, which is a conserved feature to many ZFs (e.g., ZF2 and ZF4). The Arg394 in turn recognizes the 3' Gua. Mutations of both restudies will affect sequence specificity and DNA binding affinity. (**g**) A 18% SDS-PAGE showing examples of the purified proteins used in this study, with each lane containing approximately 4 μg protein.

Supplemental text. WT1 Q369P is not a DDS mutation

Fw: HGMD comments

From: Andrew Phillips <PhillipsAD@cardiff.ac.uk>
Sent: Thursday, January 16, 2014 4:11 AM
To: Zhang, Xing
Subject: Fw: HGMD comments

Dear Prof. Zhang

Thank you for your message (reproduced below) via the HGMD comment form regarding the *WT1* substitution reported in Ohta (2000) J Urol 163, 1857, which is included in HGMD as accession number CM004287. The reference does indeed report a Gln to Arg substitution (described as occurring at amino acid 369, which is equivalent to residue 437 in the latest version of the cDNA reference sequence - NM_024426.4). I think the error has arisen because the nucleotide alteration is given in the **CASE REPORT** section of the paper as "an A to C mutation at nucleotide 1106" and this substitution would result in a CCG codon for Proline. However, the text accompanying Figure 2 gives the change as "A/G (nt.1106)" and "Gln369Arg (A/G at nucleotide 1106), which means new Msp I (CCGG) site". Consequently, I have amended the mutation entry to CAG>CGG Gln-Arg at codon 437 (and included an explanatory note in the Comments); please note that these amendments will not be reflected on the public site until the next update, due at the end of March.

Thank you for bringing this error to our attention and please accept my apologies for any inconvenience it may have caused you. If you have any further queries on this matter please do not hesitate to contact me.

Best wishes

Andrew

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-----Forwarded by Andrew Phillips/wmgadp/CardiffUniversity on 16/01/2014 08:09AM -----

To: phillipsAD@cardiff.ac.uk, stensonPD@cardiff.ac.uk, shawk3@cardiff.ac.uk
From: apache@mampwww03.cf.ac.uk (Apache)
Date: 15/01/2014 10:13PM
Subject: HGMD comments

HGMD comments from IP address 10.255.232.55 on Wednesday 15th of January 2014 10:13:54 PM

Name: xing zhang

Email address:

xzhan02@emory.edu

Comments:

This concerns CM004287 of WT1 gene. The entry states a Gln to Pro mutation, yet the reference refers to a Gln to Arg mutation. Could you please clarify the discrepancy?

Supplementary Table S1. Statistics of X-ray diffraction and Refinement

WT1 Protein	Q369 (Wild-type)	Q369H	Q369H (F=5fC)	Q369H (X=5caC)	Q369R	Q369R (X=5caC)
DNA sequence (M=5mC)	3'- TGAGGGTGCGA-5' 5'-TACTCCCACGC -5'	3'- TGAGGGTGCGA-5' 5'-TACTCCCACGC -5'	3'- TGFGGGTGCGA-5' 5'-TACGMCCACGC -5'	3'- TGXGGGTGCGA-5' 5'-TACGMCCACGC -5'	3'- TGGGGGTGCGA-5' 5'-TACCCCCACGC -5'	3'- TGXGGGTGCGA-5' 5'-TACGMCCACGC -5'
PDB code	5KL2	5KL3	5KL4	5KL5	5KL6	5KL7
Diffraction Data collection (APS SER-CAT; wavelength=1Å)						
Beamline	22-ID	22-ID	22-ID	22-BM	22-ID	22-ID
Space Group	*C*2	*C*2	*P*1	$P2_12_12$	*C*2	$P2_12_12$
Unit cell (a, b, c (Å))	70.1, 65.1, 35.7	75.2, 66.1, 35.6	35.8, 43.9, 54.8	67.5, 77.2, 35.8	76.0, 66.0, 35.6	67.5, 77.8, 35.7
α, β, γ (°)	90.0, 93.4, 90.0	90.0, 91.7, 90.0	82.0, 88.6, 88.5	90, 90, 90	90.0, 90.5, 90.0	90, 90, 90
Resolution (Å) *	29.12-1.69 (1.75-1.69)	29.22-1.45 (1.50-1.45)	29.56-1.79 (1.85-1.79)	29.26-2.29 (2.37-2.29)	29.0-1.64 (1.70-1.64)	29.24-1.59 (1.65-1.59)
[a] R-merge *	0.106 (0.424)	0.082 (0.610)	0.073 (0.540)	0.114 (0.511)	0.075 (0.439)	0.089 (0.464)
[b] <I/σI> *	16.3 (2.2)	16.3 (1.9)	10.7 (1.7)	15.4 (2.5)	20.0 (2.1)	17.7 (2.8)
Completeness (%) *	97.8 (84.2)	88.0 (43.3)	92.9 (69.6)	98.5 (84.4)	91.6 (50.4)	99.9 (99.6)
Redundancy *	6.3 (2.9)	4.6 (2.4)	3.6 (2.4)	6.4 (4.2)	6.0 (1.7)	6.3 (3.4)
CC1/2, CC *	(0.948 / 0.987)	(0.668 / 0.895)	Not available	Not available	(0.794 / 0.941)	Not available
Obs. Reflections	110,696	126,357	105,691	56,117	119,132	165,448
Unique reflections *	17,475 (1,503)	27,228 (1338)	29040 (2155)	8,763 (720)	19,712 (1085)	26,124 (2556)
Refinement						
Resolution (Å)	1.69	1.45	1.79	2.29	1.64	1.59
No. Reflections	17,401	27,161	28,983	8,708	19,659	26,017
[c] R-work/ [d] R-free	0.153 / 0.204	0.150 / 0.183	22.0 / 26.5	19.7 / 25.4	0.162 / 0.209	14.6 / 19.0
No. Atoms						
Protein	740	741	1444	752	745	752
DNA	462	496	921	449	506	506
Zn	3	3	6	3	3	3
Solvent	133	181	180	92	125	136
B-factors ($Å^2$)						
Protein	26.3	24.4	29.3	31.3	33.7	24.5
DNA	25.7	22.9	28.6	30.8	33.8	25.6
Zn	23.7	21.8	27.5	27.8	31.4	19.8
Solvent	35.9	33.2	31.7	32.1	38.1	32.8
R.m.s. deviations						
Bond length (Å)	0.013	0.014	0.012	0.004	0.012	0.012
Bond angles (°)	1.4	1.4	1.4	0.6	1.3	1.5
All atom clashscore	3.1	3.1	5.0	0.0	3.1	3.0
Ramachandran (%)						
Favored	100.0	98.9	99.4	98.9	100.0	100
Allowed	0.0	1.2	0.6	1.2	0.0	0.0
Rotamer outliers (%)	0	0	0	0	0	0
C_β deviation	0	0	0	0	0	0

*Values in parenthesis correspond to highest resolution shell;

[a] $R_{merge} = \Sigma \mid I - \langle I \rangle \mid / \Sigma I$, where I is the observed intensity and <I> is the averaged intensity from multiple observations;

[b] $\langle I/\sigma I \rangle$ = averaged ratio of the intensity (I) to the error of the intensity (σI);

[c] $R_{work} = \Sigma \mid Fobs - Fcal \mid / \Sigma \mid Fobs \mid$, where Fobs and Fcal are the observed and calculated structure factors, respectively;

[d] R_{free} was calculated using a randomly chosen subset (5%) of the reflections not used in refinement.