11. Selenomethionyl RRF was purified and crystallized as described for wild-type *T. maritima* RRF [A. Kaji, in preparation; M. Selmer, S. Al-Karadaghi, G. Hirokawa, A. Kaji, A. Liljas, *Acta Crystallogr.* **D55**, 2049 (1999)] except that 5 mM dithiothreitol was used throughout purification and crystallization. Selenomethionine incorporation at all four positions was confirmed by electrospray mass spectroscopy (EMS). MAD data at the selenium edge were collected at BM14, European Synchrotron Radiation Facility (ESRF), France, on a MAR345 Image Plate detector. MAD data were processed with Mosflm [A. G. W. Leslie, *CCP4 ESF-EACMB Newslett. Protein Crystallogr.* **26** (1992)], SCALA, and TRUNCATE [CCP4, *Acta Crystallogr.* **D50**, 760 (1994)]. Three selenium sites were found with SHELXS-97 (Patterson superposition) [G. M. Sheldrick, *Methods Enzymol.* **276**, 628 (1997)] and SHELXC (dual-space methods) [G. M. Sheldrick, in *Direct Methods for Solving Macromolecular Structures*, S. Fortier, Ed. (Kluwer, Dordrecht, Netherlands, 1998), pp. 401–411] and were used to calculate phases to 4.0 Å with MLPHARE (CCP4). Solvent flattening and phase extension to 2.9 Å with DM (CCP4) improved the map and allowed tracing of 184 amino acid residues in the $p4_32_12$ map. Model building was performed with the program O [T. A. Jones, J.-Y. Zou, S. W. Cowan, M. Kjeldgaard, *Acta Crystallogr.* **A47**, 110 (1991)]. The model was refined with CNS [A. T. Brünger *et al.*, *Acta Crystallogr.* **D54**, 905 (1998)] against the 2.55 Å native data. The final model includes 184 protein residues, 2 acetate ions, and 65 water molecules. The current R factor and R_{free} (using 5% of the data) are 23.0 and 27.7%, respectively. The refined model has no residues in the disallowed region of the Ramachandran plot according to PROCHECK [R. A. Laskowski, M. V. MacArthur, D. S. Moss, J. M. Thornton, *J. Appl. Crystallogr.* **26**, 283 (1993)].
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Microglial Activation Resulting from CD40-CD40L Interaction After β-Amyloid Stimulation

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Alzheimer's disease (AD) has a substantial inflammatory component, and activated microglia may play a central role in neuronal degeneration. CD40 expression was increased on cultured microglia treated with freshly solublized amyloid-β (Aβ, 500 nanomolar) and on microglia from a transgenic murine model of AD (Tg APP_{sw}). Increased tumor necrosis factor α production and induction of neuronal injury occurred when Aβ-stimulated microglia were treated with CD40 ligand (CD40L). Microglia from Tg APP_{sw} mice deficient for CD40L demonstrated reduction in activation, suggesting that the CD40-CD40L interaction is necessary for Aβ-induced microglial activation. Finally, abnormal tau phosphorylation was reduced in Tg APP_{sw} animals deficient for CD40L, suggesting that the CD40-CD40L interaction is an early event in AD pathogenesis.

It has been suggested that Aβ activation of microglial cells may be involved in the inflammatory component of AD (*1*, *2*). However, the mechanisms of microglial activation by Aβ remain speculative. As CD40, an important receptor involved in cellular signaling and activation, plays a key role in inflammation (*3*), we investigated whether the interaction of CD40-CD40L with Aβ activates microglia. We used reverse transcriptase polymerase chain reaction (RT-PCR), protein immunoblot, and flow cytometry [fluorescence-activated cell sorting (FACS)] to determine whether Aβ could specifically induce CD40 expression on cultured microglial cells after treatment with a low dose (500 nM) of freshly solublized $Aβ_{1-40}$ or $Aβ_{1-42}$. The potentially pathogenic peptides $Aβ_{1-40}$ or $Aβ_{1-42}$ induced CD40 expression on cultured microglial cells when compared with Aβ-free, reverse Aβ ($Aβ_{40-1}$) or the 695 isoform of soluble amyloid precursor protein (sAPPα-695) (*4*, *5*) (Web fig. 1) (*6*). To see whether endogenous overexpression of Aβ could lead to microglial CD40 expression, we measured CD40 expression on microglia from a transgenic mouse model of AD (Tg APP_{sw}, overexpressing $Aβ_{1-40}$ and $Aβ_{1-42}$) (*7*) and control (wild-type) littermates (*4*). Microglia from Tg APP_{sw} newborn mice had markedly increased levels of soluble $Aβ_{1-40}$ compared with control littermates (*8*), and the CD40-expressing cell fraction was markedly increased in primary cultured microglia from Tg APP_{sw} mice compared with microglia from control littermates (Web fig. 2) (*6*). CD40 expression was increased in microglia from control littermates exposed to $Aβ_{1-42}$ and in Tg APP_{sw} microglia reexposed to exogenous $Aβ_{1-42}$ compared with those exposed to $Aβ_{40-1}$ or sAPPα-695 (Web fig. 2) (*6*). These data show that Aβ peptides specifically induce CD40 expression in primary cultured and N9 microglia.

