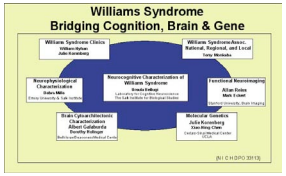
POSTER SYMPOSIUM: SOCIETY FOR NEUROSCIENCES, 2007

Symposium Title: GENES, NEURAL SYSTEMS, AND SOCIAL BEHAVIOR

Supported by NICHD PPG 33113
"Williams Syndrome: Bridging Cognition, Brain and Gene"

Session Number: 696
Date: Tuesday, November 6th 2007



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2. **Autonomic Correlates of Processing Upright and Inverted Affective Faces in Williams Syndrome**
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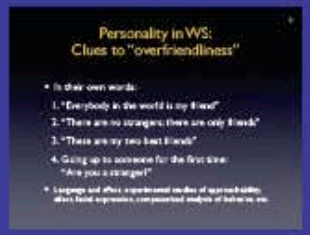
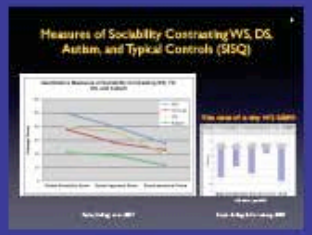
Defining the Social Phenotype in Williams Syndrome

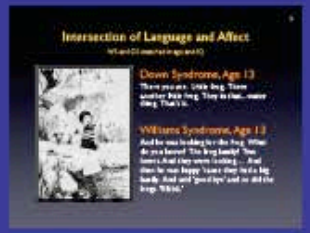
U. Bellugi, A. Järvinen-Pasley, J. Reilly, D. Mills, Y. Searcy, A. Yam, K. Hill, I. Fishman, A. Reiss, J. Korenberg

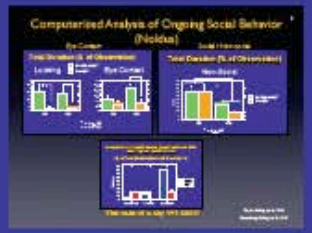
Salk Institute, La Jolla, CA; Emory Univ. Atlanta ,GA, Stanford Univ., Palo Alto, CA, Cedars-Sinai Medical Center, Los Angeles, CA

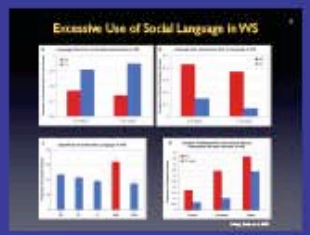
Converging Behavioral Measures

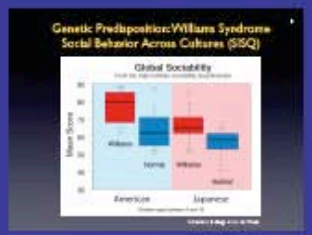




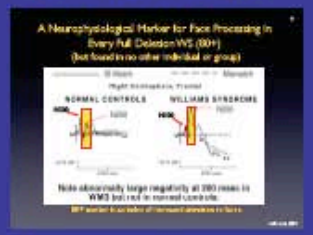


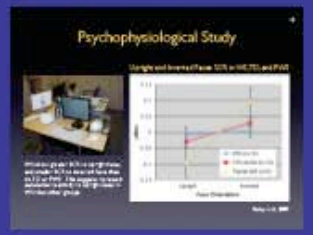


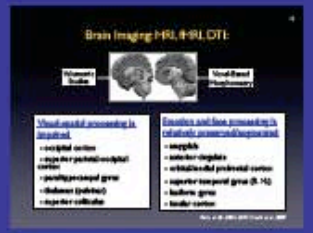




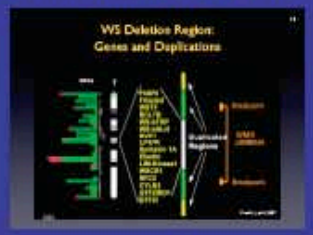
Clues from Imaging

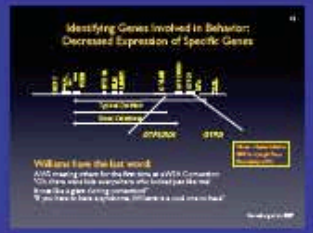


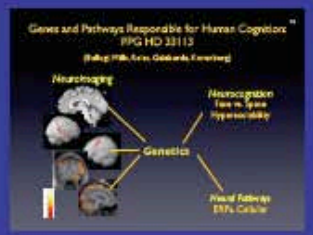




Clues from Molecular Genetics







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"Williams Syndrome: Bridging Cognition, Brain and Gene"

No. 696.1

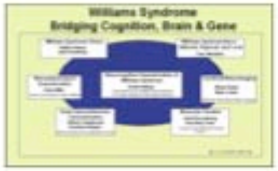
DEFINING THE SOCIAL PHENOTYPE IN WILLIAM SYNDROME

(U. Bellugi, A. Järvinen-Pasley, J. Reilly, Y.M. Searcy, K.J. Hill, A. Galaburda, A.L. Reiss, & J.R. Korenberg)

Williams Syndrome (WS) is a rare, genetic disorder, caused by a hemideletion of about 25 genes on chromosome 7q11.23, resulting in an unusual social phenotype. Individuals with WS are overly-friendly, engage in interactions freely (sometimes irrepressibly) with strangers, and yet display other non-social anxieties. In this poster, we provide an overview of the social phenotype of WS.

I. Converging behavioral measures: a) Parental questionnaires: WS are rated high on overall sociability, tendency to approach strangers, lack of shyness, empathy; b) Social use of language: WS exhibit higher than normal use of affective evaluative language for initiating and maintaining social contexts (Reilly et al); c) Experimental paradigm: WS indicate higher than normal willingness to approach and interact with strangers; d) Affective prosody: WS measure higher than normal on computer-based analysis; e) Computer-based ethogram of non-verbal social behaviors: WS are significantly higher than contrast groups on duration and frequency of eye contact, interpersonal proximity, initiation of social contacts. Together these results indicate an unusual but consistent social phenotype, including strong affiliative drive. In this poster symposium, overall, converging results for a social phenotype of WS are presented in the context of brain imaging and molecular genetics from a multi-disciplinary program project.

II. Neurophysiological findings. Mills shows that during face processing, WS exhibit an unusual lelectrophysiological (ERP) 'signature' that may index their increased attention to faces; and furthermorethat WS and Autism show contrasting ERP activity during face processing. New ERP studies are presented to positive and negative emotional facial expressions in WS (Mills et al, this symposium). III. High resolution brain imaging studies. Reiss et al found relative enlargement in the amygdala and ventralanterior orbital frontal cortex (PFC) (Reiss et al 2004). We now report a positive association of PFC with the use of hypersocial language (Searcy et al, this symposium). Moreover, fMRI studies of face and gaze processing suggest increased activation in the "mirror neuron" system in WS (Hoeft et al, this symposium). IV. Molecular genetic findings. Importantly, Korenberg reports for the first time molecular genetic clues to the consistent hypersocial behavior, brain structure and function in WS, implicating specific genes within the WS syndrome region (Korenberg et al). (See other symposium submissions titled: Genes, Neural Systems, and Social Behavior). Supported in part by HD33113.



Genes, neural systems, and social behavior: Autonomic correlates of processing upright and inverted affective faces in Williams syndrome

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Introduction

Williams syndrome (WS) is a neurogenetic disorder caused by a hemizygous deletion of approximately 25 genes on chromosome 7q11.23. Behaviorally, WS is associated with heightened appetitive social drive (e.g., tendency to indiscriminately approach strangers), a preference for viewing and increased skill in identifying faces, and language features that increase the likelihood of social interaction with others (e.g., Bellugi et al., 2007; Järvinen-Pasley et al., in press; Meyer-Lindenberg et al., 2006).

Neuroanatomical data indicate relative enlargement in the amygdala and prefrontal cortical structures (Reiss et al., 2004). Emerging functional evidence indicates that disturbances in amygdala regulation by OFCs are also implicated in the unusual social profile of WS (Meyer-Lindenberg et al., 2005). Moreover, face processing appears to be sustained by deviant electrophysiological activity in the brain (Mills et al., 2000).

Almost nothing is known about autonomic nervous system function in WS. In typical development (TD), different temperament profiles have been linked to distinct patterns of autonomic responding that reflect individual differences in cortical arousal, thereby regulating social and emotional behavior (Eysenck, 1967, 1981). Extraverts are characterized by chronic underarousal, and subsequently seek out stimulation (e.g., social) in the environment, while introverts show the opposite pattern of autonomic arousal and subsequent behavior.

