

# Genetic polymorphisms influence children's susceptibility to organophosphates

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# NEUROTOXIC EFFECTS OF LOW-LEVEL LONG-TERM EXPOSURE TO ORGANOPHOSPHATES

- a. Past (1980's): Epidemiologic studies on effects of everyday low-level exposures in workers and residents
- b. Re- examination of the same adult cohort with the same and newer methods
- c. Examination of the offspring with same and newer methods

**Kiryat Shmona**

**Sde Nechemia**

Map

Satellite

**Amir**

**Kfar Blum**

**Shamir**

**Neot Mordechai**

**The Jordan River**



Google

2 mi  
2 km

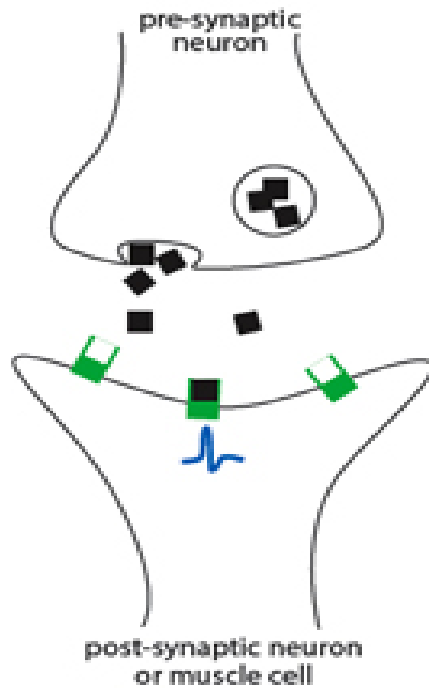
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# BACKGROUND

Our previous studies showed the neurobehavioral effects of repeated annual seasonal exposures to organophosphorous (OP) pesticides in children in kibbutz communities in Hula Valley.

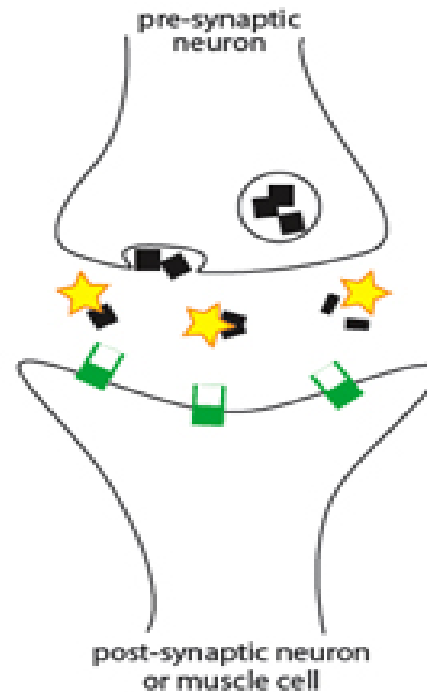
# BACKGROUND

## Acetylcholine signaling at synapse



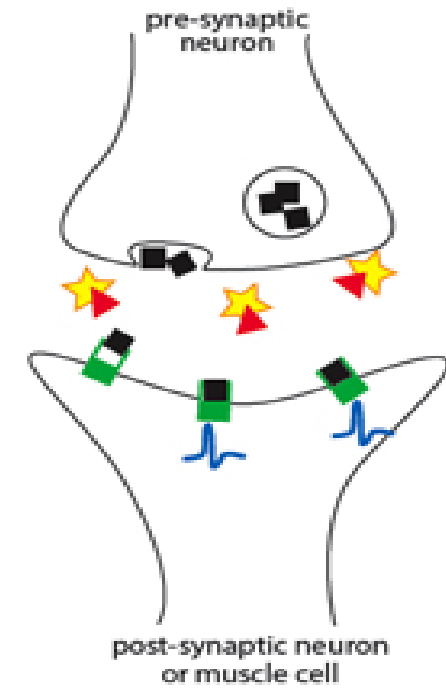
- Acetylcholine (ACh)
- U ACh Receptor
- ⚡ Signal transmission

## ACh Esterase STOPS signaling process



- ACh
- U ACh Receptor
- ⚡ Signal transmission
- ★ ACh Esterase

## OP's inhibit ACh Esterase



- ACh
- U ACh Receptor
- ⚡ Signal transmission
- ★ ACh Esterase
- ▲ Organophosphate pesticide (OP)

# BACKGROUND:

Mutations in PON1 gene are expressed in Paraoxonase activity variants which may affect several phenotypes as well as the susceptibility to OP neural effects.

Genetic polymorphisms may be observed in SNP`s (Single Nucleotide Polymorphisms) located on the gene encoding PON1 enzyme or its promoter.

