Exploring Causal Links in the Gut-Brain Axis: A Mendelian Randomization Study of Gut Microbiota, Metabolites, and Cognition

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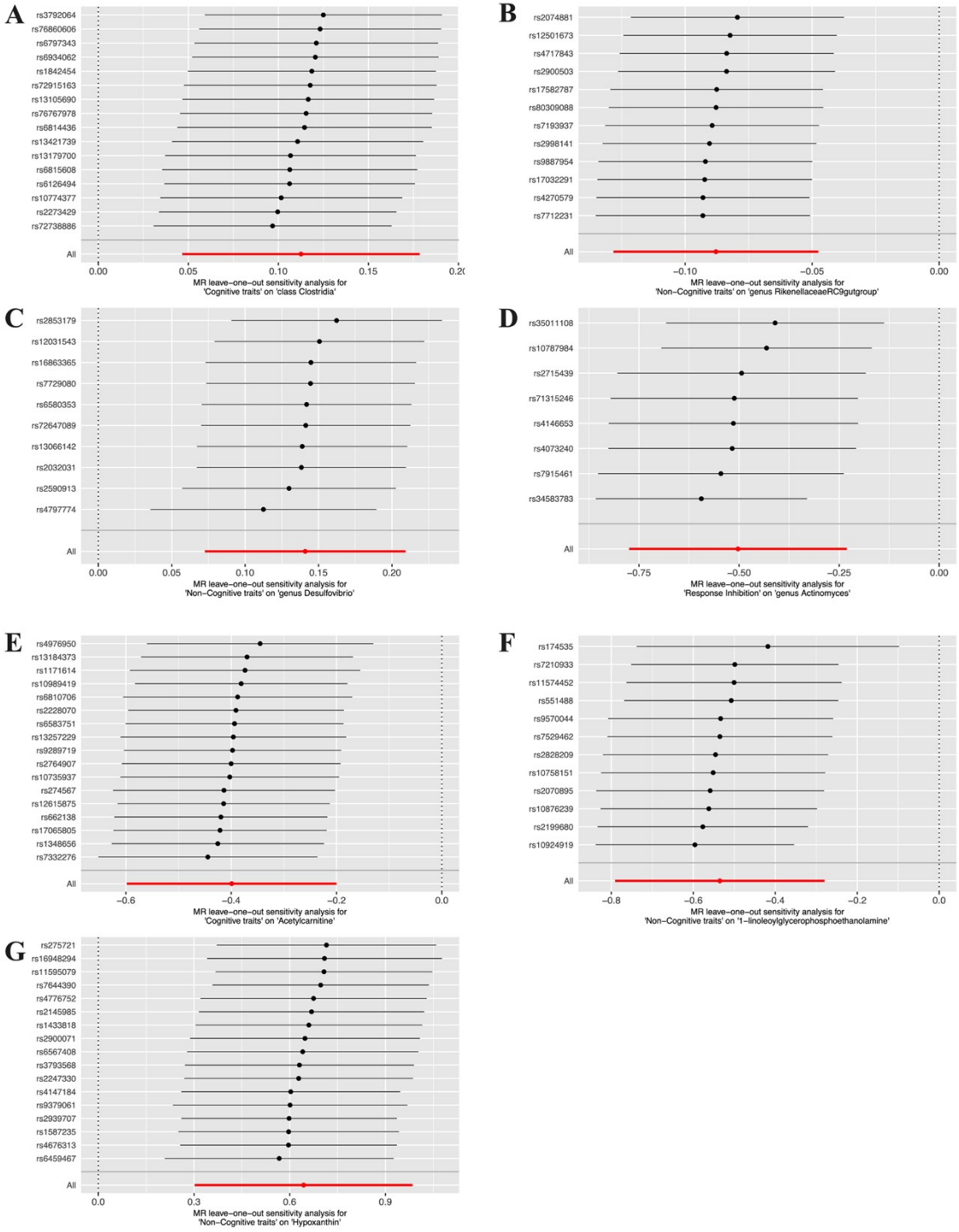