The present study sought to examine autonomic responsivity (skin conductance responses, SCR; heart rate, HR) to face stimuli in individuals with WS. **We hypothesized that individuals with WS, as a group, would show similar autonomic profile to extraverts with regard to social stimuli, relatively manifested as small SCRs, low HR reactivity, and low overall skin conductance level.**

Methods

Participants

21 individuals with WS, for whom FISH analyses confirmed a full deletion of the band 7q11.23 on Chromosome 7; 21 typical chronological age (CA)- and gender-matched controls (TD); and 5 siblings with a a subset of WS features associated with a smaller deletion in the WS region (Partial WS, PWS). We used multicolor FISH to show a 500 kb deletion including the genes ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, WBSCR1, LAT2, and RFC2 in all family members (Korenberg et al., 2007). Combining genetic and cognitive data strongly support the hypothesis that the genes deleted in this family contribute more subtlety than GTF2IRD1 or GTF2I to the typical cognitive and physical features of WS.

	WS	TD control	PWS
PARTICIPANTS	n = 21	n = 21	n = 5
GENDER	7 M, 14 F	8 M, 13 F	1 M, 4 F
AGE yrs M(SD)	22.8 (6.7)	24.6 (6.4)	11.7 (2.6)
AGE yrs range	14.5 - 33.4	18.5 - 34.7	9.5 - 15.7
Verbal IQ M(SD)	73 (10.9)	107 (10.7)	78 (5.8)
Full scale IQ M(SD)	68 (12.0)	105 (11.0)	77 (5.9)

Design

- Stimuli: 46 upright expressions of facial affect from Ekman & Friesen (1976), and the same images inverted (total stimuli N=92)
- An equal number of images of males and females exhibiting an equal number of happy, sad, angry, afraid, surprised, disgusted, and neutral expressions were included
- The paradigm was adapted from Adolphs et al. (1998)
- Stimulus items are randomized between subjects with respect to face orientation (inverted/upright) and affect valence

- Experimental task: label the emotion in each face: "Decide how the person is feeling", while SCR and HR simultaneously recorded
- **Participants indicate their responses verbally**
- Stimuli are presented using MatLab®, and MatLab sends a digital pulse to BioPac® computer at the onset of each stimulus
- Stimulus items appear sequentially on screen for 1s, followed by a response screen listing the seven possible emotions

- Psychophysiology: to monitor SCR, silver/silver chloride electrodes filled with isotonic NaCl unibase eletrolyte jelly are placed on the first 2 fingers of non-dominant hand
- HR is recorded via electrodes attached to right forearm and left ankle
- Testing sessions begin with a 5-minute period, during which baselines are established

Data Analysis

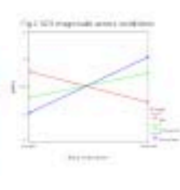
Psychophysiology: The SCR data were normalized according to Lykken (1972). Using AcqKnowledge software (BioPac Systems, Goleta, CA), the SCR and HR for each stimulus was determined, as well as the baseline skin conductance level. The SCR data were highpass filtered at 0.05Hz, and responses occurring within 2-7s after the stimulus delivery were considered as being caused by the stimulus. The mean value of the response was calculated. The remaining activity was averaged and considered as the baseline electrodermal level. For HR, responses occurring within 1-5s after the stimulus delivery were considered as being caused by the stimulus. The mean value of the response was again calculated. Separate ANOVAs were carried out on the SCR and HR data, with group entered as the between-subjects factor, and face orientation (2 levels) and affect category (7 levels) as within-subjects factors.

Behavioral data: The accuracy of responses was determined within each affect category across the upright and inverted conditions. A 3x2x7 repeated measures ANOVA was conducted.

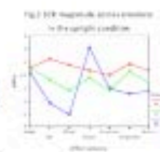
Results

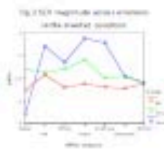
1. Magnitude of SCR

ANOVA yielded a significant main effect of face orientation (p=.013), and a face orientation by group interaction (p<.001) (Fig.1). Post-hoc tests (Bonferroni) showed that the **WS group was associated with a greater SCR to the upright stimuli than both TD (p<.001) and PWS (p<.001)**, which did not differ (p=.259). In contrast, **WS group was associated with a smaller SCR to the inverted stimuli than both TD (p<.001) and PWS (p<.001)**, which did not differ (p=.355).



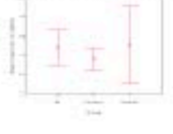
Although the main effect of emotion (p=.272), and emotion by group (p=.415), and emotion by face orientation (p=.171) interactions failed to reach significance, Figs. 2 and 3 show the SCR changes across the affect categories in the upright and inverted conditions.





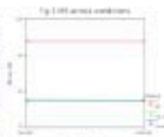
2. Baseline skin conductance level

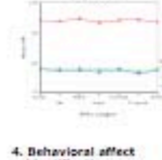
The groups did not differ in the mean baseline skin conductance level (p=.453) (Fig.4).

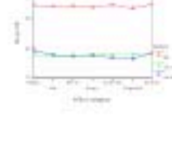


3. Heart rate reactivity

ANOVA revealed a significant main effect of group only (p<.001) (Fig.5), with **WS exhibiting higher HR overall** than both TD (p<.001) and PWS (p=.007) groups, which did not differ (p=1.0). Figs. 6 and 7 show the HR reactivity across the different emotions in the upright and inverted conditions.

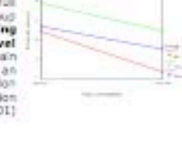


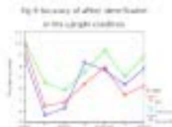


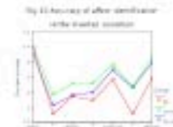


4. Behavioral affect identification

ANOVA revealed a significant main effect of face orientation (p<.001), with all groups performing better with the upright than the inverted stimuli (Fig.8); a main effect of group (p<.001), with **TD performing at a significantly higher level than WS** (p<.001); a main effect of emotion (p<.001); an emotion by group interaction (p=.006); and a face orientation by emotion interaction (p<.001) (Figs. 9,10).







Post-hoc tests (Bonferroni) revealed that:

- Happy upright stimuli: PWS<TD (p=.011)
- Sad upright stimuli: WS<TD (p=.036)
- Surprised upright stimuli: WS<TD (p=.039)
- Neutral upright stimuli: WS<TD (p=.003)
- Sad inverted stimuli: WS<TD (p=.004)
- Angry inverted stimuli: WS<TD (p=.029)
- Disgusted inverted stimuli: WS<TD (p<.001), and WS<PWS (p=.004)
- Neutral inverted stimuli: WS<TD (p=.002)

Summary

Participants with WS uniquely showed a greater mean SCR to the upright than to the inverted faces. Whereas individuals with WS, relative to both TD and PWS, showed the greatest magnitude of SCR to the upright face and the lowest magnitude to the inverted face, both TD and PWS groups showed the opposite pattern, with **the PWS group presenting the most dramatic contrast to the WS group**. At the same time, **WS group showed significantly poorer affect identification ability compared to the TD controls.**

As SCRs also index cognitive effort, it is unsurprising that both TD and PWS groups exhibited significantly greater SCRs in the inverted than upright condition.

Significantly increased SCR to the upright face in individuals with WS suggests **heightened sensitivity to social stimuli**, converging with behavioral findings (e.g., Järvinen-Pasley et al., in press).

The current findings suggest an atypical organization of autonomic function in WS, with increased autonomic responsiveness to upright faces. **Together with the near typical baseline skin conductance level, and significantly increased HR, the autonomic profile of individuals with WS is different to that seen in extraverted TD individuals.** The marked differences between the WS and PWS groups show that the **atypical pattern observed in WS is not due to intellectual impairment alone**. Further, this pattern of autonomic function appears to the linked to the full WS deletion.

Given that WS is associated with widespread structural and functional aberrations in the amygdala and the OFC (Meyer-Lindenberg et al., 2005; Reiss et al., 2004), i.e., in the pathways, which modulate emotional arousal and expression including autonomic nervous system reactivity, it may be that the underlying autonomic function is also abnormal in individuals with WS, as reported in this study. These data provide promising initial evidence of increased autonomic reactivity to upright faces in individuals with the full WS deletion, which may be linked to their unique social phenotype.