# RESEARCH OBJECTIVES :

To estimate relationship between PON1 genetic outcomes of and performance of cognitive tasks in a group of 8-12 years-old children in Hula Valley.

## GROUP STUDIED

8-12 years-old schoolchildren in kibbutzim  
“second generation” in families with 30-50 y  
in exposed settings.



## Schoolchildren (N=96) studied: two sub-groups:

- Children (N=51) who reside and attend school in Hula valley.
- Children (N=45) residing in hills around Hula valley and attend school in valley.

Comparison group (N=40) included age- and gender-matched children residing in a different kibbutz in Jordan Rift Valley in which use of pesticides has been minimal (“organic agriculture”) for decades.

# The Jordan Rift Valley

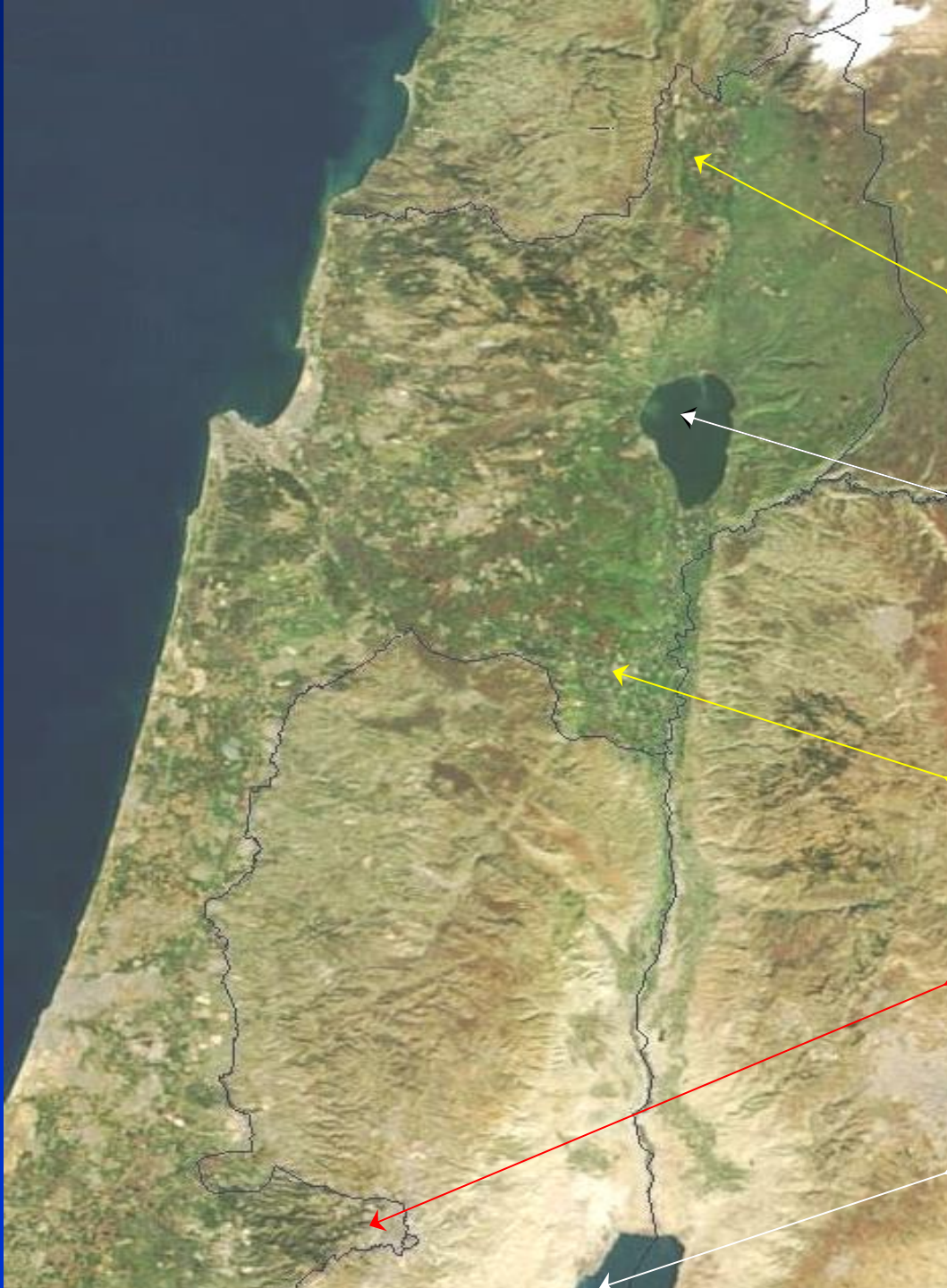
Hula Valley

Sea of Galilee

Beit Shean Valley

Jerusalem

Dead Sea



# Cognitive Tests for Executive Skills

- **Trail Making Tests A and B** for screening, attention and graphomotor ability
- **Digit Span Test (forward and backward)** for auditory memory involving attention (WISC-III)
- **Digit Symbol Test** for eye-hand coordination in new learning processes (WISC-III)
- **Arithmetic Test** (WISC-III)
- **Bender–Gestalt Test** for visual-motor Gestalt
- **Digit Cancellation Test** as a measure of short-term memory and reaction time
- **Diamond Test** for screening and attention ability
- **Rapid Automated Naming** for reading competence
- **Purdue Pegboard** for manual dexterity

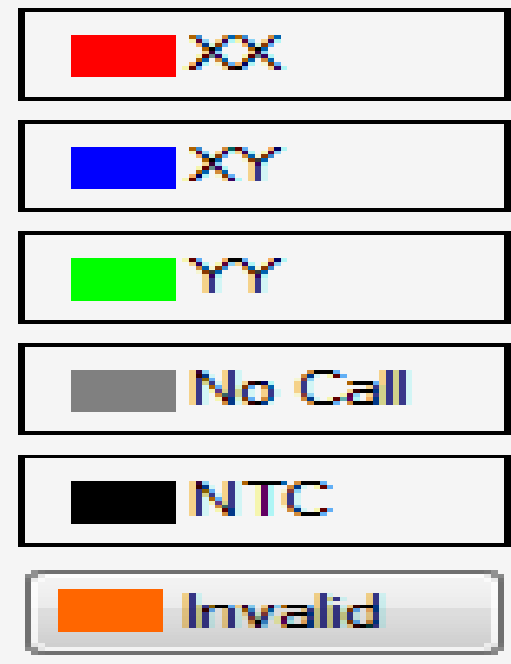
# Genetic analysis

- Saliva samples were collected and extracted using Oragene DNA according to the manufacturer's protocol.