To examine whether proinflammatory cytokines could regulate Aβ-dependent CD40 expression, we treated Aβ-challenged microglia with low doses of interleukin-1β (IL-1β), IL-2, IL-4, IL-6, IL-12, or interferon-γ (IFN-γ) (*4*), as the expression of CD40 has been shown to be variously regulated by these cytokines on human thymic epithelial cells, human endothelial cells, and keratinocytes (*9*). Only a low dose

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(10 U/ml) of IFN-γ synergistically enhanced Aβ-dependent CD40 expression on cultured microglial cells (Web fig. 3) (*6*). Mononuclear cells from AD patients secrete markedly higher amounts of IFN-γ compared with age-matched controls (*10*). Furthermore, increased amounts of IFN-γ have been shown to activate microglia after stimulation with Aβ (*1*). Our data suggest that IFN-γ may increase Aβ's effect on activation of microglia through induction of the CD40 receptor.

Activation of microglial cells results in an increase in tumor necrosis factor α (TNF-α) release (*1*, *11*), and high doses (>11 μM) of $Aβ_{1-42}$ are able to produce increased TNF-α production in microglial cells (*1*). Such doses of $Aβ_{1-42}$ rapidly produce large amounts of Aβ fibrils and loss of Aβ solubility in vitro (*12*). A much lower dose of freshly solubilized $Aβ_{1-42}$ (500 nM) did not induce TNF-α release from primary cultured microglia (*4*) (Web fig. 4) (*6*). However, when cultured microglia were pretreated with the same low dose of $Aβ_{1-42}$, addition of CD40L synergistically increased TNF-α release (Web fig. 4) (*6*). With the use of a monoclonal antibody to CD40, which extinguishes CD40L-induced TNF-α release in a dose-dependent manner, the CD40-CD40L interaction was attenuated in microglia, and TNF-α release was significantly reduced after $Aβ_{1-42}$ treatment (Web fig. 4) (*6*). This result indicates that CD40L mediates its effects through the CD40 receptor.

Having shown that Aβ peptides activate microglia through the CD40 pathway, we then investigated whether activation of Aβ-pretreated microglia by ligation of CD40 could mediate neuronal cell injury (*4*). Activated microglial cells (treated with $Aβ_{1-42}$ followed by CD40L immediately) promoted injury of primary cultured cortical neurons (Fig. 1). Exogenous TNF-α at similar concentrations as those produced by microglia activated with $Aβ_{1-42}$ and CD40L dose dependently induced neuronal injury and death as measured by lactate dehydrogenase (LDH) release assay (*13*), suggesting a mechanism by which the CD40-CD40L interaction is responsible for the effect. Blockade of CD40 with antibody to CD40 (anti-CD40) (1:200 dilution) significantly reduced neuronal cell injury (Fig. 1, B and C), showing that the interaction of CD40L with CD40 on Aβ-pretreated microglia is crucial for induction of neuronal injury.

To evaluate the possibility that Aβ might lead to CD40 pathway–mediated microglial activation in vivo, we crossed Tg APP_{sw} mice with mice deficient in CD40L (*14*) and measured TNF-α production in primary cultured microglial cells from these animals (*4*). Results show increased production of TNF-α mRNA and protein in microglia from Tg APP_{sw} mice compared with control littermates (Fig. 2). Furthermore, TNF-α production was significantly attenuated in Tg APP_{sw}/CD40L–deficient microglia compared with Tg APP_{sw} microglial cells (Fig. 2), suggesting that attenuation of the CD40-CD40L interaction results in reduced microglial activation in a transgenic model of AD. Moreover, rechallenge of Tg APP_{sw}/CD40L–deficient microglia with CD40L resulted in recovery of the Tg APP_{sw} phenotype (Fig. 2), further confirming the requirement of CD40 pathway stimulation in mediating Aβ's bioactive response.