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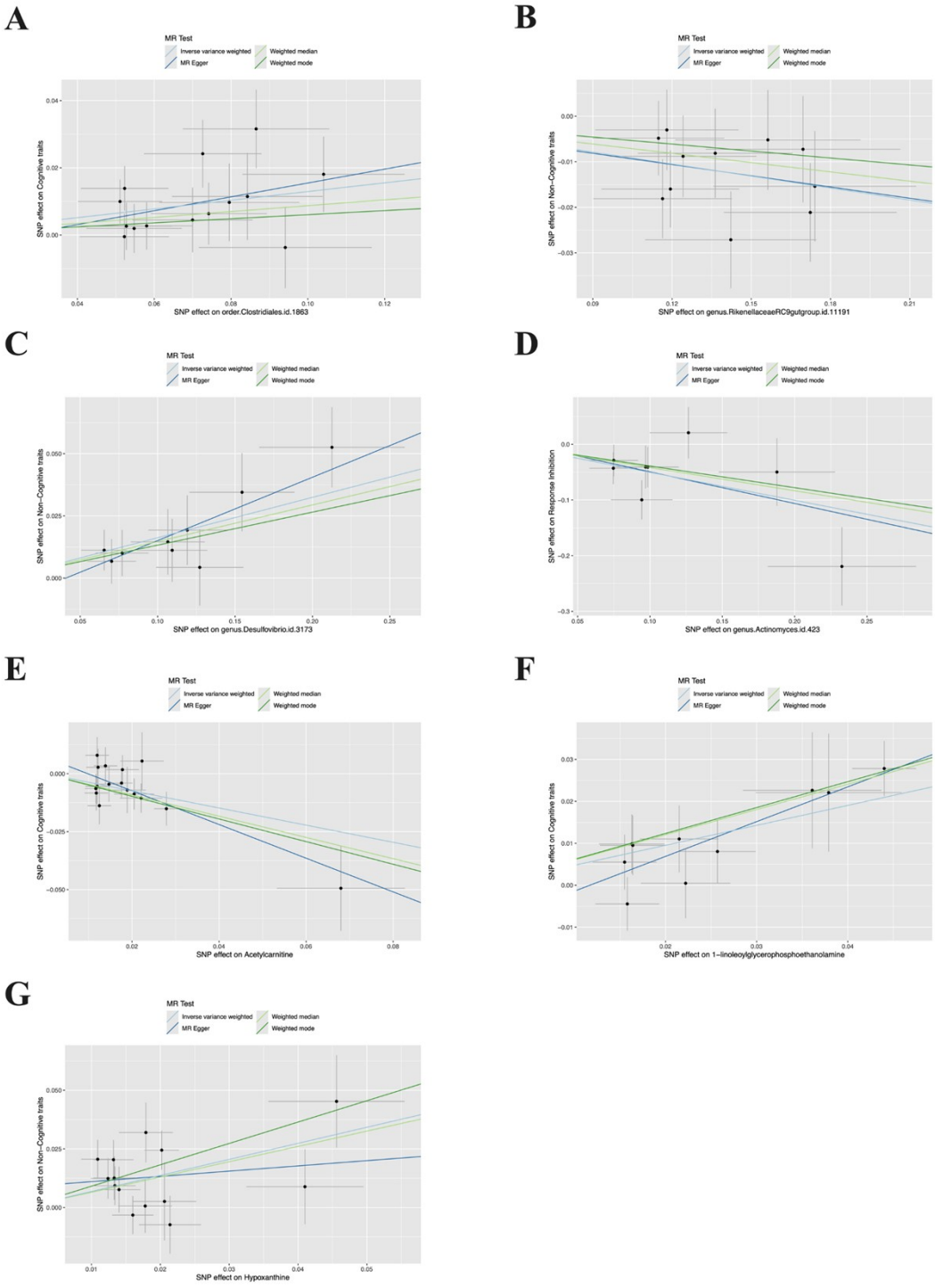