- SNP genotype (TaqMan) was tested for 17 known genomic alterations in the PON-1 gene and screened using Nano-Fluidic Chip 96.96CS Genotyping Dynamic Array IFC and BioMark™ HD System (Fluidigm).
- SNP genotyping was performed according to the manufacturer's protocol and analyzed by Fluidigm Analysis Software V.3

# Fluidigm Arrays - raw results

Rows: SNP Assays  
Lines: Samples

Genotypes:



Chip #1	
Sample	Assay
1	15: BRCA1 rs26889C
2	25: POINT1102V
3	85: POINT1102V
4	85: POINT1102V
5	74: POINT1102V
6	74: POINT1102V
7	74: POINT1102V
8	74: POINT1102V
9	74: POINT1102V
10	74: POINT1102V
11	74: POINT1102V
12	74: POINT1102V
13	74: POINT1102V
14	74: POINT1102V
15	74: POINT1102V
16	74: POINT1102V
17	74: POINT1102V
18	74: POINT1102V
19	74: POINT1102V
20	74: POINT1102V
21	74: POINT1102V
22	74: POINT1102V
23	74: POINT1102V
24	74: POINT1102V
25	74: POINT1102V
26	74: POINT1102V
27	74: POINT1102V
28	74: POINT1102V
29	74: POINT1102V
30	74: POINT1102V
31	74: POINT1102V
32	74: POINT1102V
33	74: POINT1102V
34	74: POINT1102V
35	74: POINT1102V
36	74: POINT1102V
37	74: POINT1102V
38	74: POINT1102V
39	74: POINT1102V
40	74: POINT1102V
41	74: POINT1102V
42	74: POINT1102V
43	74: POINT1102V
44	74: POINT1102V
45	74: POINT1102V
46	74: POINT1102V
47	74: POINT1102V
48	74: POINT1102V
49	74: POINT1102V
50	74: POINT1102V
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80	74: POINT1102V
81	74: POINT1102V
82	74: POINT1102V
83	74: POINT1102V
84	74: POINT1102V
85	74: POINT1102V
86	74: POINT1102V
87	74: POINT1102V
88	74: POINT1102V
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91	74: POINT1102V
92	74: POINT1102V
93	74: POINT1102V
94	74: POINT1102V
95	74: POINT1102V
96	74: POINT1102V
97	74: POINT1102V
98	74: POINT1102V
99	74: POINT1102V
100	74: POINT1102V

