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The Existence Of An Androgen Responsive Transcriptome In The Peripheral Blood Of Boys Extends The Utility Of The HCG Stimulation Test

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Background

- The hCG stimulation test is a valuable method for assessing androgen production but there is a need to explore its utility in assessing androgen responsiveness and long-term prognosis
- Our aim was to explore the effect of hCG stimulation on the peripheral transcriptome in boys undergoing investigation for DSD

Methods

- 13 boys undergoing investigation for 46XY DSD received IM hCG 1500u on 3 consecutive days and had blood sampling on D0 and D3
- RNA was extracted from peripheral blood mononuclear cells on the Qiacube using RNA Blood Mini Kit with an incorporated DNase step. Microarray hybridisation was performed on 13 paired samples using the Affymetrix Human Transcript Array (HTA) 2.0
- Gene expression fold change was calculated and corrected for those boys who did not have a testosterone rise

Week	Wk1				Wk2		Wk3		Wk4	>Wk8
Date										
Day	Mon	Tue	Wed	Thu	Mon	Thu	Mon	Thu	Mon	
HCG 1500 im	*	*	*		*	*	*	*		
Serum Testos, SHBG [1]	*			*					*	
Salivary Testosterone (optional) [2]	*	*	*	*					*	
Serum Androstenedione, DHT, DHEAS, Store [3]	*			*						
Serum AMH [4]	*									
Urine Steroid Profile [4]	*									
LHRH Stim Test (0,20,60min)	*									
Karyotype & DNA [4]	*									
Ultrasound scan of Testes & Renal Tracts [5]									*	
Stretched Penile length	*			*					*	
Examine for Testes (Scrotal, Ing, Abdo, Absent)	*			*					*	
Endocrine Follow-up										*

Figure 1: The hCG Stimulation Test

Results

- Median age (range) at test was 0.83yrs(0.18-11.23) with a median External Masculinisation Score of 9(6-11)
- 3 boys had isolated proximal hypospadias, 6 had bilateral undescended testes and 4 had a combination of hypospadias, impalpable testes or micropenis
- Median pre and post hCG testosterone were <0.5nmol/l (<0.5-6) and 7.9nmol/l (<0.5-31.5), respectively
- Median fold change of testosterone was 6.8 (1-26.6) and 3(23%) boys did not demonstrate a testosterone rise (non-responders)
- Median AMH in the responders was 688pmol/l (24, 1628) and in the non-responders was <4pmol/l (<4,256)
- White cell counts for the samples are shown in Figure 2

Results cont

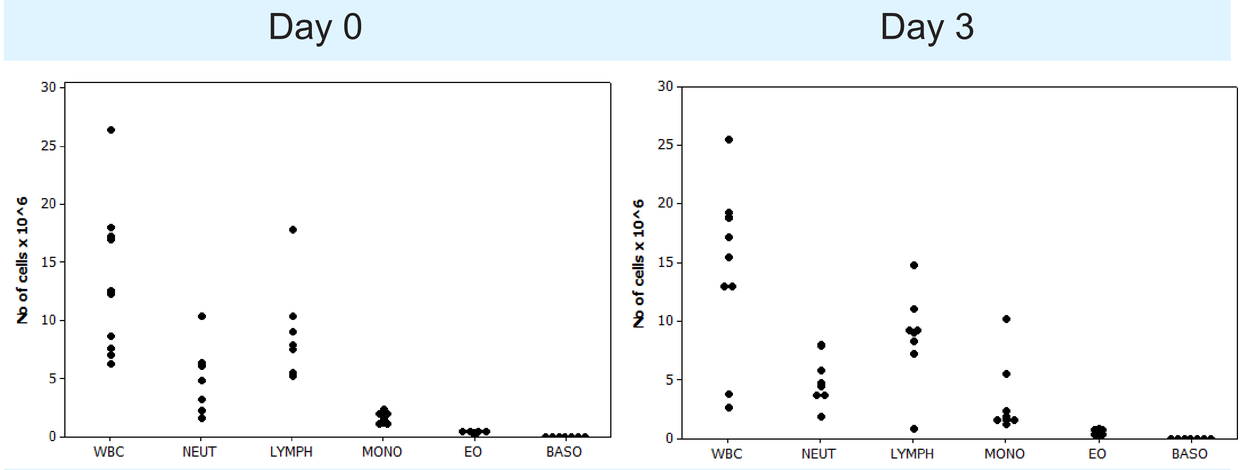

Figure 2: White cell counts in peripheral blood samples

- 8(80%) of the responders and 2(66%) of the non-responders had AR mutation analysis performed and had no variant detected
- When corrected for gene expression changes in the non-responders, all 10 of the responders demonstrated a 20% or greater increase in the expression of piR-37150, a non-coding piwi-interacting RNA
- 8(80%), 6(60%) and 4(40%) of the responders demonstrated a 30%, 40% and 50% rise respectively in a total of 5 piRNAs