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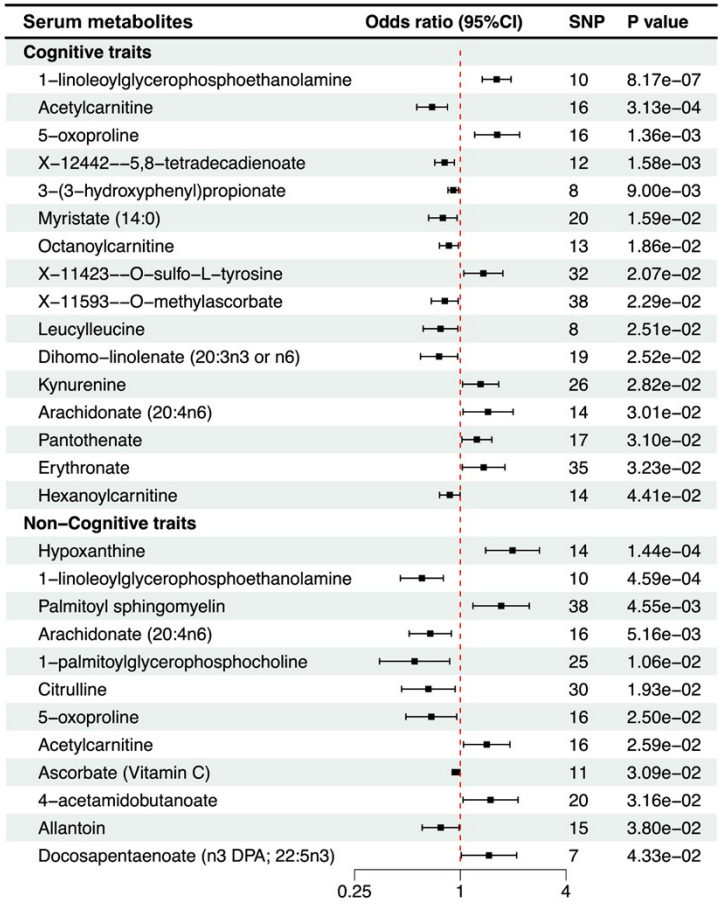
A