Our data thus far show that Aβ initiates microglial activation through the CD40 pathway, an effect that was attenuated in Tg APP_{SW} mice deficient for CD40L. To assess the

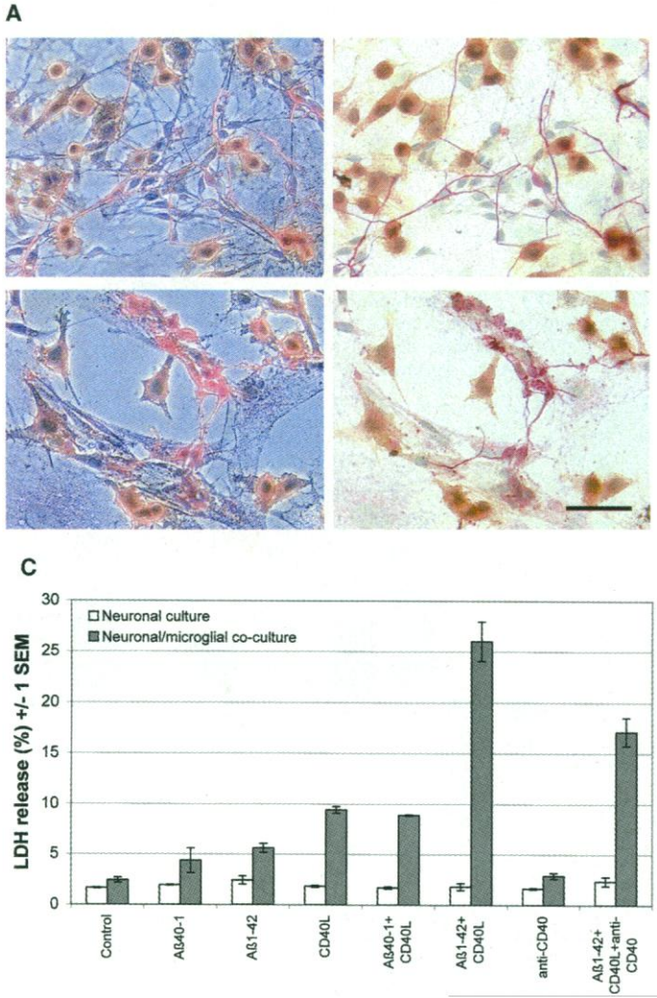


Fig. 1. Treatment of primary cultured microglia with $Aβ_{1-42}$ results in cortical neuron cell injury. (**A**) Micrographs showing phase contrast views before (top left) and after (bottom left) cotreatment with CD40L and $Aβ_{1-42}$. Bright-field micrographs of identical fields are also displayed before (top right) and after (bottom right) cotreatment. CD45-positive cells (brown) are microglia, whereas neurofilament L-staining cells (red) are neurons. Bar denotes 50 μm (calculated for each panel). Neuronal, but not microglial, degeneration is apparent only after cotreatment and is not detectable after treatment with CD40L or $Aβ_{1-42}$ alone. Results shown are from one of three independent experiments with similar results. (**B**) Primary cultured microglia-mediated neuronal cell injury is further evidenced by ^{51}Cr release assay. Data are reported as mean ^{51}Cr release values ± 1 SEM, and $n = 9$ for each condition presented. ANOVA showed main effects of effector:target ratio ($P < 0.001$), CD40L ($P < 0.001$), anti-CD40 ($P < 0.001$), and $Aβ_{1-42}$ ($P < 0.001$), but not for reverse $Aβ_{40-1}$ ($P = 0.752$). ANOVA also revealed an interactive term among $Aβ_{1-42}$, CD40L, and effector:target ratio ($P < 0.001$), indicating a ratio-dependent decrease in percentage of cell lysis as a result of the interaction between $Aβ_{1-42}$ and CD40L. Post hoc comparison showed a difference across ratios between $Aβ_{1-42}$ + CD40L and $Aβ_{1-42}$ + anti-CD40 + CD40L experimental conditions ($P < 0.001$), indicating an overall decrease in percentage of cell lysis as a result of addition of the anti-CD40. (**C**) LDH assay showing similar results to (B).

possibility that the Aβ-CD40-CD40L pathway might be an early stimulant of pathology associated with AD, we examined tau phosphorylation status in our transgenic mice, hyperphosphorylation of which is known to be associated with neurofibrillary tangles in AD brains and with AD-like pathology in transgenic models of the disease (*15*). Brain homogenates from Tg APP_{sw} mice deficient for CD40L demonstrated both a faster tau electrophoretic mobility (Fig. 3A) and a significant reduction in phospho-tau immunoreactivity at multiple sites (by protein immunoblotting) compared with those from Tg APP_{sw} mice alone (*4*) (Fig. 3, B to E). All of the animals in these experiments were 8 months old, an age before which significant Aβ deposition occurs in the Tg APP_{sw} mouse line used (*7*), suggesting that these events may occur early in the processes that mediate AD neuropathology.