References

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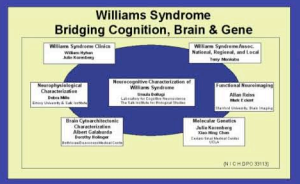
AUTONOMIC CORRELATES OF PROCESSING UPRIGHT AND INVERTED AFFECTIVE FACES IN WILLIAMS SYNDROME

(A. Järvinen-Pasley, N. Tsuchiya, M.K. Leonard, A. Yam, K.J. Hill, A. Galaburda, D. Mills, A.L. Reiss, J.R. Korenberg, & U. Bellugi)

Williams syndrome (WS) is a neurogenetic disorder caused by a hemizygous deletion of approximately 25 genes on chromosome 7q11.23. Behaviorally, WS is associated with heightened appetitive social drive (e.g., tendency to indiscriminately approach strangers), a preference for viewing and increased skill in identifying faces, and language features that increase the likelihood of social interaction with others (e.g., Bellugi et al, 2007; Meyer-Lindenberg et al, 2006). Neuroanatomical data indicate relative enlargement in the amygdala and prefrontal cortical structures (Reiss et al, 2004). Almost nothing is known about autonomic nervous system function in WS. In typical development, different temperament profiles have been linked to distinct patterns of autonomic responding that reflect individual differences in cortical arousal, thereby regulating social and emotional behavior (Eysenck, 1967, 1981). It may therefore be hypothesized that individuals with WS show similar autonomic responding to extroverts with regard to social stimuli, manifested as smaller skin conductance responses (SCRs), lower heart rate (HR) reactivity, lower overall skin conductance level, and faster electrodermal habituation, compared to introverts. We examined autonomic responses (SCR, HR) of 9 individuals with WS, 10 typically developing controls (TD), and 5 individuals with a partial WS deletion (PWS), to 92 upright and inverted affective and neutral faces (Ekman & Friesen, 1976). The experimental groups exhibited significantly different patterns of autonomic reactivity across the upright and inverted stimuli, with individuals with WS uniquely showing a greater SCR to the upright than the inverted faces. Whereas individuals with WS, relative to both TD and PWS, showed the greatest magnitude of SCR to the upright face and the lowest magnitude of SCR to the inverted face, those with TD and PWS showed the opposite pattern, with the PWS group presenting the most dramatic contrast to the WS group. There was a trend toward a main effect of affect category, and no significant between-group differences were found in baseline skin conductance level or HR reactivity. This pattern suggests atypical organization of autonomic function in WS, with increased responsiveness to upright faces, and decreased responsiveness to inverted faces. Marked differences between the WS and PWS groups show that the atypical pattern observed in WS is not due to intellectual impairment alone.
Supported in part by HD33113



Genes, Neural Systems, and Social Behavior

Neural activity to positive and negative emotional expressions in Williams syndrome

D. L. Mills[1], A. Yami[2], A. Hood[1], E. Sheehan[1], A. Galaburda[3], A. L. Reiss[4], J. R. Korenberg[5], and U. Bellugi[2]

[1]Emory University*, [2]The Salk Institute for Biological Studies,, [3]Beth-Israel Deaconess Hospital, [4]Stanford University [5]Cedars-Sinai Medical Center, UCLA

INTRODUCTION

Williams Syndrome (WS) is a neurodevelopmental disorder caused by the deletion of approximately 25 genes on chromosome 7. The typical WS socio-cognitive phenotype is characterized by increased affiliative drive and attention to faces, with relative strengths in face recognition and language processing, and relative deficits in spatial processing.

People with WS are often described as "hypersocial", empathetic, and show increased responsiveness to others in distress. Behavioral evidence suggests that people with WS are less accurate at identifying negatively valenced facial expressions such as fear; and fMRI studies have shown decreased activation of the amygdala in response to fearful expressions. Yet there is very little known about the neural mechanisms underlying processing of different emotional expressions in WS.

In this study we tested the hypothesis that brain activity to positive emotional expressions would be enhanced individuals with WS, whereas brain activity to fearful expressions would be attenuated.

Stimuli

The stimuli consisted of 200 different photographs (half male / half female):

- 50 faces with happy expression
- 50 faces with a fearful expression
- 50 faces with a neutral expression
- 50 scrambled faces

100 faces of each expression were rated by 40 Emory undergraduate students on a Likert-type scale for each emotional category. Only the best exemplars of each category were used as stimuli.

Participants

	WS n=26	TD n=25	DD n=11
Participants			
Sex	12M, 14F	11M, 13F	5M, 5F
Mean age	30	30	34
Age Range	17-48y	18-48y	19-55y
FSIQ	65	106	65
VIQ	70	108	69
PIQ	65	103	66

FISH analyses confirmed a full deletion of the band 7q11.23 on Chromosome 7 for all WS participants (Korenberg, et al. 2001).

Developmentally Delayed (DD) participants included two individuals with Down syndrome and nine individuals with mental retardation and developmental delay without a known etiology.

Procedure

EEG was recorded from 64 channels using an EGI sensor net with a bandpass of .1 to 100 Hz. ERPs were averaged separately to pictures of happy, fearful, and neutral emotional expressions and to scrambled faces.

Each picture was presented for 1750 milliseconds. A fixation cross was shown during the interstimulus interval (ISI). The duration of the ISI was jittered with a mean of 1000 ms plus or minus 200 ms. The subject's task was to press a button as quickly and accurately as possible to determine if the photograph was of a male or female.

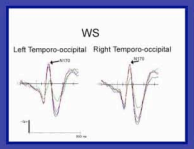
Group	% correct	Std. Dev
WS	89	18.91
TD	98	4.11
DD	89	9.46

The TD group performed significantly better than the TD and DD groups. There were no other group differences.

Results - N170

The N170 component is typically larger to faces than non-face stimuli.

In the figures below, ERPs to happy faces are depicted by blue lines, to neutral expressions by black lines, and to scrambled faces by green lines. Negative voltage is plotted up.



For adults with WS, the N170 was symmetrical, was larger to faces than scrambled faces (p=.03), and did not differ by emotion

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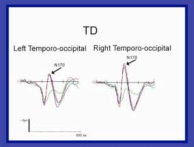
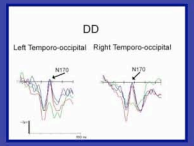


For TD adults, the N170 was larger over the right than left hemisphere (p=.02), was larger to faces than scrambled faces (p=.002), and did not differ by emotion.

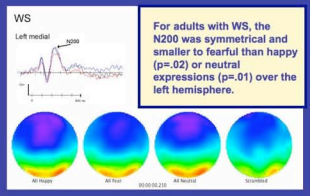


For DD adults like WS, the N170 was symmetrical, but did not significantly differ for faces and scrambled faces (p=.2), or emotional expression.

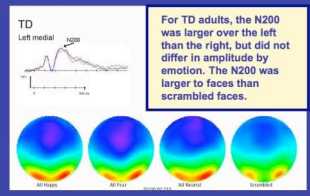
The N170 tended to be larger for the WS than other groups, but only approached significance (p=.06). However, there was significant group x emotion interaction (p=.03). For happy faces, the N170 was larger for WS than TD adults over the left hemisphere; for fearful expressions the N170 was larger for WS than DD individuals. In spite of apparent group differences in amplitude on the plots, there were no significant differences for neutral or scrambled faces.

Results- N2

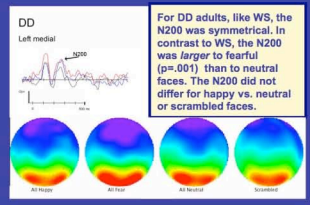
In our previous studies the N2 to faces was larger in WS than TD or adults with autism. The findings were interpreted as showing increased attention to faces in WS. We hypothesized that for individuals with WS the N2 amplitude would be reduced to fearful expressions and larger to happy faces relative to neutral expressions.



For adults with WS, the N200 was symmetrical and smaller to fearful than happy (p=.02) or neutral expressions (p=.01) over the left hemisphere.



For TD adults, the N200 was larger over the left than the right, but did not differ in amplitude by emotion. The N200 was larger to faces than scrambled faces.



For DD adults, like WS, the N200 was symmetrical. In contrast to WS, the N200 was *larger* to fearful (p=.001) than to neutral faces. The N200 did not differ for happy vs. neutral or scrambled faces.

The results are consistent with the hypothesis that individuals with WS show reduced activity to fearful expressions independent of IQ effects.