B

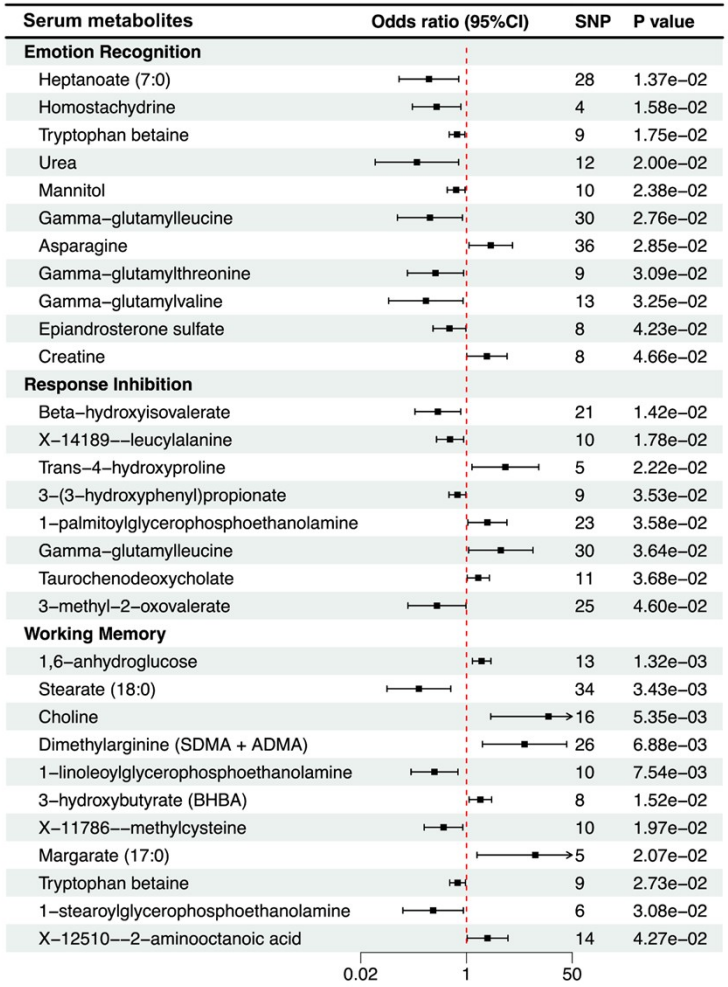
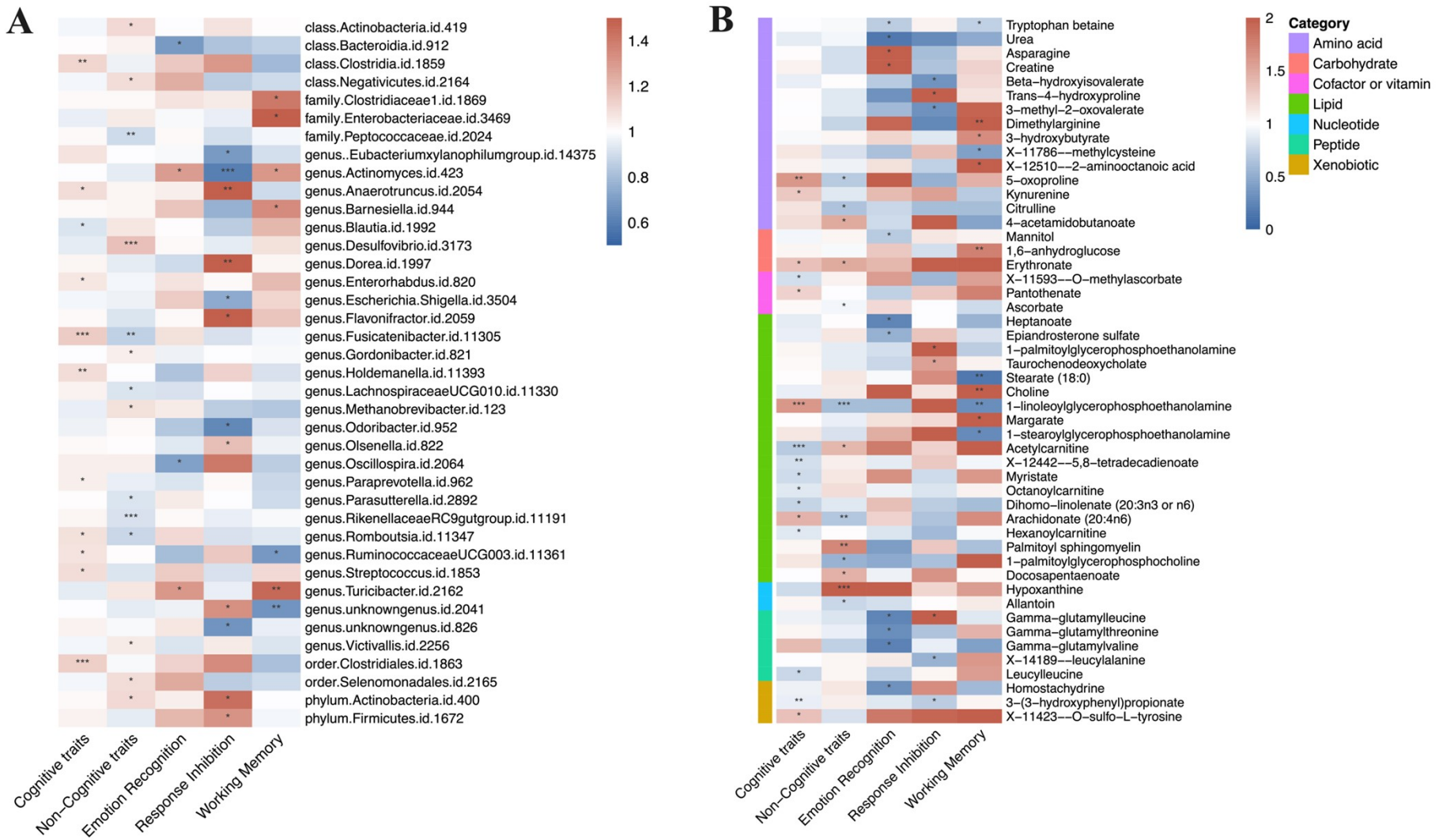


Figure S4 IVW MR associations of gut microbiota (A) and metabolites (B) on different cognitive phenotypes. (Exposure has suggestive causal associations with at least one cognitive feature, IVW<0.05). Significance label was based on IVW results (***: P<0.001, **: P<0.01, *: P<0.05)



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* Detailed results pertaining to Tables S3-S5 are presented in the accompanying "Supplementary material - Table S3-5" file due to the extensive data.