Chip #2	
Sample	Assay
1	15: BRCA1 rs26889C
2	25: POINT1102V
3	85: POINT1102V
4	85: POINT1102V
5	74: POINT1102V
6	74: POINT1102V
7	74: POINT1102V
8	74: POINT1102V
9	74: POINT1102V
10	74: POINT1102V
11	74: POINT1102V
12	74: POINT1102V
13	74: POINT1102V
14	74: POINT1102V
15	74: POINT1102V
16	74: POINT1102V
17	74: POINT1102V
18	74: POINT1102V
19	74: POINT1102V
20	74: POINT1102V
21	74: POINT1102V
22	74: POINT1102V
23	74: POINT1102V
24	74: POINT1102V
25	74: POINT1102V
26	74: POINT1102V
27	74: POINT1102V
28	74: POINT1102V
29	74: POINT1102V
30	74: POINT1102V
31	74: POINT1102V
32	74: POINT1102V
33	74: POINT1102V
34	74: POINT1102V
35	74: POINT1102V
36	74: POINT1102V
37	74: POINT1102V
38	74: POINT1102V
39	74: POINT1102V
40	74: POINT1102V
41	74: POINT1102V
42	74: POINT1102V
43	74: POINT1102V
44	74: POINT1102V
45	74: POINT1102V
46	74: POINT1102V
47	74: POINT1102V
48	74: POINT1102V
49	74: POINT1102V
50	74: POINT1102V
51	74: POINT1102V
52	74: POINT1102V
53	74: POINT1102V
54	74: POINT1102V
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57	74: POINT1102V
58	74: POINT1102V
59	74: POINT1102V
60	74: POINT1102V
61	74: POINT1102V
62	74: POINT1102V
63	74: POINT1102V
64	74: POINT1102V
65	74: POINT1102V
66	74: POINT1102V
67	74: POINT1102V
68	74: POINT1102V
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73	74: POINT1102V
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75	74: POINT1102V
76	74: POINT1102V
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78	74: POINT1102V
79	74: POINT1102V
80	74: POINT1102V
81	74: POINT1102V
82	74: POINT1102V
83	74: POINT1102V
84	74: POINT1102V
85	74: POINT1102V
86	74: POINT1102V
87	74: POINT1102V
88	74: POINT1102V
89	74: POINT1102V
90	74: POINT1102V
91	74: POINT1102V
92	74: POINT1102V
93	74: POINT1102V
94	74: POINT1102V
95	74: POINT1102V
96	74: POINT1102V
97	74: POINT1102V
98	74: POINT1102V
99	74: POINT1102V
100	74: POINT1102V

Chip #3	
Sample	Assay
01	101
13	102
25	103
37	104
48	105
61	106
73	107
85	108
02	109
14	110
26	111
38	112
21	113B
50	114
62	115
74	116
86	120
03	128
45	137
15	201
27	202
39	203
51	205
33	205B
63	206
75	208
82	21102
87	301
04	302
16	304
28	305
09	306
52	307
64	308
76	308
83	310
05	312
17	401
29	402
41	403
53	404
65	405
77	406
89	407
06	410
18	411
30	412
42	501
54	502
66	503
78	505
90	506
07	507
19	508
31	508
43	510
55	511
67	513
79	514
91	515
08	516
20	517
32	518
44	519
56	520
68	521
80	522
57	6403
69	7301
81	7302
40	NTC

# RESULTS

Association was found between the **codon region L55M** and the performance of **Trail Making B** and **Digit Cancellation B Tests**:

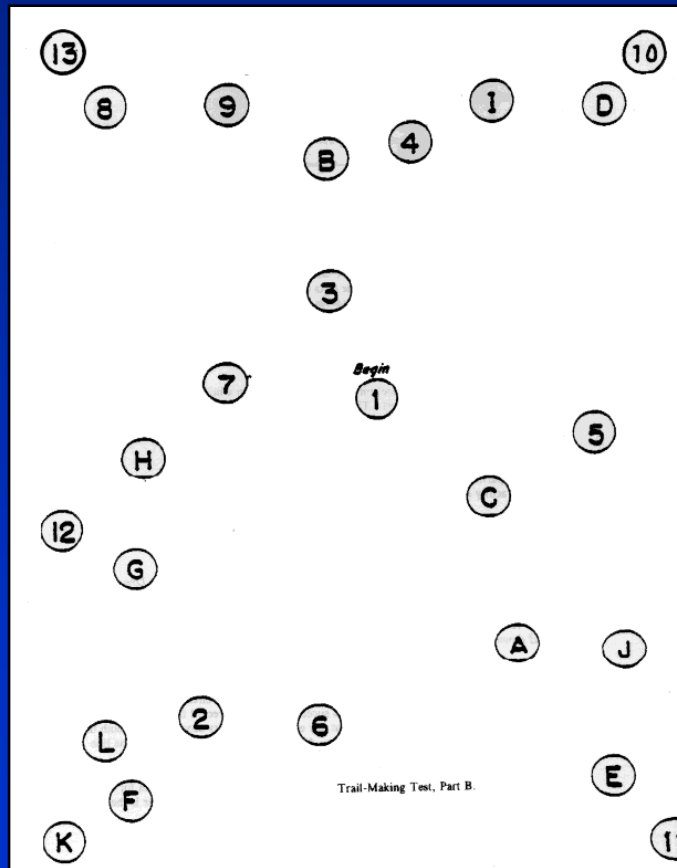
The individuals with the heterozygote variant **A:T** scored the least in both tests.