DISCUSSION

The N170 is a face sensitive component most likely generated in the fusiform gyrus. The N170 was larger to faces than scrambled faces for the TD and WS groups but not for the DD adults. The results also showed that the typical right greater than left N170 asymmetry was apparent only for the TD adults. This suggests that in spite of increased interest and experience with faces, individuals with WS do not show a normal pattern of right hemisphere specialization for faces.

The amplitude of the N200 differed by group in responsiveness to emotional expressions. As predicted for the participants with WS, the N200 was smaller in amplitude to fearful than neutral or happy expressions. In contrast, the DD controls showed increased neural activity to fearful expressions. The atypical pattern observed in WS is not due to retardation alone because of the differences between the WS and IQ matched DD groups.

Reduced activity to fearful expressions in individuals with WS is consistent with previous behavioral, MRI and fMRI data. These data are consistent with previous research suggesting abnormal reponsiveness of the amygdala to fearful expressions in WS (Myer-Lindenberg, et al., 2005, Mobbs et al. 2004).

The predicted greater activity to happy vs. neutral expressions was not observed for the WS group. This suggests that the social phenotype observed in WS may not be due to hypersensitivity to positive emotions.

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POSTER SYMPOSIUM: SOCIETY FOR NEUROSCIENCES, 2007

Symposium Title: GENES, NEURAL SYSTEMS, AND SOCIAL BEHAVIOR

Stemming from NICHD PPG 33113
"Williams Syndrome: Bridging Cognition, Brain and Gene"

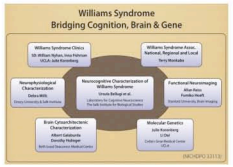
No. 696.7

NEURAL ACTIVITY TO POSITIVE AND NEGATIVE EMOTIONAL EXPRESSIONS IN WILLIAMS SYNDROME

(D. Mills, A. Yam, A. Hood, E. Sheenan, A. Galaburda, A.L. Reiss, J.R. Korenberg, & U. Bellugi)

Williams Syndrome (WS) is a neurodevelopmental disorder caused by the deletion of approximately 25 genes on chromosome 7. The typical WS socio-cognitive phenotype is characterized by increased affiliative drive and attention to faces, relative strengths in face recognition and language processing, and relative deficits in spatial processing. People with WS are often described as "hypersocial", empathetic, and show increased responsiveness to others in distress. Behavioral evidence suggests that people with WS are less accurate at identifying negatively valenced facial expressions such as fear; and fMRI studies have shown decreased activation of the amygdala in response to fearful expressions. Yet, there is very little known about the neural mechanisms underlying processing of different emotional expressions in WS. The present study used the event-related potential (ERP) technique to examine neural activity to pictures of faces displaying happy, fearful and neutral expressions and to scrambled faces, in adults with WS, age- and gender-matched typically developing adult controls (TD), and age, gender, and IQ-matched developmentally delayed controls (DD). The task required participants to sort face stimuli by gender with a button press. The results showed that a negative going component at 200 ms, N200, was larger to neutral than to scrambled faces for the TD and WS groups. The opposite pattern was observed for the DD controls. For the happy vs. fear comparison, the N200 did not differ by emotional expression for the TD participants. However the N200 was larger to both happy and neutral than to fearful expressions for individuals with WS. The reduced activity to fearful expressions is consistent with previous research based on behavioral, MRI and fMRI data. In stark contrast, the DD controls showed increased neural activity to fearful expressions. Our working hypothesis is that abnormal responsiveness to the fearful stimuli may be related to abnormal amygdala function to faces in WS. The N170 component has been shown to be larger to faces than other stimuli. For all groups the N170 was larger to faces than to scrambled faces. Emotional expression modulated the amplitude of the N170 for both the TD and DD control groups, but not for the WS participants. This pattern suggests atypical organization of emotional processing in WS, with decreased responsiveness to fearful expressions, and increased responsiveness to positive expressions. Moreover, the atypical pattern observed in WS is not due to retardation alone as evidenced by the marked differences between the WS and DD groups. (See also other symposium submissions titled: Genes, Neural Systems, and Social Behavior).

Supported in part by HD33113



696.8/P16

GENES, NEURAL SYSTEMS and SOCIAL BEHAVIOR: Association between Cerebral Shape and Social Use of Language in Williams Syndrome

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Introduction

Williams syndrome (WS)

• A neurogenetic disorder resulting from a ~1.6Mb chromosomal microdeletion (~28 genes) at band 7q11.23 [1]

• A multisystem disorder characterized by aberrant development of the brain and a unique profile of cognitive and behavioral features [2]

Cognitive & behavioral profile in WS

▪ 'overly friendly', increased interest in social interactions
▪ IQ typically in Mild to Moderate range of mental retardation
▪ major deficits in spatial processing
▪ relative strengths in language and face processing
▪ increased use of affective prosody and social language in narratives

Neuroanatomical abnormalities in WS [3, 4]

▪ overall brain volume decreased by about 10%, particularly attributable to reductions in parietal and occipital lobe volumes

▪ regions with relatively increased gray matter volume: ventral prefrontal cortex, superior temporal gyrus, amygdala and posterior vermis of the cerebellum

▪ decreased parieto-occipital lobe volumes relative to frontal volumes, thought to lead to a more flat bending angle of the corpus callosum and cerebrum compared to typical

Two key brain networks involved in regulating social behavior [5, 6]

1. Ventral circuit monitoring social cognition includes:
   ▪ amygdala, anterior cingulate, superior temporal gyrus and mediodorsal nucleus of the thalamus

   ▪ social information processed in the amygdala is transferred to the orbitofrontal cortex where social responses and behaviors are selected

2. Striatal-thalamocortical loop:
   ▪ prefrontal regions- the orbital and lateral cortex and anterior cingulate

   ▪ in charge of behavioral inhibition and includes prefrontal regions: the orbital and lateral cortex and anterior cingulate

Purpose of the present study

▪ Employ signal detection method (QROC) to identify brain regions most strongly associated with WS vs typically developing controls (TD) [7]

▪ Evaluate the relationship between these brain regions, and a salient feature of the WS social phenotype: the atypically high use of social and affective language (social engagement devices, SED) in narratives

Abbreviations used:
PFC: prefrontal cortex
QROC: quality receiver operating characteristic curve
SED: social engagement devices
STG: superior temporal gyrus
TD: typically developing controls
VAPFC: ventral anterior prefrontal cortex
WS: Williams syndrome

Methods

Participants

	WS	TD	Evaluative Language Subset WS	Evaluative Language Subset TD
	n = 41	n = 40	n = 27	n = 12
GENDER	19 M, 22 F	16 M, 24 F	15 M, 12 F	2 M, 10 F
AGE years M (SD)	29.4 (9.0)	27.5 (7.4)	28.1 (10.0)	26.0 (7.0)
Age range (years)	12.4 - 50.4	18.2 - 49.2	12.4 - 50.4	18.5 - 41.5
Verbal IQ	72.0 (7.5)	~100.0 (10.0)	72.7 (8.1)	102.4 (10.8)
Performance IQ	67.5 (8.7)	~100.0 (10.0)	66.6 (9.4)	104.5 (13.2)

Materials & Procedures

MRI

• Participants were scanned with a General Electric 1.5 Tesla Signa Scanner. Sagittal brain images were acquired with a three-dimensional (3D) volumetric radio frequency spoiled gradient echo pulse sequence using the following scan parameters: repetition time, 24 ms; echo time, 6 ms; flip angle, 45°; slice thickness, 1.2 mm; field of view, 24 cm; and matrix size, 256 x 192 for 124 contiguous slices

•Data processing steps following a protocol including removal of non-brain tissues from the images, correction of equipment related image artifacts, and separation (segmentation) of tissue components (grey, white, CSF) [8]

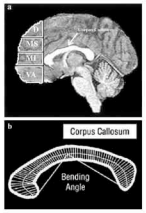


Figure 1:

a- subdivisions of the prefrontal cortex:
D = dorsal, MS = middle-superior,
MI = middle-inferior, VA = ventral-anterior

b- The bending angle of the corpus callosum: the angle created by lines connecting the most anterior and posterior points of the corpus callosum with the midline

Social Language

• Participants were presented with the 24-page wordless picture book "Frog, where are you?" [9], and asked to tell the story to the experimenter

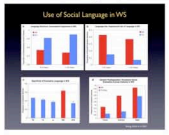
• Stories were transcribed and coded for **evaluative language** [10]: elements within the story that convey the narrator's perspective on events

  • cognitive inferences: inferences about the characters and their motivations e.g. "The *boy was happy* to see his frog"

  • **social engagement devices** (SED): phrases that add interest, serving to attract and maintain the listener's attention: e.g. sound effects, intensifiers (*very, so*, "The boy was *rea…lly* sad"); and character speech ("*The boy said, 'Oh, little froooggie…'*); "*All of the sudden…*"

• SED scores reflect the proportion of SED to the total use of evaluation devices

More about "the frog story" and the use of social evaluation devices in WS:



The extensive use of social devices to engage the listener is a consistent finding, and is a central feature of the unique social behavior of individuals with WS.