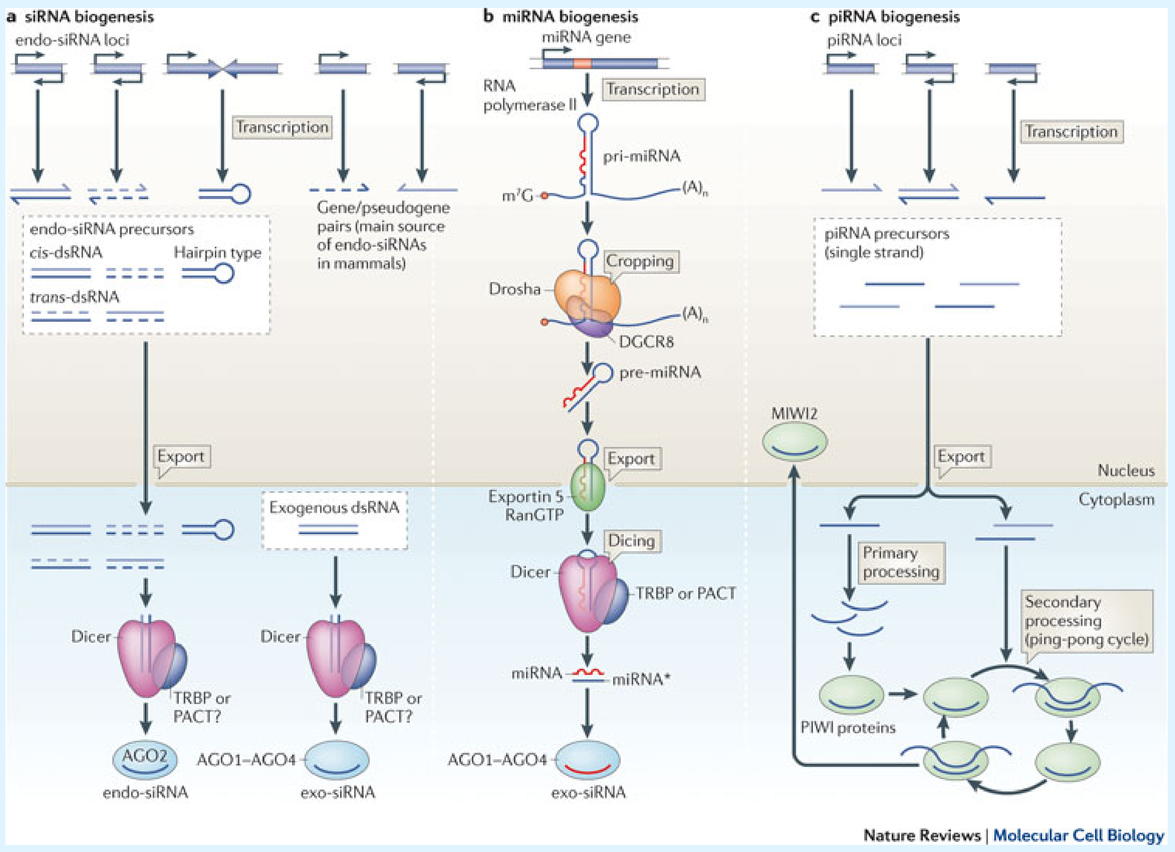

Figure 3: small interfering RNA Biogenesis[1]

Conclusions

- The identification of a dynamic peripheral transcriptome that is associated with an androgen response following hCG stimulation extends the potential value of this clinical test
- The role of piRNAs as a diagnostic and prognostic marker of gonadal function needs further investigation

[1] Siomi MC, Sato K et al. PIWI-interacting small RNAs: the vanguard of genome defence. Nature Reviews Mol Cell Biol. 2011;12:246-256

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