We propose three possible scenarios for how microglial activation may occur in AD. In AD, it has been shown that activated microglia are colocalized with perivascular Aβ (*16*) and endothelial and smooth muscle cells constitutively express CD40L at low levels (*17*). The activation of perivascular microglia may result from an interaction between the smooth muscle or endothelial cell–derived CD40L and Aβ-dependent microglial CD40. Furthermore, perivascular microglial activation could contribute to the perivascular neuronal cell death that occurs in AD and the vascular damage associated with cerebral amyloid angiopathy that occurs in 83% of AD cases (*18*). T cell–associated antigens have been found in AD brains, and activated T cells have been found in AD brain tissue closely associated with reactive microglia (*19*). As activated $CD4^+$ T cells express CD40L, it is possible that activated T cells are able to provide CD40L for the activation of microglia. Finally, a recent study reported that activated platelets express CD40L (*20*), and activation of platelets is increased in AD patients (*21*). These findings raise the possibility that Aβ-induced CD40-expressing microglial cells might receive soluble CD40L from activated platelets.

Taken together, these data show that low doses of Aβ lead to microglial activation only after ligation of CD40. Previous findings have shown that Aβ can act directly on neurons to promote oxidative stress and increase their vulnerability to excitotoxicity and apoptosis. The present findings suggest that, in addition to such direct actions on neurons, Aβ may indirectly endanger neurons by inducing microglial responsiveness to CD40 ligand, resulting in the production of neurotoxic molecules by microglia. Noteworthy is the finding that Tg APP_{sw} mice do not demonstrate marked neuronal cell

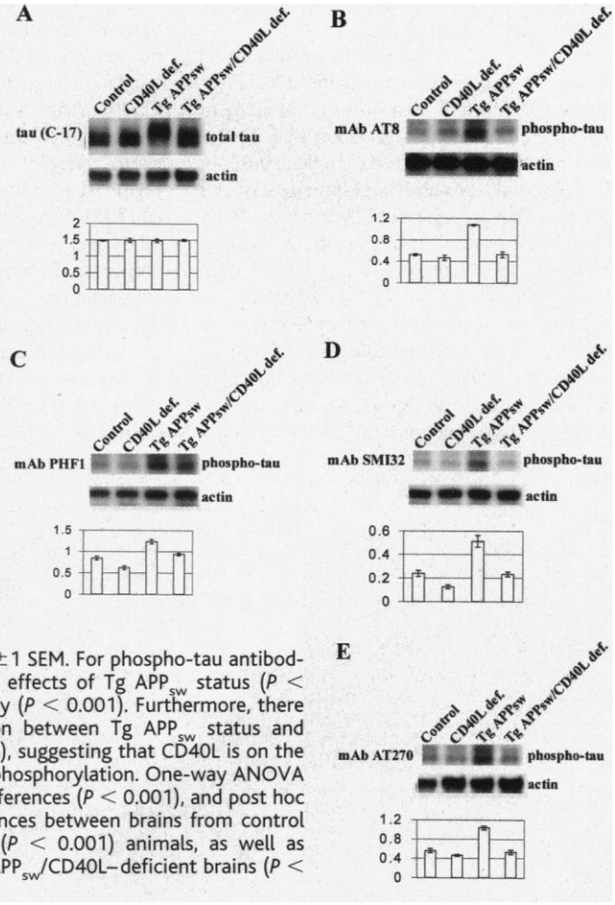
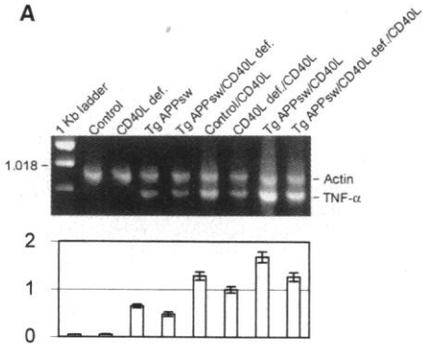