[10] **Figure 2**: 1. Mastery of morpho-syntax in narratives of children with WS and TD (1a), and use of social evaluation in narratives of children with WS and TD (1b). 1c. The specificity of evaluative language in WS. WS exhibit greater use of social language than TD, those with early focal lesions (FL), language impairment (LI), and high functioning autism (HFA). 1d. Excessive social affective evaluation in WS across languages and cultures, underscoring a genetic predisposition for use of social language in WS

Results

The Quality Receiver Operating Characteristic Curve (QROC) analysis

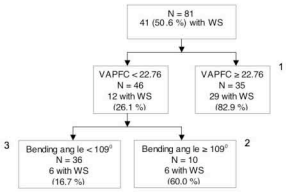


Figure 3:

• The QROC was applied to identify the brain regions that most sensitively and specifically distinguished WS from controls. Brain regions included in this analysis have been reported to be abnormal in subjects with WS .

• Gray matter volumes included in the QROC analysis: ventral and dorsal anterior prefrontal regions, dorsal-anterior cingulate, parietal and occipital lobes, superior temporal gyrus, cerebellum, amygdala, hippocampus, and thalamus. The bending angle of the corpus callosum also was included [11].

• The end result of the QROC partition yielded three groups:
  •(1) VAPFC ≥ 22.76 cm³, 82.9% with WS;
  •(2) VAPFC < 22.76 cm³ and bending angle ≥ 109°, 60.0% with WS;
  •(3) VAPFC < 22.76 cm³ and bending angle < 109°, only 16.7% with WS.

• **The combination of large or small VAPFC and large bending angle had 85.4% sensitivity and 75.0% specificity in distinguishing WS from controls.**

Association between social evaluation device scores and VAPFC size

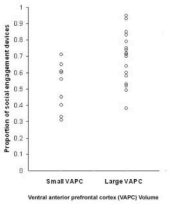


Figure 4:

The WS subgroup with large VAPFC had significantly higher SED scores than the subgroup with small VAPFC (0.68 ± 0.16 vs. 0.51 ± 0.15, t = -02.5, P < 0.05). There was also a significant positive correlation between adjusted VAPFC volume and SED scores of the WS group (r = 0.50, P < .01).

• **Linear regression indicated that only adjusted VAPFC volume significantly predicted SED scores** (b = 0.52, r2 change = 0.28, P = 0.01), whereas amygdala volume (b = -0.19, P = 0.32) and STG volume (b = 0.08, P = 0.67) did not.

• There were no significant correlations between VAPC volume and other aspects of the WS phenotype such as VIQ (r = 0.10, P = 0.63) or PIQ (r = 0.33, P = 0.09).

Discussion

Brain regions that distinguish Williams syndrome

• We utilized a signal detection method (QROC) that has rarely been used in imaging studies. QROC was instrumental in the identification of specific brain measures that are most closely associated with WS.

• Using the QROC we identified the VAPFC, and consequently elucidate its association with the use of social language in WS.

• The VAPFC, as well as the bending angle of the corpus collusum, are strong distinguishing characteristics of individuals with WS.

The relationship between brain and behavior

• The orbitofrontal cortex, which partially overlaps with the VAPFC region defined here, is a pivotal part of the ventral circuit that monitors social cognition and regulates emotional states and behavior.

• The abnormal morphology of the VAPFC may be associated with enhanced use of social language in individuals with WS.

• Thus our results associating the VAPFC with abnormal social use of language in WS provide support for the important role of prefrontal cortex abnormalities to the social phenotype in WS.

Genes to Brain to Behavior

• Our findings support that aberrant neurodevelopment of the ventral anterior region of the prefrontal cortex is an important factor contributing to the unique cerebral morphology of individuals with WS, as a consequence of haploinsufficiency of genes from the deleted region.

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POSTER SYMPOSIUM: SOCIETY FOR NEUROSCIENCES, 2007

Symposium Title: GENES, NEURAL SYSTEMS, AND SOCIAL BEHAVIOR

Stemming from NICHD PPG 33113
"Williams Syndrome: Bridging Cognition, Brain and Gene"

No. 696.8

ASSOCIATION BETWEEN CEREBRAL SHAPE AND SOCIAL USE OF LANGUAGE IN WILLIAMS SYNDROME

(Y.M. Searcy, D. Gothelf, J. Reilly, U. Bellugi, T. Larne-Amos, D. Mills, J.R. Korenberg, A. Galaburda, & A. Reiss)

Williams syndrome is a neurogenetic disorder resulting from a hemizygous microdeletion at band 7q11.23. It is characterized by aberrant development of the brain and a unique profile of cognitive and behavioral features. We sought to identify the neuroanatomical abnormalities that are most strongly associated with Williams syndrome employing signal detection methodology. Once identified with a Quality Receiver Operating Characteristic Curve, we hypothesized that brain regions distinguishing subjects with Williams syndrome from controls would be linked to the social phenotype of individuals with this disorder. Forty-one adolescents and young adults with Williams syndrome and 40 typically developing controls matched for age and gender were studied. The Quality Receiver Operating Characteristic Curve identified a combination of an enlarged ventral anterior prefrontal cortex and large bending angle of the corpus callosum to distinguish between Williams syndrome and controls with a sensitivity of 85.4% and specificity of 75.0%. Within the Williams syndrome group, bending angle significantly correlated with ventral anterior prefrontal cortex size but not with other morphometric brain measures. Ventral anterior prefrontal size in subjects with Williams syndrome was positively associated with the use of social engagement devices in a narrative task assessing the use of social and affective language. Our findings suggest that aberrant morphology of the ventral anterior prefrontal cortex is a pivotal contributing factor to the abnormal size and shape of the cerebral cortex and to the social-linguistic phenotype of individuals with Williams syndrome. (See also other symposium submissions titled: Genes, Neural Systems, and Social Behavior).
Supported in part by HD33113

The Mirror Neuron System And "Hypersociability" In Williams Syndrome

696.3

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INTRODUCTION

Striking Feature of Williams syndrome (WS)
Known for excessive sociability and empathy for others [1], but still controversial [2].

Neural Systems Subserving Empathy and Social Cognition mPFC, temporal pole (TP), posterior STG, and the Mirror Neuron System (MNS) including the IFG and rostral IPL [3,4]

Goal of Study To investigate these brain systems in a meta-analysis of 4 fMRI studies related to social cues (face, gaze and affect processing).

METHODS

Participants
- WS: n=36, age=30.9±12.0, F:M=30:6, FSIQ: 65.6±11.3
- CONTROLS: n=37, age33.1±10.5, F:M=28:9, FSIQ: 117.1±11.0

fMRI Tasks
1. Matching emotions of faces (block), 2. Matching gender of neutral faces (block), 3. Matching gender of faces with emotions (event-related), 4. Judging gaze direction and face orientation of neutral faces (block)

fMRI Data Collection, Processing & Analyses
1. DATA COLLECTION: 1.5 & 3.0T GE, TR 2s, whole-brain, **2. PREPROCESSING:** (Slice-time correction), realignment, normalization and smoothing (8mm-FWHM). **3. INDIVIDUAL SJ STATS:** Fixed effects GLM comparing all face stimuli to resting or scrambled faces. **4. GROUP STATS:** Random effects GLM within and between subjects (p=0.05 corrected).