Table S1. The measurement methods of hot and cold cognition

Cognitive measurement	Details
Working Memory	Working memory, the ability to temporarily store and manipulate information, was assessed using the N-back task (two-back condition), a widely used measure (Kirchner, 1958; Meule, 2017; Gajewski et al., 2018; Slaney et al., 2023). In each trial, a number is briefly presented (500 ms), and participants must report whether it matches the number presented two trials earlier. This task comprises 48 trials without feedback (8 of which are matches), preceded by 12 practice trials with feedback. The primary outcome measure was the discriminability index (d'), an overall performance estimate where a higher d' indicates better working memory. Of the 3312 participants assessed with cognitive tasks at age 24, 182 did not provide any data, and 70 were omitted due to negative d' scores and/or not responding to more than 50% of the trials, resulting in a final sample of 3242 (mean = 2.75, SD = 0.81). Individuals who did not respond on more than 50% of trials or had a negative d' were excluded from the analyses (N = 78).
Emotion recognition	Emotion recognition, the ability to identify emotion expressions, was assessed using a six-alternative forced choice (6AFC) emotion recognition task (Penton-Voak et al., 2012). This task, comprising 96 trials (16 for each emotion), measures the ability to identify emotions in facial expressions. In each trial, a face displaying one of six basic emotions (happiness, sadness, anger, fear, disgust, or surprise) is briefly presented (200 ms) and then immediately covered up. Participants then report which emotion was displayed using six labels. Each emotion is presented at eight levels of intensity. The primary outcome measure was the number of facial emotions accurately identified (hits), with a higher score indicating better emotion recognition. Emotion recognition data were available for 3368 participants (mean = 0.69, SD = 0.08).
Response inhibition	Response inhibition, the ability to suppress a prepotent response, was assessed using the Stop-Signal Task (Logan et al., 1984). The task comprised 256 trials, with a 4:1 ratio of trials without stop signals to those with stop signals. In each trial, participants were shown a letter (X or O) for 1,000 ms and asked to identify it as quickly as possible. On 25% of the trials, a stop signal (a tone) was presented after the letter, requiring participants to inhibit their response. Mean response times were calculated, and the primary outcome measure was the stop-signal reaction time (SSRT), a reliable indicator of inhibitory control. Shorter SSRTs signify faster inhibition. SSRT data were available for 3201 participants (mean = 258.60, SD = 53.19).

Table S2. Detailed information on gut microbiota, serum metabolites, and cognitive phenotype genome-wide association studies (GWAS) data.

Trait		Sample Size	Population	Data source (PMID/DOI)
Gut microbiome	9 Phyla 16 Classes 20 Orders 35 Families 131 Genera	18340	European (16 cohorts, N = 13,266), Middle Eastern (1 cohort, N = 481), East Asian (1 cohort, N = 811), American Hispanic/Latin (1 cohort N = 1097), African American (1 cohort, N = 114), multi-ancestry (4 cohorts, N = 2571)	MiBioGen consortium: www.mibiogen.org (PMID:33462485)
Serum Metabolites	452 metabolites	7824	European (German, British)	IEU open GWAS (ID: met-a-303 ~ met-a-754) (PMID: 24816252)
Cognitive traits	/	257700	European ancestry individuals	GWAS catalog: GCST90011875 (PMID: 33414549)
Non-cognitive traits	/	510795	European ancestry individuals	GWAS catalog: GCST90011874 (PMID: 33414549)
Specific cognitive traits	Working memory	2471	European (United Kingdom)	Avon Longitudinal Study of Parents and Children (DOI: 10.5523/bris.2ux5exb501kds2pq7wv8o6dv85)
	Response inhibition	2446	European (United Kingdom)	Avon Longitudinal Study of Parents and Children (DOI: 10.5523/bris.2nu9kzcjeyuu72hvi24968pe66)
	Emotion recognition	2560	European (United Kingdom)	Avon Longitudinal Study of Parents and Children (DOI: 10.5523/bris.2774f89r0hf0a2t76atdeu753c)

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