Fig. 3. Tau protein phosphorylation is significantly reduced in brains from Tg APP_{sw} mice deficient for CD40L. Protein immunoblotting showing (**A**) faster electrophoretic mobility of total tau (tau-1 and tau-2, region shown is from ~40 to 70 kD) and (**B** to **E**) decreased phospho-tau signal (region shown is from ~50 to 65 kD) in brain lysates from Tg APP_{sw}/CD40L–deficient mice compared with Tg APP_{sw} animals. Protein immunoblots for identical samples were probed with the indicated tau-specific antibodies (mAb is monoclonal antibody), stripped, and reprobed with actin antibody (top). The histogram represents the ratio of total or phospho-tau to actin signal (bottom), with $n = 3$ for each group ±1 SEM. For phospho-tau antibodies, ANOVA revealed main effects of Tg APP_{sw} status ($P < 0.001$) and CD40L deficiency ($P < 0.001$). Furthermore, there was a statistical interaction between Tg APP_{sw} status and CD40L deficiency ($P < 0.01$), suggesting that CD40L is on the mediating pathway to tau phosphorylation. One-way ANOVA revealed between-group differences ($P < 0.001$), and post hoc comparison showed differences between brains from control littermates and Tg APP_{sw} ($P < 0.001$) animals, as well as between Tg APP_{sw} and Tg APP_{sw}/CD40L–deficient brains ($P < 0.001$).



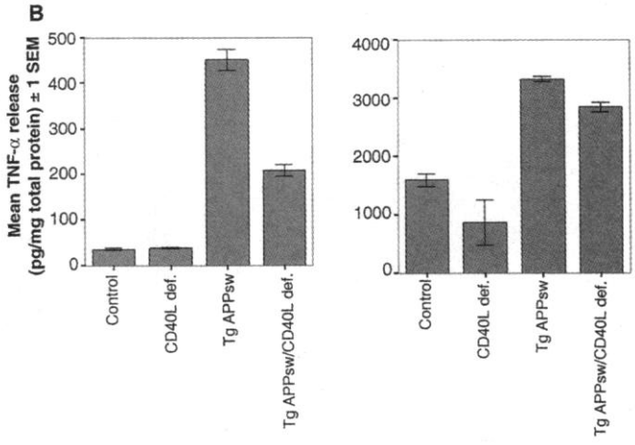


Fig. 2. Microglial activation is significantly reduced in primary cultured microglial cells from Tg APP_{sw}/CD40L–deficient mice. (**A**) RT-PCR showing increased expression of TNF-α mRNA in primary cultured microglia from Tg APP_{sw} mice before and after CD40 ligation. Histogram represents the ratio of the TNF-α signal to the actin signal; $n = 3$ for each condition ±1 SEM. CD40 ligation of Tg APP_{sw}/CD40L–deficient microglia raises TNF-α mRNA levels to near that of Tg APP_{sw} microglia. (**B**) TNF-α ELISA before (left) or after (right) CD40 ligation. In (A) and (B) before CD40 ligation, ANOVA revealed main effects of Tg APP_{sw} status ($P < 0.001$) and CD40L–deficient status ($P < 0.02$), as well as an interaction between these terms ($P < 0.02$), indicating interactive blockade of TNF-α release in Tg APP_{sw}/CD40L–deficient microglia. In (A) and (B) after CD40 ligation, ANOVA revealed main effects of microglia from Tg APP_{sw} ($P < 0.01$) and CD40L-deficient mice ($P < 0.02$), but no interaction between them ($P > 0.10$), indicating recovery of the Tg APP_{sw} phenotype in Tg APP_{sw}/CD40L–deficient microglia.

loss (7), as opposed to our in vitro data showing Aβ-induced, microglial CD40-mediated neuronal degeneration. This difference may be due to other cell types (including astroglia and cerebral vascular cells) and extracellular systems available in vivo in the central nervous system that are likely to mitigate the tendency to neuronal death induced by Aβ and CD40L. In our experimental models, the CD40-mediated neurotoxic pathway appears to be activated quite early in the pathogenic cascade, suggesting that therapeutic agents that block the CD40 signaling pathway may suppress neurodegeneration in AD.