Interpersonal Reactivity Index (IRI) [5]
WS vs. Controls: all P's > 0.1
Regressing out IQ did not change the results

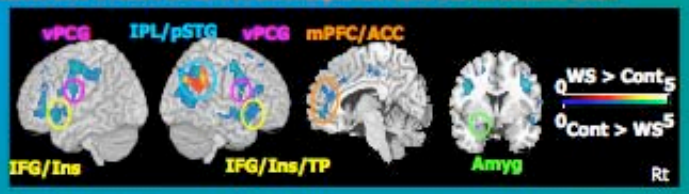
	Perspective taking	Fantasy	Empathic concern	Personal distress
WS (n=10)	13.4(1.9)	12.7(3.0)	21.3(1.8)	15.5(3.1)
Controls (n=10)	17.1(1.3)	16.6(2.4)	20.7(1.7)	11.8(1.9)

ABBREVIATIONS ACC: anterior cingulate cortex, AF: arcuate fasciculus, Amyg: amydgala, FG: fusiform gyrus, IFG: inferior frontal gyrus, Ins: insula, IPL: inferior parietal lobule, mPFC: medial prefrontal cortex, SLF: superior longitudinal fasciculus, STG: superior temporal gyrus, TP: temporal pole, vPCG: ventral precentral gyrus

RESULTS

MNS & 'Social Brain' Networks In WS And Controls

Controls Activate These Systems More Than WS



Controlling for 1. age, gender and task performance, and 2. voxel-based gray matter morphometry showed similar results.

Covariation Between Brain Activation And Empathy Is Different In Controls & WS

Higher empathy scores associated positively with activation in:

CONTROLS anterior MNS and 'social brain' regions (mPFC, IFG/PCG/insula, STG)

WILLIAMS more posterior regions (IPL, occipital, thalamus)

CONCLUSIONS

- Brain regions associated with the MNS and the Social Brain are dysfunctional in WS 'compared to healthy controls'.
- IPL hyperactivation in face of anterior/limbic hypoactivation in WS suggest a disconnect between these systems (see SFN Poster 696.4 & [6] for right SLF/AF DTI dysfunction in WS).
- Results from correlating empathy scores and brain activation suggest the reliance of visual-spatial and perceptual processing in WS.

ONGOING ANALYSES

1. Confirm analyses in native space.
1. Functional connectivity.

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POSTER SYMPOSIUM: SOCIETY FOR NEUROSCIENCES, 2007

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Stemming from NICHD PPG 33113
"Williams Syndrome: Bridging Cognition, Brain and Gene"

No. 696.3

THE MIRROR NEURON SYSTEM REFLECTS HYPERSOCIABILITY IN WILLIAMS SYNDROME

(F. Hoeft, D. Ng, A. Karchemskiy, N. Kobayashi, J.C. Bavinger, A. Galaburda, D. Mills, J.R. Korenberg, U. Bellugi, & A.L. Reiss)

Williams syndrome (WS) is a neurodevelopmental disorder caused by a hemizygous deletion of approximately 25 genes on chromosome 7q11.23. One striking feature of the syndrome that distinguishes it from other disorders is excessive sociability and empathy for others (Meyer-Lindenberg et al. Nature Reviews Neuroscience 2006). The mirror neuron system (MNS) including the inferior frontal and inferior parietal regions, and the superior temporal region which provides visual input to the MNS, have been linked to empathy and socialization (Rizzolatti and Craighero, Annual Review of Neuroscience, 2004). It may therefore be hypothesized that individuals with WS show greater recruitment of regions implicated in the MNS, especially during processing of facial stimuli that convey social cues. In this preliminary study, we pooled previously collected functional magnetic resonance imaging (fMRI) data from 4 studies of affect and gaze processing. We compared 43 WS and 38 typically developing (TD) individuals. Relative to TD individuals the WS group showed increased activation in the left inferior frontal, bilateral rostral inferior parietal and right superior temporal regions during the perception of affective and gaze facial stimuli. On the other hand, compared to WS, the TD group showed increased activation in the right inferior frontal region only. This study shows promising initial results suggesting putative neural systems associated with excessive sociability in WS. Future studies using tasks that more effectively target the mirror neuron system as well as examination of associations between brain activation in these regions and behavioral measures of empathy are warranted. (See also other symposium submissions titled: Genes, Neural Systems, and Social Behavior). Supported in part by HD33113

White matter abnormalities in Williams Syndrome as Measured by Diffusion Tensor Imaging (DTI)

696.4

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INTRODUCTION

- Williams syndrome is a genetic disorder associated with deficits in visuospatial processing, [1].
- The (right > left) Superior Longitudinal Fasciculus (SLF) is related to the dorsal stream and is important for visuospatial attention / processing.
- SLF connects the posterior parietal cortex (PPC) and the posterior lateral prefrontal cortices (PFC) [2].
- Diffusion Tensor Imaging (DTI) provides the ability to assess white matter integrity in pathways within the brain[3,4].
- We predicted that Williams subjects exhibit aberrant white matter integrity in the (right) SLF but not in the Inferior Longitudinal Fasciculus (ILF).

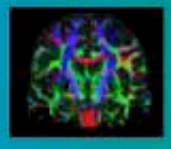
METHODS

Participants

- 10 Williams (WMS): 6 males, 10 Typically Developing (TD): 7 males and 10 Developmentally Delayed (DD): 3 males.
- Age (WMS): *M* = 26.28, *SD* = 7.5; (TD): *M* = 27.8, *SD* = 9.5; (DD): *M* = 23.2, *SD* = 5.5.
- IQ (WMS): *M* = 65.4, *SD* = 10.6, (TD): *M* = 114.3, *SD* = 12.8, (DD) *M* = 71.1, *SD* = 16.2.

Imaging Parameters

- 3T Signa LX Scanner
- Single-shot spin-echo echo-planar sequence
- TE=68.4 ms, Flip=90 degrees
- 33 slices, parallel to AC-PC: 3.8 mm thick
- FOV=240 mm x 240 mm



TBSS

- Tract-Based Spatial Statistics (5)
- Group whole brain analysis of FA differences

ROQS

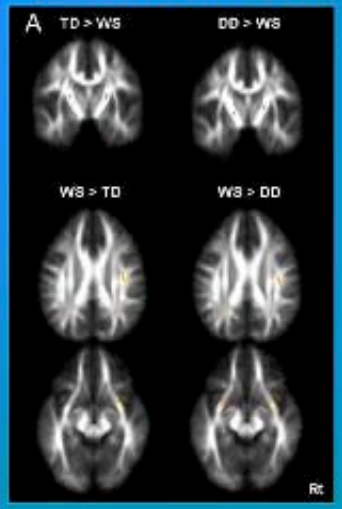
- Reproducible Objective Quantification Scheme (6)
- Semiautomatically segments white matter based on user-selected seed pixels

Fiber-Tracking

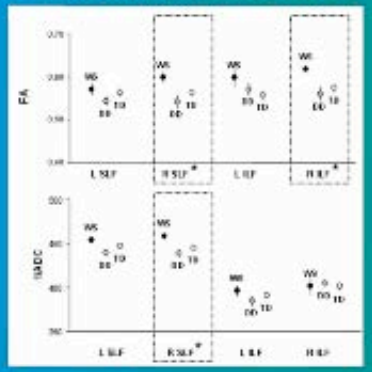
- User defined Regions of Interest
- Tracts fibers based on *a priori* defined FA and turning angle threshold

RESULTS

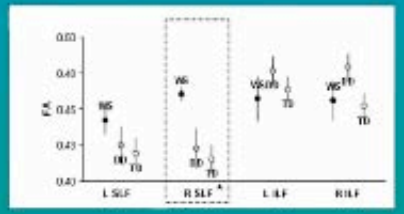
A. WMS vs. Controls: TBSS

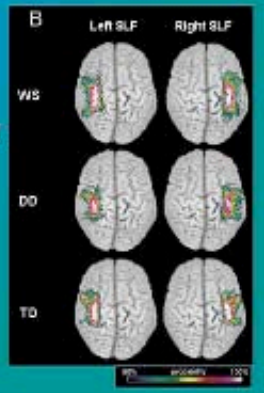


B. WMS vs. Controls: ROQS



C. WMS vs. Controls: Fiber Tracking





CONCLUSIONS

- Data provide a neural correlate to the link between visuospatial dysfunction in WMS with structural abnormality within the Rt SLF.
- Increased FA may be indicative of reduced branching of this pathway.
- Future directions: investigate how specific genes (partial deletions) affect SLF white matter integrity.