The individuals with the homozygote variant **A:A** obtained the highest scores in **Digit Cancellation B Test**.

Both homozygote variants **A:A** and **T:T** obtained similar scores in **Trail Making B Test**.

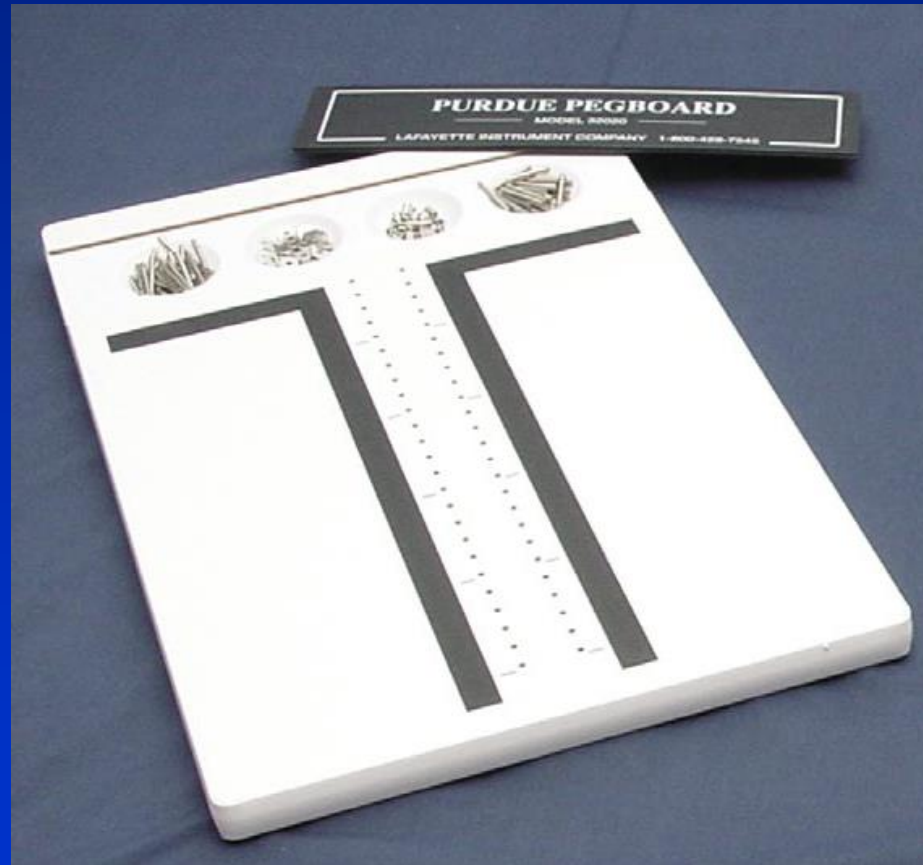
# Trail Making B Test

for screening, attention and graphomotor ability



# Purdue Pegboard Test

for manual dexterity





# RESULTS

The performance of **Purdue Pegboard Test** for manual **dexterity** was associated with **SNP** located at **G909C**:

The individuals with the heterozygote variant **C:G** scored the least in Purdue Pegboard Test of manual dexterity comprising all its sub-tests –Rt. hand, Lt. hand and both hands.

All these associations were standardized to different levels of exposure to OP drift.

# CONCLUSIONS

Several PON1 polymorphisms are associated with the cognitive abilities of manual dexterity and executive skills: first and foremost in the multi-channel attention span, visual scanning, task switching and execution speed.

There may be environmental-susceptibility interactions and epigenetic effects in children with low-level endemic exposure to OP drift.

# Current Paradigm:

A **genetic** factor may influence children's susceptibility to organophosphates.

**PON-1 gene polymorphisms** might reflect **genetic susceptibility** of children to effects of long-term low-level exposure to organophosphates.

# **This study was supported by:**

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**The Chief Scientist, Israel Ministry of  
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