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4. See supplementary data at www.sciencemag.org/feature/data/1041359.
5. All analyses were performed with SPSS for Windows, release 7.5.1. Data were analyzed with analysis of variance (ANOVA) followed by post hoc comparisons of means where appropriate with Bonferonni's method. Single mean comparisons were performed with the *t* test for independent samples. Alpha levels were set at 0.05 for each analysis.
6. Web figures are available at www.sciencemag.org/feature/data/1041359.
7. Tg APP_{sw} mice are the 2576 line crossed with C57B6/SJL as described [K. K. Hsiao *et al.*, *Science* **274**, 99 (1996); K. K. Hsiao *et al.*, *Neuron* **15**, 1203 (1995)]. To further characterize adult mice, we analyzed CD40 and TNF-α protein expression by protein immunoblot (densitometric signal ratio to actin) in brain lysates from 12-month-old Tg APP_{sw} or control littermates. CD40 and TNF-α protein levels were significantly ($P < 0.01$) increased in Tg APP_{sw} mice compared with control littermates (CD40, mean = 1.01 ± 0.03 SEM compared with 0.75 ± 0.03; TNF-α, 1.04 ± 0.01 compared with 0.81 ± 0.04).
8. Soluble $A\beta_{1-40}$ was quantified in the culture media of Tg APP_{sw} or control littermate–derived microglia 48 hours after plating with the Aβ40 enzyme-linked immunosorbent assay (ELISA) kit (QCB, Hopkinton, MA), in strict accordance with the manufacturer's instruction. Data showed a significant ($P < 0.001$) increase in soluble $A\beta_{1-40}$ from Tg APP_{sw} microglia (mean = 291.90 ± 21.81 SEM pg of $A\beta_{1-40}$ per milligram of total cellular protein) compared with control littermate–derived microglia (23.41 ± 7.75).
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Cognitive Modularity and Genetic Disorders

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This study challenges the use of adult neuropsychological models for explaining developmental disorders of genetic origin. When uneven cognitive profiles are found in childhood or adulthood, it is assumed that such phenotypic outcomes characterize infant starting states, and it has been claimed that modules subserving these abilities start out either intact or impaired. Findings from two experiments with infants with Williams syndrome (a phenotype selected to bolster innate modularity claims) indicate a within-syndrome double dissociation: For numerosity judgments, they do well in infancy but poorly in adulthood, whereas for language, they perform poorly in infancy but well in adulthood. The theoretical and clinical implications of these results could lead to a shift in focus for studies of genetic disorders.

Many theorists use the adult neuropsychological model of impaired and intact cognitive modules to explain genetic disorders. Adults whose brains have developed normally but who later suffer brain damage are often left with a pattern of dissociations between different cognitive modules. They may function normally in several domains but, for instance, display impaired performance solely in the domain of face processing or in the domain of syntax. This leads theorists to claim that human (adult) cognition is characterized by independently functioning modules that subserve different domains and can be differentially impaired. When similar dissociations are found in children with genetic disorders, the use of the same adult model is either explicitly recommended (*1, 2*) or implicit in the argumentation from an uneven performance profile to underlying brain structure (*3–5*). It is also assumed that if the phenotypic outcome in a genetic disorder presents an uneven cognitive profile, then this will also characterize the initial state in infancy. In other words, it is claimed that atypically developing children start out with a fractionated pattern of impaired and intact modules. This claim is then used to bolster theoretical arguments in favor of the prespecified modular structure of the human mind/brain.

A syndrome that has attracted much attention in this respect is Williams syndrome (WS). WS is a rare neurodevelopmental disorder caused by a submicroscopic deletion on chromosome 7q11.23. Although a number of genes have now been identified in the deleted region, original claims as to their cognitive function in WS (*6*) have been challenged by our more recent work (*7*). WS occurs in 1 in 20,000 live births. Its clinical features include several physical abnormalities accompanied by mild to moderate mental retardation and a specific personality profile. The interest in WS among cognitive neuroscientists stems from the very uneven cognitive profile displayed in the phenotypic outcome (*8*). Many theorists have used this uneven pattern of abilities and impairments in WS to support claims about the existence of innately specified cognitive modules, some of which are spared (language and face processing) and others impaired (number and visuospatial processing) (*4, 5*). This view tends to ignore the dynamics of development [see (*8*) for discussion]. With the use of new data from infants, we argue that one cannot assume that the infant starting state can be directly inferred from the phenotypic outcome.

To assess our hypotheses and to explore the infant origins of the cognitive outcome in WS, we specifically chose two cognitive domains with different phenotypic end states. By middle childhood or adulthood, individuals with WS

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