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POSTER SYMPOSIUM: SOCIETY FOR NEUROSCIENCES, 2007

Symposium Title: GENES, NEURAL SYSTEMS, AND SOCIAL BEHAVIOR

Stemming from NICHD PPG 33113
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WHITE MATTER ABNORMALITIES IN WILLIAM SYNDROME AS MEASURED BY DIFFUSION TENSOR IMAGING (DTI)

(A.L. Reiss, N. Barnea-Golary, B. Haas, G. Golarai, D. Ng, A. Karchemskiy, A. Galaburda, J.R. Korenberg, U. Bellugi, & F. Hoeft)

Williams syndrome (WS) is a neurodevelopmental disorder caused by a hemizygous deletion of approximately 25 genes on chromosome 7q11.23. One of the characteristic features of the syndrome is severe visuospatial construction deficits with relatively good face and object processing skills as well as overly-social behavior. Associated functional and gray matter abnormalities have been reported in the dorsal visual pathway with an intact ventral visual pathway. Whether there are associated abnormalities in white matter integrity is unknown. Here we examined 10 individuals with WS compared with 10 typically developing (TD) and 10 developmentally delayed (DD) controls using diffusion tensor imaging (DTI).

Three DTI analysis methods were used to ensure the reliability of the results: (1) voxel-based analysis using tract-based spatial statistics (TBSS), (2) tractography using DtiStudio, and (3) Reproducible Objective Quantification Scheme (ROQS). Highly convergent results were generated using these independent analytical methods. Significantly "greater" fractional anisotrophy (FA) was observed in theright superior longitudinal fasciculus (SLF) in individuals with WS compared to both control groups but not in the left SLF. Greater FA in the right SLF was associated with worse visuospatial skills in subjectswith WS indicating that the findings are clinically relevant. The right inferior LF (ILF) were significantly different from the TDs and DDs in TBSS and ROQS but not fiber-tracking, which could be related to the superior face processing skills in WS. While the exact mechanism of the increased FA is unclear, the results indicate that individuals with WS show abnormalities in white matter regions associated with visuospatial skills, as consistent with their behavioral profile/phenotype. (See also other symposium submissions titled: Genes, Neural Systems, and Social Behavior). Supported in part by HD33113

Genes, neural systems, and social behavior: Genetic origins of sociability in Williams syndrome

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Abstract

Genetic contributions to human cognition and behavior are intuitively clear but fiendishly difficult to define. The genetic deletion of about 25 genes in Williams syndrome (WS) provides a unique model to relate the role of single genes or gene clusters to the shift in social behavior described as hypersociability, increased gaze and attention to strangers (see Bellugi et al, this symposium). To narrow the gene(s) responsible for WS behavior, we initially established and applied genomic maps and reagents spanning the WS region to analyze a large cohort of families, to define a region commonly deleted. This provided the basis for establishing the variation in cognition and behavior typical of WS with full deletions. Rare individuals with smaller deletions provide the opportunity to ask whether a subset of genes may contribute to a subset of WS features. In the current work, we report genetic, developmental, physical, cognitive and behavioral data to show that an individual deleted for all of the typical WS genes except for the telomeric region, shows less hypersociability and less prolonged eye contact than typical WS. The individual was ascertained at 14 months as atypical for WS, and was studied through age 4 4/12 y, with normal developmental milestones but the small size and supravalvular aortic stenosis seen in WS. Genetic analyses included breakpoint determination using high resolution oligonucleotide arrays, confirmation by multicolor fluorescence hybridization with a panel of 45 BACs, somatic cell hybrids, and quantitative gene expression in LB cell lines using qRT-PCR of 12 WS genes. Deletion retained FKBP6-region distal to CYLN2. Social behavior was evaluated using the SISQ (Salk Institute Sociability Questionnaire) at 2 7/12 y, #5889 differed from WS ($p < 0.05$) and was in the normal range for Global Sociability and Approach Strangers. Further analyses utilized an computer based ethogram in which behavior was evaluated in a free play situation with a novel adult. In contrast to a WScohort, #5889 was less likely to prolong eye contact and less likely to spend time close to the novel adult, and less likely to engage in social interaction. These results suggest genes distal to CYLN2 contribute to WS behavior as measured in #5889 and provide the basis to reason from molecular genetics and cellular detail to the brain networks modulating human social behavior.

Goal

To understand the neural pathways underlying social behavior in WS, to identify genes responsible for the variability and to evaluate primate models. Studies such as this with atypical deletions help to dissect and reveal distinctive behaviors. These will allow us to infer anatomic and physiologic interconnections and to understand the molecular basis of human cognition and behavior.

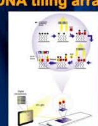
Introduction

Williams syndrome (WS) provides a remarkable model for studying human cognition because a hemizygous deletion of merely ~20 genes in chromosome band 7q11.23 is responsible for the unusual array of characteristics present in WS. This unique circumstance enables us to elucidate pathways that lead from genes to behavior; however, the contribution of each gene to the WS phenotype remains unknown. The distinctive pattern of physical, cognitive, and behavioral traits shown in WS is one facet that allows the parsing of the WS profile and so careful comparisons of differences between individuals with WS. Coupled with better methods that sharpen breakpoint analyses of individuals with atypical deletions, we have recently seen the dawn of drawing conclusions about specific genes and the phenotypes for which they are responsible. In this poster, we aim to draw another such conclusion, this time in regards to the connection between GTF2I and WS social behavior

Techniques

I. Oligonucleotide Isothermal High Resolution DNA tiling array

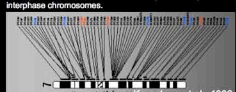
Chromosomal breakpoints were mapped to sub-kilobase resolution using high resolution DNA tiling arrays. DNA from cell lines or individuals was used to probe a custom high density array of chromosome 7 with a median distance of 1/90 bp ;the array contained 45-85 bp oligonucleotides probes which were adjusted in their C+G content to yield similar optimum hybridization temperatures (isothermal array). The probes tiled all of chromosome 7, including the short arm to allow the identification of breakpoints at 200-300 bp resolution (Urban et al., 2006; Korbel et al., 2007). DNA from the patients was labeled with Cy3 and hybridized to the array along with DNA from a reference pool of 7 individuals that was labeled with Cy5. Copy number changes were called using the BreakPtr algorithm.

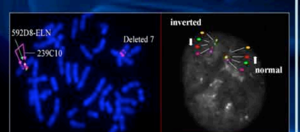


Competetive hybridization schemes utilize similar approaches for BAC arrays and oligonucleotide arrays. These are symbolized at the right. On the left is shown the approach for oligonucleotide array generation using maskless sequencing.

II. Whole Genome BAC tiling Array: High Resolution Fluorescence in situ hybridization (FISH)

The chromosomes were prepared from the peripheral blood by following the protocols as described by Korenberg et al. (1995). Four-color FISH analysis was performed with BACs using a method as described previously with some modifications (Korenberg et al. 2003). Briefly, the BAC DNAs were isolated using a Qiagen Midprepkit and DNAs were directly labeled FITC, Texas Red, Cy3, and Cy5 and hybridized to metaphase and interphase chromosomes.





III. Southern blotting and PCR

Procedures for DNA isolation and digestion, agarose gel construction, Southern Blotting, probe labeling, hybridization, and autoradiogram development were conducted as described by Korenberg et al. (1989). All probes were isolated as DNA fragments. The blots were hybridized with PCR fragments generated from large scale sequence analyses at 5 kb intervals throughout the cosmid 183E01. Densitometric analyses were performed by using the Alpha Innotech density measurement program (Alpha Innotech Corporation, San Leandro, CA).PCR was preformed using the DNAs extracted from the hybrid cell line with a total of 39 pairs of customer designed primers covering the region in between TFII-IRD1 and TFII-I.

V. Psychometric Testing

All subjects underwent a battery of neurocognitive measures over a 3-day period. Both standardized and experimental measures were used to assess four neurocognitive domains: general intelligence, language, visuospatial, and face processing. Measures included the Preschool Language Scale, 3rd edition, the Salk Institute Sociability Questionnaire (SISQ), and the ethogram described below. Individual scores from patient 5889 were compared to those of the full deletion WS group (n = 101) by conversion to z-scores with a mean of zero and standard deviation of one. Z-scores of 2.00 or higher were considered to be statistically significant.

Behavioral data – Ethogram:

The Noldus observer was used to code toddlers in free play situations either with their mothers or with a novel adult (the experimenter). Coding was done by two undergraduates blind to the study goals and unfamiliar with WS. These raters were trained on videotapes of typically developing toddlers until their inter-rater reliability was consistently > 0.85. Behaviors rated are noted below.

- Gaze location (looking, eye contact, Toy/Play, other)
- Interactive behaviors (imitating, offering, pointing, rejecting, requesting, showing, vocalising, sign gestures)
- Interaction type (dyadic, triadic joint, mediated joint, non-social)
- Play style (symbolic play, non-specific)
- Physical location (far away, near, touching)
- Affect (neutral affect, negative affect, positive affect)
- Change in Proximity (approach, withdraw)

Results

I. Subject

	5889
GROWTH	
Feeding Difficulty	-
Post term birth (>41 wks)	
Failure to Thrive	+
Vomiting or GI Reflux	-
Prolonged Colic (>4mo)	-
Chronic Constipation	-
FACIAL FEATURES	
Bitemporal Narrowing	+
Epicanthal folds	+
Strabismus	+
Short Nose	+
Full Cheeks	+
Long Philtrum	+
Small, spaced teeth	+
Periorbital Fullness	+
Stellate Iris	+
Bulbous Nasal Tip	+
Malar hypoplasia (abnormal cheekbones)	+
Prominent Lips	+
Malocclusion	-
Medial Eyebrow flare	+
CARDIOVASCULAR DEFFECTS	
Supravalvular aortic stenosis	+
Peripheral Pulmonic Stenosis	-
Other Cardiac Defects	
Other Vascular Stenosis	
Supravalvular pulmonic stenosis	-
Hypertension	-

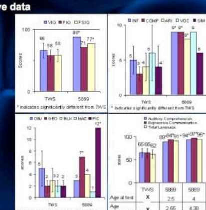
The family of subject 5889 reported in this study gave informed consent for genetic, clinical and neuropsychologic studies, as approved by the IRB of Cedars-Sinai Medical Center, Los Angeles and The Salk Institute. The clinical features were evaluated by experienced geneticists longitudinally from 14 months through 4 years.

II. Psychometric testing

The physical development of 5889 follows typical WS patients for her age; however, her language abilities were in the normal range for age, her visual-spatial performance relatively high and she was not indiscriminantly hypersocial with strange adults, which is extremely atypical of WS individuals.

Consequently subject 5889 is unlike individuals with WS (and putatively typical deletions) who exhibit delay in but later, relative strengths in language combined with weaknesses in aspects of visual spatial processing, as well as a typical idiosyncratic "hypersocial" behavior and increased interest in non-familiar individuals.

1. Cognitive data

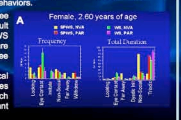


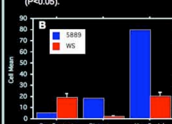
2. Social behavior test

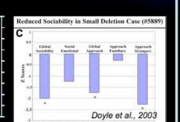
A. Behavioral Ethogram for Patient 5889 (SPWS) and Typical WS Individuals (WS) in Presence of Parent (PAR) or a Novel Adult (NVA). Left: frequency of specified behaviors; right: duration of specified behaviors.

B. Ethogram Showing Duration of Three Behaviors in Presence of a Novel Adult for Patient 5889 and Typical WS Children of Similar Age. Differences are highly significant (P<0.01) for all three behaviors.

C. Difference Between #5889 and Typical WS Children on the SISQ. Differences on Global Sociability and Approach Strangers are statistically significant (P<0.05).





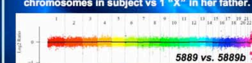


III. Molecular analysis

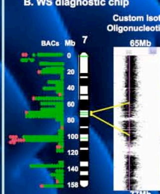
Breakpoint was determined using high resolution oligonucleotide arrays, confirmation by multicolor fluorescence hybridization with a panel of 45 BACs, somatic cell hybrids, and quantitative gene expression in LB cell lines using qRT-PCR of 12 WS genes.

1. Isothermal Oligonucleotide array

A. Whole genome chip shows sensitive quantitation of 2 "X" chromosomes in subject vs 1 "X" in her father.

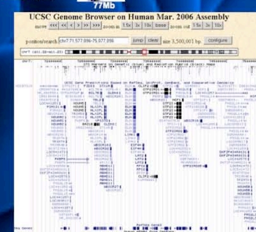


B. WS diagnostic chip



Custom Isothermal Oligonucleotide Array
- Array coverage on chromosome band 7q11.23
- Array > 285,000 Isothermal oligonucleotides
- Oligonucleotide size 45-85bp
- 1 oligonucleotide probe/100bp

(designed by JR Korenberg)

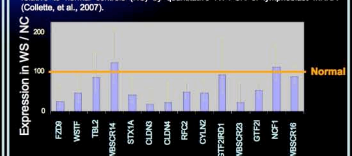


2. Southern blotting

Probe: STS3 of General Transcription Factor 2 (GTF2I), with diverse digestion enzyme. All lanes are as indicated. Arrow shows an extra band present in the patient's sample (STS3 break region). A: PstI, B: HindIII, C: PvuII.

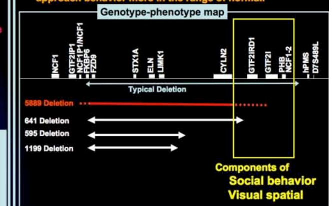
3. Gene expression level analysis

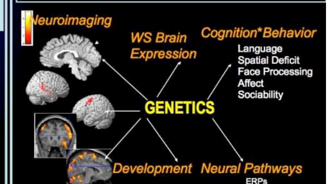
We showed that 14 WS genes have decreased expression in the WS population relative to normal controls (NC) by quantitative RT-PCR of lymphoblast mRNA (Collette, et al., 2007).



Summary

1. Multi-dimensional genetic, clinical, psychometric analysis of a large cohort of WS persons reveals 1-2% with smaller deletions.
2. Development of hierarchical high resolution genetic approaches reveal and validate an infant with WS and a small deletion.
3. Longitudinal psychometric and clinical analysis reveals visuospatial function above typical WS and social approach behavior more in the range of normal.





Conclusions

Integration of neurogenetic analyses of rare individuals provides powerful clues to the neurobiology of human social behavior. Our results suggest that variation in expression of genes distal to CYLN2, Gtf2IRD1, Gtf2I contribute to WS behavior as measured in #5889 and provide the basis to reason from molecular genetics and cellular detail to the brain networks modulating human social behavior.

The impact of rare human events studied at multidimensional levels should be considered in solving the neurobiology of human behavior.

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POSTER SYMPOSIUM: SOCIETY FOR NEUROSCIENCES, 2007

Symposium Title: GENES, NEURAL SYSTEMS, AND SOCIAL BEHAVIOR

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No. 696.2

GENETIC ORIGINS OF SOCIABILITY IN WILLIAMS SYNDROME

(J.R. Korenberg, U. Bellugi, X.N. Chen, l. Salandanani, T. Tirosh-Wagner, A. Galaburda, L. Reiss, D. Mills, & T. Doyle)

Genetic contributions to human cognition and behavior are intuitively clear but fiendishly difficult to define. The genetic deletion of about 25 genes in Williams syndrome (WS) provides a unique model to relate the role of single genes or gene clusters to the shift in social behavior described as hypersociability, increased gaze and attention to strangers (see Bellugi et al, this symposium). To narrow the gene(s) responsible for WS behavior, we initially established and applied genomic maps and reagents spanning the WS region to analyze a large cohort of families, to define a region commonly deleted. This provided the basis for establishing the variation in cognition and behavior typical of WS with full deletions. Rare individuals with smaller deletions provide the opportunity to ask whether a subset of genes may contribute to a subset of WS features. In the current work, we report genetic, developmental, physical, cognitive and behavioral data to show that an individual deleted for all of the typical WS genes except for the telomeric region, shows less hypersociability and less prolonged eye contact than typical WS. The individual was ascertained at 14 months as atypical for WS, and was studied through age 4 4/12 y, with normal developmental milestones but the small size and supravalvular aortic stenosis seen in WS. Genetic analyses included breakpoint determination using high resolution oligonucleotide arrays, confirmation by multicolor fluorescence hybridization with a panel of 45 BACs, somatic cell hybrids, and quantitative gene expression in LB cell lines using qRT-PCR of 12 WS genes. Deletion retained FKBP6-region distal to CYLN2. Social behavior was evaluated using the SISQ (Salk Institute Sociability Questionnaire) at 2 7/12 y, #5889 differed from WS ($p < 0.05$) and was in the normal range for Global Sociability and Approach Strangers. Further analyses utilized an computer based ethogram in which behavior was evaluated in a free play situation with a novel adult. In contrast to a WS cohort, #5889 was less likely to prolong eye contact and less likely to spend time close to the novel adult, and less likely to engage in social interaction. These results suggest genes distal to CYLN2 contribute to WS behavior as measured in #5889 and provide the basis to reason from molecular genetics and cellular detail to the brain networks modulating human social behavior. (See also other symposium submissions titled: Genes, Neural Systems, and Social Behavior). Supported in part by HD33113